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WNT/FZD6 SIGNALING IN HEMATOPOIETIC STEM CELL EMERGENCY RESPONSE

Par

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RÉSUMÉ

Les cellules souches hématopoïétiques (CSH) sont chargées de produire tous les types de cellules sanguines tout au long de la vie de l'organisme. Les CSH sont maintenues dans la moëlle osseuse à l'état de repos dans un microenvironnement spécial nommé niche. Cette niche régule la réponse des CSH aux changements environnementaux. Un récepteur de la famille des Frizzled (Fzd), Fzd6, est exprimé sur les CSH et leurs descendants. Nous avons déjà démontré que Fzd6 joue un rôle important en régulant l'expansion des CSH et leur maintien à long terme. Nous voulons maintenant mieux comprendre les mécanismes par lesquels Fzd6 régule les CSH pendant l'hématopoïèse en situation de stress induite par lipopolysaccharide (LPS). Nous observons une fréquence significativement plus élevée de cellules de type CSH chez les souris WT comparativement aux souris Fzd6 KO. Cependant, il y a moins de CSH KO au stade G0 du cycle cellulaire, suggérant qu'elles sont plus fortement activées que les CSH WT. Lorsque les cellules WT sont transplantées dans un environnement KO, elles réagissent au LPS au moins aussi fortement que dans un environnement WT. De plus, leur capacité à reconstituer un hôte secondaire n'est pas affectée. Ces résultats montrent que le défaut dans l'expansion des CSH Fzd6 KO est intrinsèque aux cellules hématopoïétiques et qu'il n'est pas dû à une réponse inefficace au LPS. Nous montrons également que la sortie des CSH Fzd6 KO de la moëlle osseuse vers le sang périphérique est altérée à la suite de l'injection de G-CSF et nous avons identifié MIP1α, qui est produit par les macrophages, comme un candidat prometteur responsable de la suppression des CSH KO. Ces études suggèrent que Fzd6 a un effet protecteur dans la moëlle osseuse et pourrait aider à prévenir une suractivation de la réponse inflammatoire dans un contexte d'infection systémique.

ABSTRACT

Hematopoietic stem cells (HSCs) are in charge of the production of all blood cell types throughout the organism's lifetime. HSCs are maintained in the bone marrow in a quiescent state in a special microenvironment called niche that regulates the HSCs response to environmental changes. The Frizzled (Fzd) family receptor Fzd6 is expressed on HSCs and their downstream progeny. We have previously shown that Fzd6 plays a pivotal role in regulating HSC expansion and long-term maintenance. Now we sought to better understand the mechanisms by which Fzd6 regulates HSCs during LPS-induced stress hematopoiesis. We observed a significantly higher frequency of HSC-like cells in WT mice as compared to Fzd6 KO mice. However, fewer KO HSCs were found in the G0 stage of cell cycle, suggesting that they were more strongly activated. Also, when WT cells were transplanted in a KO environment, they responded to LPS at least as strongly as in WT environment. Moreover, their ability to reconstitute a secondary host was not impaired. These results show that the defect in Fzd6 KO HSC expansion is intrinsic to hematopoietic cells and is not due to an inefficient response to LPS. We further report that Fzd6 KO HSCs show impaired egress from bone marrow to peripheral blood after G-CSF administration and identify macrophage-derived MIP1α as a promising candidate responsible for KO HSC suppression. These studies suggest that Fzd6 has a protective effect in the bone marrow and may help prevent an overactivation of the inflammatory response in the context of systemic infections.

RÉSUMÉ RÉCAPITULATIF

INTRODUCTION

Le sang est un composant essentiel de la vie. Il est composé de nombreux types de cellules générées dans la MO à partir des cellules souches hématopoïétiques (CSH) et qui circulent dans les veines et les artères jusqu'aux organes périphériques pour devenir des cellules spécialisées. Chaque jour, plus de 100 milliards (Boulais *et al.*, 2015) de cellules sanguines matures sont produites par le système hématopoïétique pour remplir de nombreuses fonctions importantes, telles que le transport de l'oxygène dans les organes périphériques, la surveillance des agents pathogènes étrangers et la régénération des organes.

À l'état basal, les CSH à long terme (CSH-LT) se différencient séquentiellement en CSH à court terme (CSH-CT) et en progéniteurs multipotents (MPP) qui ont une capacité d'auto-renouvellement limitée (Figure 1.1). Toutefois, le potentiel des MPP de se différencier en tous les types de cellules sanguines reste inchangé. Les MPP génèrent des progéniteurs lymphoïdes multipotents (LMPP). Ensemble, ces quatre types de cellules forment la population de cellules souches/progénitrices hématopoïétiques (CSPH), qui sont identifiées par la combinaison de marqueurs $Lin^-(B220^-CD3e^-CD11b^-GR1^-Ter119^-)$ $Sca1^+cKit^+$ (LSK) chez la souris. Les LMPP sont définis comme étant des LSK $Flt3^+$ et représentent jusqu'à 25% des LSK (Adolfsson *et al.*, 2005). Ils ont le potentiel de produire les lignées lymphoïdes et myéloïdes, mais ne produiront pas de mégakaryocytes ou de cellules érythroïdes (Adolfsson *et al.*, 2005, Kohn *et al.*, 2012). Les MPP2 et les MPP3 sont caractérisés comme étant des sous-populations des LSK $Flt3^-$ et les LMPP sont aussi nommés MPP4 (Wilson *et al.*, 2008). Les LMPP/MPP4 incluent les progéniteurs lymphoïdes précoces $Rag1^+$ (Igarashi *et al.*, 2002), qui migrent de la moëlle osseuse au thymus pour établir une population de *early thymocyte progenitor* (ETP) qui ont le potentiel de produire un grand nombre de lymphocytes T, mais qui ont une capacité restreinte de produire des cellules *Natural killer* (NK), des cellules dendritiques (DC) et des cellules B (Bell *et al.*, 2008). Les progéniteurs lymphoïdes communs (CLP) génèrent des cellules B, des NK et des DC dans la moëlle osseuse (Kondo *et al.*, 1997, Mansson *et al.*, 2010). Les CLP pourraient aussi avoir le potentiel de se différencier en lymphocytes T. En aval des MPP2/MPP3 se trouvent les progéniteurs myéloïdes communs (CMP) (Akashi *et al.*, 2000, Manz *et al.*, 2002), qui donnent naissance à des progéniteurs de granulocytes et macrophages (GMP) et à des progéniteurs de mégakaryocytes et érythrocytes (MEP) (Nakorn *et al.*, 2003,

Pronk *et al.*, 2007). Les granulocytes et les macrophages sont rapidement générés à partir des GMP (Akashi *et al.*, 2000, Manz *et al.*, 2002).

Le système hématopoïétique à la capacité d'accroître rapidement et considérablement la production de cellules sanguines pour compenser une perte de celles-ci en conditions de stress, telles qu'une infection virale ou bactérienne, une irradiation, une chimiothérapie, etc. Dans ces cas, le système hématopoïétique est activé pour s'adapter à une demande croissante de générer des nouvelles cellules sanguines. L'expression « hématopoïèse d'urgence » est utilisée pour distinguer les conditions causées par le stress et l'hématopoïèse de base.

L'hématopoïèse d'urgence est caractérisée par l'expansion des CSPH et de leurs progénitures, en nombre et en proportion, en réponse à des conditions de stress dans la moëlle osseuse causées par des signaux environnementaux, tels que des cytokines inflammatoires ou des produits microbiens (Baldridge *et al.*, 2010, Boettcher *et al.*, 2014, Kwak *et al.*, 2015). L'infection systémique par différents agents pathogènes d'origine virale, bactérienne ou parasitaire peut changer le débit normal de l'hématopoïèse dans la moëlle osseuse. Des infections *in vivo* avec des agents causatifs du paludisme ou de la toxoplasmose changent l'hématopoïèse de base avec une augmentation spectaculaire du nombre de granulocytes dans la moëlle osseuse tout en inhibant temporairement la différenciation des lymphocytes, des érythrocytes et des mégakaryocytes (Chou *et al.*, 2012, Villeval *et al.*, 1990). L'infection par *Plasmodium chabaudi* ou *Pneumocystis carinii* accroît les populations de CSH et de LSK dans la moëlle osseuse et le sang périphérique. Les progéniteurs qui découlent de ces populations étaient également plus nombreux (Belyaev *et al.*, 2010, Shi *et al.*, 2011). De plus, dans un modèle de souris, à la suite d'une infection chronique avec *Mycobacterium avium*, le niveau d'IFN-γ avait dramatiquement augmenté dans le sérum des animaux infectés, menant à la prolifération des CSH-LT et des CSH-CT (Baldridge *et al.*, 2010). Peu de temps après l'activation des CSH, elles passent de la moëlle osseuse au sang périphérique pour migrer vers différents organes dont fait partie la rate, ou les organes infectés, où elles débutent l'hématopoïèse extra-médullaire et se différencient directement en cellules requises (Abidin *et al.*, 2017, Burberry *et al.*, 2014, Griseri *et al.*, 2012, Haas *et al.*, 2015).

Plus spécifiquement, une augmentation rapide de la granulopoïèse a été décrit dans différentes infections bactériennes. À la suite de l'infection, l'hôte produit du G-CSF, ce qui déclenche la granulopoïèse d'urgence (Demetri *et al.*, 1991, Lieschke *et al.*, 1994). De la même manière, mimer une infection bactérienne systémique aigue *in vivo* en administrant du lipopolysaccharide (LPS) chez la souris mène à une augmentation des neutrophiles (Boettcher

et al., 2014). La granulopoïèse d'urgence est définie comme étant la génération de neutrophiles *de novo* dans la MO pour remédier à la demande du système sanguin à la suite d'une infection bactérienne ou à une inflammation (Manz *et al.*, 2014). La cascade d'urgence peut se diviser en trois parties. Premièrement, la présence d'agents pathogènes invasifs doit être détectée pour informer le système immunitaire et hématopoïétique de l'urgence. Deuxièmement, la situation d'urgence doit être transmise via des signaux moléculaires pour induire la production de neutrophiles dans la MO. Troisièmement, le système hématopoïétique doit récupérer et retourner à l'état basal (Manz *et al.*, 2014). L'homéostasie sera rétablie à la suite de l'élimination des organismes pathogènes ou de la disparition des conditions de stress (Manz *et al.*, 2014).

Lorsque l'invasion d'un pathogène devient systémique chez un organisme vivant, la transmission de signaux pour alerter le système immunitaire est l'étape cruciale pour déclencher la cascade de la granulopoïèse d'urgence. Cela nécessite qu'un ou plusieurs types de cellules rencontrent des organismes étrangers et disposent d'un mécanisme permettant de détecter et traduire le signal en granulopoïèse d'urgence. Heureusement, des récepteurs qui reconnaissent certains motifs, tels que les récepteurs de type Toll (TLR), détectent et répondent à une variété de classes de motifs moléculaires conservés associés à un agent pathogène (PAMP). Deux modèles sont proposés pour détecter les signaux des agents pathogènes : une détection directe par les CSH et les progéniteurs exprimant des TLR et une détection indirecte transmise via des cellules hématopoietiques matures ou non-hématopoïétiques. Il a été démontré que les CSH-LT et leur descendance expriment des TLR qui peuvent directement répondre aux agents pathogènes chez l'humain (Chicha *et al.*, 2004, De Luca *et al.*, 2009) et la souris (Nagai *et al.*, 2006, Schmid *et al.*, 2011). La liaison des TLR sur les CSPH mène à leur prolifération, leur migration vers les organes périphériques et leur différenciation, en plus de contribuer à la réponse du système immunitaire (Manz *et al.*, 2014). À la suite de la stimulation des TLR, les CSH deviennent plus nombreuses et subissent une différenciation spécifique vers la lignée myéloïde (De Luca *et al.*, 2009, Nagai *et al.*, 2006, Schmid *et al.*, 2011). La transmission directe des signaux via les TLR permettent aux CSH de combler rapidement la perte de cellules de l'immunité innée causée par l'infection. Bien que la fonction des TLR dans la détection des pathogènes est bien décrite dans les cellules hématopoïétiques, les cellules non-hématopoïétiques expriment aussi des TLR et contribuent à la propagation des signaux inflammatoires. Par exemple, les acariens provoquent de l'asthme allergique via le signal du TLR4 qui est exprimé sur des cellules épithéliales structurales des voies respiratoires (Hammad *et al.*, 2009). Schilling et al. ont démontré que les souris déficientes pour le récepteur TLR4 dans les cellules épithéliales de la vessie étaient incapables d'amorcer une réponse protectrice

pour contrôler une infection précoce par *Escherichia coli*, bien que les cellules hématopoïétiques de ces souris exprimaient TLR4 (Schilling *et al.*, 2003). L'étude montre également que les cellules épithéliales TLR4+ ne peuvent pas activer une réponse inflammatoire à elles seules, ce qui suggère que les signaux de TLR4 dans les cellules épithéliales et les cellules hématopoïétiques sont nécessaires pour activer une réponse immunitaire efficace. Ces résultats démontrent que les cellules épithéliales de la vessie jouent un rôle crucial dans l'immunité innée médiée par TLR4 pour éliminer *E. coli* (Schilling *et al.*, 2003).

Le lipopolysaccharide (LPS) est une endotoxine et un composant de la membrane externe des bactéries à Gram négative. Le LPS comprend une région oligo- ou polysaccharide ancrée dans la membrane externe de la bactérie par une queue de lipide A. Le lipide A est la principale partie immunostimulante du LPS (Erridge *et al.*, 2002). Le LPS est utilisé dans plusieurs modèles pour induire une inflammation systémique pour mimer un choc septique. Le LPS est reconnu par TLR4 qui est exprimé à la surface de différents types de cellules tels que les CSPH, les macrophages, les cellules endothéliales et les cellules mésenchymateuses (MSC) (Nagai *et al.*, 2006, Vaure *et al.*, 2014). Lorsque le LPS est lié à son récepteur, la signalisation de TLR4 dans les macrophages mène à l'activation des macrophages et à la sécrétion de médiateurs inflammatoires et de chimiokines. Ces chimiokines sont des chimio-attractants qui permettent le recrutement des neutrophiles, des lymphocytes T, des monocytes et des cellules dendritiques au site de l'inflammation (Amanzada *et al.*, 2014, Tirosh *et al.*, 2010) . L'administration de LPS *in vivo* chez la souris mène à la liaison du LPS avec le TLR4 exprimé sur les CSPH et les cellules endothéliales, permettant la prolifération des CSPH, la sécrétion de G-CSF et une augmentation significative de la production de neutrophiles. Par conséquent, l'hématopoïèse basal passe à la granulopoïèse d'urgence (Boettcher *et al.*, 2014). L'administration répétée de LPS entraîne l'activation des CSH au repos et leur entrée dans le cycle cellulaire. Cela se traduit par une capacité d'auto-renouvellement restreinte des CSH via la signalisation TLR4-TRIF-FOS-p38 (Takizawa *et al.*, 2014). Le choc septique peut être induit chez la souris par une injection intrapéritonéale de LPS de *Pseudomonas aeruginosa* à 3mg/kg, ce qui inhibe la production de cellules myéloïdes et mène à l'épuisement des CSH (Zhang *et al.*, 2016).

Les membres de la famille des Frizzled (Fzd) sont connus pour être les récepteurs principaux impliqués dans les voies de signalisation de Wnt. Fzd6 est exprimé sur les CSH, les progéniteurs lymphoïdes précoces (ELP), les CLP et les CMP (Yokota *et al.*, 2008). Fzd6 est

aussi exprimé sur les MSC, les cellules endothéliales dans la moëlle osseuse (Dosen *et al.*, 2006) et dans différents organes chez l'humain tels que le cœur, le cerveau, le placenta, les poumons, le foie, les muscles squelettiques, les reins, le pancréas, le thymus, la prostate, les testicules, les ovaires, le petit intestin et le colon (Tokuhara *et al.*, 1998). Fzd6 est impliqué dans la voie de polarité planaire de la cellule, qui est nécessaire pour la transmission du signal de polarité dans les cellules épithéliales de l'épiderme (Wang *et al.*, 2006). Récemment, Corda *et al.* ont montré une incidence accrue de l'expression de Fzd6 dans le sous-type du cancer du sein triple-négatif. En contrôlant l'assemblage du cytosquelette d'actine, Fzd6 régule la motilité et l'invasion de lignées cellulaires du cancer du sein (Corda *et al.*, 2017). Fzd6 est un récepteur de Wnt4 pour les cellules progénitrices hématopoïétiques immatures, basé sur la réduction de l'expansion des CSPH médié par Wnt4 en absence de Fzd6 (Heinonen *et al.*, 2011b). Fzd6 a également un rôle dans la régulation de la survie et l'expansion des cellules souches et progénitrices hématopoïétiques (Abidin *et al.*, 2015). Cependant, jusqu'à maintenant, les mécanismes fonctionnels de Fzd6 dans les CSH restent obscurs.

RÉSULTATS

Dans l'étude précédente, nous avons montré que Fzd6 contrôle l'expansion des CSH en réponse au LPS (Abidin *et al.*, 2015). Il y avait moins de CSH dans la moëlle osseuse (MO) des souris $Fzd6^{-/-}$ que chez les souris $Fzd6^{+/+}$ trois jours après l'administration du LPS. Nous nous sommes alors demandé si ce résultat était dû à l'inefficacité des souris KO à répondre au LPS ou si les CSH KO ne peuvent pas maintenir la prolifération en raison d'un auto-renouvellement défectueux. Pour répondre à cette interrogation, nous avons administré du LPS à des souris WT et KO à deux reprises à raison de 1mg/kg/jour. Nous avons laissé 48 heures entre les deux injections et nous avons évalué les proportions de CSH toutes les 24 heures. Nous n'avons observé aucune différence dans la réponse au LPS des CSH KO en comparant avec les CSH WT à 24 et 48 heures (Figure 3.1B, C). Cependant, après 48 heures, le nombre de CSH KO a diminué rapidement comparativement au nombre de CSH WT (Figure 3.1B). Il n'y avait aucune différence dans les populations MPP KO lorsqu'elles sont comparées à leur contrôle WT. Nous avons également découvert que le nombre de CSH KO dans la phase G1 du cycle cellulaire est significativement plus élevé que pour les CSH WT (Figure 3.1E). À la suite des résultats obtenus, nous concluons que les CSH KO ont effectivement perdu leur fonction.

Pour étudier le rôle du microenvironnement dans l'activation des CSH, nous avons greffé des CSH WT à des souris receveuses WT et KO (Figure 3.2A). Chez les greffes

primaires, les CSH WT reconstituaient la MO et le sang périphérique des hôtes WT et KO de manière équivalente (Figure 3.2B, C). Les CSH WT s'expandent aussi bien suite à l'injection du LPS dans un environnement KO que dans un environnement normal. De plus, le pourcentage et le nombre de CSH greffées étaient similaires à la 16^{e} semaine suivant la greffe (Figure 3.2D, E). Les colonies générées à partir des cellules de la MO venant des souris receveuses KO étaient moins nombreuses que les colonies venant des souris WT. Cependant, les nombres de colonies générées à partir de cellules spléniques étaient inversés par rapport aux colonies de MO (Figure 3.2F), ce qui suggère que la MO KO est plus inflammatoire que la MO WT tandis que la rate des souris KO favorise la croissance des cellules formant des colonies. Nous concluons que l'impact de Fzd6 sur la réponse des CSH est intrinsèque aux cellules hématopoiétiques. Pour vérifier la capacité des CSH activées dans un environnement KO à générer le système sanguin chez des souris irradiées, nous avons greffé des cellules de la MO provenant des greffes primaires ayant reçu du LPS dans des souris congéniques (B6.SJL) (Figure 3.3A). Les CSH activées dans un environnement WT ou KO reconstituent le sang périphérique et la MO des hôtes secondaires avec une efficacité similaire (Figure 3.3B-E).

Nous observons une concentration de G-CSF plus élevée dans la MO de souris KO que dans la MO WT à la suite de l'administration de LPS (données non montrées). G-CSF est aussi utilisé cliniquement pour la mobilisation des CSH (Anasetti *et al.*, 2012). En se basant sur cela, nous voulions savoir si les CSH KO étaient mobilisées plus efficacement dans le sang et la rate. Nous avons administré du G-CSF à des souris WT et KO pendant trois jours consécutifs à 25mg/kg/jour et nous avons ensuite analysé la MO, la rate et le sang périphérique des souris. Nous n'avons détecté aucune différence dans l'expansion des CSH WT et KO dans la MO (Figure 3.5A, B). Il y avait une diminution significative de la mobilisation des CSH KO dans le sang et dans la rate en comparant avec les CSH WT (Figure 3.5B, C). Le phénotype s'est confirmé avec les essais de formation des colonies à partir de cellules de la MO et la rate (Figure 3.5D). Finalement, nous concluons que Fzd6 contrôle la motilité des CSH de la MO.

Pour étudier quels facteurs hématopoïétiques contrôlent la réponse des CSH lorsque la MO est en condition de stress, nous avons effectué une analyse par puce à protéines avec du surnageant de MO provenant de groupes de souris traités et non-traités avec le LPS. Nous avons observé une augmentation significative de l'expression de CCL3, CXCL2 et TNF des échantillons de souris Fzd6$^{-/-}$ injectées avec le LPS (Figure 3.6A). CCL3/MIP1α a été démontré comme un facteur inhibiteur des cellules souches. Nous avons alors sélectionné MIP1α pour les analyses plus approfondies. Il y a une augmentation significative du niveau de MIP1α au 3^{e} jour

après un traitement de LPS dans la MO Fzd6$^{-/-}$ (Figure 3.6B). Nous avons également découvert que les macrophages sont une source importante de MIP1α (Figure 3.6C). MIP1α inhibe la croissance des colonies hématopoïétiques lorsque les cellules de MO WT sont mises en culture avec un surnageant de macrophages stimulés avec LPS (Figure 3.6D).

DISCUSSION ET CONCLUSION

Nous avons étudié le rôle fonctionnel de Fzd6 sur les CSH en utilisant un modèle de granulopoïèse d'urgence. Nos résultats montrent que l'impact de Fzd6 sur les CSH pour effectuer la granulopoïèse d'urgence induite par LPS est intrinsèque aux cellules. Nous montrons également que Fzd6 contrôle la migration des CSH vers le sang périphérique et la rate. De plus, MIP1α est un candidat qui supprime la réponse des CSH Fzd6$^{-/-}$ dans la MO en condition de stress.

Récemment, Baba *et al.* ont rapporté que MIP1α, qui est produit par les basophiles dans la MO, est un régulateur négatif de la prolifération des CSPH (Baba *et al.*, 2016), tandis que Rhonda *et al.* ont démontré que MIP1α régule le nombre de CSH et la différenciation des progéniteurs myéloïdes (Staversky *et al.*, 2018a). Dans notre étude, nous avons observé que les souris KO produisent un niveau significativement plus élevé de MIP1α dans la MO que les souris WT (Figure 3.6B). Nos résultats montrent aussi que le nombre de CSH KO diminue rapidement 3 jours après l'administration de LPS (Figure 3.1B). Cela concorde avec le fait que MIP1α inhibe la prolifération des CSPH (Broxmeyer *et al.*, 1990, Graham *et al.*, 1990). Dans notre modèle, nous montrons que les macrophages dérivés de la moëlle osseuse produisent une grande quantité de MIP1α après une stimulation avec LPS (Figure 3.6C). En utilisant les essais de colonies, nous avons mis en culture des cellules de MO avec du surnageant de macrophages dérivés de la MO stimulés avec LPS et nous avons évalué la production de colonies myéloïdes. Nos résultats confirment que le surnageant des macrophages, qui contient une concentration élevée de MIP1α, restreint la formation de colonies myéloïdes (Figure 3.6D). Ces résultats suggèrent que MIP1α joue un rôle majeur en limitant l'expansion des CSPH dans la MO KO. Un niveau élevé de MIP1α dans la moëlle osseuse peut potentiellement créer plus d'inflammation et causer des dommages à la niche de la MO chez les souris KO.

Une régulation à la hausse de Wnt5a a également été observée dans le sang de patients en choc septique. Wnt5a promeut l'inflammation en induisant l'expression de différents gènes cibles pro-inflammatoires, tels que l'Il-6, Il-1β, TNF et peut-être MIP1α (Pereira *et al.*,

2008). Nous avons déjà publié que Fzd6 est un régulateur négatif de CDC42 dans les CSPH (Abidin *et al.*, 2015). CDC42 est un médiateur en aval de la signalisation de Wnt5a (Prasad *et al.*, 2013), ce qui suggère que la signalisation de Wnt5a peut être fortement active chez les souris déficientes en Fzd6. Ici, nous proposons que Fzd6 est un médiateur qui contrôle négativement l'axe Wnt5a/MIP1α.

Nous rapportons dans notre modèle que MIP1α est un candidat pour empêcher l'expansion des CSH *in vivo* et induire l'inflammation. Le mécanisme est similaire au mécanisme du choc septique. En ciblant MIP1α, Fzd6 pourrait être une cible thérapeutique prometteuse pour les maladies inflammatoires et le choc septique.

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LIST OF ABBREVIATIONS

ABM: Adult bone marrow

ACD: Asymmetric cell division

AEC: Epithelia type I cell

AGM: Aorta-gonad-mesonephros region

AML: Acute myeloid leukemia

Ang-1: Angiopoietin-1

APC: Adenomatous polyposis coli

Axin: The axis inhibition protein-1

Bcl-2: B cell lymphoma 2

BM: Bone marrow

BMDM: Bone marrow-derived macrophage

Ca^{2+}: Calcium

CaMKII: Ca^{2+}/calmodulin-dependent protein kinase

CAR: CXCL12-abundant perivascular cell

CDK: Cyclin dependent kinase

CDP: Common-dendritic cell progenitor

CFU: The colony-forming unit

CFU-E: Colony-forming-unit-erythroid

CFU-G: Colony-forming unit-granulocytes

CFU-GEMM: Colony-forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte

CFU-GM: Colony-forming unit-granulocyte-monocyte

CFU-M: Colony-forming unit-monocyte

CK1α: Casein kinase 1α

CLP: Cecal ligation and puncture

CLP: Common lymphoid progenitor

CML: Chronic myeloid leukemia

CMP: Common myeloid progenitor

CXCL4/pf4: Chemokine (C-X-C) motif ligand 4/ Platelet factor 4

DARC: Duffy antigen receptor for cytokines

DC: Dendritic cell

Dkk1: The Dickkopf protein -1

Dsh: Dishevelled (in Drosophila)

Dvl: Dishevelled (in vertebrates)

E: Erythrocyte

EC: Endothelial cell

EGF: Epidermal growth factor

ELP: Early-lymphoid progenitor

ETP: Early thymic progenitor

FACS: Fluorescence activated cell sorting

FBM: Fetal bone marrow

FGF: Fibroblast growth factor 1

FL: Fetal liver

FL: Flt-3 ligand

Fzd: Frizzled ortholog in mammals

G: Granulocyte

G-CSF: Granulocyte colony stimulating factor

GM-CSF: Granulocyte-monocyte colony stimulating factor

GM-CSFR: Granulocyte-macrophage colony-stimulating factor receptor

GMP: Granulocyte-monocyte progenitor

GPCR: G-protein-coupled receptor

GSK-3β: Glycogen synthase kinase-3β

HAEC: human aortic endothelial cells

HSC: Hematopoietic stem cell

HSPC: Hematopoietic stem/progenitor cell

HUVEC: Human umbilical vein endothelial cells

IFN: Interferon

IFNAR: Interferon-α/β receptor

IL: Interleukin

IRA: Innate response activator

IRF: Interferon regulatory factor

Jnk: c-Jun N-terminal kinase

LCMV: Lymphocytic choriomeningitis virus

LEF: Lymphoid enhancer binding factor

LIC: Leukemia initiating cells

LIF: Leukemia inhibitory factor

Lin: Lineage

LMPP: Lymphoid-primed multipotent progenitor

LPS: Lipopolysaccharide

LRP: Low density lipoprotein receptor-related protein

LSK: $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$

LT-HSC: Long-term hematopoietic stem cell

Ly-HSC: Lymphoid-biased HSC

M: Monocyte

MAPK: Mitogen-activated protein kinases

M-CSF: macrophage colony-stimulating factor

MEF: Megakaryocyte and macrophages

MkP: Megakaryocyte progenitor

MMP: Matrix metalloproteinase

MMTV: Mouse mammary tumor virus

MPP: Multipotent progenitor

MSC: Mesenchymal stem cell

My-HSC: Myeloid-biased hematopoietic stem cell

NK: Natural killer

OB: Osteoblast

PAMPs: Pathogen-associated molecular patterns

PCP: Planar cell polarity

PDE: phosphodiesterase

PGE_2: Prostagladin E_2

PHZ: phenyl-hydrazine

PKC: Protein kinase C

PLC: Phospholipase C

PRR: Pattern recognition receptor

PSGL-1: P-selectin glycoprotein-ligand-1

RA: Rheumatoid Arthritis

RBC: Red blood cell

ROS: Reactive oxygen species

RPE: Retinal pigment epithelium

SCD: Symmetric cell division

SCF: Stem cell factor

SDD: Symmetric differentiation division

SDF: Stromal derived factor

SFRP: Secreted Frizzled-related protein

SOCS: Suppressor of cytokine signalling

ST-HSC : Short-term hematopoietic stem cell

TCF: T-cell factor

TGF-β: Transforming growth factor-β

TLR: Toll-like receptor

TNF: Tumor necrosis factor

TPO: Thrombopoietin

VCAM-1: Vascular-adhesion protein-1

VE: Vascular endothelial

VEGF: Vascular endothelial growth factor

VEGFR: Vascular Endothelial Growth Factor Receptor

Wg: Wingless

WIF: Wnt Inhibitory Factor

YS: Yolk sac

CHAPTER 1 – INTRODUCTION

1 HEMATOPOIETIC SYSTEM

Blood is an essential component of life. It consists of many different cell types that are generated in the bone marrow from hematopoietic stem cell (HSC), circulating through veins and arteries to peripheral tissues to become terminal specialized cells. Everyday more than 100 million (Boulais *et al.*, 2015) mature blood cells are produced by the hematopoietic system to carry out many important functions such as transporting oxygen to peripheral tissues, patrolling for foreign pathogens, and remodeling tissue. HSCs are blood-isolated, bone marrow-isolated cells that have the ability to self-renew, produce terminally differentiated cells, enter blood circulation from bone marrow and undergo apoptosis (Bethesda, 2001). The HSC concept was demonstrated by Till and McCulloch (Till and McCulloch, 1961). Back in 1961, they observed forming of colonies in the spleen after 8-12 days when they transplanted bone marrow and spleen cells into lethally irradiated mice. The colonies consisted mostly of myeloid cells such as megakaryocytes, macrophages, erythrocytes, and neutrophils (Till and McCulloch, 1961). Based on this definition, HSCs have self-renewal ability and potential to differentiate into other cell types. To self-renew an HSC will divide to one or two daughter cells with the functions equivalent to mother cell via mitosis. This means that a whole genome of a mother cell and epigenetic marks will be copied to the daughter cells to preserve their stemness. Differentiation is a process where HSC gives rise to daughter cells that gradually restrict their differentiation ability, and finally become terminally differentiated cells with specialized function. There are two types of HSCs: the long-term HSCs (LT-HSCs) that are able to self-renew and differentiate into all different cell types; and the short-term HSCs (ST-HSCs) that are a progeny of LT-HSCs. This population still has the multi-lineage differentiation ability but possesses limited self-renewal potential (Bellantuono, 2004; Brown and Weissman, 2004). Mouse LT-HSCs are defined by a combination of surface markers $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}Flt3^{-}CD48^{-}CD150^{+}$ (Kiel et al., 2005).

1.1 Developmental process of hematopoietic stem cell

The development of HSCs was reported at very early stage of embryonic development. The initial site of blood production in a mouse is yolk sac (YS) which is a two-layer membrane surrounding the developing embryo. Hematopoiesis occurring in embryos is thought to comprise 2 stages: primitive and definitive. In the primitive wave, erythrocytes are generated at large numbers on day 7.5 of mouse embryos (E7.5) in the yolk sac blood islands (Medvinsky et al., 2011; Palis et al., 1999) to carry oxygen and nutrition to the developing embryos with little

activity of HSC (Mikkola and Orkin, 2006). Embryonic macrophages and megakaryocytes have also emerged at this time (Xu et al., 2001). Primitive erythrocytes are large, nucleated cells and contain embryonic globins (Palis et al., 1999). The definitive stage is characterized by the production of all the blood cell types and appearance of HSCs that have engraftment capacity. The definitive hematopoiesis takes place in several tissues over a long period of time, beginning at E8.5 in the YS and the para-aortic splanchnopleura with the appearance of erythroid-myeloid-lymphoid progenitors (Godin et al., 1995), which have the ability to give rise to both myeloid-lineage cell types and B, T progenitors. In this wave, HSCs are found in the aorta-gonad-mesonephros (AGM) region and the placenta at embryonic day 8.5 and 10, respectively, as well as in the yolk sac (Gekas et al., 2005; Medvinsky and Dzierzak, 1996; Samokhvalov et al., 2007). Later, hematopoietic progenitors migrate from YS, placenta, and AGM to colonize the fetal liver at E10.5-E11 (Gekas et al., 2005; Kumaravelu et al., 2002). By E12, the numbers of HSCs increase 14 times in placenta and fetal liver (Gekas et al., 2005; Kumaravelu et al., 2002), and fetal liver also becomes the major HSC reservoir before HSCs colonizing bone marrow at E17 (Christensen et al., 2004). From E17.5 to the first two weeks after birth, HSCs leave the liver to settle in the bone marrow.

1.2 Hematopoietic stem cell niche

Niche acts as the special microenvironment to preserve self-renewal and long-term engraftment ability of HSCs. The stem cells and niche cells interact with each other by adhesion molecules and exchange molecular signals that help to maintain HSCs function. First mentioned by Schofield (Schofield, 1978), hematopoietic stem cell niche keeping HSCs at dormant stage is the concept that is still used today. The HSC niche was proposed to be composed of osteoblasts (Calvi *et al.*, 2003), perivascular mesenchymal stromal cells, vascular endothelial cells (Ding *et al.*, 2013, Ding *et al.*, 2012, Mendez-Ferrer *et al.*, 2010) as well as hematopoietic cell types such as megakaryocytes and macrophages (Bruns *et al.*, 2014, Chow *et al.*, 2011, Winkler *et al.*, 2010, Zhao *et al.*, 2014b). The contribution of different niche cell types will be reviewed below.

1.2.1 Endothelial cells

Endothelial cells (ECs) are specialized cells that form blood vessels for blood circulation as well as transport of oxygen and nutrients to tissues (Carmeliet *et al.*, 2011). ECs are an essential

system for life, not only to promote the growth of organs via forming the vascular network (Aird, 2007, Kliche *et al.*, 2011) but also to provide the essential signals to support tissue regeneration and maintenance (Butler *et al.*, 2010, Sasine *et al.*, 2017). There are two major endothelial niches that form the endothelial network in BM: the arteriolar niche identified by surface markers VE-cadherin^{+}CD31^{+}Endomucin$^{+/-}$SCA1^{high}VEGFR3^{-}; and the sinusoidal niche that is defined by cell surface markers VE-cadherin^{+}CD31^{+} Endomucin^{+} SCA1^{low} VEGFR3^{+} (Hooper *et al.*, 2009, Poulos *et al.*, 2015). Endothelial niche can decide HSC cell fate via AKT, MAPK signaling (Kobayashi *et al.*, 2010). HSC self-renewal ability was promoted by ECs activating AKT signaling via the expression of HSC supportive factors such as stromal cell-derived factor 1 (SDF-1/CXCL12), stem cell factor (SCF/Kit ligand/KitL), and JAGGED-1 (Kobayashi *et al.*, 2010). HSC self-renewal and long-term regeneration increases profoundly after inhibition of canonical NF-kB pathway in endothelium (Poulos *et al.*, 2016). Subsequently, *in vivo* deletion of *Kitl, Cxcl12,* and *Jag1* in ECs damaged the maintenance of HSC at steady-state (Ding *et al.*, 2013, Ding *et al.*, 2012, Poulos *et al.*, 2013), and caused profound defects in regeneration capacity following myelo-suppressive injury (Poulos *et al.*, 2013). Additionally, deletion of pleiotrophin (Ptn) (Himburg *et al.*, 2010), epidermal growth factor (Egf) (Doan *et al.*, 2013), and E-selectin expressed on endothelial cells in vascular HSC niche of the bone marrow (Winkler *et al.*, 2012) destroys the regenerative capacity of HSCs. In conclusion, these data suggest that the endothelial niche cell plays a vital role to preserve homeostasis.

1.2.2 Macrophages

Macrophages are an essential cell type of HSC niche, and HSPCs in BM are found in contact with these cells. Macrophages in BM secrete an unknown soluble factor that induces MSC production of CXCL12/SDF1. In turn CXCL12 anchors HSCs to the niche and maintain them at quiescent stage in the BM (Chow *et al.*, 2011, Christopher *et al.*, 2011). Depletion of CD169^{+} (Siglec1) macrophages in mice leads to the mobilization of HSPCs from BM to the bloodstream (Chow *et al.*, 2011). Winkler showed that G-CSF administration into the mice rapidly depleted endosteal macrophages, inhibited formation of endosteal bone, down-regulated expression of factors that are required for retention and self-renewal of HSCs and consequently promoted mobilization of HSPCs (Winkler *et al.*, 2010). In addition, α-smooth muscle actin–positive (α-SMA+) macrophages produce prostaglandin E_2 (PGE_2), leading to decreased generation of reactive oxygen species (ROS) and thus preserving HSPC function in the BM (Ludin *et al.*, 2012). A subset of BM macrophages also express the Duffy antigen receptor for

cytokines (DARC), which directly makes contact with long-term HSCs (LT-HSCs) through interaction with CD82/KAI1 that is highly expressed on LT-HSCs. This interaction maintained HSCs at dormant stage (Hur *et al.*, 2016). Recently, Luo established the role of different macrophage polarization stages on HSC function ex vivo (Luo *et al.*, 2018). In this report, using *in vitro* culture system of polarized M1 (pro-inflammatory) and M2 (tissue repair) macrophages with HSCs, the authors claimed that they have opposite effects on mouse and human HSC self-renewal and expansion: while M1 macrophages inhibit HSC expansion and self-renewal via secretion of NO, M2 macrophages show promotion of HSC self-renewal and expansion (Luo *et al.*, 2018).

1.2.3 Megakaryocytes

HSPCs are also observed in direct contact with megakaryocytes. Megakaryocytes are an essential component of HSC niche, and contribute to maintain HSC quiescence during steady-state and promote HSC regeneration after injury. Conditional depletion of megakaryocytes results in the loss of HSC quiescence, and data suggest that megakaryocytes directly preserve HSC self-renewal and long-term regeneration capacity via secreting cell cycle regulators like C-X-C motif ligand 4 (CXCL4, also named platelet factor 4, Pf4), transforming growth factor β1 (TGF-β1) and TPO (Bruns *et al.*, 2014, Nakamura-Ishizu *et al.*, 2014, Zhao *et al.*, 2014b). In addition, under bone marrow stress conditions, such as treatment of mice with a chemotherapeutic agent, 5-fluoruracil, megakaryocytes down-regulate TGF-β1 and produce fibroblast growth factor 1 (FGF-1) to stimulate the expansion of HSPCs (Zhao *et al.*, 2014b).

1.2.4 Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are plastic adherent cells with a spindle shape, isolated from BM, adipose and other tissues. MSCs are thought to have a capacity of differentiation into bone, cartilage, marrow stromal cells, adipocytes, muscle and connective tissue (Caplan, 1991). MSCs have the surface phenotype $CD105^{+}CD73^{+}CD90^{+}CD45^{-}CD34^{-}CD14^{-}CD19^{-}CD3^{-}$, $MHCII^{-}$ and are known to express MHC class I molecules *in vitro* (Barry *et al.*, 2001, Barry *et al.*, 1999, Pittenger *et al.*, 1999). MSCs secrete several growth factors and chemokines like SDF-1/CXCL12, KitL, Flt-3 ligand (FL), IL-6, IL-11, leukemia inhibitory factor (LIF), macrophage colony-stimulating factor (M-CSF), tumor necrosis factor (TNF)-α, and TGF-β1 (Jeltsch *et al.*, 2011, Majumdar *et al.*, 2000, Mishima *et al.*, 2010). Evidence illustrates that

MSCs are a key component of HSC niche in bone marrow and contribute to homeostasis (Valtieri *et al.*, 2008). Depletion of MSCs leads to reduction of HSCs in the bone marrow (Mendez-Ferrer *et al.*, 2010). Undifferentiated osteoblasts B10, a human MSC cell line, and osteoblast-differentiated B10 (Ost-B10) secreted SCF, FL, TPO that effectively supported HSC expansion ex vivo (Mishima *et al.*, 2010). Additionally, culture of human HSCs isolated from cord blood cells in presence of stromal cell with TPO and IL6 can control HSC expansion and differentiation ex vivo (Rappold *et al.*, 1999, Zhang *et al.*, 2008).

1.2.5 Osteoblasts

Schofield and Lord reported that hematopoietic stem/progenitor cells (HSPCs; identified as colony-forming unit-spleen at the time) preferentially located near the bone surface (Schofield *et al.*, 1986). Later, Benayahu demonstrated that osteoblasts support hematopoiesis (Benayahu *et al.*, 1992, Benayahu *et al.*, 1989) by the production of hematopoietic growth factors like G-CSF and hepatocyte growth factor (HGF) (Taichman *et al.*, 1996, Taichman *et al.*, 2001). Transplantation of HSPCs into non-ablated recipient mice results in selective distribution of HSPCs in the endosteal region of bone marrow (Nilsson *et al.*, 2001). Osteoblasts also contribute to maintaining HSCs at a quiescent stage. Using immunohistochemical staining, Calvi et al. indicated the expression of CXCL12, SCF, IL-6 and Jagged 1, known as the Notch ligand on osteoblasts (Calvi *et al.*, 2003). Increased Notch signaling results in elevation of LT-HSC numbers, whereas inhibition of Notch pathway decreases LT-HSC numbers (Karanu *et al.*, 2000, Stier *et al.*, 2002). Arai et al. showed that angiopoietin-1 expressed by osteoblasts binds to Tie2 receptor on HSPCs to promote HSPC quiescence, and maintenance of HSPCs *in vitro* (Arai *et al.*, 2004). Osteoblasts also express thrombopoietin (TPO) which interacts with MPL receptor on quiescent HSCs. This interaction results in up-regulation of beta1-integrin and cyclin-dependent kinase inhibitors in HSCs, and subsequently induction of quiescence of LT-HSCs. Inhibition of TPO/MPL pathway by using anti-MPL neutralizing antibody, AMM2, or by decreasing TPO levels decreases the number of HSPCs at quiescent stage (Qian *et al.*, 2007, Yoshihara *et al.*, 2007). The other factor that regulates HSCs activity in osteoblastic niche is osteopontin, which restricts HSC expansion in bone marrow (Stier *et al.*, 2005). In the absence of osteopontin in culture conditions, human $CD34^+$ cells increase Jagged1-Notch signaling by up-regulating stromal Jag1, angiopoietin-1, and Notch1 receptor (Iwata *et al.*, 2004). Taken together, angiopoietin-1, osteopontin, and TPO are involved in the maintenance of the HSPC compartment and prevention of HSPC exhaustion.

1.3 Steady-state hematopoiesis

After birth, the murine bone marrow is the primary site of hematopoiesis. To maintain the HSC pool, HSC will enter cell cycle and divide by symmetric or asymmetric division. There are three types of HSC division: 1) symmetric self-renewal, that is, one mother stem cell gives rise to two daughter cells with the phenotype and function which are identical to mother cell (Yamamoto *et al.*, 2013); 2) asymmetric self-renewal occurs when one daughter cell remains stem cell and the other becomes a committed progenitor cell; and 3) symmetric commitment that occurs when both two daughter cells are committed progenitor cells (Ito *et al.*, 2003, Yamamoto *et al.*, 2013).

At steady-state, LT-HSCs differentiate sequentially into ST-HSCs and multi-potent progenitor cells (MPPs) that are the progeny of LT-HSCs with restriction in self-renewal ability, but their differentiation into all blood cell types is unchanged (Figure 1.1). MPPs generate lympho-myeloid primed MPPs (LMPPs), which have the potential to produce lymphoid and myeloid lineages except megakaryocyte and erythroid (Adolfsson *et al.*, 2005, Kohn *et al.*, 2012). The development process from LT-HSCs to ST-HSCs to LMPP to GMPs also reported in Nestorowa et al. (Nestorowa *et al.*, 2016). The percentage of LMPP population is up to 25% in $Lin^-(B220^-CD3e^-CD11b^-GR1^-Ter119^-)Sca1^+cKit^+$ (LSK) population in bone marrow. The LMPP subset is defined as $Flt3^+LSK$ (Adolfsson *et al.*, 2005). Recently, MPP2 and MPP3 were characterized as sub-populations in $Flt3^-LSK$ fraction, with the $Flt3^+LSK$ fraction renamed MPP4 (Wilson *et al.*, 2008). LT-HSCs give rise independently to MPP1 (known as ST-HSCs), MPP2, MPP3 and LMPP (Pietras *et al.*, 2015). MPP2 and MPP3 both have potential to produce myeloid output (Cabezas-Wallscheid *et al.*, 2014). MPP2 and MPP3 were confirmed to produce all myeloid lineages in clonogenic methylcellulose assays. However, MPP2 showed extensive potential to give rise to megakaryocytes meanwhile MPP3 displayed a dominant GM progeny (Pietras *et al.*, 2015). In addition, MPP2 and MPP3 populations could also able to differentiate into B, T cell when co-cultured with BM stromal cell line OP9. In vivo experiments showed MPP2 and MPP3 have distinct function from LMPP and these populations are myeloid-biased MPP subsets within LSK (Pietras *et al.*, 2015). LMPP/MPP4s also include the $Rag1^+$ early lymphoid progenitor population (Igarashi *et al.*, 2002), which migrate from bone marrow to thymus to establish an early thymocyte progenitor (ETP) population that has the potential to efficiently boost up T cell numbers, but little ability to give rise to natural killer cell (NK), dendritic cell (DC) and B cells (Bell *et al.*, 2008). Common lymphoid progenitors (CLPs) generate B cells, NK cells and DCs in the bone marrow (Kondo *et al.*, 1997, Mansson *et al.*, 2010). CLPs are also

thought to have some potential to develop into T cells. Further downstream of MPPs are common myeloid progenitors (CMPs) (Akashi *et al.*, 2000, Manz *et al.*, 2002), which in turn give rise to granulocyte-macrophage progenitors (GMPs) and megakaryocyte/erythrocyte progenitors (MEPs) (Nakorn *et al.*, 2003, Pronk *et al.*, 2007). Granulocytes and macrophages are rapidly created from GMPs (Akashi *et al.*, 2000, Manz *et al.*, 2002). Recently, research using different kinds of techniques such as single-cell transcriptome analysis, and Transposon (Tn) tagging has confirmed that Erythrocytes (E), Granulocytes (G), and Monocytes (M) share a common origin (Rodriguez-Fraticelli *et al.*, 2018). The study also reveals that LT-HSCs display Mk-biased priming (Rodriguez-Fraticelli *et al.*, 2018, Sanjuan-Pla *et al.*, 2013) and Mk lineage is largely produced independently from other hematopoietic lineages (Rodriguez-Fraticelli *et al.*, 2018).

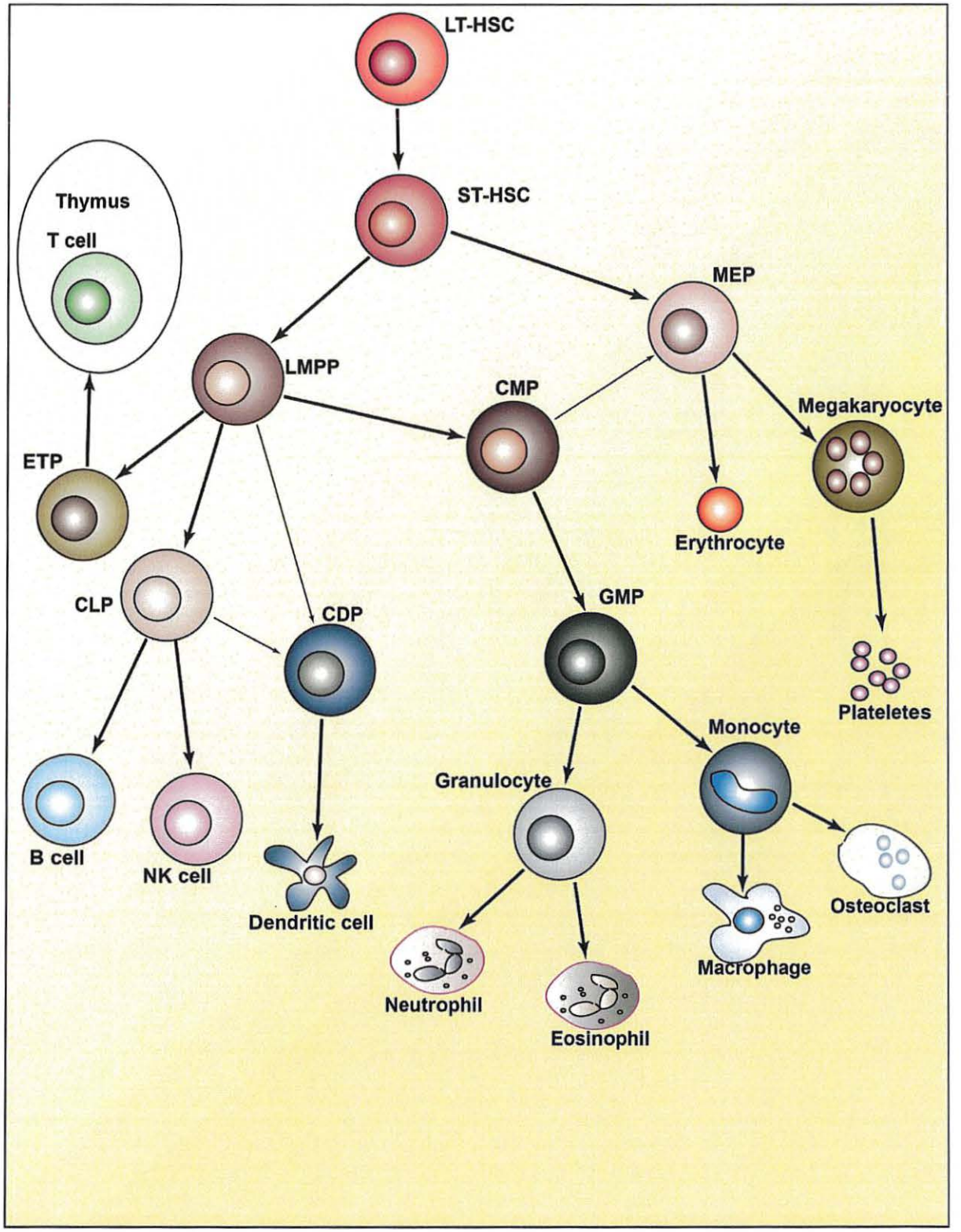
LT-HSC
ST-HSC
Thymus
T cell
MEP
LMPP
CMP
Megakaryocyte
ETP
Erythrocyte
CLP
CDP
GMP
Plateletes
Monocyte
Granulocyte
B cell
NK cell
Dendritic cell
Osteoclast
Macrophage
Neutrophil
Eosinophil

Figure 1.1 The adult hematopoietic hierarchy

Long-term hematopoietic stem cells lie at the apex of the hierarchy, giving rise to all blood cell types that are shown in this model by bold arrow and potential alternative differentiation routes are shown by thin arrows.

1.4 Emergency hematopoiesis

Different from steady-state hematopoiesis, the hematopoietic system has the capability of rapidly and dramatically boosting up its downstream cellular output to compensate for blood cell loss during stress conditions such as viral or bacterial infections, irradiation, chemotherapy, etc. In these cases, the hematopoietic system is activated to adapt to the increased demand for generating new blood cells. The term emergency hematopoiesis has been used to distinguish stress-induced conditions from steady-state hematopoiesis.

Emergency hematopoiesis is characterized by the expansion of HSPCs and their progeny in both proportion and numbers in response to bone marrow stress conditions via the guidance of environmental signals such as inflammatory cytokines and pathogen products (Baldridge *et al.*, 2010, Boettcher *et al.*, 2014, Kwak *et al.*, 2015). Systemic infection with various pathogens, such as bacterial, viral and parasitic organisms could change the normal output of BM. *In vivo* infection with *Plasmodium chabaudi adami* and *Toxoplasma gondii* switched from steady-state hematopoiesis to a dramatic increase of granulocyte numbers in the BM, and temporarily inhibited lymphocyte, erythrocyte and megakaryocyte differentiation (Chou *et al.*, 2012, Villeval *et al.*, 1990). Infection with *Plasmodium chabaudi* or *Pneumocystis carinii* could expand HSC and LSK populations in the BM and peripheral blood. The downstream MMPs were also expanded (Belyaev *et al.*, 2010, Shi *et al.*, 2011). Furthermore, following chronic infection with *M. avium* in a mouse model, IFN-γ is dramatically increased in the sera of infected animals leading to the proliferation of LT-HSCs, and ST-HSCs (Baldridge *et al.*, 2010). Shortly after HSCs become activated, they enter peripheral blood from bone marrow to migrate to extra-medullary sites such as spleen, inflamed tissues where they directly differentiated to the demanded target cells (Abidin *et al.*, 2017, Burberry *et al.*, 2014, Griseri *et al.*, 2012, Haas *et al.*, 2015).

Rapid increase in granulopoiesis has been described in various bacterial infections. Following infection host produces G-CSF that triggers emergency granulopoiesis (Demetri *et al.*, 1991, Lieschke *et al.*, 1994). Similarly, mimicking acute bacterial infection *in vivo* by injection of lipopolysaccharide (LPS) to experimental mice revealed a significant elevation in the neutrophil

number output (Boettcher *et al.*, 2014). Homeostasis will be reestablished following the clearance of infected pathogens or the disappearance of stress conditions (Manz *et al.*, 2014). These processes will be discussed in greater detail below.

2 THE WNT SIGNALING PATHWAY

In 1982, Roel Nusse and Harold Varmus were the first to describe mouse mammary tumor virus (MMTV) inducing a tumor in the mammary gland in mice by an unknown gene called *Int1* (Integration 1) (Nusse *et al.*, 1982). In 1973, Sharma had reported a fly gene named *Wingless* (Wg) whose mutation caused *Drosophila melanogaster* lack wings, and this gene was homologous to *Int1* in mammals (Cabrera et al., 1987; Rijsewijk et al., 1987). Mutations in this gene also caused defects in the segmentation of Drosophila embryos. Based on the conservation in amino acid sequences, a novel family of genes was identified as Wnt (Nusse *et al.*, 1991), and this family has expanded to comprise a number of proteins across vertebrate and invertebrate species. There are 19 genes encoding for Wnt proteins in human and mouse genome that have been characterized so far, and this number in the zebrafish is 15 and 8 in Drosophila (Miller, 2002). The Wnt ligands are secreted proteins containing a cysteine-rich motif and require post-translational modification by adding sugars and lipids (Willert and Nusse, 2012). Wnt proteins consist of 350 to 400 amino acids in length and have a conserved region of 22-24 cysteine residues, named cysteine-rich domain (CRD) (Takada et al., 2006). The Xenopus Wnt8 (XWnt8) crystal structure has 3.25Å in size and is composed of amino-terminal (NTD) and carboxyl-terminal domains (CTD). The larger NTD comprises a six α-helix core that contains 10 conserved cysteine residues to form 5 disulfide bridges, whereas CTD brings together two β-sheets with 6 disulfide bridges (Bazan *et al.*, 2012, Chu *et al.*, 2013). Wnts undergo posttranslational modifications by porcupine (Porcn), a Wnt-specific enzyme which is in charge for Wnt acetylation (Takada *et al.*, 2006). Adding lipid to a serine residue in a conserved region, for example Ser209 of Wnt3a, is indispensable for processing before secretion and biological activity of almost all Wnts (Takada et al., 2006). On the other hand, glycosylation is not strictly required. When a Wnt protein is processed in the Endoplasmic Reticulum (ER) and Golgi, a multispan transmembrane protein encoded by *Wntless* gene (*Wls*) binds to Wnt protein and brings Wnt to the cell surface for secretion (Banziger *et al.*, 2006, Goodman *et al.*, 2006). Once Wnt is secreted from the cell, Wls is recycled for a new cycle of Wnt secretion (Belenkaya *et al.*, 2008, Coudreuse *et al.*, 2006).

Frizzled family is known as the main receptors for Wnt proteins, while lipoprotein-receptor-related protein (LRP)-5 and LRP6 are co-receptors participating in the canonical Wnt pathway (MacDonald *et al.*, 2012). Fzd proteins are seven-transmembrane receptors, which belong to G protein-coupled receptor family. Fzd receptors are composed of 3 different domains, extracellular tail, transmembrane and cytoplasmic domains. There are 10 Fzd receptors included from Fzd1-10 in mammals (Willert *et al.*, 2012). Wnt signaling can be divided into canonical and non-canonical pathways. Canonical Wnt pathway or Wnt/β-catenin pathway, is the best documented Wnt signaling pathway, especially in hematopoietic cells. In the abscence of Wnt protein ligand (Figure 1.2A, left), β-catenin in cellular cytoplasm is targeted for degradation by a destruction complex composing of glycogen synthase kinase 3β (GSK3β), casein kinase 1α (CK1α), the axis inhibition protein 1 (AXIN1), and the adenomatous polyposis coli (APC) (Stamos *et al.*, 2013). CK1 and GSK3β continuously phosphorylate β-catenin at several points leading to ubiquitination of phosphorylated β-catenin and subsequent β-catenin degradation by the proteosome. In the nucleus, a repressor complex will be formed to silence target genes of Wnt pathway. Wnt protein engages the Fzd receptor and LRP5 or LRP6 at the cell membrane, leading to the recruitment of Axin to the membrane and thus inhibiting the formation of destruction complex. This leads to the accumulation of β-catenin in the cytoplasm and its translocation to nucleus (Stamos *et al.*, 2013). Upon arriving in nucleus, β-catenin binds to T cell factor (TCF) to activate transcription of target Wnt genes including cyclin D1, c-Myc, Cox2 and NOS (Staal *et al.*, 2008) (Figure 1.2A, right). The canonical Wnt pathway includes Wnts-1, -2, -3, -8a, -8b, -10a, and -10b and non-canonical includes Wnts-4, -5a, -5b, -6, 7a, -7b, and -11 (Siar *et al.*, 2012).

In contrast to canonical pathway, non-canonical signalling does not require expression of β-catenin and the co-receptor LRP5/6 but it needs Fzd receptor and the intracellular component Dvl (Figure 1.2). This signalling is subdivided into planar cell polarity (PCP) and Wnt/Ca^{2+} pathway. In the Wnt/$Ca2^{+}$ pathway, interaction of Wnt-Fzd complex leads to activation of phospholipase C (PLC). As a result the PLC cleaves phosphatidylinositol-4, 5bisphosphate (PIP_2) to inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 activates downstream signal to release Ca^{2+} to cytoplasm. Increasing intracellular Ca^{2+} subsequently leads to translocation of nuclear factor of activated T cells (NFAT) and transcription of target genes. The target genes differer between cell types (Figure 1.2B, right) (Staal *et al.*, 2008). Wnt protein exerts its signal through DVL-Fzd complex and activates RAC1, JUN N-terminal kinase (JNK) in case of PCP pathway. Activation of JNK influences the cellular cytoskeleton and cell shape. DVL-Fzd complex also activates Dishevelled-associated activator of morphogenesis (DAAM1) and RAS

homologue gene-family member A (RHOA), whose downstream effector RHO-associated coiled-coilcontaining protein kinase (ROCK), then guides reassembly of cell cytoskeleton (Figure 1.2B, left) (Endo *et al.*, 2005).

Wnt signaling has been well documented in many different developmental processes, from establishing the polarity in a single cell in a tissue to specifying the mammalian body axis. Many researches have shown that Wnt signaling regulates stem cell self-renewal, apoptosis, and motility (MacDonald *et al.*, 2009, Staal *et al.*, 2016, Staal *et al.*, 2005).

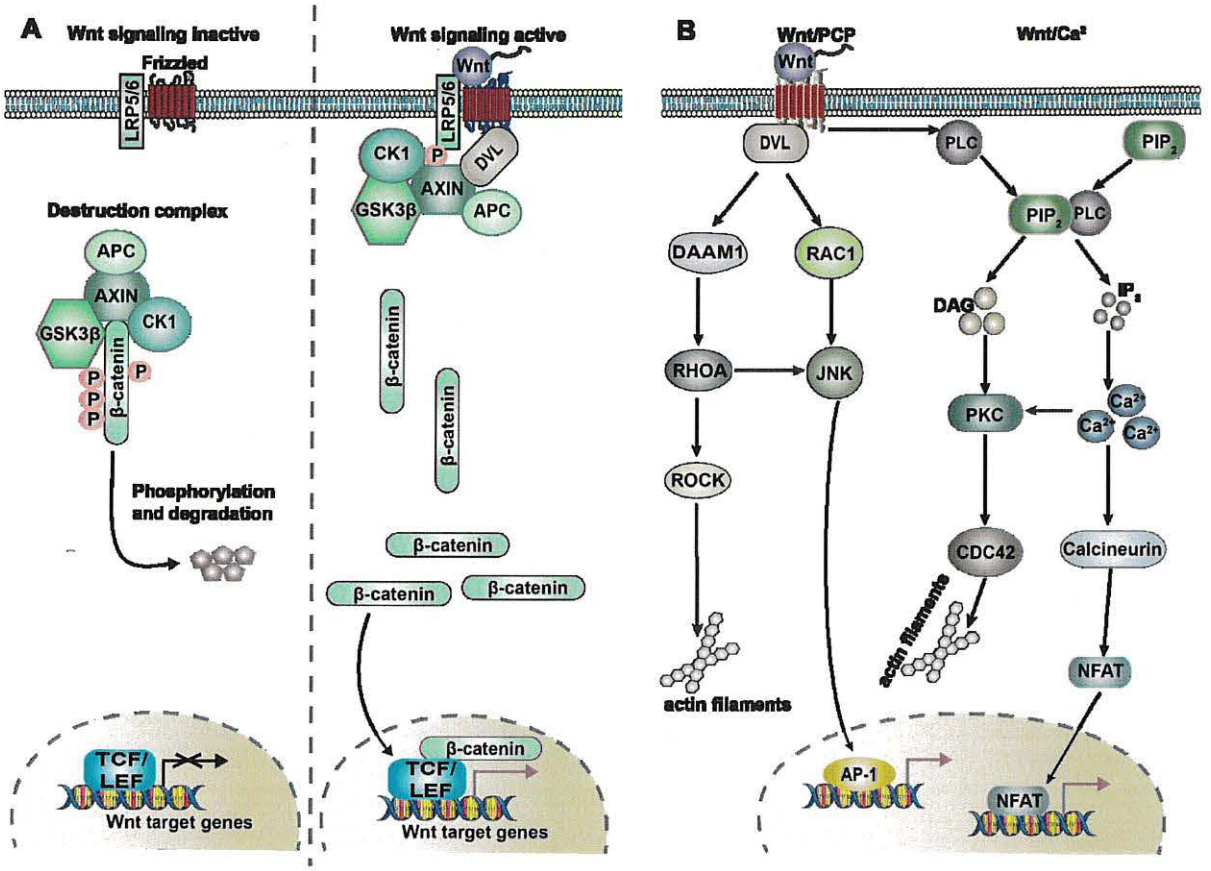

Figure 1.2 Overview of Wnt canonical and non-canonical pathway

A, In the absence of Wnt ligand, β-catenin will be targeted for degradation by destruction complex (Wnt pathway in inactive stage, left). The interaction of Wnt protein with Fzd, and LRP co-receptor mediates increasing of cellular β-catenin, subsequently translocation of β-catenin to nucleus in order to turn on target genes expression (panel A, right). B, Wnt non-canonical pathway divides into Wnt PCP and Wnt Ca^{2+} signaling. Engaged of Wnt and Fzd receptor leads to activate DVL and RAC1, JNK in PCP (panel B, left). Wnt/Fzd complex also triggers Ca^{2+} signaling to translocate NFAT to nucleus and transcribe cytoskeleton-related genes (panel B, right). Adapted from "Rapp et al., Zhan et al." (Rapp *et al.*, 2017, Zhan *et al.*, 2016).

2.1 Wnt signaling in embryonic hematopoiesis

The first evidence of Wnt signaling in HSPCs was showed by Austin *et al.* (Austin *et al.*, 1997). Using reverse transcriptase-polymerase chain reaction, they showed the expression of *Wnt-5a* and *Wnt-10b* in murine hematopoietic tissue and HSPCs at E11 in the YS, and E14 in the fetal liver. They also reported the expression of four different murine Frizzled homologs (mFzd3, 4, 5, 7) in the YS and fetal liver. Treatment of HSC population with recombinant Wnt5a resulted in improved cell expansion, proliferation, and survival (Austin *et al.*, 1997). This evidence supports the idea that Wnt pathway may be involved in controlling fetal HSCs. Later, the Wnt/β-catenin pathway was found to be active in the YS blood islands of *Xenopus* and mouse embryos (Cheng *et al.*, 2008, Tran *et al.*, 2010). The components of Wnt signaling such as Dishevelled, TCF, and β–catenin, have been found at the time of HSC appearance around E10-E12 in AGM. In addition, expression of nuclear β–catenin is restricted in endothelial cells in AGM, specifically intra-aortic hematopoietic clusters that contain HSC activity (Herguido *et al.*, 2012). When they cultured AGM isolated from mouse embryos with GSK3 inhibitor, SB216763, which increases stability of β–catenin, they observed an increase in HSC activity. In contrast, inhibiting the interaction of β–catenin with TCF by a small molecule PKF-115-584 decreased HSC activity in irradiated mice (Herguido *et al.*, 2012). β–catenin and *Wnt3a* are reported to be expressed in the fetal liver at E12.5, when HSCs boost up their numbers (Luis *et al.*, 2010). Knockout of *Wnt3a* gene in mice caused prenatal death at E12.5 due to severe defects in many developmental processes. *Wnt3a* deficient mice had reduced numbers of HSPCs in the fetal liver, and displayed poor long-term reconstitution ability in secondary recipient (Luis *et al.*, 2009). Other Wnt ligands such as Wnt3, 3a, 4, 5a, 5b, 8a, LRP5 and Wnt16 are up-regulated at primitive wave at YS and AGM of murine mouse embryos. Wnt3a, 5b, 6, 7a, 7b, and 10b increased expression in the definitive phase at the AGM and early liver (Corrigan *et al.*, 2009). Frizzled receptors were also expressed in the murine YS, AGM, fetal liver such as Fzd5, Fzd4 thereby supporting Wnt signaling related to the developmental hematopoiesis (Corrigan *et al.*, 2009). More recently, using zebrafish embryonic model Wnt16 ligand is observed in the somites that controlled a regulatory network required for HSC differentiation (Clements *et al.*, 2011).

2.2 Wnt signaling pathway in niche

The interaction of HSCs with their niche is crucial to maintain self-renewal and quiescence over a long period of time. Wnt signaling has been described to influence HSC

function and self-renewal (Richter *et al.*, 2017). The question is whether Wnt pathway plays a role in the organization / maintenance of the HSC niche. Wnt signaling in stem cell niche has begun to be addressed in the last decade. Inhibition of Wnt signaling in bone marrow microenvironment by using transgenic mice overexpressing DKK1, an antagonist of Wnt pathway, specifically in osteoblasts resulted in the loss of HSC quiescence, and a severe decline in HSC self-renewal activity (Fleming *et al.*, 2008). Secreted frizzled-related protein 1 (Sfrp1) is expressed in stromal cells, and is a natural inhibitor of Wnt signaling. Sfrp1-deficient mice have a reduction of HSC numbers in G0 stage, and defects in self-renewal (Renstrom *et al.*, 2009). Wnt inhibitor factor 1, Wif1, expressed by osteoblasts has been used as another model of natural Wnt inhibitor. HSCs from *Wif1*$^{-/-}$ mice displayed an increased cycling activity under systemic stress by chemotherapy or after a transplant of wild type HSCs into *Wif1*$^{-/-}$ recipient mice, consequently resulting in the exhaustion of the HSC pool (Schaniel *et al.*, 2011).

Studies in adult mouse bone marrow have described the expression of Wnt signaling component in the microenvironment surrounding the HSCs. Wnt10b is expressed in erythrocytes, immature B cells and myeloid cells (Congdon *et al.*, 2008), whereas Wnt-5a was found in mature B cells (Liang *et al.*, 2003). Wnt10b, Wnt2b, and Wnt5a are produced by bone marrow stromal cell (BMSC) from fetal bone marrow (FBM) and adult bone marrow (ABM). Wnt5a is manifested in populations that are enriched for HSPCs in human bone marrow (Van Den Berg *et al.*, 1998). Wnt5a, Wnt10b, and Wnt3a are expressed in the hematopoietic microenvironment in murine bone marrow at homeostatic condition (Reya *et al.*, 2000) Wnt10b expression is increased in the injured bone marrow microenvironment in mouse after chemotherapy (Congdon *et al.*, 2008). There is also a relatively recent report on the expression of a variety of different Wnts in the bone. Wnt10b and Axin2 produced by Osterix-expressing osteolineage cells involve in regulation of bone proliferation and differentiation (Tan *et al.*, 2014).

2.3 Wnt signaling in hematopoietic stem and progenitor cells

In order to understand the role of Wnt signalling in HSPC developmental process, proliferation and specification, there is a massive number of gain and loss of of function studies that have been conducted in mouse, zebrafish models as well as in human cells. There are several approaches to investigate Wnt pathway such as treatment with soluble Wnt ligands, activation of translocation of β-catenin to nucleus, and inactivation of downstream regulators of the Wnt pathway such as GSK-3β. Supplementation of embryonic stem cells in culture with Wnt3a leads

to their proliferation and increased output of murine hematopoietic lineages; this canonical Wnt was also reported to promote the expansion of human embryonic progenitor cells in culture (Corrigan *et al.*, 2009). Austin et al. reported that culturing hematopoietic progenitors extracted from fetal liver with Wnt10b induced proliferation and enhanced hematopoietic lineage output (Austin *et al.*, 1997). Vijayaragavan et al. discovered that Wnt11 has the ability to promote the expansion of embryonic HSPC numbers in culture, suggesting the possibility that the non-canonical Wnt signaling could also have a role in hematopoietic lineage fate-choice (Vijayaragavan *et al.*, 2009). By culturing $Fzd6^{-/-}$ and $Fzd6^{+/+}$ LSK from fetal liver in presence of Wnt4, Heinonen et al. demonstrated that Wnt4 mediates LSK expansion through planar cell polarity pathway with the participation of Fzd6 receptor (Heinonen *et al.*, 2011b).

Disinhibiting β-catenin is a common strategy that is used in both murine and human HSC research. The principle of this method is pharmacological inhibition of GSK-3β that leads to the stabilization of β-catenin and results in its translocation to nucleus to activate Wnt target genes. In this direction, *in vivo* administration of GSK-3β inhibitors to immune-compromised recipient mice increased HSCs reconstitution capacity and sustained long-term repopulation (Trowbridge *et al.*, 2006). Pre-treatment with an inhibitor of GSK-3β of human HSCs improves myeloid and lymphoid engraftment of *in vitro* expanded of HSC in xeno-graft transplantation models (Ko *et al.*, 2011). Studies have further shown that inhibition of GSK-3β by administration of SB216763 24 hours after total-body irradiation mitigated hematopoietic acute radiation injury and improved recovery of hematopoietic system in a mouse model (Lee *et al.*, 2014). GSK-3β inhibition has also been described to delay human cord blood cell cycling, reduce apoptosis, promote tight adherence to bone marrow stromal cells *in vitro*, and preserve the HSCs ability to reconstitute recipient mice (Holmes *et al.*, 2008). Completed deletion of *GSK-3a* and *GSK-3b* by genetic knock-out induced acute myeloid leukemia (AML) disease in mouse *in vivo*. *GSK-3b* deficiency alone in mice impairs hematopoiesis by inducing myelo-dysplasia (Guezguez *et al.*, 2016).

The functional role of canonical Wnt signalling on hematopoiesis was also studied by directly targeting β-catenin. Transduction of the active form of β-catenin by using retrovirus into sorted HSCs from mice expressing Bcl-2 protein increases phenotypic HSCs numbers in culture setting and enhances long-term reconstitution *in vivo (Reya et al., 2003)*. Stabilized active β-catenin in lymphoid or myeloid progenitors in culture arrests differentiation of these cells and re-enforces their potential to give rise to multiple lineages. For instance, *in vitro* expression of stable β-catenin in CLPs generates phenotypic myeloid cells such as granulocytes, macrophages and the inverse results are seen when myeloid progenitors are cultured with β-

catenin (Baba *et al.*, 2005, Baba *et al.*, 2006). Strikingly, mice with inducible expression of constitutively active β-catenin in hematopoietic system showed HSC expansion in number but blocked differentiation in lymphoid and myeloid progenitors, subsequently leading to the failure to reconstitute the hematopoietic system (Kirstetter *et al.*, 2006, Scheller *et al.*, 2006). Wnt signalling combined with inhibition of apoptotic signalling guides HSC self-renewal and long term repopulation abilities (Perry *et al.*, 2011, Reya *et al.*, 2003). Therefore the cellular level of β-catenin or β-catenin in combination with apoptosis signalling may decide the fate of HSCs. In a recent study, different mutations in Adenomatous polyposis coli gene, a negative regulator of β-catenin, were used to generate mice that express different levels of β-catenin. Using this model, Luis et al. demonstrated that HSC expansion or exhaustion depends on the level of active β-catenin *in vivo* (Luis *et al.*, 2011).

To further investigate the physiological role of Wnt signalling in various HSC aspects such as growth, specification and function, many studies have been conducted where β-catenin and the components of Wnt signalling are targeted for deletion. Wnt-3a deficient mice show a dramatic decrease of HSPCs numbers in the fetal liver and die before birth at day 12.5 of embryonic development (Luis *et al.*, 2009). Besides this, transplantation of purified LSK cells from Wnt-3a null mice to normal wild type recipient displays severe reduction in reconstitution ability. Moreover, lack of Wnt-3a affects the development of myeloid lineages but not lymphoid cells at the progenitor level, suggesting a role of Wnt-3a with stem cell differentiation. Conditional deletion of β-catenin in hematopoietic system using Vav-Cre transgene in developing mice supported these findings. β-catenin deficient mice could form HSCs, but these cells displayed self-renewal and long-term reconstitution defects (Zhao *et al.*, 2007). Interestingly, deletion of β-catenin in adult bone marrow progenitor cells by using IFN-induced Mx-Cre transgene did not result in defects in HSC self-renewal or maintenance of hematopoietic lineages (Cobas *et al.*, 2004). This result suggests that β-catenin does not impair the self-renewal ability of adult HSCs.

2.4 Wnt signaling in inflammation

Wnt signaling has a wide range of effects in tissue regeneration, survival, proliferation and differentiation. Accumulating experimental evidence recently has shown that in addition to the roles mentioned previously, Wnt signaling also participates in both promoting inflammation and controlling inflammation. The evidence for Wnt signaling involved in inflammatory responses came from the study of Sen et al. who observed an increased level of Wnt5a protein

in synovial tissue of rheumatoid arthritis (RA) patients and assigned a functional role for Wnt5a in RA pathogenesis (Sen *et al.*, 2000). Later, Blumenthal et al. showed that Wnt5a and its receptor Fzd5 were found in lung biopsies of patients diagnosed with Tuberculosis. Wnt5a was secreted by macrophages after mycobacteria infection in a Toll-like receptor (TLR)-NF-κB dependent manner (Blumenthal *et al.*, 2006). Wnt5a expression was also found to be enhanced in different inflammatory diseases such as cutaneous lichen planus (Zhang *et al.*, 2015), lesional skin of patients with psoriasis vulgaris (Reischl *et al.*, 2007), and in the sera of septic patients (Pereira *et al.*, 2008). Indeed, pathogen sensing through TLRs subsequently leads to production of Wnt5a by both murine and human macrophages following infection with Mycobacteria, or *Leishmania major, Leishmania donovani* (Chaussabel *et al.*, 2003, Nau *et al.*, 2002). Similarly, induction of Wnt5a upon infection was also observed in *Mycobacterium marinum*-infected zebrafish model (van der Sar *et al.*, 2009). Wnt5a mainly enhances pro-inflammatory cytokine secretion not only in non-immune cells (Kim *et al.*, 2010, Sen *et al.*, 2001), but also in a variety of immune cell types (Blumenthal *et al.*, 2006, Ghosh *et al.*, 2009, Ouchi *et al.*, 2010, Pereira *et al.*, 2008). Wnt5a was reported to up-regulate rapid expression of inflammatory genes in specific human aortic endothelial cells (HAECs) and HUVECs, and activate NF-κB pathway in these cells (Kim *et al.*, 2010). Moreover, Wnt5a treatment inhibits β-catenin signaling in human retinal pigment epithelium (RPE) through promoting phosphorylation of β-catenin leading to degradation. Reduction of β-catenin signaling in RPE results in reducing the expression level of pro-inflammatory factors, such as TNF-α, NF-κB and vascular endothelial growth factor (VEGF) (Kim *et al.*, 2015). More recently, Jang et al. reported an increase in the expression levels of β-catenin Wnt3a, and Wnt5a when stimulating BEAS-2B human bronchial epithelial cells with LPS. Treatment of this cell line with commercial Wnt3a, and Wnt5a resulted in increasing expression level of pro-inflammatory cytokines. Knock-down of Wnt3a, and Wnt5a expression blocked the inflammatory response, and treatment with Wnt antagonist DKK-1 or Porcn inhibitor LGK974 inhibited of inflammatory response (Jang *et al.*, 2017). From the evidence mention above, canonical Wnt signaling shows the ability to promote inflammation.

In immune cells, for example, Wnt5a activates the Wnt/Ca^{2+} pathway in macrophages that leads to elevated Ca^{2+} level in the cytoplasm, thereby activating Calcineurin and Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) (Pereira *et al.*, 2008). Under homeostatic condition, Wnt5a signaling was shown to sustain function of macrophages in the immune system. It was demonstrated that Wnt5a promotes phagocytosis, but it does not affect bacterial killing activity of macrophages (Maiti *et al.*, 2012). Wnt5a and its receptor Fzd5 are expressed in

human mononuclear cells in peripheral blood. The expression of Wnt5a is dependent on the activation of NF-κB and signaling through TLR in sustaining innate immune macrophage response to E.coli/ LPS (Naskar *et al.*, 2014). Functional studies showed that Wnt5a and Fzd5 regulate the antibacterial activity of IL-12 on dendritic cells (DCs) and the production of IFNγ from stimulated T cells (Cooper *et al.*, 1995). These findings suggest that Wnt5a and its receptor bridge the innate and adaptive immune responses against bacterial infection (Cooper *et al.*, 1995). In addition, Wnt5a signaling has been linked to pathogenesis of chronic diseases such as obesity (Ouchi *et al.*, 2010), colitis (Sato *et al.*, 2015), and psoriasis (Kim *et al.*, 2016). Furthermore, Wnt5a is required for CXCL12-CXCR4 signaling and migration of murine and human T cells (Ghosh *et al.*, 2009).

In addition to induction of inflammation in experimental animal models, Wnt signaling also plays role in repressing inflammation. The evidence of this fate comes from study of Neumann et al. where they infected mice with *M. tuberculosis* via aerosol route (Neumann *et al.*, 2010). The authors detected all 19 Wnt genes at different time points analyzed, and a significant reduction in the mRNA level of Wnt2, 2a, 3a, 4, 5a, 7a, 8a and 10b during the course of infection (Neumann *et al.*, 2010). On the other hand, Wnt 5b, 8b, 9a, 9b, 11 and Wnt16 remained unchanged. From day1 to day 21 post-infection, there was a rapid increase of bacterial numbers in the lungs, and this number remained stable until day 42. Fzd1 receptor was up-regulated in lungs of the infected animals and also *in vitro* in macrophages cultured with *M. tuberculosis*. Moreover, *in vitro* culture of macrophages with IFN-gamma led to increased expression of Fzd1 on the surface. The authors also stated that the levels of IFNγ, TNF, and pro-inflammatory genes were upregulated, while β-catenin levels decreased in the lung of infected mice at day 21, 42 post-infection (Neumann *et al.*, 2010). Expression of Axin2, a β-catenin target gene declined 70% at day 21, 42 post-infection (Neumann *et al.*, 2010). Reduction in the β-catenin activity was also reported in the lung of mice infected with *Streptococcus pneumonia* (Hoogendijk *et al.*, 2011). Pre-incubation of macrophages with Wnt3a following infection with *M. tuberculosis* resulted in a significant decrease of TNF production by up to 45% at 24h post-infection. In a separate setting, inhibition of GSK3β in macrophages leads to enhance expression level of Axin2, which was accompanied by decreased TNF production (Neumann *et al.*, 2010). This clearly demonstrated that Wnt/β-catenin signaling suppresses inflammation induced by *M. tuberculosis*. P2X7 receptor (P2X7R), a cell death receptor, is highly expressed on epithelial type I and type II cells in alveolar (AEC I, II). Using P2X7R agonist 2'-3'-O-(4-benzoylbenzoyl)-ATP (BzATP) to induce acute lung injury in a rat model lead to AEC I death by suppressing Wnt/β-catenin pathway through increasing GSK-3β

activity. Treatment of rodents with Wnt3a or GSK-3β inhibitor reduced AEC I death. Wnt3a also attenuated the AEC I damage in mouse acute lung injury model induced by LPS. These results suggest that Wnt3a recovers Wnt/β-catenin signaling by blocking the effect of P2X7R, and prevents AEC I death in animal models (Guo *et al.*, 2014). Another canonical Wnt ligand, Wnt2 has been indicated to inhibit inflammation induced by *Salmonella* and *Escherichia coli* in intestinal epithelial cells (Liu *et al.*, 2012).

Augmented Wnt/β-catenin signaling was described in sepsis, a dysregulated inflammatory response to infection that is life threatening without effective available therapy. Using small molecule inhibition of Wnt/β-catenin activity shows a reduction of organ injury and inflammation in a cecal ligation and puncture (CLP)-induced sepsis model. iCRT3, a chemical agent called 2-(((2-(4-Ethylphenyl)-5-methyl-1,3-oxazol-4-yl)methyl)sulfanyl)-N-(2-phenylethyl) acetamide, that is a small compound with cell-permeable ability. This molecule binds directly to β-catenin and blocks the interaction of β-catenin and TCF, subsequently shutting down gene expression. iCRT3 was demonstrated to inhibit the production of pro-inflammatory cytokines in macrophages treated with LPS (Sharma *et al.*, 2017). Treatment of septic mice with iCRT3 mitigated systemic inflammation and attenuated lung injury. A significant decrease of neutrophil recruitment in the lungs and inflammation as well as apoptosis was also described (Sharma *et al.*, 2017). Multiple Wnt ligands were found with differential expression levels in blood of sepsis patients. LPS-induced systemic inflammation in mouse model also displayed a network of Wnt expression in the mouse serum that was partially similar to the results observed in human blood. Inhibition of Wnt signaling by targeting Wnt acylation and β-catenin activity using pharmacological compounds dampens IL-6 and TNF in serum of LPS-injected mice that demonstrated a positive correlation with sepsis (Gatica-Andrades *et al.*, 2017). In another model of septic shock, male Wistar rats received high dose of LPS to induce endotoxemia. In the study, Wnt3a and Wnt5a exhibited distinct expression patterns in the lung of endotoxemic animals. 6 hours following administration, Wnt5a, Fzd5, and CaMKII were up-regulated in the lung of experimental animals whereas Wnt3a, phosphorylated GSK-3β at Ser9, total β-catenin, and nuclear β-catenin, Fzd1, and Dsh1 were down-regulated (Hii *et al.*, 2015). Furthermore, Wnt5a signaling was described to be activated very early in the lungs of animal model of sepsis as well as from patients diagnosed with septic shock. Specifically, after stimulation with LPS in human bronchial epithelial BEAS-2B cells and human lung MRC-5 fibroblasts, Villar et al. observed a significant increase of Wnt5a, total β-catenin, non-phospho β-catenin, and cyclin D1 and matrix metalloproteinase-7 (MMP7), two downstream target genes of the Wnt pathway. They also demonstrated that Wnt5a and MMP7 protein expression levels increased in rat lungs

18h after sepsis induction. In septic patients who died within the first 24h, the levels of Wnt5a, and MMP7 were also markedly elevated in the lung biopsies (Villar *et al.*, 2014).

2.5 Frizzled receptor 6

Fzd6 belongs to Frizzled family that is known as the main receptor for Wnt pathway. Fzd6 is expressed on HSCs, early lymphoid progenitors (ELP), CLPs and CMPs (Yokota et al., 2008). In addition, Fzd6 is also expressed on MSCs, endothelial cells in BM (Dosen *et al.*, 2006) and in different tissues such as the heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, thymus, prostate, testis, ovary, small intestine, and colon of human (Tokuhara *et al.*, 1998). Fzd6 gene is located on human chromosome 8q22.3-q23.1, and chromosome 15 in mouse (Tokuhara *et al.*, 1998).

Fzd6 is involved in the planar cell polarity (PCP) signaling pathway, which is required for polarity signal transmission in epidermal epithelial cells (Wang *et al.*, 2006). Fzd3 and Fzd6 are expressed in inner-ear sensory epithelia in an asymmetric arrangement that correlates with the axis of cell polarity. These proteins have been reported to control neural tube closure and orientation of hair bundles on a subset of auditory and vestibular sensory cells (Wang *et al.*, 2006). In another report, while the authors demonstrated that the absence of Fzd6 contributes to neural tube defects in human, they could not find evidence to link this kind of syndrome with Fzd3 (De Marco *et al.*, 2011). Fzd6 expression has been shown in skin and hair follicles and it controls hair patterning in mouse (Chang *et al.*, 2016, Guo *et al.*, 2004, Wang *et al.*, 2010). A wide range of developmental defects, such as broadening of neural plate, neural tube defects and shortened body axis have been demonstrated with mutations in PCP genes (Murdoch *et al.*, 2014). In case of deletion of Fzd6 gene in mice, male newborns displayed abnormal claw morphology, suggesting that Fzd6 regulates differentiation process of claw/nail formation (Cui *et al.*, 2013, Frojmark *et al.*, 2011), and a similar phenotype was seen in humans (Frojmark *et al.*, 2011, Naz *et al.*, 2012, Wilson *et al.*, 2013). The asymmetrical distribution of Fzd6 in dental lamina correlates with their growth direction during tooth development, especially in species with both temporary and permanent teeth (Putnová *et al.*, 2017).

There is some evidence of Fzd6 being involved in cancer development such as its overexpression in colorectal cancer (Caldwell *et al.*, 2008), and down-regulation in acute myeloid leukemia (AML) cell lines (Garcia *et al.*, 2013). Recently, Corda et al. have illustrated an increased incidence of Fzd6 expression in the triple-negative breast cancer subtype. By

controlling assembly of actin cytoskeleton, Fzd6 regulated motility and invasion of breast cancer cell lines (Corda *et al.*, 2017).

Fzd6 is a negative regulator of β-catenin transcriptional activity in human colon cancer cell lines SW480 (Golan *et al.*, 2004). Fzd6 activates TAK1/NLK kinases, subsequently declines activation of target genes of β-catenin via phosphorylation of nucleus transcription factor TCF/LEF (Golan *et al.*, 2004). Down-regulation of Fzd6 in HelaS3 cells did not lead to increased nuclear levels of β-catenin, or activation of RAC after exposure to canonical Wnt ligand Wnt3a, suggesting that Fzd6 is involved with non-canonical pathway (Sato *et al.*, 2010). Fzd6 also inhibited gastric cancer cell proliferation and migration through activation of a non-canonical Wnt pathway (Yan *et al.*, 2016). In contrast, Fzd6 expression pattern was associated with increasing β-catenin levels in chronic B lymphocytic leukemia mouse model (Wu *et al.*, 2009). The expression level of Fzd6 is positively correlated with canonical Wnt-3a in human mesenchymal stem cells (Kolben *et al.*, 2012).

Fzd6 is known as a receptor for Wnt4 in immature hematopoietic progenitor cells, based on a reduction in the expansion of HSPCs mediated by Wnt4 in the absence of Fzd6 (Heinonen *et al.*, 2011a). Fzd6 has also been shown to have a role in regulating the survival and expansion of HSPCs observed in competitive transplantation assays (Abidin *et al.*, 2015). However, until now, the mechanisms of Fzd6 function in HSCs remain obscure.

3 EMERGENCY GRANULOPOIESIS

Neutrophils are known as critical effector cells in the innate immune system that participate in patrolling organisms for microbial pathogens. When they detect signs of pathogen, neutrophils destroy them by releasing intracellular secretory granules, phagocytose them, or capture the pathogen by release nuclear materials to form neutrophil extracellular traps. (Rosales, 2018). They have a short lifetime, ranging from one to four days and they are constantly produced in the bone marrow from HSPCs, with the number of up to 10^{11} cells per day in steady-state (Chistiakov *et al.*, 2015). Under steady-state conditions, neutrophils enter the circulation, migrate to peripheral tissues and are finally eliminated by macrophages to finish their lifecycle. In emergency conditions such as local or systemic infections and inflammation, the organism consumes a huge number of neutrophils to sustain homeostasis. This requires production of large numbers of neutrophils to meet the body's demands (Chistiakov *et al.*, 2015).

Emergency granulopoiesis is defined as the *de novo* generation of neutrophils in the bone marrow to fulfill the demands of the blood system in response to infection or inflammation (Manz *et al.*, 2014). The emergency cascade can be divided into three phrases. Firstly, the presence of invading pathogens needs to be sensed to inform the immune and hematopoietic system of the emergency events. Second, the emergency case needs to be translated into molecular signals to induce production of neutrophils in the bone marrow. Third, the hematopoietic system needs to recover and return to steady-state conditions after clearance of an infectious pathogen (Manz *et al.*, 2014).

LPS, also named endotoxin is a component of the outer membrane of Gram-negative bacteria. LPS includes an oligo- or polysaccharide region anchored in the outer bacterial membrane by a lipid A tail. The lipid A component is the major immune-stimulatory part of LPS (Erridge *et al.*, 2002). LPS is used in many models to mimic sepsis or inflammation (Fink, 2014, Nemzek *et al.*, 2008). LPS can be recognized by TLR4 that is expressed on the surface of various cell types such as HSPCs, macrophages, MSCs (Nagai *et al.*, 2006, Vaure *et al.*, 2014). Once LPS binds to its receptors, TLR4 on macrophages results in macrophage activation and secretion of inflammatory mediators and chemokines. In turn, those chemokines act as chemo-attractants to recruit neutrophils, T lymphocytes, monocytes, dendritic cells to the site of inflammation (Amanzada *et al.*, 2014, Tirosh *et al.*, 2010). *In vivo* administration of LPS in the

mouse model leading to LPS ligation with TLR4 expressed on HSPCs, results in proliferation of HSPCs and a significant increase in the output of neutrophils. As a consequence, steady-state hematopoiesis is switched to emergency granulopoiesis (Boettcher *et al.*, 2014). Repetitive challenge with LPS leads to the activation of quiescent HSCs and their entry to cell cycle though TLR4 pathway. This results in the restriction of HSC self-renewal ability via TLR4-TRIF-ROS-p38 signalling (Takizawa *et al.*, 2014). Sepsis induced in mouse model by intra-peritoneal injection of 3mg/kg *Pseudomonas aeruginosa* LPS leads to suppressed generation of myeloid cells and exhaust of HSCs (Zhang *et al.*, 2016). In addition to bacterial products, viruses and parasites also promote emergency myelopoiesis. In a previous study, we showed that *Leishmania donovani*, a protozoan parasite that causes a chronic infection induces the proliferation of HSPCs in the BM and spleen, especially increase myelopoiesis with emergency GMP and HSC-like output during the course of infection. In addition, increased parasite burden was correlated with enhanced myelopoiesis, which mostly produces granulocytes and monocytes which are downstream of GMP (Abidin *et al.*, 2017). Mice infected with *Toxoplasma gondii*, another protozoan parasite, displayed a blockage in the generation of Erythrocytes, the precursor of red blood cells (RBC) and increased GMP progeny. However, the authors showed only a significant increase in monocyte numbers and a little change in granulocyte output (Chou *et al.*, 2012).

3.1 Sensing of pathogens

When invading pathogens become systemic in a living organism, transmission of signals to alert the immune system is a crucial step to start the emergency granulopoiesis cascade. This requires a cell type or several cell types encounter the foreign organisms and have a machinery to detect and translate the signal into emergency granulopoiesis. Fortunately, pattern recognition receptors, such as TLRs, detect and respond to a variety of classes of conserved pathogen-associated molecular patterns (PAMPs).

3.1.1 Direct sensing

LT-HSCs and their downstream progeny in both humans (Chicha *et al.*, 2004, De Luca *et al.*, 2009) and mice (Nagai *et al.*, 2006, Schmid *et al.*, 2011) have been shown to express TLRs that can directly respond to pathogens. TLR ligation on HSPCs leads to their proliferation, migration to peripheral tissues and differentiation, and therefore contributes to the response of

the immune system (Manz *et al.*, 2014). Following TLR stimulation, human (De Luca *et al.*, 2009) HSCs as well as murine HSCs (Nagai *et al.*, 2006, Schmid *et al.*, 2011) expand their numbers and undergo specific differentiation to myeloid lineages. Direct transmission of signals through TLRs enables HSCs to immediately reconstitute cells of the innate immune system after a rapid depletion during infection. Indeed, there is evidence that both migratory HSCs and bone marrow resident HSCs can respond directly to TLR danger signals (Manz *et al.*, 2014). *In vitro* stimulation of mouse HSPCs with TLR2 and TLR4 agonists leads to their proliferation in a cytokine-independent manner and promotes their differentiation to myeloid cells (Nagai *et al.*, 2006). Similarly, human $CD34^+$ HSPCs subjected to *in vitro* TLR ligation enhances myeloid differentiation of these cells (De Luca *et al.*, 2009, Sioud *et al.*, 2007). There is a small proportion of HSCs that constantly enters peripheral blood circulation from the bone marrow and re-enters the bone marrow after that (Wright *et al.*, 2001). Although it is not clear how important this event is, it is thought to be involved in patrolling for invading pathogens in remote tissues. After encountering foreign microorganisms, HSPCs could directly differentiate in their progeny to fight against pathogens at the site of infection and support tissue repair (Manz *et al.*, 2014). Ex vivo pre-stimulation of HSPCs with LPS followed by transplantation under the kidney capsule, and continued *in vivo* LPS stimulation has been indicated to generate a myeloid cell cluster within the kidney (Massberg *et al.*, 2007). Furthermore, early treatment of HSPCs with TLR-agonist results in increased secretion of different hematopoietic growth factors including IL-6. *In vivo* treatment with 5-fluorouracil (5-FU) or ionizing radiation stimulates HSPCs to produce hematopoietic growth factors that enhance generation of myeloid lineages. In addition, combining type I interferon and TLR7 signaling on common myeloid progenitors was reported to promote monocyte/macrophage differentiation (Buechler *et al.*, 2016). BM WT was transplanted into TLR4 deficient mice (WT->Tlr4 KO), and vice versa TLR4 deficient BM was transplanted into WT mice (Tlr4 KO->WT) (Liu *et al.*, 2015). The authors then injected recipients with LPS low dose and observed a significant increase in HSPCs in KO mice, while there was no change in HSPC numbers in WT mice (Liu *et al.*, 2015). Chimeric mice with a 60%:40% mixture of WT/KO was generated. At 12 week post-engraftment, mice were challenged with LPS. WT HSCs increased 3 fold compared to unchanged in Tlr4 deficient HSCs. In vitro experiment where sorted-HSCs were cultured with LPS also displayed significant expansion 12 hours post stimulation (Liu *et al.*, 2015). These results suggest that the effect of LPS on HSCs was direct via activation of TLR4 on HSCs. Taken together, the experimental evidence clearly proves that HSPCs express TLRs and can thus detect and respond directly to pathogens.

3.1.2 Indirect sensing

Although TLR function in pathogen sensing is well described in hematopoietic cells, non-hematopoietic cells also have TLRs and contribute to propagating inflammatory signals. For example, house dust mites induce allergic asthma via TLR4 signal that is expressed on structural airway cells (Hammad *et al.*, 2009). Schilling et al. demonstrated that mice that are deficient in TLR4 in bladder epithelial cell were unable to prime a protective response to control the early infection with *E. coli* although hematopoietic cells expressed TLR4 in these mice (Schilling *et al.*, 2003). The study also showed TLR4+ epithelial cells alone could not activate inflammatory response, suggesting that TLR4 signals both on epithelial cells and hematopoietic cells were required for activation of the immune response. These results demonstrated that bladder epithelial cells play a crucial role in innate immunity mediated via TLR4 to eliminate *E. coli* (Schilling *et al.*, 2003). In another model, when $Tlr4^{-/-}$ bone marrow was transplanted into $Tlr4^{+/+}$ mice, and all resident macrophages and circulating leukocytes thus lacked *Tlr4*, these cells were completely non-responsive to LPS. However, when $Tlr4^{+/+}$ bone marrow cells were transplanted to $Tlr4^{-/-}$ environment ($Tlr4^{-/-}$ endothelial cells), followed by LPS-induced systemic inflammation, the mice displayed little increase in the number of neutrophil migrating into lungs. This phenomenon suggests that endothelial cells were important for sensing and translating signal of LPS (Andonegui *et al.*, 2003). TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, and TLR9 expression was also reported in mesenchymal stromal cells, and bone marrow stromal cells promote monocyte egress from bone marrow in response to TLR stimulation with its agonist (Hwa *et al.*, 2006, Pevsner *et al.*, 2007, Tomchuck *et al.*, 2008). More recently, Manz et al. indicated that endothelial cells drive LPS-induced emergency granulopoiesis by signalling through TLR- myeloid differentiation primary response protein 88 (Myd88) axis. Subsequently, following LPS stimulation, signal was translated into G-CSF production by the endothelial cells to switch bone marrow output from steady-state to emergency granulopoiesis. This study also identified bone marrow endothelial cells as the main source of G-CSF, suggesting this cell type is a critical regulator of emergency granulopoiesis (Boettcher *et al.*, 2014).

In addition to non-hematopoietic cells, mature hematopoietic cells are also proposed as a model for indirect sensing of pathogen. Monocytes and macrophages are the initial cell types which sense the signal of pathogens in both local and systemic bacterial infection models (Hamilton, 2008, Kaushansky, 2006). This activity is largely dependent on TLR signaling, which leads to the production of inflammatory cytokines and hematopoietic growth factors (De Waal Malefyt *et al.*, 1991, He *et al.*, 2009, Nishizawa *et al.*, 1990). These characteristics make them

ideal cell types for pathogen sensing. However, the idea of monocytes/macrophages sensing the invading pathogen signals leading to proliferation of HSPCs and subsequently inducing emergency myelopoiesis has not been demonstrated yet in an *in vivo* experimental model.

3.2 Cytokines regulating myelopoiesis

3.2.1 Colony-stimulating factors

Hematopoiesis is a process regulated by various hematopoietic growth factors including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Hamilton, 2008, Kaushansky, 2006). G-CSF plays an important role in the production of granulocytes during steady state granulopoiesis, emergency granulopoiesis, and it is an important hematopoietic cytokine promoting HSC mobilization from the bone marrow (Demetri *et al.*, 1991, Duhrsen *et al.*, 1988). Initially, G-CSF was used to induce neutrophil production in patients whose neutrophils are depleted as a side effect of using chemotherapy (Gabrilove *et al.*, 1988). G-CSF is also used in clinic because it promotes rapid mobilization of HSC from bone marrow to peripheral blood; mobilized HSCs are then collected for several purposes such as transplantation (Anasetti *et al.*, 2012). G-CSF signals via its homo-dimeric receptor G-CSFR, which is mainly expressed on hematopoietic cells (Liu *et al.*, 2000). Many cell types such as macrophages, epithelial cells, endothelial cells and fibroblasts secrete G-CSF in response to LPS, IFN-β, and IL-17 (Fossiez *et al.*, 1996, Sano *et al.*, 2007). G-CSF release from these cells boosts the generation of neutrophils within bone marrow and their mobilization to peripheral blood. Vast production of neutrophils and their progenitors creates a proteolytic environment due to the secretion of matrix metallo-proteinases leading to the degradation of adherent molecules that anchor HSCs with their niche, and subsequent HSC mobilization to peripheral blood (Pelus *et al.*, 2004). G-CSF or G-CSFR null mice had 90% reduction in circulating neutrophils (Lieschke *et al.*, 1994, Liu *et al.*, 1996). The precise impact of G-CSF directly on HSCs during emergency hematopoiesis remains unclear. Studies of Zhan et al. showed a mitigated response of granulocytes and increased lethality of G-CSF deficient mice compared to their counterparts during infection with *Listeria monocytogenes* (Lieschke *et al.*, 1994, Zhan *et al.*, 1998). On the other hand, there was no difference between G-CSF null mice and control mice in a model of *Candida albicans*-induced emergency granulopoiesis (Basu *et al.*, 2000). Administration of G-CSF activates quiescent HSCs and increases the proportion of HSCs, promoting emergency granulopoiesis (Tamura *et al.*, 1987). G-CSF is proposed to have

ability to induce both quiescent normal and malignant HSCs enter cell cycling (Saito *et al.*, 2010, Wilson *et al.*, 2008). Recent researches show G-CSF promotes dormant HSCs migration from BM to peripheral blood without proliferation (Bernitz *et al.*, 2017, Kovtonyuk *et al.*, 2016). G-CSF stimulates $CD41^{+}$HSCs proliferation, which is short-term HSCs with primarily potential to regenerate myeloid lineages (Bernitz *et al.*, 2017). However, G-CSF produced by endothelial cells is required for the induction of emergency granulopoiesis in response to LPS, promoting accelerated generation of granulocyte-monocyte progenitors (Boettcher *et al.*, 2014). Expression of G-CSFR on HSCs was not required for HSCs mobilization by G-CSF, suggesting G-CSFR-dependent signals act in trans to mobilize HSCs from bone marrow to peripheral blood most likely by decreasing adhesion molecule CXCL12 (Liu *et al.*, 2000). The available evidence proposes that G-CSF mostly acts downstream of HSCs such as on CMPs, GMPs, and granulopoietic progenitors but not on HSCs (Kovtonyuk *et al.*, 2016).

GM-CSF enables HSPC differentiation toward myeloid lineages (Gasson, 1991, Lang *et al.*, 1987). GM-CSF deficient mice presented a significant depletion of myeloid cells in the bone marrow and in inflamed tissues, resulting in a failure to control *L. monocytogenes* infection (Zhan *et al.*, 1998). In addition, in a model of chronic infection with *Mycobacterium avium* GM-CSF null mice were unable to increase myeloid progenitor numbers following infection (Zhan *et al.*, 2000). Taken together, GM-CSF seems to be important for emergency myelopoiesis.

3.2.2 Interleukin-3

IL-3 has a function in promoting steady-state hematopoiesis. In the early 1990s, Yang et al. showed that a gibbon T cell line secreted a hematopoietic growth factor, which stimulates formation of colony in culture setting. This factor was later identified homologous to murine IL-3 (Yang *et al.*, 1986). Culture of human bone marrow cells with recombinant gibbon IL-3 in methylcellulose promoted the growth of myeloid progenitors. This finding suggests that IL-3 stimulates the proliferation of myeloid lineages (Yang *et al.*, 1986). Recent studies have shown the functional activity of IL-3 in emergency hematopoiesis. In a cecal ligation and puncture model of sepsis, it was observed that IL-3 contributes to the enhancement of leukocyte numbers in bone marrow and peripheral blood (Weber *et al.*, 2015). Moreover, innate response activator (IRA) B cells developed from B1a B cells were identified as the main source of IL-3 this model (Weber *et al.*, 2015). In that setting, IL-3 enhanced the generation of Ly-$6C^{high}$ monocytes and neutrophils that switched from steady-state to emergency myelopoiesis. The high level of IL-3 in serum also enhanced the cytokine storm that leads to an increase in the death rate. Therefore

inhibition of IL-3 production enhanced the survival of septic mice. However, the functional role of IL-3 from IRA B cells during more general emergency hematopoiesis still needs to be investigated (Weber *et al.*, 2015).

3.2.3 Interleukin-6

IL-6 is a cytokine that has many different functions. IL-6 plays crucial roles in several different biological processes, including hematopoiesis as well as the immune response. IL-6 receptor is a protein complex consisting of two subunits: a ligand-binding α chain (IL-6Rα; also known as CD126) and a signal-transmitting β subunit glycoprotein 130 (gp130; or CD130) (Schaper *et al.*, 2015, Scheller *et al.*, 2011). T cells and macrophages secrete IL-6 in response to infection, trauma, and tissue damage (Scheller *et al.*, 2011). Osteoblasts secrete IL-6 to support the formation of osteoclasts. IL-6 is not important for steady-state hematopoiesis (Kopf *et al.*, 1994), and the expression of other cytokines could compensate for the loss of IL-6, but it has a critical role in the development of lineages under stress conditions such as inflammation (Maeda *et al.*, 2005). Treatment of mice with phenyl-hydrazine (PHZ), which is known to lyse erythrocytes, led to severe hemolytic anemia, with depletion of white blood cells, and granulocyte/monocyte progenitors. IL-6 deficient mice displayed defects in hematopoietic recovery compared to wild type mice, indicating a role of IL-6 in the regulation of HSPC proliferation and differentiation (Bernad *et al.*, 1994). Likewise, IL-6 deficient mice showed a severe impairment of neutrophil response to systemic *Candida albicans* infection. This defect is reversed by the administration of IL-6 (Romani *et al.*, 1996). MPPs and myeloid progenitors express IL-6Rα, while LT-HSCs and ST-HSCs do not have this receptor (Reynaud *et al.*, 2011). When these MPP populations are stimulated with IL-6, there is a rapid increase of myeloid cell lineages and blockade of $CD19^+$ B cell formation (Reynaud *et al.*, 2011). In a model of emergency granulopoiesis where both G-CSF and GM-CSF, and IL-6 were deleted, the triple mutant mice displayed a significant decrease of neutrophil activity in response to LPS. This impairment is recovered following exposure to IL-6, suggesting that IL-6 has a role in controlling emergency granulopoiesis (Walker *et al.*, 2008). Recently, Schürch et al. reported that $CD8^+$ cytotoxic T cells secrete IFN-γ during acute viral infection, which induced MSCs in the bone marrow to secrete hematopoietic cytokines including IL-6. IL-6 secretion by MSCs subsequently promotes HSPC proliferation and differentiation into myeloid cell lineages (Schurch *et al.*, 2014). Furthermore, Zhao et al. showed direct pathogen sensing through TLRs led to IL-6 secretion by HSPCs. The authors used chimeric mice transplanted with wild type and

IL-6 null HSPCs to eliminate the effect of mature myeloid cells, which are the major source of IL-6. IL-6 competent wild type HSPCs produced myeloid cells effectively in response to LPS as compared to IL-6 null HSPCs. The outcome of this study suggests that HSPC-derived IL-6 plays a role in emergency response to bone marrow stress conditions (Zhao *et al.*, 2014a).

3.2.4 Interferon gamma

IFNs are known as inflammatory cytokines that have well-studied functions in the immune system against a large number of pathogens. IFN-γ has several effects on HSC production, proliferation and function. It was shown that inflammatory signaling positively regulates the formation of embryonic HSPCs in both mouse and zebrafish models. In the absence of IFN-γ signalling, there was a significant decrease of HSPCs in AGM. Knock-down of IFN regulator factor 2 (IRF2), which is known as negative regulator of IFN signaling, resulted in increasing expression of IFN target genes and HSPC production in the embryonic zebrafish. IFN-γ signaling does not seem to impact HSC survival or proliferation in AGM (Li *et al.*, 2014). Besides that, there are several studies demonstrating that IFN-γ directly activates quiescent HSCs that express IFN-γ receptor on their surface. Chronic infection with *Mycobacterium* in mice shows LT-HSC expansion in both number and proportion during the course of the infection. The authors report increased output of HSCs in the course of infection. Completely knocking-out the *Ifng* gene results in less proliferative activity of LT-HSCs compared to wild type mice in steady-state and chronically infected conditions. The outcome of these experiments suggests that the response of LT-HSCs to a chronic infection requires IFN-γ signalling (Baldridge *et al.*, 2010). Another study in favour of this hypothesis is that an acute *Ehrlichia muris* infection transiently converts dormant HSCs to an active stage in IFN-γ-dependent fashion (MacNamara *et al.*, 2011). During an acute infection with LCMV, the total HSC pool does not increase even though increasing in cell division, suggesting that most cells division does not contribute to self-renewal event during LCMV infection, but the divided cells contribute to differentiation and migration to peripheral blood (Matatall *et al.*, 2014). The study also reported that IFN-γ plays a more important role in myeloid-biased HSCs (My-HSCs) than lymphoid-biased HSCs (Ly-HSCs) based on increasing proliferation of My-HSCs post recombinant IFN-γ injection (Matatall *et al.*, 2014). On the other hand, IFN-γ induced cell division promotes differentiation and inhibits self-renewal. Mice repeatedly infected with *Mycobacterium avium* developed pancytopenia, characterized by decreased red and white blood cell and platelet counts following exposure with pathogen. A significant increase in the levels of IFN-γ in serum of infected mice sustained 6

months post infection lead to HSCs depletion through impaired self-renewal ability, and enhanced expression of BATF2 that leads to increased terminal differentiation (Matatall *et al.*, 2016). Bruin et al. published an article in contradiction with these findings, as the authors illustrate that IFN-γ reduces the proliferative capacity of HSCs in mouse model. Culture of purified HSCs with recombinant IFN-γ caused a significant reduction in HSPC percentage and numbers. Indeed, transplantation of cultured HSCs in competition with WT cells into recipient mice showed long-term reconstitution failure of IFN-γ-treated HSCs compared to wild type cells (De Bruin *et al.*, 2012). Furthermore, IFN-γR1$^{-/-}$ HSCs recover to normal levels much better compared to their counterparts on day 8 of infection with the acute Armstrong strain of LCMV (De Bruin *et al.*, 2013). To exclude the indirect effect of IFN-γ on HSCs, IFN-γR1 chimeric mice (WT:IFN-γR1$^{-/-}$) were generated and challenged with Armstrong strain. Similarly, IFN-γR1$^{-/-}$ HSCs recovered better than WT ones. The authors concluded that IFN-γ inhibits HSC recovery following viral infection. IFN-γ also enhances suppressor of cytokine signalling (SOCS) 1 level in HSCs, which inhibits phosphorylation of STAT5 via TPO, therefore down-regulating key cell-cycle genes, *Ccnd1 (Cyclin D1)* and *p57* (De Bruin *et al.*, 2013). IFN-γ is also reported to indirectly influence HSPCs. This conclusion comes from the study of Schurch et al. where the authors described that cytotoxic CD8^{+} T lymphocytes produced IFN-γ, mediating the secretion of IL-6 from bone marrow MSCs and thus promoting myeloid lineage differentiation (Schurch *et al.*, 2014).

3.2.5 Interferon type I-α, β

IFNs are anti-viral cytokines that are secreted by host cells in response to tumors, and pathogens such as viruses, bacteria, and parasites (Chiba *et al.*, 2018). Type I IFNs include IFNα and IFNβ, which are produced by different cell types such as fibroblasts and monocytes. The mechanism of anti-viral activity of these cytokines is to inhibit the replication and production of viral genome. Type I IFNs engage with their cell surface receptor complex that is composed of IFN-α receptor 1 (IFNAR1) and IFNAR2 subunits (Chiba *et al.*, 2018). The role of IFN-α/β on hematopoiesis mostly came from the research of viral infection using lymphocytic choriomeningitis virus (LCMV) model and injection of mice with poly I:C. treatment of HSPCs with IFN-α or IFN-α/β deficient mice, concluded that type I IFNs repress progenitor cell proliferation and differentiation (Chiba *et al.*, 2018). Mice treated with poly I:C every 2 days for a month showed detectable level of IFN-Is from day 3 to 30 in sera. Along with chronic production of type I IFNs, bone marrow cells such as B lymphocytes, and granulocytes were depleted. This

depletion was dependent on IFNAR (Pietras *et al.*, 2014). Chronic exposure to IFN-I led to a transient augmentation in HSC proliferation but the HSC pool remained unchanged in number. Importantly, HSCs exposed to IFN-I rapidly re-enter quiescent state (Pietras *et al.*, 2014). Infection with non-cytopathic LCMV leads to greatly depleted hematopoietic progenitor numbers in the bone marrow of wild type mice (Sato *et al.*, 2009). Using IFN regulator factor 2 (Irf2) deficient mice, a transcriptional suppressor of type I IFN signalling expressed in HSCs, the authors demonstrated that Irf2 preserves HSC self-renewal and reconstitution of multi-lineage differentiation ability (Sato *et al.*, 2009). Injection of IFNα *in vivo* caused HSC conversion from dormant stage to active cell cycle (Essers *et al.*, 2009). In response to IFNα treatment, HSCs up-regulated the expression of downstream targets of IFNα genes, phosphorylation of STAT1, and PKB/Akt signalling, and increased the expression of stem cell antigen-1 (Sca-1). At steady state, Foudi et al. characterized approximately 1% phenotypic HSCs enter cell cycle by using label retention assay (Foudi *et al.*, 2009). Following injection with poly I:C the percentage of HSCs cycle was six to seven fold higher for up to 3 days, led to accumulation of reactive oxygen species and DNA damage in remaining HSCs. DNA damage thus induced HSCs senescence via IFN-dependent signaling.

3.2.6 Macrophage inflammatory Protein -1 alpha

Macrophage inflammatory protein-1 alpha (MIP-1α, also known as CCL3) is a chemokine that is secreted by activated macrophages. Human and mouse MIP-1α engages with its receptors CCR1, CCR5, and D6. Mouse MIP-1α also binds to CCR3 (Nibbs *et al.*, 1997). CCR1 is in charge of the mobilization of HSPCs via CCL3 (Broxmeyer *et al.*, 1999). CCL3 shows potential ability in hematopoietic regulation in physiologic conditions, and regulates HSPC proliferation in BM (Cook *et al.*, 1995). MIP-1α cDNA was first cloned as an endogenous inflammatory mediator from RAW264.7 mouse macrophage cell line that was stimulated with LPS (Davatelis *et al.*, 1988). Immediately after the identification of *CCL3* cDNA, Graham et al. described CCL3 as a stem cell inhibitor when they observed the inhibition of colony formation when primitive BM cells were cultured with supernatant from the J774.2 macrophage cell line (Graham *et al.*, 1990). Subsequent research illustrates that CCL3 could inhibit the formation of colony and proliferation of HSPCs both *in vitro* and *in vivo* (Broxmeyer *et al.*, 1990, Graham *et al.*, 1990). CCL3 inhibits primitive progenitor cell proliferation but induces more mature progenitor proliferation (Broxmeyer *et al.*, 1989, Broxmeyer *et al.*, 1990). Moreover, CCL3 blocks cell cycle entry and therefore maintains HSCs at quiescent stage (Verfaillie *et al.*, 1994). Injection of high

dose of CCL3 to mice (Lord *et al.*, 1995) and humans (Broxmeyer *et al.*, 1998) causes rapid mobilization of HSCs from BM to peripheral blood. Recently, Staversky et al. by using CCL3 deficient mice demonstrated that CCL3 regulates HSC numbers and myeloid differentiation in steady-state (Staversky *et al.*, 2018a). Moreover, Baba et al. revealed basophils consitutively produce CCL3 at steady-state to negatively control normal hematopoiesis, especially basophil-derived CCL3 regulates HSCs regeneration post transplantation (Baba *et al.*, 2016). Using model of chronic myeloid leukemia (CML) the study also showed that basophil-like leukemic cells generate CCL3 in BM, and in turn this molecule impacts their biological function on CCR1, CCR5-expressing non-leukemic cells to maintain leukemia initiating cells (LICs), which are formed by transformation of normal HSCs (Baba *et al.*, 2016). CCL3 could act as an inducer of normal HSPC mobilization from BM to peripheral blood (Baba *et al.*, 2013), reorganizing leukemic niche cells in BM to support the maintenance of LICs (Schepers *et al.*, 2013).

MIP-1α regulates acute and chronic inflammatory responses of host organisms at the site of infection or injury by recruitment of pro-inflammatory cells (Maurer *et al.*, 2004). MIP-1α is expressed in the lung of mice after injection with LPS in a time-dependent fashion, resulting in the infiltration of neutrophils and macrophages in the lungs. Administration of anti-MIP-1α antibody reduces neutrophil and macrophage infiltrate and decreases lung permeability (Standiford *et al.*, 1995). Hsieh et al., 2008 demonstrated that MIP-1α has an important function in mediating the acute inflammatory response following trauma-hemorrhage. MIP-1α null mice show attenuated acute inflammatory responses, rapid recovery, and less remote organ injury after trauma (Hsieh *et al.*, 2008).

CHAPTER 2 – HYPOTHESIS AND OBJECTIVES

Hematopoietic stem cell lies at the apex of hematopoietic hierarchy that is in charge for all blood cell types production throughout the lifetime of the organism. Most HSCs remain in a quiescent stage that helps them to avoid aberrant proliferation leading to DNA damage and exhaustion. Boettcher et al. showed in 2014 that systemic inflammation caused by LPS, a component of the gram-negative bacterial coat, was sensed by endothelial cells. Subsequently, endothelial cells translate the pathogen signal into a switch from steady-state to emergency granulopoiesis by secreting granulocyte-colony stimulating factor (G-CSF). G-CSF promotes the differentiation of granulocyte-monocyte progenitor cells to finally meet the high demand of neutrophil output after LPS injection *in vivo*. Although the mechanisms promoting HSPC activation and egress are well characterized, it is less clear what promotes their survival and return to the bone marrow.

In a previous study, our laboratory has shown that Fzd6, a non-canonical Wnt receptor expressed in HSPCs, regulated self-renewal and long-term engraftment of hematopoietic stem cells in irradiated recipient mice. Using the LPS-induced emergency granulopoiesis model, Fzd6 deficient HSCs failed to expand after the second LPS injection compared to their counterpart wild type HSCs (Abidin *et al.*, 2015). Thus, we seek to understand why Fzd6 null HSCs could not expand in response to the bone marrow inflammatory stress. We also want to know the molecular mechanisms that regulate Fzd6 deficient HSC behavior and evaluate the contribution of Fzd6-deficient environment. In competitive transplantation assays, where we transplanted Fzd6 KO or WT BM mixed 1:1 with competitor BM into irradiated recipients, Fzd6 KO HSCs showed poor reconstitution ability at 4 weeks after transplantation. The defects in resconstition became more pronounced at week 12 and 16 postransplant. These results suggest that the impairment in regeneration ability observed in Fzd6 KO HSCs is cell intrinsic (Abidin *et al.*, 2015). We therefore hypothesize that the impact of Fzd6 in hematopoietic stem cells is hematopoietic cell intrinsic. We proposed two objectives:

1. To study the mechanism of Fzd6 signaling during steady-state and HSC activation.
2. To investigate the role of Fzd6-deficient microenvironment in HSC response to LPS.

CHAPTER 3 – PUBLICATION

FRIZZLED-6 PROMOTES HEMATOPOIETIC STEM/PROGENITOR CELL MOBILIZATION AND SURVIVAL DURING LPS-INDUCED EMERGENCY MYELOPOIESIS

Running title: Fzd6 protects HSPCs during stress hematopoiesis

Keywords: hematopoietic stem cells, emergency hematopoiesis, Wnt signaling, Frizzled-6, lipopolysaccharide, macrophages

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Authors' contribution:

BMA: designed and conducted experiments, analyzed data and prepared figures and figure legends.

THN: designed and conducted experiments, analyzed data and prepared figures and figure legends.

KMH: conceived the project, designed the experimental approach, analyzed data and wrote the manuscript.

Experiments:

Figure 1, 4, 6A: BMA performed the experiments

Figure 2, 3, 5, 6B-D: THN performed the experiments

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1 SUMMARY

Emergency hematopoiesis involves the activation of bone marrow hematopoietic stem/progenitor cells (HSPCs) in response to systemic inflammation. HSPCs are maintained in the bone marrow in a special microenvironment that regulates their response to environmental changes. We have previously shown that Fzd6 plays a pivotal role in regulating HSPC expansion and long-term maintenance. Now we sought to better understand the underlying mechanisms. Using the LPS-induced emergency granulopoiesis model, we show that the defect in HSPC expansion was intrinsic to Fzd6-deficient hematopoietic cells and not due to inefficient LPS sensing. Rather, Fzd6-deficient HSPCs were more strongly activated, but their differentiation and mobilization to peripheral blood were impaired. Fzd6-deficient macrophages further contributed to HSPC loss by secreting myelosuppressive MIP-1α. These studies suggest that Fzd6 has a protective effect in the bone marrow to prevent an overactive inflammatory response. We further propose that mobilization may promote HSPC survival during bone marrow inflammation.

2 INTRODUCTION

Emergency hematopoiesis is characterized by the expansion of hematopoietic stem/progenitor cells (HSPCs) and their progeny in response to a combination of signals from their immediate environment, such as inflammatory cytokines and pathogen-associated molecular patterns (Baldridge *et al.*, 2010, Boettcher *et al.*, 2014, Burberry *et al.*, 2014, Haas *et al.*, 2015, Kwak *et al.*, 2015, Manz *et al.*, 2014). In the prototypical model, Toll-like receptor (TLR)-4 ligation on HSPCs by lipopolysaccharide (LPS) leads to HSPC proliferation, promotes myeloid differentiation, and ultimately results in functional HSPC exhaustion in the presence of a persistent stimulus (Liu *et al.*, 2015, Takizawa *et al.*, 2017). In contrast, HSPC migration to peripheral sites, such as spleen, is not dependent on direct pathogen sensing by the HSPCs but rather results from the secretion of granulocyte colony-stimulating factor (G-CSF) by the endothelial cells in the bone marrow hematopoietic niche (Boettcher *et al.*, 2014, Burberry *et al.*, 2014, Haas *et al.*, 2015). The concomitant increase in granulocyte differentiation in response to G-CSF and their release to circulation serves to replace these short-lived cells that are being recruited to the site of infection or inflammation. Moreover, bone marrow myeloid cells will also signal back to HSPCs by producing reactive oxygen species that will further stimulate HSPC proliferation and differentiation (Kwak *et al.*, 2015, Zhu *et al.*, 2017).

HSPC activation generally corresponds to changes in Wnt signaling activity, with the various intracellular signaling pathways promoting either activation or quiescence (Abidin *et al.*, 2015, Lento *et al.*, 2014, Staal *et al.*, 2016, Sugimura *et al.*, 2012). Different Wnt signaling pathways have also been linked to the regulation of inflammation: while the canonical Wnt/β-catenin signaling appears to promote the secretion of pro-inflammatory cytokines in response to LPS and to induce lung injury in sepsis (Gatica-Andrades *et al.*, 2017, Sharma *et al.*, 2017), the prototypical non-canonical ligand Wnt5a has been shown to stimulate phagocytosis and cytokine secretion by macrophages (Maiti *et al.*, 2012), but also to promote the differentiation of tolerogenic dendritic cells (Oderup *et al.*, 2013, Valencia *et al.*, 2011). However, the precise role of Wnt signaling in emergency myelopoiesis is not well established. We have shown in a previous study that the Wnt/polarity receptor Fzd6 is necessary for the efficient HSPC expansion after competitive transplant and in response to LPS (Abidin *et al.*, 2015), but the underlying cellular and molecular mechanisms remained unclear.

We now show here that Fzd6 simultaneously protects HSPCs and promotes their mobilization to the periphery, most likely by a combination of cell-autonomous and indirect mechanisms mediated by other hematopoietic cells, such as bone marrow macrophages. *Fzd6*$^{-/-}$ HSPCs display an efficient immediate-early response that cannot be sustained as the cells become more rapidly exhausted. Furthermore, they mobilize less efficiently to the spleen and generate fewer progeny *in situ* in the bone marrow. *Fzd6*$^{-/-}$ macrophages contribute to the inefficient response by secreting large quantities of myelosuppressive factors, such as MIP-1α. These results show that hematopoietic Fzd6 is required for normal emergency granulopoiesis and lead us to propose that HSPC mobilization could actually promote their survival by removing them from an inflammatory bone microenvironment.

3 RESULTS

3.1 Initial HSC response to LPS is intact in *Fzd6*$^{-/-}$ bone marrow

Our previous data showed a decreased expansion of *Fzd6*$^{-/-}$ CD150^{+} Lin^{-} Sca-1^{+} cKit^{hi} (LSK) HSPCs in the bone marrow on day three after the first injection of LPS (Abidin *et al.*, 2015). This could be explained either by defective LPS sensing or lack of HSPC self-renewal, translating into exhaustion, cell differentiation, or cell death. To better discriminate between the two possibilities, we analyzed HSPC activation also at earlier time points at 24h and 48h after the first injection (Fig. 3.1A). There was no difference in HSPC expansion on day 1 (Fig. 3.1B),

indicating that the initial LPS sensing was not affected by the absence of Fzd6. However, the decline in HSPC numbers following the second injection was much more pronounced in the *Fzd6*$^{-/-}$ bone marrow, resulting in the overall deficit in HSPC number as compared to *Fzd6*$^{+/+}$ controls. This was accompanied by an initial decrease in the proportion of CD150^{+}CD48^{-} LSKs that are enriched in hematopoietic stem cells (HSCs) when compared to CD150^{+}CD48^{+} multipotent progenitors (MPPs); however, in contrast to *Fzd6*$^{+/+}$ controls in which the proportions returned to normal by day 3, the relative frequency and number of CD150^{+}CD48^{-} LSKs were decreased in *Fzd6*$^{-/-}$ bone marrow, suggesting a potential defect in the ability of the bone marrow to return to quiescence after stimulation (Fig. 3.1C, 3.1D).

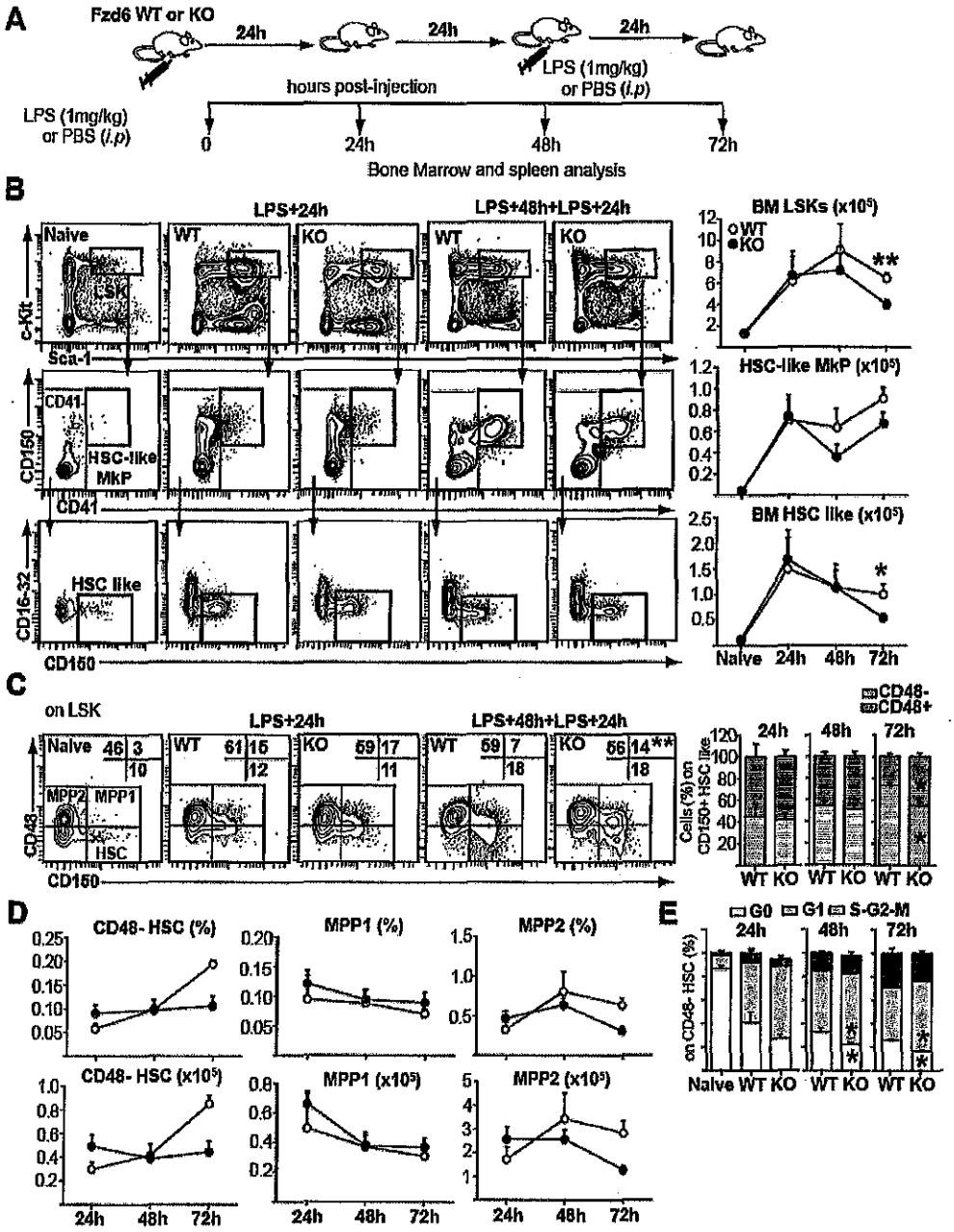

Figure 3.1 Fzd6-deficiency impairs LPS-induced HSC expansion.

(A) Experimental design for LPS-induced emergency granulopoiesis. **(B)** Representative flow cytometry data and gating strategy of LPS-treated BM HSPCs of *Fzd6*$^{+/+}$ (WT) and *Fzd6*$^{-/-}$ (KO) mice at various time points. BM cells were first gated on Lin$^-$ (B220$^-$ CD3ε$^-$ CD11b$^-$ GR1$^-$ Ter119$^-$) and identified according to Sca-1 and c-Kit (CD117) expression. Stem cell-like megakaryocyte progenitors (MkP) were identified as CD41$^+$ CD150$^+$ within the Lin$^-$cKit^{hi} Sca1$^+$ (LSK) population. HSC-like cells were defined as CD150$^+$ CD16/CD32$^-$ CD41$^-$ LSKs. Graphs show the absolute numbers of different subpopulations in the bone marrow (two tibiae + two femora). **(C)** Representative flow cytometry data showing CD150 and CD48 expression on LSKs. Bar graphs on the right show the contribution of CD48$^-$ HSCs and CD48$^+$ myeloid-biased MPP2 cells to CD150$^+$ LSKs. **(D)** Graphs show the percentage contribution and absolute numbers

of the different bone marrow LSK subpopulations as defined by CD150 and CD48 expression. **(E)** Ki-67/Hoechst co-staining was used to distinguish the G0, G1, and S/G2/M cell cycle phases of $CD150^+$ $CD48^-$ HSCs at different time points after LPS injection. All graphs represent mean + SEM, pooled from at least three independent experiments with a total of at least 5 mice per group $*P<0.05$; $**P<0.01$; $***P<0.001$.

Although the vast majority of adult HSCs are usually in a quiescent state in the G0 phase of the cell cycle, they can be activated in response to myeloablation or the production of inflammatory cytokines, such as seen in the emergency response to LPS (Baldridge *et al.*, 2010, Liu *et al.*, 2015, Pietras *et al.*, 2016, Takizawa *et al.*, 2017, Wilson *et al.*, 2008). The proportion of $CD150^+CD48^-$ LSKs in G0 decreased significantly in $Fzd6^{+/+}$ controls as well as in $Fzd6^{-/-}$ mice in response to LPS (Fig. 3.1E); however the relative frequency of quiescent HSCs was lower in the $Fzd6^{-/-}$ bone marrow at 48h and 72h. Together with the decrease in cell numbers, these results point toward an overactivation and potential exhaustion of HSCs, rather than inefficient expansion due to impaired LPS sensing.

3.2 HSC exhaustion is dependent on *Fzd6*$^{-/-}$ hematopoietic cells

Although repeated exposure to LPS can lead to HSC exhaustion directly via TLR4 expression on HSCs themselves (Liu *et al.*, 2015, Takizawa *et al.*, 2017), emergency granulopoiesis and LPS-induced extramedullary hematopoiesis in the spleen are dependent on non-hematopoietic cells, such as endothelial cells (Boettcher *et al.*, 2014, Boettcher *et al.*, 2012). Given that *Fzd6* expression has been reported in the bone marrow stromal compartment (Lento *et al.*, 2013), we wished to evaluate whether the apparent induction of HSC exhaustion in $Fzd6^{-/-}$ bone marrow was due to functional deficiencies in non-hematopoietic cells. We therefore reconstituted irradiated $Fzd6^{+/+}$ and $Fzd6^{-/-}$ recipient mice with congenic ($CD45.1^+$ $CD45.2^+$) bone marrow cells (Fig. 3.2A) and evaluated their emergency response 16 weeks after transplant. We first confirmed that there was no difference in the proportion of peripheral blood cells (Fig. 3.2B) or bone marrow progenitor cells before LPS injection (Fig. 3.2C). There was also no difference in the proportion or number of bone marrow HSPCs between $Fzd6^{+/+}$ and $Fzd6^{-/-}$ hosts on day 3, after the induction of emergency myelopoiesis (Fig. 3.2D, 3.2E), suggesting that $Fzd6^{-/-}$ stroma was fully able to support both normal and emergency hematopoiesis from wild-type HSPCs. Although there was a slight decrease in the number of granulocyte-monocyte colonies obtained from the bone marrow of $Fzd6^{-/-}$ recipients when compared to their $Fzd6^{+/+}$ counterparts, this difference was not statistically significant (Fig. 3.2F). Moreover, there was actually an increase in the number of colonies obtained from the

spleen of *Fzd6*$^{-/-}$ hosts, suggesting that the decrease in bone marrow colony formation could be due to an increased release of HSPCs to the periphery (Fig. 3.2F).

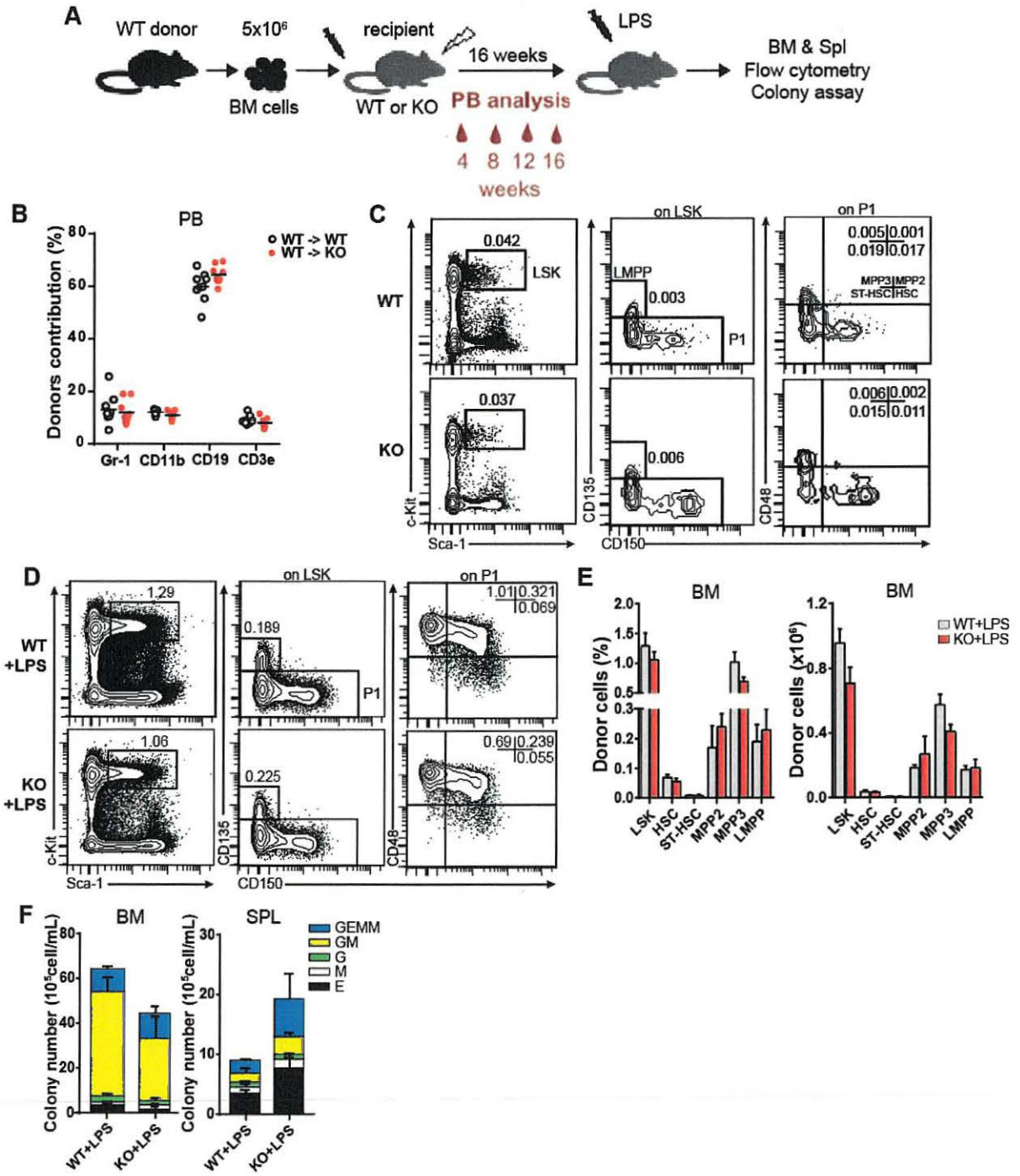

Figure 3.2 **Normal emergency hematopoiesis in Fzd6-deficient hosts reconstituted with congenic Fzd6-sufficient bone marrow.**

(A) Experimental design for primary transplants. **(B)** Peripheral blood analysis of recipient mice 16 weeks after transplant, showing the proportion of different blood cells within donor-derived cells (overall peripheral blood chimerism was >95% for all recipients). Data are pooled from two independent transplants, with each individual dot representing one mouse. Horizontal lines represent the sample mean. Similar results were obtained in a third independent experiment. **(C)** Representative flow cytometry analysis of bone marrow aspirates 16 weeks after transplant, showing the proportion of donor-derived bone marrow HSPCs. The numbers within different quadrants depict mean percentage within donor-derived cells. **(D)** Representative flow cytometry analysis of LPS-injected chimeras on day 3. **(E)** Percentages and absolute numbers of donor-derived HSPCs in the bone marrow on day 3. Data are pooled from two independent transplants and shown as mean + SEM (n=6 for *Fzd6*$^{+/+}$ and n=8 for *Fzd6*$^{-/-}$ hosts). Similar results were obtained in a third independent experiment. **(F)** Numbers of myeloid colonies derived from bone marrow (left) and spleen (right) of LPS-injected chimeras. Data are pooled from two independent transplants and shown as mean + SEM (n=6 for *Fzd6*$^{+/+}$ and n=8 for *Fzd6*$^{-/-}$ hosts).

Given that increased mobilization could lead to the loss of bone marrow HSCs with the ability to reconstitute secondary hosts, we further transplanted bone marrow cells isolated from LPS-injected chimeras into congenic CD45.1^{+} recipients (Fig. 3.3A). The secondary recipients showed no evidence of differentiation bias induced by *Fzd6*$^{-/-}$ environment (Fig. 3.3B). Moreover, there was no decrease in donor chimerism within peripheral blood granulocytes, suggesting that bone marrow cells recovered from *Fzd6*$^{-/-}$ hosts had no major repopulation defects (Fig. 3.3C). Lastly, we also determined the frequency and number of donor-derived HSPCs in the bone marrow and observed no major difference between cells derived from *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ primary hosts (Fig. 3.3D, 3.3E). These results demonstrate that the *Fzd6*$^{-/-}$ environment alone is not sufficient to induce defective HSC expansion and function during LPS-induced emergency hematopoiesis. They rather confirm our previous findings (Abidin *et al.*, 2015), indicating that the defect is hematopoietic cell-autonomous.

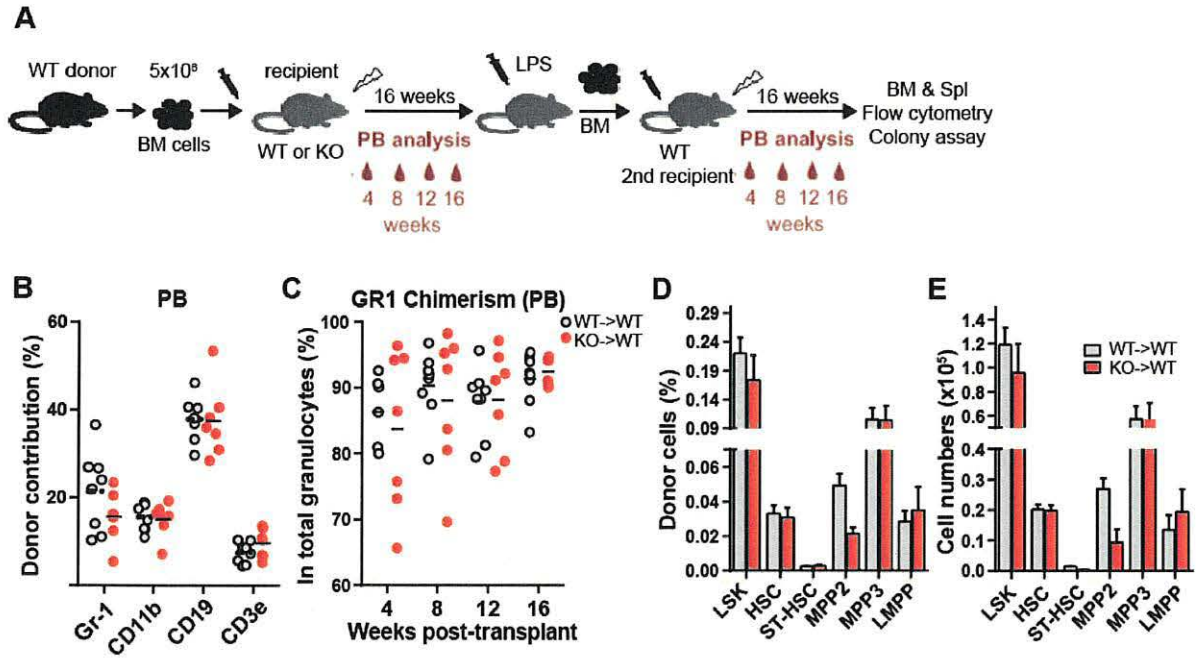

Figure 3.3 Hematopoietic Fzd6 is sufficient to maintain HSPC repopulation potential in Fzd6-deficient environment

(A) Experimental design for secondary transplants. **(B)** Peripheral blood analysis of recipient mice 16 weeks after transplant, showing the proportion of different blood cells within primary donor-derived cells (overall peripheral blood chimerism was >90% for all recipients). **(C)** Peripheral blood donor chimerism in secondary recipients within $GR1^{hi}$ SSC^{hi} granulocytes at different times after transplant. Data in **(B)** and **(C)** are pooled from two independent transplants, with each individual dot representing one mouse. Horizontal lines represent the sample mean. **(D)** Proportion of various HSPC populations within donor-derived cells in the bone marrow of secondary recipients at 16 weeks post-transplant. **(E)** Absolute numbers of donor-derived HSPCs in the bone marrow of secondary recipients at 16 weeks post-transplant. Data in **(D)** and **(E)** are pooled from two independent transplants and shown as mean + SEM (n=9 for both groups). None of the differences were statistically significant.

3.3 Fzd6 promotes LPS-induced bone marrow emergency myelopoiesis

One potential explanation for decreased HSC expansion in the *Fzd6*$^{-/-}$ bone marrow could be their accelerated differentiation. LPS injection induces a rapid release of neutrophil granulocytes and inflammatory Ly-$6C^{hi}$ monocytes from the bone marrow, followed by an upregulation of myeloid differentiation programs and a shutdown of B lymphopoiesis (Boettcher *et al.*, 2014, Liu *et al.*, 2015). To evaluate the potential for accelerated myeloid differentiation in *Fzd6*$^{-/-}$ mice, we determined the number of granulocyte-monocyte progenitor cells (GMPs; $CD16/CD32^{+}$ $cKit^{+}$ $CD41^{-}$ $CD150^{-}$ Lin^{-}) at different times after LPS injection. There was no difference between *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ bone marrow at steady state (Fig. 3.4A, 3.4B), as we have previously published (Abidin *et al.*, 2017). Conventional $Sca\text{-}1^{-}$ GMPs peaked at 48h and

returned back to baseline levels by 72h (Fig. 3.4A, 3.4B); however, the peak numbers tended to remain lower in the absence of Fzd6. A similar trend could also be seen for Sca-1^{+} emergency GMPs, which were still detectable in *Fzd6*$^{+/+}$ bone marrow at 72h, but whose numbers were approximately 3-fold lower in *Fzd6*$^{-/-}$ individuals (Fig. 3.4A, 3.4B). The initial release of myeloid cells from the bone marrow was highly similar in both groups, once more suggesting that the initial LPS sensing was intact even in the absence of Fzd6. However, there was a decrease in granulocyte and monocyte accumulation in the *Fzd6*$^{-/-}$ spleen (Fig. 3.4C, 3.4D), and the surge of myeloid differentiation seen at 72h in *Fzd6*$^{+/+}$ bone marrow was absent in *Fzd6*$^{-/-}$ individuals (Fig. 3.4D). These results demonstrated that the impaired emergency response in *Fzd6*$^{-/-}$ bone marrow was not restricted to HSCs but impacted also their downstream differentiation.

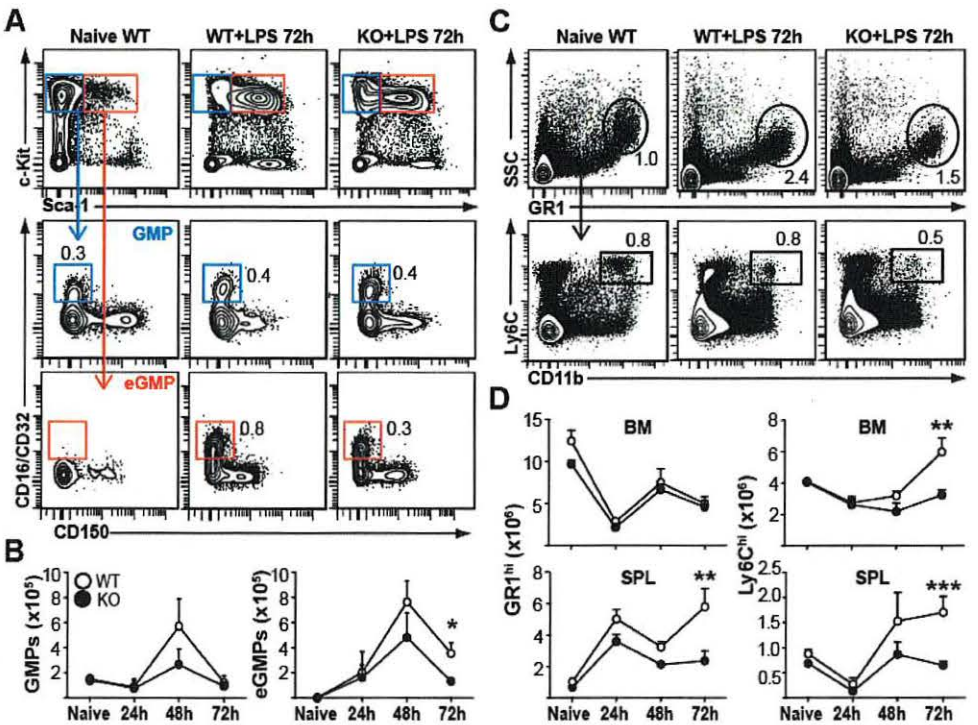

Figure 3.4 Decreased emergency myelopoiesis in *Fzd6*$^{-/-}$ mice.

A) Representative flow cytometry data and gating strategy for bone marrow myeloid progenitor cells. Conventional GMPs were first gated on Lin^{-} Sca-1^{-} cKit^{hi} and then identified as CD16/CD32^{+} CD150^{-} and CD41^{-}. Emergency GMPs (eGMPs) were defined as CD16/CD32^{+} CD150^{-} CD41^{-} LSKs. Mean percentage for each cell subset is indicated within flow cytometry plots. **(B)** Numbers of GMPs and eGMPs in *Fzd6*$^{+/+}$ (WT) and *Fzd6*$^{-/-}$ (KO) mice at various time points. **(C)** Representative flow cytometry data and gating strategy for myeloid cell subsets in the spleen. The same strategy was used for bone marrow as well. Neutrophil granulocytes were defined as SSC^{hi} GR1^{hi}. Ly-6C^{hi} CD11b^{+} monocytes were selected within the "NOT granulocyte" population. Mean percentage for each cell subset is indicated within flow cytometry plots. **(D)** Numbers of GR1^{hi} granulocytes and Ly-6C^{hi} monocytes in bone marrow and spleen in *Fzd6*$^{+/+}$ (WT) and *Fzd6*$^{-/-}$ (KO) mice at various time points. All graphs represent mean + SEM pooled

from at least three independent experiments with a total of at least 5 mice per group. *P<0.05; **P<0.01; ***P<0.001.

3.4 Fzd6 is necessary for efficient HSC mobilization in response to G-CSF

LPS-induced emergency myelopoiesis is largely mediated by G-CSF, secreted by endothelial cells in response to TLR4-mediated signals (Boettcher *et al.*, 2014, Boettcher *et al.*, 2012). G-CSF induces granulocyte and HSPC mobilization, activates bone marrow macrophages, and promotes granulopoiesis in the bone marrow. Therefore, a deficient emergency response as seen in *Fzd6*$^{-/-}$ bone marrow could be due to a decrease in G-CSF production or an inability to properly react to G-CSF. Given that the *Fzd6*$^{-/-}$ environment was able to support emergency hematopoiesis from wild-type HSPCs, the former possibility seemed unlikely. We rather administered G-CSF intraperitoneally on three consecutive days, and then analyzed HSPC activation and mobilization to evaluate the potential of *Fzd6*$^{-/-}$ bone marrow cells to respond to G-CSF.

There was no difference in the relative proportion or absolute number of HSPC subsets in the bone marrow between *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ mice (Fig. 3.5A, 3.5B), suggesting that they were able to respond to G-CSF mediated activation. However, the proportion of HSCs that were mobilized to the peripheral blood was decreased in *Fzd6*$^{-/-}$ mice when compared to controls (Fig. 3.5C). This difference was even further accentuated in the spleen, where the number of HSCs was 2-fold lower after G-CSF treatment (Fig. 3.5B). Similar pattern was also seen for downstream MPPs (Fig. 3.5B) and for myeloid colony-forming cells (Fig. 3.5D), whose recruitment to the spleen was significantly reduced in the absence of Fzd6. These results indicate that *Fzd6*$^{-/-}$ HSPCs become normally activated in the bone marrow, but are then less efficiently mobilized to the periphery. Rather, they undergo cell death as shown by the increased frequency of cells with pyknotic nuclei (Fig. 3.5E).

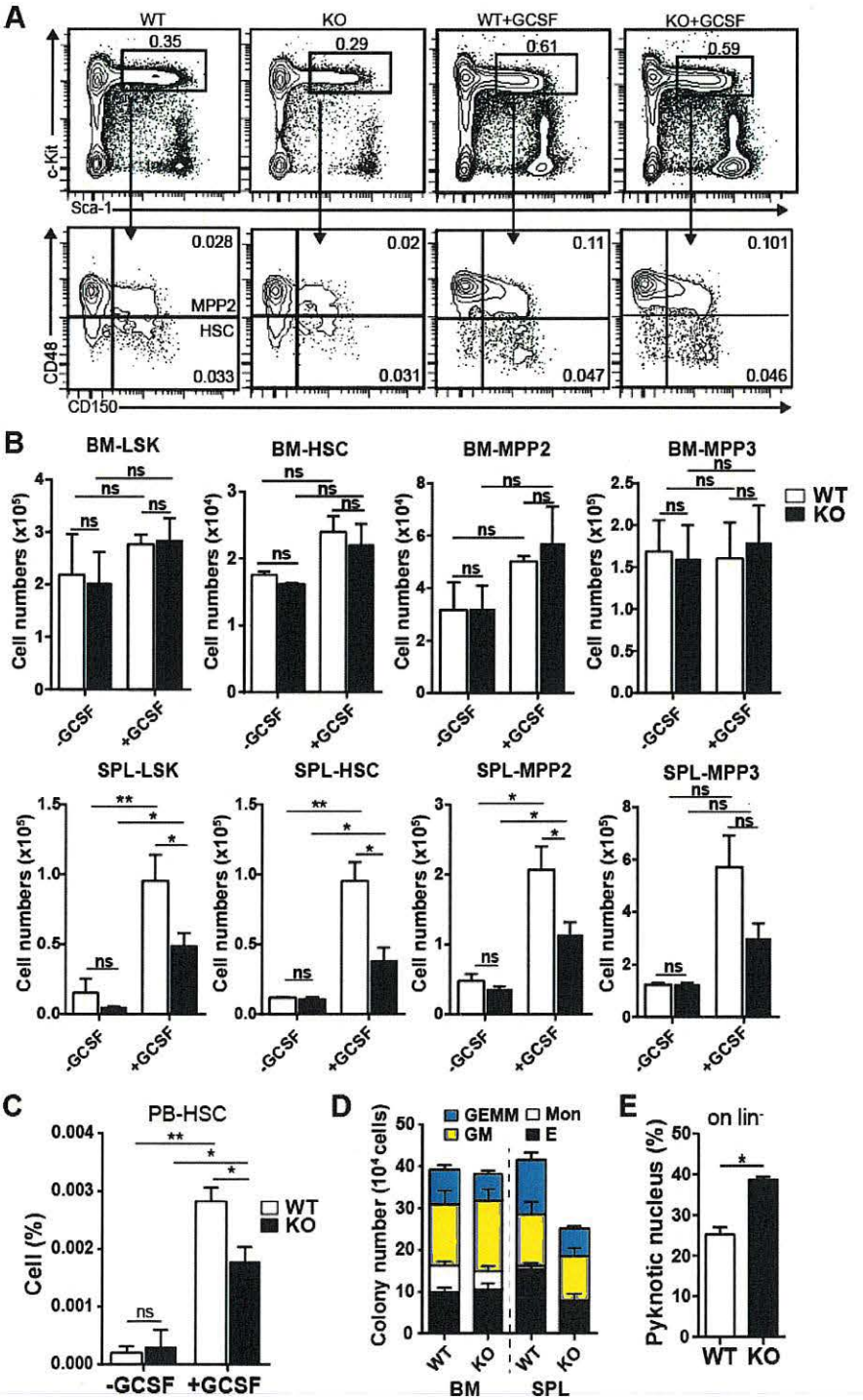

Figure 3.5 Fzd6 promotes G-CSF-induced HSC mobilization to peripheral blood and spleen

(A) Representative flow cytometry data for LSKs and HSPC subsets as identified by CD150 and CD48 staining in the bone marrow of PBS- and G-CSF-treated *Fzd6*$^{+/+}$ (WT) and *Fzd6*$^{-/-}$ (KO) mice. Numbers within panels represent mean percentage within total bone marrow. **(B)** Numbers of HSPCs in the bone marrow (top) and spleen (bottom) from PBS- and G-CSF-treated mice. **(C)** Relative frequency of HSCs in

peripheral blood of PBS- and G-CSF-treated mice. **(D)** Myeloid colony-forming assay from bone marrow and spleen of G-CSF-treated *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ mice. **(E)** Imaging flow cytometry analysis of pyknotic nuclei with condensed chromatin as shown by morphology and DAPI staining intensity. All bar graphs represent mean + SEM with 3 mice per group for mock and 5 per group for G-CSF-treated mice pooled from three independent experiments. *P<0.05; **P<0.01.

3.5 *Fzd6*$^{-/-}$ bone marrow macrophages inhibit HSPC expansion

The decrease in HSPC survival could be due to overactivation, as suggested by the cell cycle analysis in Figure 3.1. An alternative, but not mutually exclusive, explanation for the loss of *Fzd6*$^{-/-}$ HSPCs could be the presence of myelosuppressive factors in the bone marrow environment. Our previously published results in the context of a chronic parasitic infection suggested that soluble factors present in the *Fzd6*$^{-/-}$ bone marrow could suppress HSPC proliferation and myeloid differentiation (Abidin *et al.*, 2017). To address this question in LPS-induced emergency myelopoiesis, we analyzed the soluble factors present in bone marrow cell suspensions from LPS-treated *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ mice as well as *Fzd6*$^{-/-}$ hosts reconstituted with congenic wild type hematopoietic cells by protein arrays. We identified three factors that appeared upregulated by at least two-fold in the *Fzd6*$^{-/-}$ bone marrow following LPS administration, and whose upregulation was dependent on *Fzd6*$^{-/-}$ hematopoietic cells: CCL3/MIP-1α, CXCL2/MIP-2α, and TNF-α (Fig. 3.6A). We focused on CCL3/MIP-1α, as we also found it upregulated in *Leishmania*-infected *Fzd6*$^{-/-}$ bone marrow (Abidin *et al.*, 2017), and it is well known for its inhibitory effects on HSPC function *in vivo* and *in vitro* (Baba *et al.*, 2013, Broxmeyer *et al.*, 1990, Schepers *et al.*, 2013, Staversky *et al.*, 2018b).

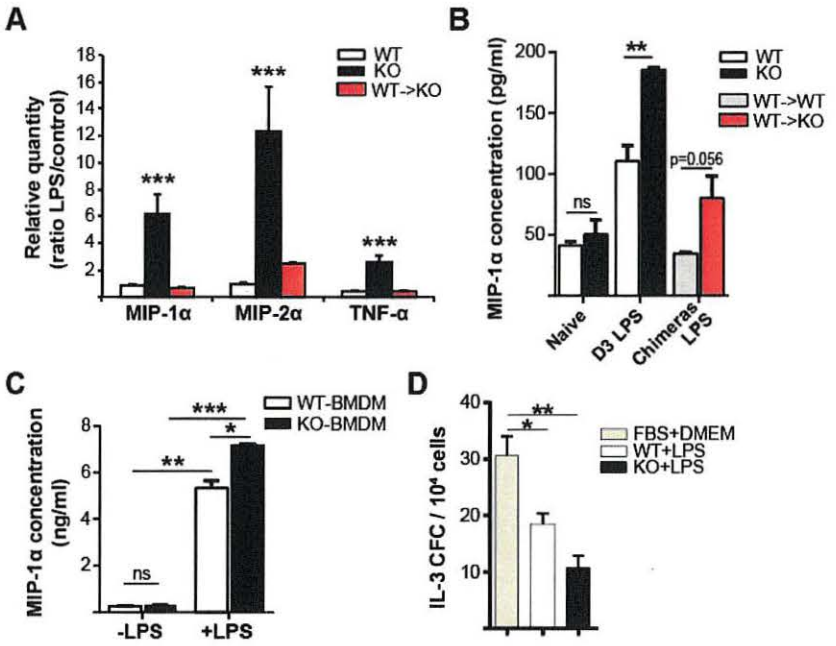

Figure 3.6 ***Fzd6*$^{-/-}$ bone marrow macrophages secrete more MIP-1α in response to LPS and inhibit HSPC proliferation**

(**A**) Analysis of soluble pro-inflammatory factors from bone marrow of LPS-injected and control mice by protein array. Results are presented as ratio over naïve control bone marrow. (**B**) Quantification of CCL3/MIP-1α by ELISA from bone marrow extracellular fluid. (**C**) CCL3/MIP-1α secretion by bone marrow derived macrophages stimulated with 100 ng/ml LPS for 24h. (**D**) IL-3-dependent colony formation by WT bone marrow progenitors in the presence of supernatants obtained from LPS-stimulated macrophages. All bar graphs represent mean + SEM from three independent experiments. *P<0.05; **P<0.01; ***P<0.001.

We first went on to better quantify CCL3/MIP-1α levels in the bone marrow by ELISA and confirmed the results obtained from the protein array (Fig. 3.6B). The concentration of MIP-1α was significantly higher in *Fzd6*$^{-/-}$ bone marrow during LPS-induced emergency myelopoiesis as compared to *Fzd6*$^{+/+}$ mice. Moreover, MIP-1α concentration was decreased in LPS-treated chimeric mice, and the stronger responses corresponded to individuals with a larger proportion of remaining *Fzd6*$^{-/-}$ host hematopoietic cells (not shown). These results strongly suggested that the increased MIP-1α production was in large part due to *Fzd6*$^{-/-}$ bone marrow hematopoietic cells.

Next we wanted to directly investigate the contribution of bone marrow macrophages. We first generated bone marrow-derived macrophages (BMDMs) in culture from *Fzd6*$^{-/-}$ and *Fzd6*$^{+/+}$ progenitors. We have previously shown that there is no major difference in BMDM maturation in culture between *Fzd6*$^{+/+}$ and *Fzd6*$^{-/-}$ progenitors (Abidin *et al.*, 2017). However, when stimulated

with LPS, *Fzd6*$^{-/-}$ BMDMs secreted more MIP-1α (Fig. 3.6C), indicating that *Fzd6*$^{-/-}$ macrophages may be more sensitive to LPS and could thus contribute to the strong initial bone marrow response seen *in vivo*. Furthermore, we tested the growth-inhibitory potential of the *in vitro*-stimulated BMDMs and saw that the *Fzd6*$^{-/-}$ BMDM-conditioned medium was very effective in suppressing HSPC proliferation and colony formation in response to IL-3 (Fig. 3.6D). Collectively these results show that *Fzd6*$^{-/-}$ hematopoietic cells, such as macrophages, secrete large amounts of pro-inflammatory cytokines and chemokines, including MIP-1α, which may then contribute to the establishment of a myelosuppressive bone marrow environment that will prevent HSPC expansion and differentiation during LPS-induced emergency hematopoiesis.

4 DISCUSSION

Emergency myelopoiesis in acute infections serves to replace short-lived myeloid cells that have been recruited to peripheral tissues to counter the infectious organism. It can be conceptually divided in three parts: 1) mobilization of granulocytes and monocytes from the bone marrow; 2) activation and mobilization of bone marrow HSPCs; and 3) preferential differentiation of HSPCs into myeloid cells. Each of these parts is regulated by a combination of signals provided by microbial products, immune cells and non-immune cells. We have examined here the implication of Wnt signaling, and more specifically the importance of the Wnt receptor Fzd6, in LPS-induced emergency granulopoiesis. Our results show that *Fzd6*$^{-/-}$ HSPCs' expansion, differentiation and mobilization to peripheral blood were impaired. The defect was hematopoietic-intrinsic, most likely the result of HSPC-intrinsic exhaustion combined with macrophage-derived myelosuppressive factors, such as MIP-1α. These results support the hypothesis that non-canonical Wnt signalling protects bone marrow HSPCs from an overactive inflammatory response.

The impact of Wnt signalling on HSPC proliferation and self-renewal is dose-dependent (Luis *et al.*, 2011, Sugimura *et al.*, 2012). Low levels of canonical, β-catenin-dependent Wnt signalling enhance HSC self-renewal and bone marrow repopulation (Lento *et al.*, 2014, Luis *et al.*, 2011), while sustained β-catenin activity promotes myeloid proliferation and differentiation but leads to HSC exhaustion (Hérault *et al.*, 2017, Luis *et al.*, 2011, Scheller *et al.*, 2006). We have previously shown that Fzd6 was necessary for HSC self-renewal and bone marrow repopulation after transplant (Abidin *et al.*, 2015). Our data further suggested that Fzd6 was a negative regulator of both canonical and Wnt/Ca^{2+}/Cdc42 pathways. Increased Wnt/Ca^{2+} signalling also results in HSC activation and loss of quiescence (Florian *et al.*, 2013, Sugimura

et al., 2012). Lack of Fzd6 could thus result in loss of quiescence due to a decreased HSC activation threshold. Similar to the first week after transplant in our previous study (Abidin *et al.*, 2015), our results here indicate that *Fzd6*$^{-/-}$ HSCs and progenitor cells become readily activated and enter cell cycle after LPS stimulation, but their capacity to maintain the response is impaired and they become prematurely exhausted and more prone to cell death.

Circulating HSPCs have been suggested to patrol extramedullary tissues and have the capacity to either settle down to differentiate or return to the bone marrow (Massberg *et al.*, 2007). Upon stimulation with LPS, the increase in bone marrow G-CSF concentrations together with the concomitant decrease in CXCL12 will accentuate HSPC and granulocyte release into circulation (Boettcher *et al.*, 2014), but the functional importance of HSPC mobilization in emergency hematopoiesis is still unknown. We show here that *Fzd6*$^{-/-}$ bone marrow HSPCs expand in number after G-CSF administration but accumulate less in peripheral blood and spleen, suggesting that their mobilization is impaired. Instead, they are more prone to cell death. The mobilization defect could be HSPC-intrinsic or dependent on the environment. Based on our results with the chimeric mice, *Fzd6*$^{-/-}$ bone marrow non-hematopoietic cells respond normally, and our protein array data suggest that there is no deficit in G-CSF production. However, we cannot completely exclude the possibility that *Fzd6*$^{-/-}$ myeloid cells respond inadequately to G-CSF, even though their release into circulation is not impaired. They could therefore indirectly regulate HSPC mobilization, for example at the level of CXCL12 cleavage and disruption of the CXCL12/CXCR4 interaction (Levesque *et al.*, 2003). On the other hand, Wnt5a/CDC42-mediated F-actin reorganization regulates HSPC adhesion and migration toward CXCL12 (Schreck *et al.*, 2017), and it is not inconceivable that the enhanced CDC42 polarization in *Fzd6*$^{-/-}$ HSPCs could also influence their association with bone marrow niches (Abidin *et al.*, 2015). *Fzd6*$^{-/-}$ cells display no defect in bone marrow homing after transplant, and we cannot exclude the possibility that a subset of HSPCs could associate even more strongly with the surrounding niches in an intact, non-irradiated bone marrow, thus preventing their mobilization.

Irrespective of the mechanism that retains *Fzd6*$^{-/-}$ HSPCs in the bone marrow, their ability to contribute to emergency myelopoiesis is impaired. In addition to the HSC-intrinsic self-renewal defect, the *Fzd6*$^{-/-}$ bone marrow environment appears proinflammatory, further promoting HSC exhaustion (Abidin *et al.*, 2017). Repeated LPS administration has been shown to result in the loss of functional HSCs directly via TLR4 signalling in HSCs (Liu *et al.*, 2015, Takizawa *et al.*, 2017), and a prolonged exposure to proinflammatory cytokines and reactive

oxygen species produced by immune cells may also have the same effect (Ito *et al.*, 2006, Matatall *et al.*, 2016, Pietras *et al.*, 2016, Zhu *et al.*, 2017). The decreased mobilization of *Fzd6*$^{-/-}$ HSPCs could thus increase their exposure to the proinflammatory environment and contribute to their decreased expansion and survival.

The mechanistic underpinnings of the enhanced MIP-1α secretion by *Fzd6*$^{-/-}$ bone marrow macrophages are still unclear. We have previously shown that *Fzd6*$^{-/-}$ BMDMs expressed the same level of maturation markers as their *Fzd6*$^{+/+}$ counterparts, and appeared to respond normally to IFN-γ-mediated priming; however, their ability to control the proliferation of intracellular pathogens was improved (Abidin *et al.*, 2017). These data would indicate that Fzd6 may not be directly involved in macrophage differentiation but would rather regulate their function, possibly at the level of activation and polarization. Pro-inflammatory M1 macrophages will secrete larger quantities of pro-inflammatory cytokines, such as TNF and IL-1β, as well as reactive oxygen species, crucial for their microbicidal function (Murray *et al.*, 2011). Such a profile would promote HSC activation and expansion at the expense of their self-renewal (Luo *et al.*, 2018), and in combination with the reduced self-renewal capacity of *Fzd6*$^{-/-}$ HSPCs may result in stunted emergency myelopoiesis as observed in our study.

The overall role of Wnt signalling in macrophage polarization likely varies depending on the model system as well as the cell of origin. For example, the prototypical non-canonical ligand Wnt5a has been shown to enhance pro-inflammatory cytokine secretion by peritoneal macrophages *in vitro* (Maiti *et al.*, 2012), and its expression can be induced by TLR signalling (Blumenthal *et al.*, 2006), but its reported *in vivo* effects vary from tolerogenic to bactericidal (Bergenfelz *et al.*, 2012, Jati *et al.*, 2018, Valencia *et al.*, 2011). Our results suggest that Fzd6 is more likely to play a tolerogenic role, at least in the context of sterile, LPS-induced inflammation, and pharmacological activation of Fzd6 could thus be an interesting option for limiting systemic inflammation. However, response to LPS does not predict susceptibility to infection (Fensterheim *et al.*, 2017), and it would be necessary to verify the importance of Fzd6 signalling in sepsis, for example, by using infectious models.

In summary, we have shown here that Fzd6 not only protects HSPCs during LPS-induced emergency hematopoiesis but also enhances their differentiation and mobilization to the periphery. The impact of Fzd6 is dependent on hematopoietic cells, such as bone marrow macrophages. Collectively these data are in line with previous results demonstrating that non-canonical Wnt signalling protects bone marrow HSPCs from undue activation. They further

prompt us to hypothesize that mobilization may serve to promote HSPC survival during bone marrow inflammation.

5 EXPERIMENTAL PROCEDURES

5.1 Mice

C57BL/6 (B6; CD45.2$^+$) and B6.SJL-PtprcaPep3b/BoyJ (Ly5a) (B6.SJL; CD45.1$^+$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). *Fzd6*$^{-/-}$ mice were first backcrossed onto C57BL/6 background and maintained as *Fzd6*$^{+/-}$ intercrosses as previously detailed (Abidin *et al.*, 2015). Mice were kept under specific free pathogen conditions in sterile ventilated racks at the animal facility of Institut National de la Recherche Scientifique (INRS)-Institut Armand-Frappier (Centre National de Biologie Expérimentale). Sex-matched *Fzd6*$^{+/+}$ littermates were used as controls. All mice used for the experiments were between 7 and 12 weeks of age unless otherwise specified. All procedures involving animals were done according to the Canadian Council on Animal Care guidelines and approved by the Comité institutionnel de protection des animaux of INRS.

5.2 Transplantation assay

For primary transplantation experiments, 5×10^6 B6xB6.SJL F1 BM cells (CD45.1$^+$/CD45.2$^+$) were injected intravenously through the lateral tail vein into lethally irradiated *Fzd6*$^{-/-}$ or *Fzd6*$^{+/+}$ recipient mice (CD45.2$^+$). For secondary transplants, 5×10^6 total bone marrow cells (>95% CD45.1$^+$/CD45.2$^+$) from pooled LPS-treated primary recipients were injected into lethally irradiated congenic B6.SJL (CD45.1$^+$) secondary recipients. In order to determine peripheral donor chimerism, we collected blood from the cheek every 4 weeks to analyze by flow cytometry. Sixteen weeks after secondary transplant mice were euthanized and we analyzed their bone marrow and spleen for reconstitution.

5.3 LPS and G-CSF injections

Mice were injected intraperitoneally with γ-irradiated LPS (*E. coli* 0111:B4; Sigma-Aldrich, Oakville, ON, Canada) twice with a 48-hour interval at a dose of 1mg/kg body weight (*Fzd6*$^{-/-}$ and *Fzd6*$^{+/+}$ mice) or 0.5mg/kg body weight (transplant recipients) per injection. Mice

were euthanized at indicated time points with CO_2 and their bone marrow and spleen harvested for analysis.

Recombinant murine G-CSF (Stem Cell Technologies, Vancouver, BC, Canada) was injected intraperitoneally to *Fzd6*$^{-/-}$ and *Fzd6*$^{+/+}$ mice for 3 consecutive days at a dose of 25 µg per mouse per day. Twenty-four hours after the last injection, peripheral blood was collected by cardiac puncture under isoflurane-induced anesthesia into EDTA-tubes. Mice were then euthanized and their bone marrow and spleen harvested for analysis.

5.4 Flow cytometry

Bone marrow was harvested by flushing femora and tibiae with sterile 1X PBS using a 25-gauge needle to obtain a single cell suspension. Spleen was ground in PBS with a sterile plunger of a 3ml syringe and the cell suspension passed through a 100µm filter mesh. PBS was supplemented with 0.1% bovine serum albumin (BSA) and 0.5mM ethylene-diamine-tetra-acetic acid (EDTA) for flow cytometry staining. See Table S3.1 for a complete list of antibodies. For intracellular staining, surface stained cells were fixed and permeabilized using the Foxp3 staining kit (eBioscience, San Diego, CA) and then incubated with appropriate antibodies. For cell cycle analysis, cells were first incubated for 30 min at 37°C with Hoechst #33342 (Sigma-Aldrich) in DMEM supplemented with 10% Premium FBS (Wisent Bioproducts, St-Bruno, QC, Canada) and 1 mM HEPES (Life Technologies, Burlington, ON, Canada), followed by staining with surface antibodies and intracellular anti-Ki67 as described above. Samples were acquired with a four-laser LSR Fortessa flow cytometer (BD Biosciences, Mountain View, CA) and analyzed using BD FACS Diva software (BD Biosciences) or FlowJo (for histogram overlays; Tree Star).

5.5 Imaging flow cytometry (Amnis ImageStream)

BM cells were harvested as above and enriched for HSPCs using EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit (Catalog 19856, Stem Cell Technologies, Vancouver, BC). Cells were washed in PBS and stained with surface antibodies anti-CD150 Alexa Fluor 647, anti-CD117 PE, and anti-Sca1 PE-Cy7, after which stained cells were fixed and permeabilized at 4°C for 45 minutes. Next, cells were stained with DAPI (Life Technologies), washed in PBS and image acquisition was performed with Amnis ImageStream

Mark II imaging flow cytometer (EMD Millipore). Raw data were analyzed with IDEAS v6.1 software using the apoptotic wizard.

5.6 Bone marrow–derived macrophage cultures

Bone marrow suspension was prepared in sterile HBSS (Life Technologies) and $1.5x10^6$ cells were plated in 10 ml DMEM (Life Technologies) supplemented with 10% Premium FBS and 20% L929-conditioned medium in non-adherent petri dishes (Heinonen *et al.*, 2006). The cultures were incubated at 37°C, 5% CO_2 for 7 days with a full medium change on day 4. Macrophages were harvested on day 7, and seeded at $1.2\text{-}1.5\times10^6$ cells/well in DMEM/10% FBS in a 6-well plate. Macrophages were stimulated with LPS at concentration of 100 ng/ml for 24h. Supernatants were collected by centrifugation and stored at -80°C until use.

5.7 Colony-forming unit assays

Cell suspension was diluted in IMDM (Invitrogen) containing 10% Premium FBS, and seeded into 35 mm non-adherent petri dishes in semi-solid methylcellulose medium (Methocult GF M3434; Stem Cell Technologies) at a concentration of 10^4 cell/dish. The dishes were incubated at 37°C, 5% CO_2 for 7-10 days, and colonies were counted based on morphology under an inverted microscope.

For the growth inhibition assay, freshly isolated wildtype bone marrow cells were seeded at a density of 10^4 cells/35 mm dish in base methylcellulose medium (Methocult GF M3231), supplemented with 10 ng/ml recombinant IL-3 (Peprotech, Rocky Hill, NJ) and 20% conditioned culture medium as indicated. The dishes were incubated at 37°C, 5% CO_2 for 7-10 days, and colonies were counted based on morphology under an inverted microscope.

5.8 Bone marrow cytokine/chemokine analysis

Bone marrow supernatant was collected by harvesting cells from both hind legs into 2 ml sterile PBS, followed by centrifugation, and snap-frozen and stored at -80°C until use. Conditioned culture medium from LPS-treated macrophages was collected and stored at -80°C until use. Supernatants were pooled from at least four mice per sample, and analyzed using a membrane-based Proteome profiler mouse cytokine/chemokine array kit (R&D Systems). Array images were further analyzed using the NIH ImageJ image analysis software. Samples were

normalized by subtracting pixel intensities from negative controls, and the fold changes for treated mice were determined as a ratio over untreated mice of the same genotype. Macrophage inflammatory protein 1 alpha level was measured using MIP1α Quantikine ELISA kits (R&D Systems) following manufacturer's instructions.

5.9 Statistical analysis

Two-tailed Student's *t* test was used to compare between different experimental groups. P value < 0.05 was considered as significant.

6 AUTHORSHIP CONTRIBUTIONS

B.M.A. and T.H.N. designed and conducted experiments, analyzed data and prepared figures and figure legends. K.M.H. conceived the project, designed the experimental approach, analyzed data and wrote the manuscript.

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8 SUPPLEMENTARY INFORMATION

Table S3.1 List of antibodies used for flow cytometry

Antibody name	Clone	Flourochrome	Dilution	Company
B220 (CD45R)	RA3-6B2	Biotin	1/400	BD Biosciences
CD3e	145-2C11	Biotin	1/400	BD Biosciences
CD11b	M1/70	Biotin	1/400	BD Biosciences
Gr1 (Ly6C/G)	RB6-8C5	Biotin	1/400	BD Biosciences
Ter119	TER-119	Biotin	1/800	BD Biosciences

Fc Block (anti-CD16/CD32)	2.4G2	Biotin	1/100	BD Biosciences
Streptavidin	561419	V500	1/1600	BD Biosciences
CD11b	M1/70	A647	1/800	BD Biosciences
CD16/32	93	PerCP-Cy5.5	1/100	BD Biosciences
CD117 (c-Kit)	2B8	PE	1/800	BD Biosciences
Ki67	SolA15	PE-eFluor610	1/200	eBioscience
Ly-6A/E (Sca-1)	D7	PE-Cy7	1/800	BD Biosciences
CD135 (Flt3)	A2F10	PerCP-eFluor 710	1/400	eBioscience
CD41	MWGReg30	FITC	1/200	BD Biosciences
CD48	HM48-1	Brilliant Violet 421	1/400	BD Biosciences
CD150	TC15-12F12.2	Alexa Fluor 647	1/400	Biolegend
CD45.1	A20	FITC	1/200	eBioscience
CD45.2	104	eFluor 450	1/200	eBioscience
CD3	145-2C11	PE-Cy7	1/200	BD Biosciences
CD19	1D3	PE	1/800	eBioscience
GR1	RB6-8C5	APC-Cy7	1/800	eBioscience
Hoechst		33342		Sigma-Aldrich
Foxp3				eBioscience

CHAPTER 4 – DISCUSSION

We have examined here the functional role of Fzd6 on HSCs using emergency granulopoiesis model. Our results showed that the influence of Fzd6 on HSCs response to LPS-induce emergency granulopoiesis is hematopoietic intrinsic. We also reported that Fzd6 controls HSCs migration from BM to peripheral blood and spleen. Moreover, we determined MIP1α as a candidate that suppresses *Fzd6*$^{-/-}$ HSCs response in BM stress condition.

Evidence from studies by Graham et al. showed that MIP1α acts as a stem cell inhibitor that inhibits the growth of myeloid colonies when they cultured primary BM cells with supernatant from the J774.2 macrophage cell line (Graham et al., 1990). MIP1α inhibits proliferation of HSPCs both *in vitro* and *in vivo* (Graham et al., 1990, Broxmeyer et al., 1990). Recently, Baba et al. Reported that MIP1α produced by basophils in the BM is a negative regulator of HSPC proliferation (Baba et al., 2016), while Rhonda et al. demonstrated that MIP1α regulates hematopoietic stem cell numbers and differentiation of myeloid progenitors (Staversky *et al.*, 2018a). In our study, we observed that *Fzd6*$^{-/-}$ mice produced significant higher levels of MIP1α in the BM than *Fzd6*$^{+/+}$ mice at day 3 post LPS injection (Figure 3.6B). Our results also showed that the numbers of KO HSCs declined rapidly on day 3 post LPS treatment (Figure 3.1B). This is in agreement with the fact that MIP1α inhibits HSPC proliferation (Graham et al., 1990, Broxmeyer et al., 1990). In previous studies, inflammatory macrophages and monocytes are known to produce MIP1α (Koch *et al.*, 1994, Nibbs *et al.*, 1997). In our model, we showed that bone marrow-derived macrophages from Fzd6 KO mice produce larger amounts of MIP1α than those from WT mice after LPS stimulation (Figure 3.6C). By using a colony assay, we cultured BM cells with LPS-stimulated bone marrow-derived macrophage supernatant and scored the myeloid colony output. Our result confirmed that LPS-treated KO macrophage supernatant, which contains a high concentration of MIP1α, restricted myeloid colony formation (Figure 3.6D). However, there was no significant difference between KO macropahe supernatant and WT group (Figure 3.6D). In the future, we would need to repeat this experiment to confirm the data. These results suggest that MIP1α could play a major role in limiting HSPC expansion in *Fzd6*$^{-/-}$ bone marrow. In order to confirm MIP1α role in suppressing the expansion of HSPC, we need to set up a competitive transplantation, in which *MIP1α*$^{-/-}$ BM and *MIP1α*$^{+/+}$ are mixed in the ratio 1:1 and transplanted in irradiated mice. Four weeks after transplantation, LPS-induced emergency granulopoiesis will be performed in transplanted mice as well as analyzed BM. We also could culture BM cells with LPS-treated BMDM supernatant in presence of MIP1 α neutralizing antibody and score the colonies output.

MIP1α is best known as a chemokine produced by activated macrophages that modulates acute or chronic inflammatory host responses at the site of infection or injury by recruitment of inflammatory cells like macrophages, monocytes, and neutrophils (Maurer et al., 2004). After administration of LPS to mice, MIP1α levels become elevated in the lung of LPS-treated mice. As a result, neutrophils and macrophages infiltrate the lung of injected mice (Standiford et al., 1995). Administration of an anti- MIP-1α antibody reduces neutrophil and macrophage infiltrate and decreases lung permeability (Standiford *et al.*, 1995). Hsieh et al. demonstrated that MIP-1α has an important function in mediating the acute inflammatory response following trauma/hemorrhage. MIP-1α null mice showed attenuated acute inflammatory responses, rapid recovery, and less remote organ injury after trauma (Hsieh *et al.*, 2008). Elevated MIP1α in the bone marrow could potentially create more inflammation and cause BM niche damage in *Fzd6*$^{-/-}$ mice.

We also showed that the total number of hematopoietic colonies from WT cells in Fzd6$^{-/-}$ BM environment appeared lower than the colonies from Fzd6$^{+/+}$ environment. This suggests that KO environment may be more inflammatory than WT. However, the results for colonies obtained from the spleen of transplanted mice were inverted with the BM, suggesting that a large proportion of colony-forming cells had migrated out of the bone marrow and to the spleen (Figure 3.2F). In addition, MIP1α has been demonstrated to modify BM microenvironment and support the maintenance of chronic myeloid leukemia (Baba et al., 2013). We hypothesize that uncontrolled inflammatory environment has a negative effect on HSPCs. Using ImageStream technique we observed that Fzd6 KO cells are susceptible to cell death (Figure 3.5E). It is worth to look for apoptosis by Annexin V, or necrosis in the future. MIP1α protein mediates its biological function by interaction with cell surface receptors CCR1, CCR5, and CCR9 (Lentszch et al., 2003). MIP1α appears in our model as an inflammatory inducer, that caused inhibition of HSPCs expansion, formation of myeloid colonies and promoted cell death. Based on these results, we plan to block receptor CCR1, known as one of the receptors for MIP1α *in vivo* (Ottersbach *et al.*, 2001) to reduce the inflammation and rescue fully or partly KO HSC expansion. By blocking receptor of MIP1α *in vivo*, we could demonstrate whether MIP1α is a key factor in our model.

Sepsis is a clinical syndrome that is caused by dysregulation of systemic immune response to infection and leads to failure of multiple organs. Wnt signaling participates in both promoting inflammation and controlling inflammation. The study describes an increased level of Wnt5a protein in synovial tissue of rheumatoid arthritis (RA) patients and assigned a functional

role for Wnt5a in RA pathogenesis (Sen *et al.*, 2000). Wnt5a expression was also reported to increase in the lung of patients with Tuberculosis, and in macrophages of patients infected with mycobacteria (Blumenthal et al., 2016). Gatica et al showed that a systemic challenge of mice with LPS to mimic sepsis led to an elevated Wnt5a in the serum (Gatica et al., 2017). Wnt5a up-regulation was also found in blood of septic patients, promoted inflammation by inducing the expression of target pro-inflammatory genes such as IL6, IL-1β, and TNF and perhaps MIP1α (Pereira et al., 2008). We published that Fzd6 is a negative regulator of CDC42 in HSPCs (Abidin et al., 2015). CDC42 is a downstream mediator of Wnt5a signalling (Prasad *et al.*, 2013), suggesting Wnt5a-signalling could be strongly active in Fzd6 deficient mice. Wnt5a is known to be produced in the BM microenvironment by MSCs, and osteoblasts (Schreck et al., 2017). In the absence of Fzd6, the level of Wnt5a expression in BM could be enhanced, and uncontrolled levels of Wnt5a could consequently lead to the generation of MIP1α to elevate inflammation. Here, we would propose Fzd6 as a negative mediator controlling the Wnt5a/MIP1α axis. This hypothesis could be verified by measuring Wnt5a levels in BM supernatants from LPS-injected Fzd6 KO and WT mice. In addition, CDC42 expression in BM could be determined by ImageStream.

We reported in our model that MIP1α was a candidate for blocking HSC expansion *in vivo* and inducing inflammation. The mechanism in our model is similar to the mechanism in septic shock. By targeting MIP1α, Fzd6 could be a promising therapeutic target for inflammatory diseases as well as septic shock in the clinic.

G-CSF was initially used as an inducer to promote the generation of neutrophils in patients who have neutropenia as a side effect of chemotherapy (Chiba et al., 2018). Later, G-CSF was demonstrated to have the ability to induce HSC mobilization from BM into peripheral blood (Demetri et al., 1991). Subsequently, mobilized HSCs are collected for transplant into an immunocompromised patient to treat blood disorders such as leukemia, aplastic anemia, or lymphoma. Furthermore, G-CSF has been shown to activate and mobilize dormant HSCs without increasing cell cycle division (Bernitz et al., 2016). Our results in BM reveal that $Fzd6^{-/-}$ and WT HSCs were activated in a similar manner after G-SCF treatment with the expansion of the MPP2 population. There was no increase in bone marrow HSCs. We further showed that fewer $Fzd6^{-/-}$ HSCs circulate into peripheral blood and spleen compared to $Fzd6^{+/+}$ HSCs post GSCF treatment (Figure 3.5B, C, D). This suggests that Fzd6 might specifically control HSC mobility *in vivo*. In $Fzd6^{-/-}$ mice, the expression level of G-CSF in the BM is enhanced post LPS-injection (data not shown). Increased expression of G-CSF in BM of

$Fzd6^{-/-}$ compared to $Fzd6^{+/+}$ mice could compensate for defective migration. There was no difference in mobilization between $Fzd6^{-/-}$ and $Fzd6^{+/+}$ HSCs after LPS-induced emergency granulopoiesis (data not shown here). However, WT cells in the KO environment appeared to have migrated more than WT as observed with colony assay from transplanted mice (Figure 3.2F). We also observed that G-SCF induces production of granulocyte in BM and their release in peripheral blood in Fzd6-/- mice without significant difference compared to WT mice (data not shown). Increased numbers of neutrophils lead to the release of metallo-proteinases that create a proteolytic environment. In turn, those enzymes cleave adhesion molecules anchoring HSCs to the niche, to release HSCs into circulation. Fzd6 is expressed not only in hematopoietic progenitors but also in endothelial cells (ECs) and MSCs in BM. Alterations in the BM microenvironment of $Fzd6^{-/-}$ mice could be a reason for ECs to produce more G-CSF as compared to $Fzd6^{+/+}$ ECs. To test this, ECs will be sorted based on their surface markers VE-cadherin^{+} CD31^{+} Endomucin$^{+/-}$ SCA1^{high} VEGFR3^{-}, VE-cadherin^{+}CD31^{+} Endomucin^{+} SCA1^{low} VEGFR3^{+} (Hooper *et al.*, 2009, Poulos *et al.*, 2015) from $Fzd6^{-/-}$ and $Fzd6^{+/+}$ bone marrow, then sorted cells will be cultured with or without LPS to determine G-CSF expression.

By using the breast cancer model, Corda et al demonstrated that Fzd6 regulates invasion and motility of breast cancer cell lines *in vitro*. In addition, the study revealed Fzd6 controlled the metastatic process *in vivo* when they transplanted breast cancer cells to immunocompromised mice (Corda et al., 2017). They further indicated that Fzd6 regulated the extracellular matrix, particularly polymerization of extracellular fibronectin and organization of F-actin cytoskeleton. By regulation of cell cytoskeleton, Fzd6 controlled migration of breast cancer cells. Schreck et al demonstrated that Wnt5a produced by niche cells like osteoclasts regulates the assembly of F-actin cytoskeleton in HSPCs (Schreck et al., 2017). In addition, by using breast cancer cell line as well, Zhu et al demonstrated Wnt5a promotes migration of breast cancer cell via the Dvl2/Daam1/RhoA (Zhu et al., 2012, Pukrop et al., 2006). These results suggested us to look at F-actin expression and assembly in Fzd6-/- bone marrow. However, due to some technical issues, we could not have enough data to show here. However, the preliminary observation with F-actin staining is that *$Fzd6^{-/-}$* HSCs have lower MFI of Phalloidin than their counterparts. This would suggest that in the absence of Fzd6, LPS-activated HSCs have a defect in polymerization of F-actin that reduces their ability to migrate. In the next study, we will continue to look for F-actin cytoskeleton. We now hypothesize that Wnt5a acts through Fzd6/Dvl axis to control the assembly of F-actin and therefore regulation of HSC migration.

Fzd6 participates in planar cell polarity pathway, where the downstream cascade could result in cell adhesion to niche, reorganization of cellular cytoskeleton or asymmetric cell division. We also wonder whether Fzd6 regulates HSCs division in asymmetric manner. In order to answer this question, we used a combination of markers and ImageStream technique to look at how HSCs divide in mitosis. We were able to separate different phases of mitosis such as prophase, metaphase, anaphase, and telophase. However, due to some technical issues, we could not confirm that two cells binding together in telophase were true dividing cell or a doublet of two single cells attached to each other. We will need to repeat the experiment in the future to solve this technical issue. It is important to understand how HSCs divide *in vivo* under BM stress conditions. By targeting Fzd6 receptor, we could think about development of pharmacological Fzd6 agonist for therapeutic purposes, either to expand HSCs *in vitro* or to sustain long-term HSCs regeneration post transplantation or chemotherapy.

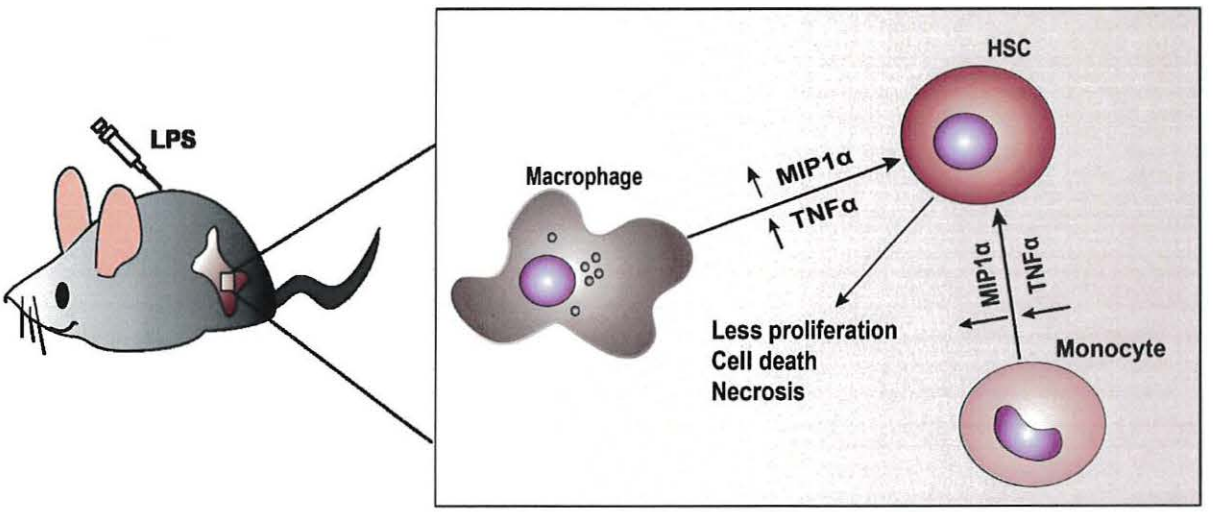

Figure 4.1 Graphical abstract

CHAPTER 5 - CONCLUSION

Fzd6 controls HSC mobilization and inflammation *in vivo*

Hematopoiesis is an essential process throughout the lifetime of an organism in which HSCs differentiate into all cell types of immune system such as lymphoid T, B cells and myeloid macrophages, monocytes, neutrophils. In order to sustain hematopoiesis for the whole life span of an organism, HSCs, a rare population, need to be maintained both in numbers and function. HSCs are used in clinical purpose to treat hematologic diseases. Thus, understanding the molecular mechanisms that regulate stem cell proliferation, and reconstitution is crucial to generate sufficient stem cell numbers for clinical goals. There are some attempts to expand HSC *ex vivo* to meet the clinical demands for hematological disorders. However, HSC expansion always comes with differentiation. This raises the question of how to increase expansion while minimizing differentiation. Wnt pathway has emerged as a critical regulator of HSC self-renewal, regeneration and motility. Here, we show that MIP1α could be a promising candidate in suppressing the expansion of HSCs in *Fzd6*$^{-/-}$ model. Put in a context of Wnt signaling, we propose that Fzd6 might be a negative regulator of Wnt5a/MIP1α signalling. Fzd6 is required for inflammatory regulation and to control migration of HSCs in order to avoid cell death. Considering from a clinical view, development of Fzd6 agonist could help to sustain HSC regeneration post transplant, self-renewal as well as *in vitro* expansion.

REFERENCES

Abidin BM, Hammami A, Stager S & Heinonen KM (2017) Infection-adapted emergency hematopoiesis promotes visceral leishmaniasis. *PLoS pathogens* 13(8).

Abidin BM, Owusu Kwarteng E & Heinonen KM (2015) Frizzled-6 Regulates Hematopoietic Stem/Progenitor Cell Survival and Self-Renewal. *J. Immunol.* 195(5):2168-2176.

Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, Bryder D, Yang L, Borge OJ, Thoren LA, Anderson K, Sitnicka E, Sasaki Y, Sigvardsson M & Jacobsen SE (2005) Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 121(2):295-306.

Aird WC (2007) Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ. Res.* 100(2):158-173.

Akashi K, Traver D, Miyamoto T & Weissman IL (2000) A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 404(6774):193-197.

Amanzada A, Moriconi F, Mansuroglu T, Cameron S, Ramadori G & Malik IA (2014) Induction of chemokines and cytokines before neutrophils and macrophage recruitment in different regions of rat liver after TAA administration. *Lab. Invest.* 94(2):235-247.

Anasetti C, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, Cutler CS, Westervelt P, Woolfrey A, Couban S, Ehninger G, Johnston L, Maziarz RT, Pulsipher MA, Porter DL, Mineishi S, McCarty JM, Khan SP, Anderlini P, Bensinger WI, Leitman SF, Rowley SD, Bredeson C, Carter SL, Horowitz MM & Confer DL (2012) Peripheral-Blood Stem Cells versus Bone Marrow from Unrelated Donors. *New Engl. J. Med.* 367(16):1487-1496.

Andonegui G, Bonder CS, Green F, Mullaly SC, Zbytnuik L, Raharjo E & Kubes P (2003) Endothelium-derived Toll-like receptor-4 is the key molecule in LPS-induced neutrophil sequestration into lungs. *J. Clin. Invest.* 111(7):1011-1020.

Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY & Suda T (2004) Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* 118(2):149-161.

Austin TW, Solar GP, Ziegler FC, Liem L & Matthews W (1997) A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. *Blood* 89(10):3624-3635.

Baba T, Naka K, Morishita S, Komatsu N, Hirao A & Mukaida N (2013) MIP-1alpha/CCL3-mediated maintenance of leukemia-initiating cells in the initiation process of chronic myeloid leukemia. *J. Exp. Med.* 210(12):2661-2673.

Baba T, Tanabe Y, Yoshikawa S, Yamanishi Y, Morishita S, Komatsu N, Karasuyama H, Hirao A & Mukaida N (2016) MIP-1alpha/CCL3-expressing basophil-lineage cells drive the leukemic hematopoiesis of chronic myeloid leukemia in mice. *Blood* 127(21):2607-2617.

Baba Y, Garrett KP & Kincade PW (2005) Constitutively active beta-catenin confers multilineage differentiation potential on lymphoid and myeloid progenitors. *Immunity* 23(6):599-609.

Baba Y, Yokota T, Spits H, Garrett KP, Hayashi S & Kincade PW (2006) Constitutively active beta-catenin promotes expansion of multipotent hematopoietic progenitors in culture. *J. Immunol.* 177(4):2294-2303.

Baldridge MT, King KY, Boles NC, Weksberg DC & Goodell MA (2010) Quiescent haematopoietic stem cells are activated by IFN-gamma in response to chronic infection. *Nature* 465(7299):793-797.

Banziger C, Soldini D, Schutt C, Zipperlen P, Hausmann G & Basler K (2006) Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* 125(3):509-522.

Barry F, Boynton R, Murphy M, Haynesworth S & Zaia J (2001) The SH-3 and SH-4 antibodies recognize distinct epitopes on CD73 from human mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* 289(2):519-524.

Barry FP, Boynton RE, Haynesworth S, Murphy JM & Zaia J (1999) The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem. Biophys. Res. Commun.* 265(1):134-139.

Basu S, Hodgson G, Zhang HH, Katz M, Quilici C & Dunn AR (2000) "Emergency" granulopoiesis in G-CSF-deficient mice in response to Candida albicans infection. *Blood* 95(12):3725-3733.

Bazan JF, Janda Claudia Y & Garcia KC (2012) Structural Architecture and Functional Evolution of Wnts. *Dev. Cell* 23(2):227-232.

Belenkaya TY, Wu Y, Tang X, Zhou B, Cheng L, Sharma YV, Yan D, Selva EM & Lin X (2008) The retromer complex influences Wnt secretion by recycling wntless from endosomes to the trans-Golgi network. *Dev. Cell* 14(1):120-131.

Bell JJ & Bhandoola A (2008) The earliest thymic progenitors for T cells possess myeloid lineage potential. *Nature* 452(7188):764-767.

Belyaev NN, Brown DE, Diaz AI, Rae A, Jarra W, Thompson J, Langhorne J & Potocnik AJ (2010) Induction of an IL7-R(+)c-Kit(hi) myelolymphoid progenitor critically dependent on IFN-gamma signaling during acute malaria. *Nat. Immunol.* 11(6):477-485.

Benayahu D, Horowitz M, Zipori D & Wientroub S (1992) Hemopoietic functions of marrow-derived osteogenic cells. *Calcif. Tissue Int.* 51(3):195-201.

Benayahu D, Kletter Y, Zipori D & Wientroub S (1989) Bone marrow-derived stromal cell line expressing osteoblastic phenotype in vitro and osteogenic capacity in vivo. *J. Cell. Physiol.* 140(1):1-7.

Bergenfelz C, Medrek C, Ekstrom E, Jirstrom K, Janols H, Wullt M, Bredberg A & Leandersson K (2012) Wnt5a induces a tolerogenic phenotype of macrophages in sepsis and breast cancer patients. *J. Immunol.* 188(11):5448-5458.

Bernad A, Kopf M, Kulbacki R, Weich N, Koehler G & Gutierrez-Ramos JC (1994) Interleukin-6 is required in vivo for the regulation of stem cells and committed progenitors of the hematopoietic system. *Immunity* 1(9):725-731.

Bernitz JM, Daniel MG, Fstkchyan YS & Moore K (2017) Granulocyte colony-stimulating factor mobilizes dormant hematopoietic stem cells without proliferation in mice. *Blood* 129(14):1901-1912.

Bethesda (2001) Hematopoietic stem cell. https://stemcells.nih.gov/info/2001report/chapter5.htm.

Blumenthal A, Ehlers S, Lauber J, Buer J, Lange C, Goldmann T, Heine H, Brandt E & Reiling N (2006) The Wingless homolog WNT5A and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood* 108(3):965-973.

Boettcher S, Gerosa RC, Radpour R, Bauer J, Ampenberger F, Heikenwalder M, Kopf M & Manz MG (2014) Endothelial cells translate pathogen signals into G-CSF-driven emergency granulopoiesis. *Blood* 124(9):1393-1403.

Boettcher S, Ziegler P, Schmid MA, Takizawa H, van Rooijen N, Kopf M, Heikenwalder M & Manz MG (2012) Cutting edge: LPS-induced emergency myelopoiesis depends on TLR4-expressing nonhematopoietic cells. *J. Immunol.* 188(12):5824-5828.

Boulais PE & Frenette PS (2015) Making sense of hematopoietic stem cell niches. *Blood* 125(17):2621-2629.

Broxmeyer HE, Cooper S, Hangoc G, Gao JL & Murphy PM (1999) Dominant myelopoietic effector functions mediated by chemokine receptor CCR1. *J. Exp. Med.* 189(12):1987-1992.

Broxmeyer HE, Orazi A, Hague NL, Sledge GW, Jr., Rasmussen H & Gordon MS (1998) Myeloid progenitor cell proliferation and mobilization effects of BB10010, a genetically engineered variant of human macrophage inflammatory protein-1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer. *Blood Cells Mol. Dis.* 24(1):14-30.

Broxmeyer HE, Sherry B, Lu L, Cooper S, Carow C, Wolpe SD & Cerami A (1989) Myelopoietic enhancing effects of murine macrophage inflammatory proteins 1 and 2 on colony formation in vitro by murine and human bone marrow granulocyte/macrophage progenitor cells. *J. Exp. Med.* 170(5):1583-1594.

Broxmeyer HE, Sherry B, Lu L, Cooper S, Oh KO, Tekamp-Olson P, Kwon BS & Cerami A (1990) Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation in vitro by bone marrow myeloid progenitor cells. *Blood* 76(6):1110-1116.

Bruns I, Lucas D, Pinho S, Ahmed J, Lambert MP, Kunisaki Y, Scheiermann C, Schiff L, Poncz M, Bergman A & Frenette PS (2014) Megakaryocytes regulate hematopoietic stem cell quiescence via Cxcl4 secretion. *Nat. Med.* 20(11):1315-1320.

Buechler MB, Akilesh HM & Hamerman JA (2016) Cutting Edge: Direct Sensing of TLR7 Ligands and Type I IFN by the Common Myeloid Progenitor Promotes mTOR/PI3K-Dependent Emergency Myelopoiesis. *J. Immunol.* 197(7):2577-2582.

Burberry A, Zeng MY, Ding L, Wicks I, Inohara N, Morrison SJ & Nunez G (2014) Infection mobilizes hematopoietic stem cells through cooperative NOD-like receptor and Toll-like receptor signaling. *Cell Host Microbe* 15(6):779-791.

Butler JM, Kobayashi H & Rafii S (2010) Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. *Nat. Rev. Cancer* 10(2):138-146.

Cabezas-Wallscheid N, Klimmeck D, Hansson J, Lipka DB, Reyes A, Wang Q, Weichenhan D, Lier A, von Paleske L, Renders S, Wunsche P, Zeisberger P, Brocks D, Gu L, Herrmann C, Haas S, Essers MAG, Brors B, Eils R, Huber W, Milsom MD, Plass C, Krijgsveld J & Trumpp A (2014) Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. *Cell Stem Cell* 15(4):507-522.

Caldwell GM, Jones CE, Soon Y, Warrack R, Morton DG & Matthews GM (2008) Reorganisation of Wnt-response pathways in colorectal tumorigenesis. *Br. J. Cancer* 98(8):1437-1442.

Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM & Scadden DT (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425(6960):841-846.

Caplan AI (1991) Mesenchymal stem cells. *J. Orthop. Res.* 9(5):641-650.

Carmeliet P & Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473(7347):298-307.

Chang H, Smallwood PM, Williams J & Nathans J (2016) The spatio-temporal domains of Frizzled6 action in planar polarity control of hair follicle orientation. *Dev. Biol.* 409(1):181-193.

Chaussabel D, Semnani RT, McDowell MA, Sacks D, Sher A & Nutman TB (2003) Unique gene expression profiles of human macrophages and dendritic cells to phylogenetically distinct parasites. *Blood* 102(2):672-681.

Cheng X, Huber TL, Chen VC, Gadue P & Keller GM (2008) Numb mediates the interaction between Wnt and Notch to modulate primitive erythropoietic specification from the hemangioblast. *Development* 135(20):3447-3458.

Chiba Y, Mizoguchi I, Hasegawa H, Ohashi M, Orii N, Nagai T, Sugahara M, Miyamoto Y, Xu M, Owaki T & Yoshimoto T (2018) Regulation of myelopoiesis by proinflammatory cytokines in infectious diseases. *Cell. Mol. Life Sci.* 75(8):1363-1376.

Chicha L, Jarrossay D & Manz MG (2004) Clonal Type I Interferon–producing and Dendritic Cell Precursors Are Contained in Both Human Lymphoid and Myeloid Progenitor Populations. *The Journal of Experimental Medicine* 200(11):1519.

Chistiakov DA, Bobryshev YV & Orekhov AN (2015) Neutrophil's weapons in atherosclerosis. *Exp. Mol. Pathol.* 99(3):663-671.

Chou DB, Sworder B, Bouladoux N, Roy CN, Uchida AM, Grigg M, Robey PG & Belkaid Y (2012) Stromal-derived IL-6 alters the balance of myeloerythroid progenitors during Toxoplasma gondii infection. *J. Leukoc. Biol.* 92(1):123-131.

Chow A, Lucas D, Hidalgo A, Mendez-Ferrer S, Hashimoto D, Scheiermann C, Battista M, Leboeuf M, Prophete C, van Rooijen N, Tanaka M, Merad M & Frenette PS (2011) Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J. Exp. Med.* 208(2):261-271.

Christopher MJ, Rao M, Liu F, Woloszynek JR & Link DC (2011) Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. *J. Exp. Med.* 208(2):251-260.

Chu Matthew L-H, Ahn Victoria E, Choi H-J, Daniels Danette L, Nusse R & Weis William I (2013) Structural Studies of Wnts and Identification of an LRP6 Binding Site. *Structure* 21(7):1235-1242.

Clements WK, Kim AD, Ong KG, Moore JC, Lawson N & Traver D (2011) An environmental Wnt16/Notch pathway specifies haematopoietic stem cells. *Nature* 474(7350):220-224.

Cobas M, Wilson A, Ernst B, Mancini SJC, MacDonald HR, Kemler R & Radtke F (2004) Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. *The Journal of experimental medicine* 199(2):221-229.

Congdon KL, Voermans C, Ferguson EC, DiMascio LN, Uqoezwa M, Zhao C & Reya T (2008) Activation of Wnt signaling in hematopoietic regeneration. *Stem Cells* 26(5):1202-1210.

Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB & Smithies O (1995) Requirement of MIP-1 alpha for an inflammatory response to viral infection. *Science* 269(5230):1583-1585.

Cooper AM, Roberts AD, Rhoades ER, Callahan JE, Getzy DM & Orme IM (1995) The role of interleukin-12 in acquired immunity to Mycobacterium tuberculosis infection. *Immunology* 84(3):423-432.

Corda G, Sala G, Lattanzio R, Iezzi M, Sallese M, Fragassi G, Lamolinara A, Mirza H, Barcaroli D, Ermler S, Silva E, Yasaei H, Newbold RF, Vagnarelli P, Mottolese M, Natali PG, Perracchio L, Quist J, Grigoriadis A, Marra P, Tutt AN, Piantelli M, Iacobelli S, De Laurenzi V & Sala A (2017) Functional and prognostic significance of the genomic amplification of frizzled 6 (FZD6) in breast cancer. *The Journal of Pathology* 241(3):350-361.

Corrigan PM, Dobbin E, Freeburn RW & Wheadon H (2009) Patterns of Wnt/Fzd/LRP gene expression during embryonic hematopoiesis. *Stem Cells Dev.* 18(5):759-772.

Coudreuse DY, Roel G, Betist MC, Destree O & Korswagen HC (2006) Wnt gradient formation requires retromer function in Wnt-producing cells. *Science* 312(5775):921-924.

Cui CY, Klar J, Georgii HP, Fröjmark AS, Baig SM, Schlessinger D & Dahl' N (2013) Frizzled6 deficiency disrupts the differentiation process of nail development. *The Journal of investigative dermatology* 133(8):1990-1997.

Davatelis G, Tekamp-Olson P, Wolpe SD, Hermsen K, Luedke C, Gallegos C, Coit D, Merryweather J & Cerami A (1988) Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. *J. Exp. Med.* 167(6):1939-1944.

De Bruin AM, Demirel O, Hooibrink B, Brandts CH & Nolte MA (2013) Interferon-gamma impairs proliferation of hematopoietic stem cells in mice. *Blood* 121(18):3578-3585.

De Bruin AM, Libregts SF, Valkhof M, Boon L, Touw IP & Nolte MA (2012) IFNγ induces monopoiesis and inhibits neutrophil development during inflammation. *Blood* 119(6):1543-1554.

De Luca K, Frances-Duvert V, Asensio MJ, Ihsani R, Debien E, Taillardet M, Verhoeyen E, Bella C, Lantheaume S, Genestier L & Defrance T (2009) The TLR1/2 agonist PAM(3)CSK(4) instructs commitment of human hematopoietic stem cells to a myeloid cell fate. *Leukemia* 23(11):2063-2074.

De Marco P, Merello E, Rossi A, Piatelli G, Cama A, Kibar Z & Capra V (2011) FZD6 is a novel gene for human neural tube defects. *Hum. Mutat.* 33(2):384-390.

De Waal Malefyt R, Abrams J, Bennett B, Figdor CG & De Vries JE (1991) Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 174(5):1209-1220.

Demetri GD & Griffin JD (1991) Granulocyte colony-stimulating factor and its receptor. *Blood* 78(11):2791-2808.

Ding L & Morrison SJ (2013) Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature* 495(7440):231-235.

Ding L, Saunders TL, Enikolopov G & Morrison SJ (2012) Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* 481(7382):457-462.

Doan PL, Himburg HA, Helms K, Russell JL, Fixsen E, Quarmyne M, Harris JR, Deoliviera D, Sullivan JM, Chao NJ, Kirsch DG & Chute JP (2013) Epidermal growth factor regulates hematopoietic regeneration after radiation injury. *Nat. Med.* 19(3):295-304.

Dosen G, Tenstad E, Nygren MK, Stubberud H, Funderud S & Rian E (2006) Wnt expression and canonical Wnt signaling in human bone marrow B lymphopoiesis. *BMC Immunol.* 7:13-13.

Duhrsen U, Villeval JL, Boyd J, Kannourakis G, Morstyn G & Metcalf D (1988) Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 72(6):2074-2081.

Endo Y, Wolf V, Muraiso K, Kamijo K, Soon L, Uren A, Barshishat-Kupper M & Rubin JS (2005) Wnt-3a-dependent cell motility involves RhoA activation and is specifically regulated by dishevelled-2. *J. Biol. Chem.* 280(1):777-786.

Erridge C, Bennett-Guerrero E & Poxton IR (2002) Structure and function of lipopolysaccharides. *Microbes Infect* 4(8):837-851.

Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA & Trumpp A (2009) IFNalpha activates dormant haematopoietic stem cells in vivo. *Nature* 458(7240):904-908.

Fensterheim BA, Guo Y, Sherwood ER & Bohannon JK (2017) The Cytokine Response to Lipopolysaccharide Does Not Predict the Host Response to Infection. *J. Immunol.* 198(8):3264-3273.

Fink MP (2014) Animal models of sepsis. *Virulence* 5(1):143-153.

Fleming HE, Janzen V, Celso CL, Guo J, Leahy KM, Kronenberg HM & Scadden DT (2008) Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. *Cell stem cell* 2(3):274-283.

Florian MC, Nattamai KJ, Dorr K, Marka G, Uberle B, Vas V, Eckl C, Andra I, Schiemann M, Oostendorp RA, Scharffetter-Kochanek K, Kestler HA, Zheng Y & Geiger H (2013) A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. *Nature* 503(7476):392-396.

Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J & Lebecque S (1996) T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* 183(6):2593-2603.

Foudi A, Hochedlinger K, Van Buren D, Schindler JW, Jaenisch R, Carey V & Hock H (2009) Defining Hematopoietic Stem and Progenitor Cell Turnover by Analysis of Histone 2B-GFP Dilution. *Nat. Biotechnol.* 27(1):84-90.

Frojmark AS, Schuster J, Sobol M, Entesarian M, Kilander MBC, Gabrikova D, Nawaz S, Baig SM, Schulte G, Klar J & Dahl N (2011) Mutations in Frizzled 6 cause isolated autosomal-recessive nail dysplasia. *Am. J. Hum. Genet.* 88(6):852-860.

Gabrilove JL, Jakubowski A, Scher H, Sternberg C, Wong G, Grous J, Yagoda A, Fain K, Moore MA, Clarkson B & et al. (1988) Effect of granulocyte colony-stimulating factor on neutropenia and associated morbidity due to chemotherapy for transitional-cell carcinoma of the urothelium. *N. Engl. J. Med.* 318(22):1414-1422.

Garcia CB, Alvarez ZM, Riveros MAR, Ortiz L, P. C., Ratkovich GS, Hernandez FG, Bravo CA, Jave SLF & Aguilar LA (2013) Restoration of WNT4 inhibits cell growth in leukemia-derived cell lines. *BMC Cancer* 13:557.

Gasson JC (1991) Molecular physiology of granulocyte-macrophage colony-stimulating factor. *Blood* 77(6):1131-1145.

Gatica-Andrades M, Vagenas D, Kling J, Nguyen TTK, Benham H, Thomas R, Korner H, Venkatesh B, Cohen J & Blumenthal A (2017) WNT ligands contribute to the immune response during septic shock and amplify endotoxemia-driven inflammation in mice. *Blood Adv* 1(16):1274-1286.

Ghosh MC, Collins GD, Vandanmagsar B, Patel K, Brill M, Carter A, Lustig A, Becker KG, Wood WW, 3rd, Emeche CD, French AD, O'Connell MP, Xu M, Weeraratna AT & Taub DD (2009) Activation of Wnt5A signaling is required for CXC chemokine ligand 12-mediated T-cell migration. *Blood* 114(7):1366-1373.

Golan T, Yaniv A, Bafico A, Liu G & Gazit A (2004) The Human Frizzled 6 (HFz6) Acts as a Negative Regulator of the Canonical Wnt·β-Catenin Signaling Cascade. *J. Biol. Chem.* 279(15):14879-14888.

Goodman RM, Thombre S, Firtina Z, Gray D, Betts D, Roebuck J, Spana EP & Selva EM (2006) Sprinter: a novel transmembrane protein required for Wg secretion and signaling. *Development* 133(24):4901-4911.

Graham GJ, Wright EG, Hewick R, Wolpe SD, Wilkie NM, Donaldson D, Lorimore S & Pragnell IB (1990) Identification and characterization of an inhibitor of haemopoietic stem cell proliferation. *Nature* 344(6265):442-444.

Griseri T, McKenzie BS, Schiering C & Powrie F (2012) Dysregulated hematopoietic stem and progenitor cell activity promotes interleukin-23-driven chronic intestinal inflammation. *Immunity* 37(6):1116-1129.

Guezguez B, Almakadi M, Benoit YD, Shapovalova Z, Rahmig S, Fiebig-Comyn A, Casado FL, Tanasijevic B, Bresolin S, Masetti R, Doble BW & Bhatia M (2016) GSK3 Deficiencies in Hematopoietic Stem Cells Initiate Pre-neoplastic State that Is Predictive of Clinical Outcomes of Human Acute Leukemia. *Cancer Cell* 29(1):61-74.

Guo N, Hawkins C & Nathans J (2004) Frizzled6 controls hair patterning in mice. *Proc. Natl. Acad. Sci. U. S. A.* 101(25):9277-9281.

Guo Y, Mishra A, Weng T, Chintagari NR, Wang Y, Zhao C, Huang C & Liu L (2014) Wnt3a mitigates acute lung injury by reducing P2X7 receptor-mediated alveolar epithelial type I cell death. *Cell Death Dis.* 5:e1286.

Haas S, Hansson J, Klimmeck D, Loeffler D, Velten L, Uckelmann H, Wurzer S, Prendergast AM, Schnell A, Hexel K, Santarella-Mellwig R, Blaszkiewicz S, Kuck A, Geiger H, Milsom MD, Steinmetz LM, Schroeder T, Trumpp A, Krijgsveld J & Essers MA (2015) Inflammation-Induced Emergency Megakaryopoiesis Driven by Hematopoietic Stem Cell-like Megakaryocyte Progenitors. *Cell Stem Cell* 17(4):422-434.

Hamilton JA (2008) Colony-stimulating factors in inflammation and autoimmunity. *Nat. Rev. Immunol.* 8(7):533-544.

Hammad H, Chieppa M, Perros F, Willart MA, Germain RN & Lambrecht BN (2009) House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* 15(4):410-416.

He RL, Zhou J, Hanson CZ, Chen J, Cheng N & Ye RD (2009) Serum amyloid A induces G-CSF expression and neutrophilia via Toll-like receptor 2. *Blood* 113(2):429-437.

Heinonen KM, Dube N, Bourdeau A, Lapp WS & Tremblay ML (2006) Protein tyrosine phosphatase 1B negatively regulates macrophage development through CSF-1 signaling. *Proc. Natl. Acad. Sci. U. S. A.* 103(8):2776-2781.

Heinonen KM, Vanegas JR, Brochu S, Shan J, Vainio SJ & Perreault C (2011a) Wnt4 regulates thymic cellularity through the expansion of thymic epithelial cells and early thymic progenitors. *Blood* 118(19):5163-5173.

Heinonen KM, Vanegas JR, Lew D, Krosl J & Perreault C (2011b) Wnt4 enhances murine hematopoietic progenitor cell expansion through a planar cell polarity-like pathway. *PLoS One* 6(4):e19279-e19279.

Hérault A, Binnewies M, Leong S, Calero-Nieto FJ, Zhang SY, Kang Y-A, Wang X, Pietras EM, Chu SH, Barry-Holson K, Armstrong S, Göttgens B & Passegué E (2017) Myeloid progenitor cluster formation drives emergency and leukaemic myelopoiesis. *Nature* 544(7648):53-58.

Herguido C, Guiu J, Altri T, Esteve J, Dzierzak E, Espinosa L & Bigas A (2012) Hematopoietic stem cell development requires transient Wnt/β-catenin activity. *The Journal of Experimental Medicine* 209(8):1457-1468.

Hii HP, Liao MH, Chen SJ, Wu CC & Shih CC (2015) Distinct Patterns of Wnt3a and Wnt5a Signaling Pathway in the Lung from Rats with Endotoxic Shock. *PLoS One* 10(7):e0134492.

Himburg HA, Muramoto GG, Daher P, Meadows SK, Russell JL, Doan P, Chi JT, Salter AB, Lento WE, Reya T, Chao NJ & Chute JP (2010) Pleiotrophin regulates the expansion and regeneration of hematopoietic stem cells. *Nat. Med.* 16(4):475-482.

Holmes T, O'Brien TA, Knight R, Lindeman R, Shen S, Song E, Symonds G & Dolnikov A (2008) Glycogen synthase kinase-3beta inhibition preserves hematopoietic stem cell activity and inhibits leukemic cell growth. *Stem Cells* 26(5):1288-1297.

Hoogendijk AJ, Diks SH, van der Poll T, Peppelenbosch MP & Wieland CW (2011) Kinase activity profiling of pneumococcal pneumonia. *PLoS One* 6(4):e18519.

Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, Kopp HG, Shido K, Petit I, Yanger K, James D, Witte L, Zhu Z, Wu Y, Pytowski B, Rosenwaks Z, Mittal V, Sato TN & Rafii S (2009) Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell* 4(3):263-274.

Hsieh C-H, Frink M, Hsieh Y-C, Kan W-H, Hsu J-T, Schwacha MG, Choudhry MA & Chaudry IH (2008) The Role of MIP-1α in the Development of Systemic Inflammatory Response and Organ Injury following Trauma Hemorrhage. *The Journal of Immunology* 181(4):2806-2812.

Hur J, Choi J-I, Lee H, Nham P, Kim T-W, Chae C-W, Yun J-Y, Kang J-A, Kang J, Lee Sang E, Yoon C-H, Boo K, Ham S, Roh T-Y, Jun Jong K, Lee H, Baek Sung H & Kim H-S (2016) CD82/KAI1 Maintains the Dormancy of Long-Term Hematopoietic Stem Cells through Interaction with DARC-Expressing Macrophages. *Cell Stem Cell* 18(4):508-521.

Hwa CH, Bae YC & Jung JS (2006) Role of toll-like receptors on human adipose-derived stromal cells. *Stem Cells* 24(12):2744-2752.

Igarashi H, Gregory SC, Yokota T, Sakaguchi N & Kincade PW (2002) Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity* 17(2):117-130.

Ito CY, Li CYJ, Bernstein A, Dick JE & Stanford WL (2003) Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A–null mice. *Blood* 101(2):517.

Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y & Suda T (2006) Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat. Med.* 12(4):446-451.

Iwata M, Awaya N, Graf L, Kahl C & Torok-Storb B (2004) Human marrow stromal cells activate monocytes to secrete osteopontin, which down-regulates Notch1 gene expression in CD34+ cells. *Blood* 103(12):4496-4502.

Jang J, Jung Y, Kim Y, Jho E & Yoon Y (2017) LPS-induced inflammatory response is suppressed by Wnt inhibitors, Dickkopf-1 and LGK974. *Sci. Rep.* 7:41612.

Jati S, Kundu S, Chakraborty A, Mahata SK, Nizet V & Sen M (2018) Wnt5A Signaling Promotes Defense Against Bacterial Pathogens by Activating a Host Autophagy Circuit. *Front. Immunol.* 9:679.

Jeltsch KS, Radke TF, Laufs S, Giordano FA, Allgayer H, Wenz F, Zeller WJ, Kögler G, Fruehauf S & Maier P (2011) Unrestricted somatic stem cells: interaction with CD34+cells in vitro and in vivo, expression of homing genes and exclusion of tumorigenic potential. *Cytotherapy* 13(3):357-365.

Karanu FN, Murdoch B, Gallacher L, Wu DM, Koremoto M, Sakano S & Bhatia M (2000) The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. *J. Exp. Med.* 192(9):1365-1372.

Kaushansky K (2006) Lineage-specific hematopoietic growth factors. *N. Engl. J. Med.* 354(19):2034-2045.

Kim, Bang SH, Choi JH, Kim CD, Won CH, Lee MW & Chang SE (2016) Interaction of Wnt5a with Notch1 is Critical for the Pathogenesis of Psoriasis. *Ann Dermatol* 28(1):45-54.

Kim, Kim J, Kim DW, Ha Y, Ihm MH, Kim H, Song K & Lee I (2010) Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. *J. Immunol.* 185(2):1274-1282.

Kim, Park S, Chung H & Oh S (2015) Wnt5a attenuates the pathogenic effects of the Wnt/beta-catenin pathway in human retinal pigment epithelial cells via down-regulating beta-catenin and Snail. *BMB Rep* 48(9):525-530.

Kirstetter P, Anderson K, Porse BT, Jacobsen SE & Nerlov C (2006) Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nat. Immunol.* 7(10):1048-1056.

Kliche K, Jeggle P, Pavenstadt H & Oberleithner H (2011) Role of cellular mechanics in the function and life span of vascular endothelium. *Pflugers Arch.* 462(2):209-217.

Ko KH, Holmes T, Palladinetti P, Song E, Nordon R, O'Brien TA & Dolnikov A (2011) GSK-3beta inhibition promotes engraftment of ex vivo-expanded hematopoietic stem cells and modulates gene expression. *Stem Cells* 29(1):108-118.

Kobayashi H, Butler JM, O'Donnell R, Kobayashi M, Ding BS, Bonner B, Chiu VK, Nolan DJ, Shido K, Benjamin L & Rafii S (2010) Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. *Nat. Cell Biol.* 12(11):1046-1056.

Koch AE, Kunkel SL, Harlow LA, Mazarakis DD, Haines GK, Burdick MD, Pope RM & Strieter RM (1994) Macrophage inflammatory protein-1 alpha. A novel chemotactic cytokine for macrophages in rheumatoid arthritis. *J. Clin. Invest.* 93(3):921-928.

Kohn LA, Hao QL, Sasidharan R, Parekh C, Ge S, Zhu Y, Mikkola HK & Crooks GM (2012) Lymphoid priming in human bone marrow begins before expression of CD10 with upregulation of L-selectin. *Nat. Immunol.* 13(10):963-971.

Kolben T, Perobner I, Fernsebner K, Lechner F, Geissler C, Ruiz-Heinrich L, Capovilla S, Jochum M & Neth P (2012) Dissecting the impact of Frizzled receptors in Wnt/beta-catenin signaling of human mesenchymal stem cells. *Biol. Chem.* 393(12):1433-1447.

Kondo M, Weissman IL & Akashi K (1997) Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91(5):661-672.

Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H & Kohler G (1994) Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* 368(6469):339-342.

Kovtonyuk LV, Manz MG & Takizawa H (2016) Enhanced thrombopoietin but not G-CSF receptor stimulation induces self-renewing hematopoietic stem cell divisions in vivo. *Blood* 127(25):3175-3179.

Kwak HJ, Liu P, Bajrami B, Xu Y, S-Y P, Nombela-Arrieta C, Mondal S, Sun Y, Zhu H, Chai L, Silberstein LE, Cheng T & Luo HR (2015) Myeloid cell-derived reactive oxygen species externally regulate the proliferation of myeloid progenitors in emergency granulopoiesis. *Immunity* 42(1):159-171.

Lang RA, Metcalf D, Cuthbertson RA, Lyons I, Stanley E, Kelso A, Kannourakis G, Williamson DJ, Klintworth GK, Gonda TJ & et al. (1987) Transgenic mice expressing a hemopoietic growth factor gene (GM-CSF) develop accumulations of macrophages, blindness, and a fatal syndrome of tissue damage. *Cell* 51(4):675-686.

Lee C-L, Lento WE, Castle KD, Chao NJ & Kirsch DG (2014) Inhibiting glycogen synthase kinase-3 mitigates the hematopoietic acute radiation syndrome in mice. *Radiat. Res.* 181(5):445-451.

Lento W, Congdon K, Voermans C, Kritzik M & Reya T (2013) Wnt signaling in normal and malignant hematopoiesis. *Cold Spring Harb. Perspect. Biol.* 5(2).

Lento W, Ito T, Zhao C, Harris JR, Huang W, Jiang C, Owzar K, Piryani S, Racioppi L, Chao N & Reya T (2014) Loss of beta-catenin triggers oxidative stress and impairs hematopoietic regeneration. *Genes Dev.* 28(9):995-1004.

Levesque JP, Hendy J, Takamatsu Y, Simmons PJ & Bendall LJ (2003) Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. *J. Clin. Invest.* 111(2):187-196.

Li Y, Esain V, Teng L, Xu J, Kwan W, Frost IM, Yzaguirre AD, Cai X, Cortes M, Maijenburg MW, Tober J, Dzierzak E, Orkin SH, Tan K, North TE & Speck NA (2014) Inflammatory signaling regulates embryonic hematopoietic stem and progenitor cell production. *Genes Dev.* 28(23):2597-2612.

Liang H, Chen Q, Coles AH, Anderson SJ, Pihan G, Bradley A, Gerstein R, Jurecic R & Jones SN (2003) Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue. *Cancer Cell* 4(5):349-360.

Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, Fowler KJ, Basu S, Zhan YF & Dunn AR (1994) Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood* 84(6):1737-1746.

Liu A, Wang Y, Ding Y, Baez I, Payne KJ & Borghesi L (2015) Cutting Edge: Hematopoietic Stem Cell Expansion and Common Lymphoid Progenitor Depletion Require

Hematopoietic-Derived, Cell-Autonomous TLR4 in a Model of Chronic Endotoxin. *J. Immunol.* 195(6):2524-2528.

Liu F, Poursine-Laurent J & Link DC (2000) Expression of the G-CSF receptor on hematopoietic progenitor cells is not required for their mobilization by G-CSF. *Blood* 95(10):3025.

Liu F, Wu HY, Wesselschmidt R, Kornaga T & Link DC (1996) Impaired production and increased apoptosis of neutrophils in granulocyte colony-stimulating factor receptor-deficient mice. *Immunity* 5(5):491-501.

Liu X, Lu R, Wu S, Zhang YG, Xia Y, Sartor RB & Sun J (2012) Wnt2 inhibits enteric bacterial-induced inflammation in intestinal epithelial cells. *Inflamm. Bowel Dis.* 18(3):418-429.

Lord BI, Woolford LB, Wood LM, Czaplewski LG, McCourt M, Hunter MG & Edwards RM (1995) Mobilization of early hematopoietic progenitor cells with BB-10010: a genetically engineered variant of human macrophage inflammatory protein-1 alpha. *Blood* 85(12):3412-3415.

Ludin A, Itkin T, Gur-Cohen S, Mildner A, Shezen E, Golan K, Kollet O, Kalinkovich A, Porat Z, D'Uva G, Schajnovitz A, Voronov E, Brenner DA, Apte RN, Jung S & Lapidot T (2012) Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. *Nat. Immunol.* 13:1072.

Luis TC, Naber BA, Fibbe WE, van Dongen JJ & Staal FJ (2010) Wnt3a nonredundantly controls hematopoietic stem cell function and its deficiency results in complete absence of canonical Wnt signaling. *Blood* 116(3):496-497.

Luis TC, Naber BA, Roozen PP, Brugman MH, de Haas EF, Ghazvini M, Fibbe WE, van Dongen JJ, Fodde R & Staal FJ (2011) Canonical wnt signaling regulates hematopoiesis in a dosage-dependent fashion. *Cell Stem Cell* 9(4):345-356.

Luis TC, Weerkamp F, Naber BA, Baert MR, de Haas EF, Nikolic T, Heuvelmans S, De Krijger RR, van Dongen JJ & Staal FJ (2009) Wnt3a deficiency irreversibly impairs hematopoietic stem cell self-renewal and leads to defects in progenitor cell differentiation. *Blood* 113(3):546-554.

Luo Y, Shao L, Chang J, Feng W, Liu YL, Cottler-Fox MH, Emanuel PD, Hauer-Jensen M, Bernstein ID, Liu L, Chen X, Zhou J, Murray PJ & Zhou D (2018) M1 and M2 macrophages differentially regulate hematopoietic stem cell self-renewal and ex vivo expansion. *Blood Adv* 2(8):859-870.

MacDonald BT & He X (2012) Frizzled and LRP5/6 receptors for Wnt/β-catenin signaling. *Cold Spring Harb. Perspect. Biol.* 4(12):a007880.

MacDonald BT, Tamai K & He X (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* 17(1):9-26.

MacNamara KC, Jones M, Martin O & Winslow GM (2011) Transient activation of hematopoietic stem and progenitor cells by IFNgamma during acute bacterial infection. *PLoS One* 6(12):e28669.

Maeda K, Baba Y, Nagai Y, Miyazaki K, Malykhin A, Nakamura K, Kincade PW, Sakaguchi N & Coggeshall KM (2005) IL-6 blocks a discrete early step in lymphopoiesis. *Blood* 106(3):879-885.

Maiti G, Naskar D & Sen M (2012) The Wingless homolog Wnt5a stimulates phagocytosis but not bacterial killing. *Proc. Natl. Acad. Sci. U. S. A.* 109(41):16600-16605.

Majumdar MK, Thiede MA, Haynesworth SE, Bruder SP & Gerson SL (2000) Human Marrow-Derived Mesenchymal Stem Cells (MSCs) Express Hematopoietic Cytokines and Support Long-Term Hematopoiesis When Differentiated Toward Stromal and Osteogenic Lineages. *J. Hematother. Stem Cell Res.* 9(6):841-848.

Mansson R, Zandi S, Welinder E, Tsapogas P, Sakaguchi N, Bryder D & Sigvardsson M (2010) Single-cell analysis of the common lymphoid progenitor compartment reveals functional and molecular heterogeneity. *Blood* 115(13):2601-2609.

Manz MG & Boettcher S (2014) Emergency granulopoiesis. *Nat. Rev. Immunol.* 14(5):302-314.

Manz MG, Miyamoto T, Akashi K & Weissman IL (2002) Prospective isolation of human clonogenic common myeloid progenitors. *Proc. Natl. Acad. Sci. U. S. A.* 99(18):11872-11877.

Massberg S, Schaerli P, Knezevic-Maramica I, Kollnberger M, Tubo N, Moseman EA, Huff IV, Junt T, Wagers AJ, Mazo IB & von Andrian UH (2007) Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell* 131(5):994-1008.

Matatall KA, Jeong M, Chen S, Sun D, Chen F, Mo Q, Kimmel M & King KY (2016) Chronic Infection Depletes Hematopoietic Stem Cells through Stress-Induced Terminal Differentiation. *Cell Rep.* 17(10):2584-2595.

Matatall KA, Shen CC, Challen GA & King K (2014) Type II interferon promotes differentiation of myeloid-biased hematopoietic stem cells. *Stem Cells* 32(11):3023-3030.

Maurer M & von Stebut E (2004) Macrophage inflammatory protein-1. *Int. J. Biochem. Cell Biol.* 36(10):1882-1886.

Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, MacArthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN & Frenette PS (2010) Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 466:829.

Miller JR (2002) The Wnts. *Genome Biol.* 3(1):reviews3001.3001-reviews3001.3015.

Mishima S, Nagai A, Abdullah S, Matsuda C, Taketani T, Kumakura S, Shibata H, Ishikura H, Kim SU & Masuda J (2010) Effective ex vivo expansion of hematopoietic stem cells using osteoblast-differentiated mesenchymal stem cells is CXCL12 dependent. *Eur. J. Haematol.* 84(6):538-546.

Murdoch JN, Damrau C, Paudyal A, Bogani D, Wells S, Greene NDE, Stanier P & Copp AJ (2014) Genetic interactions between planar cell polarity genes cause diverse neural tube defects in mice. *Dis. Model. Mech.* 7(10):1153-1163.

Murray PJ & Wynn TA (2011) Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 11(11):723-737.

Nagai Y, Garrett KP, Ohta S, Bahrun U, Kouro T, Akira S, Takatsu K & Kincade PW (2006) Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. *Immunity* 24(6):801-812.

Nakamura-Ishizu A, Takubo K, Fujioka M & Suda T (2014) Megakaryocytes are essential for HSC quiescence through the production of thrombopoietin. *Biochem. Biophys. Res. Commun.* 454(2):353-357.

Nakorn TN, Miyamoto T & Weissman IL (2003) Characterization of mouse clonogenic megakaryocyte progenitors. *Proc. Natl. Acad. Sci. U. S. A.* 100(1):205-210.

Naskar D, Maiti G, Chakraborty A, Roy A, Chattopadhyay D & Sen M (2014) Wnt5a-Rac1-NF-kappaB homeostatic circuitry sustains innate immune functions in macrophages. *J. Immunol.* 192(9):4386-4397.

Nau GJ, Richmond JF, Schlesinger A, Jennings EG, Lander ES & Young RA (2002) Human macrophage activation programs induced by bacterial pathogens. *Proc. Natl. Acad. Sci. U. S. A.* 99(3):1503-1508.

Naz G, Pasternack SM, Perrin C, Mattheisen M, Refke M, Khan S, Gul A, Simons M, Ahmad W & Betz RC (2012) FZD6 encoding the Wnt receptor frizzled 6 is mutated in autosomal-recessive nail dysplasia. *Br. J. Dermatol.* 166(5):1088-1094.

Nemzek JA, Hugunin KMS & Opp MR (2008) Modeling sepsis in the laboratory: merging sound science with animal well-being. *Comp. Med.* 58(2):120-128.

Nestorowa S, Hamey FK, Pijuan Sala B, Diamanti E, Shepherd M, Laurenti E, Wilson NK, Kent DG & Göttgens B (2016) A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. *Blood* 128(8):e20-e31.

Neumann J, Schaale K, Farhat K, Endermann T, Ulmer AJ, Ehlers S & Reiling N (2010) Frizzled1 is a marker of inflammatory macrophages, and its ligand Wnt3a is involved in reprogramming Mycobacterium tuberculosis-infected macrophages. *FASEB J.* 24(11):4599-4612.

Nibbs RJ, Wylie SM, Yang J, Landau NR & Graham GJ (1997) Cloning and characterization of a novel promiscuous human beta-chemokine receptor D6. *J. Biol. Chem.* 272(51):32078-32083.

Nilsson SK, Johnston HM & Coverdale JA (2001) Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* 97(8):2293-2299.

Nishizawa M & Nagata S (1990) Regulatory elements responsible for inducible expression of the granulocyte colony-stimulating factor gene in macrophages. *Mol. Cell. Biol.* 10(5):2002-2011.

Nusse R, Brown A, Papkoff J, Scambler P, Shackleford G, McMahon A, Moon R & Varmus H (1991) A new nomenclature for int-1 and related genes: the Wnt gene family. *Cell* 64(2):231.

Nusse R & Varmus HE (1982) Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 31(1):99-109.

Oderup C, LaJevic M & Butcher EC (2013) Canonical and noncanonical Wnt proteins program dendritic cell responses for tolerance. *J. Immunol.* 190(12):6126-6134.

Ottersbach K, Cook DN, Kuziel WA, Humbles A, Lu B, Gerard C, Proudfoot AEI & Graham GJ (2001) Macrophage inflammatory protein-1α uses a novel receptor for primitive hemopoietic cell inhibition. *Blood* 98(12):3476-3478.

Ouchi N, Higuchi A, Ohashi K, Oshima Y, Gokce N, Shibata R, Akasaki Y, Shimono A & Walsh K (2010) Sfrp5 is an anti-inflammatory adipokine that modulates metabolic dysfunction in obesity. *Science* 329(5990):454-457.

Pelus LM, Bian H, King AG & Fukuda S (2004) Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. *Blood* 103(1):110-119.

Pereira C, Schaer DJ, Bachli EB, Kurrer MO & Schoedon G (2008) Wnt5A/CaMKII signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. *Arterioscler. Thromb. Vasc. Biol.* 28(3):504-510.

Perry JM, He XC, Sugimura R, Grindley JC, Haug JS, Ding S & Li L (2011) Cooperation between both Wnt/{beta}-catenin and PTEN/PI3K/Akt signaling promotes primitive hematopoietic stem cell self-renewal and expansion. *Genes Dev.* 25(18):1928-1942.

Pevsner FM, Morad V, Cohen SM, Rousso NL, Zanin ZA, Cohen S, Cohen IR & Zipori D (2007) Toll-like receptors and their ligands control mesenchymal stem cell functions. *Blood* 109(4):1422.

Pietras EM, Lakshminarasimhan R, Techner JM, Fong S, Flach J, Binnewies M & Passegue E (2014) Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. *J. Exp. Med.* 211(2):245-262.

Pietras EM, Mirantes-Barbeito C, Fong S, Loeffler D, Kovtonyuk LV, Zhang S, Lakshminarasimhan R, Chin CP, Techner JM, Will B, Nerlov C, Steidl U, Manz MG, Schroeder T & Passegue E (2016) Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal. *Nat. Cell Biol.* 18(6):607-618.

Pietras EM, Reynaud D, Kang Y-A, Carlin D, Calero-Nieto FJ, Leavitt AD, Stuart JM, Göttgens B & Passegué E (2015) Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. *Cell stem cell* 17(1):35-46.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S & Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411):143-147.

Poulos MG, Crowley MJP, Gutkin MC, Ramalingam P, Schachterle W, Thomas JL, Elemento O & Butler JM (2015) Vascular Platform to Define Hematopoietic Stem Cell Factors and Enhance Regenerative Hematopoiesis. *Stem Cell Reports* 5(5):881-894.

Poulos MG, Guo P, Kofler NM, Pinho S, Gutkin MC, Tikhonova A, Aifantis I, Frenette PS, Kitajewski J, Rafii S & Butler JM (2013) Endothelial Jagged-1 is necessary for homeostatic and regenerative hematopoiesis. *Cell Rep.* 4(5):1022-1034.

Poulos MG, Ramalingam P, Gutkin MC, Kleppe M, Ginsberg M, Crowley MJ, Elemento O, Levine RL, Rafii S, Kitajewski J, Greenblatt MB, Shim JH & Butler JM (2016) Endothelial-specific inhibition of NF-kappaB enhances functional haematopoiesis. *Nat Commun* 7:13829.

Prasad CP, Chaurasiya SK, Axelsson L & Andersson T (2013) WNT-5A triggers Cdc42 activation leading to an ERK1/2 dependent decrease in MMP9 activity and invasive migration of breast cancer cells. *Mol. Oncol.* 7(5):870-883.

Pronk CJ, Rossi DJ, Mansson R, Attema JL, Norddahl GL, Chan CK, Sigvardsson M, Weissman IL & Bryder D (2007) Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell* 1(4):428-442.

Putnová I, Doseděllová H, Bryja V, Landová M, Buchtová M & Štembírek J (2017) Angled Growth of the Dental Lamina Is Accompanied by Asymmetrical Expression of the WNT Pathway Receptor Frizzled 6. *Front. Physiol.* 8:29.

Qian H, Buza-Vidas N, Hyland CD, Jensen CT, Antonchuk J, Mansson R, Thoren LA, Ekblom M, Alexander WS & Jacobsen SE (2007) Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell* 1(6):671-684.

Rapp J, Jaromi L, Kvell K, Miskei G & Pongracz JE (2017) WNT signaling – lung cancer is no exception. *Respir. Res.* 18(1):167.

Rappold I, Watt S, Kusadasi N, Rose-John S, Hatzfeld J & Ploemacher R (1999) Gp130-signaling synergizes with FL and TPO for the long-term expansion of cord blood progenitors. *Leukemia* 13(12):2036.

Reischl J, Schwenke S, Beekman JM, Mrowietz U, Sturzebecher S & Heubach JF (2007) Increased expression of Wnt5a in psoriatic plaques. *J. Invest. Dermatol.* 127(1):163-169.

Renstrom J, Istvanffy R, Gauthier K, Shimono A, Mages J, Jardon-Alvarez A, Kroger M, Schiemann M, Busch DH, Esposito I, Lang R, Peschel C & Oostendorp RA (2009) Secreted frizzled-related protein 1 extrinsically regulates cycling activity and maintenance of hematopoietic stem cells. *Cell Stem Cell* 5(2):157-167.

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, Willert K, Hintz L, Nusse R & Weissman IL (2003) A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 423(6938):409-414.

Reya T, O'Riordan M, Okamura R, Devaney E, Willert K, Nusse R & Grosschedl R (2000) Wnt signaling regulates B lymphocyte proliferation through a LEF-1 dependent mechanism. *Immunity* 13(1):15-24.

Reynaud D, Pietras E, Barry-Holson K, Mir A, Binnewies M, Jeanne M, Sala-Torra O, Radich JP & Passegue E (2011) IL-6 controls leukemic multipotent progenitor cell fate and contributes to chronic myelogenous leukemia development. *Cancer Cell* 20(5):661-673.

Richter J, Traver D & Willert K (2017) The role of Wnt signaling in hematopoietic stem cell development. *Crit. Rev. Biochem. Mol. Biol.* 52(4):414-424.

Rodriguez-Fraticelli AE, Wolock SL, Weinreb CS, Panero R, Patel SH, Jankovic M, Sun J, Calogero RA, Klein AM & Camargo FD (2018) Clonal analysis of lineage fate in native haematopoiesis. *Nature* 553(7687):212-216.

Romani L, Mencacci A, Cenci E, Spaccapelo R, Toniatti C, Puccetti P, Bistoni F & Poli V (1996) Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin 6-deficient mice infected with Candida albicans. *J. Exp. Med.* 183(4):1345-1355.

Rosales C (2018) Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Front. Physiol.* 9:113-113.

Saito Y, Uchida N, Tanaka S, Suzuki N, Tomizawa-Murasawa M, Sone A, Najima Y, Takagi S, Aoki Y, Wake A, Taniguchi S, Shultz LD & Ishikawa F (2010) Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nat. Biotechnol.* 28(3):275-280.

Sanjuan-Pla A, Macaulay IC, Jensen CT, Woll PS, Luis TC, Mead A, Moore S, Carella C, Matsuoka S, Bouriez Jones T, Chowdhury O, Stenson L, Lutteropp M, Green JC, Facchini R, Boukarabila H, Grover A, Gambardella A, Thongjuea S, Carrelha J, Tarrant P, Atkinson D, Clark SA, Nerlov C & Jacobsen SE (2013) Platelet-biased stem cells reside at the apex of the haematopoietic stem-cell hierarchy. *Nature* 502(7470):232-236.

Sano E, Ohashi K, Sato Y, Kashiwagi M, Joguchi A & Naruse N (2007) A possible role of autogenous IFN-beta for cytokine productions in human fibroblasts. *J. Cell. Biochem.* 100(6):1459-1476.

Sasine JP, Yeo KT & Chute JP (2017) Concise Review: Paracrine Functions of Vascular Niche Cells in Regulating Hematopoietic Stem Cell Fate. *Stem Cells Transl Med* 6(2):482-489.

Sato A, Kayama H, Shojima K, Matsumoto S, Koyama H, Minami Y, Nojima S, Morii E, Honda H, Takeda K & Kikuchi A (2015) The Wnt5a-Ror2 axis promotes the signaling circuit between interleukin-12 and interferon-gamma in colitis. *Sci. Rep.* 5:10536.

Sato A, Yamamoto H, Sakane H, Koyama H & Kikuchi A (2010) Wnt5a regulates distinct signalling pathways by binding to Frizzled2. *EMBO J.* 29(1):41-54.

Sato T, Onai N, Yoshihara H, Arai F, Suda T & Ohteki T (2009) Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. *Nat. Med.* 15(6):696-700.

Schaniel C, Sirabella D, Qiu J, Niu X, Lemischka IR & Moore KA (2011) Wnt-inhibitory factor 1 dysregulation of the bone marrow niche exhausts hematopoietic stem cells. *Blood* 118(9):2420-2429.

Schaper F & Rose-John S (2015) Interleukin-6: Biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev.* 26(5):475-487.

Scheller, Chalaris A, Schmidt-Arras D & Rose-John S (2011) The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim. Biophys. Acta* 1813(5):878-888.

Scheller M, Huelsken J, Rosenbauer F, Taketo MM, Birchmeier W, Tenen DG & Leutz A (2006) Hematopoietic stem cell and multilineage defects generated by constitutive beta-catenin activation. *Nat. Immunol.* 7(10):1037-1047.

Schepers K, Pietras EM, Reynaud D, Flach J, Binnewies M, Garg T, Wagers AJ, Hsiao EC & Passegue E (2013) Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell* 13(3):285-299.

Schilling JD, Martin SM, Hung CS, Lorenz RG & Hultgren SJ (2003) Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic Escherichia coli. *Proc. Natl. Acad. Sci. U. S. A.* 100(7):4203-4208.

Schmid MA, Takizawa H, Baumjohann DR, Saito Y & Manz MG (2011) Bone marrow dendritic cell progenitors sense pathogens via Toll-like receptors and subsequently migrate to inflamed lymph nodes. *Blood* 118(18):4829-4840.

Schofield R (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4(1-2):7-25.

Schofield R, Lord BI, Humphreys ER & Stones VA (1986) Effects of plutonium-239 on haemopoiesis. I. Quantitative and qualitative changes in CFU-S in different regions of the mouse femur and vertebrae. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 49(6):1021-1029.

Schreck C, Istvanffy R, Ziegenhain C, Sippenauer T, Ruf F, Henkel L, Gartner F, Vieth B, Florian MC, Mende N, Taubenberger A, Prendergast A, Wagner A, Pagel C, Grziwok S, Gotze KS, Guck J, Dean DC, Massberg S, Essers M, Waskow C, Geiger H, Schiemann M, Peschel C, Enard W & Oostendorp RA (2017) Niche WNT5A regulates the actin cytoskeleton during regeneration of hematopoietic stem cells. *J. Exp. Med.* 214(1):165-181.

Schurch CM, Riether C & Ochsenbein AF (2014) Cytotoxic CD8+ T cells stimulate hematopoietic progenitors by promoting cytokine release from bone marrow mesenchymal stromal cells. *Cell Stem Cell* 14(4):460-472.

Sen M, Chamorro M, Reifert J, Corr M & Carson DA (2001) Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. *Arthritis Rheum.* 44(4):772-781.

Sen M, Lauterbach K, El-Gabalawy H, Firestein GS, Corr M & Carson DA (2000) Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U. S. A.* 97(6):2791-2796.

Sharma A, Yang WL, Ochani M & Wang P (2017) Mitigation of sepsis-induced inflammatory responses and organ injury through targeting Wnt/beta-catenin signaling. *Sci. Rep.* 7(1):9235.

Shi X, Zhang P, Sempowski GD & Shellito JE (2011) Thymopoietic and Bone Marrow Response to Murine Pneumocystis Pneumonia. *Infect. Immun.* 79(5):2031.

Siar CH, Nagatsuka H, Han PP, Buery RR, Tsujigiwa H, Nakano K, Ng KH & Kawakami T (2012) Differential expression of canonical and non-canonical Wnt ligands in ameloblastoma. *J. Oral Pathol. Med.* 41(4):332-339.

Sioud M & Floisand Y (2007) TLR agonists induce the differentiation of human bone marrow CD34+ progenitors into CD11c+ CD80/86+ DC capable of inducing a Th1-type response. *Eur. J. Immunol.* 37(10):2834-2846.

Staal FJ, Chhatta A & Mikkers H (2016) Caught in a Wnt storm: Complexities of Wnt signaling in hematopoiesis. *Exp. Hematol.* 44(6):451-457.

Staal FJ & Clevers HC (2005) WNT signalling and haematopoiesis: a WNT-WNT situation. *Nat. Rev. Immunol.* 5(1):21-30.

Staal FJ, Luis TC & Tiemessen MM (2008) WNT signalling in the immune system: WNT is spreading its wings. *Nat. Rev. Immunol.* 8(8):581-593.

Stamos JL & Weis WI (2013) The β-catenin destruction complex. *Cold Spring Harb. Perspect. Biol.* 5(1):a007898.

Standiford TJ, Kunkel SL, Lukacs NW, Greenberger MJ, Danforth JM, Kunkel RG & Strieter RM (1995) Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. *The Journal of Immunology* 155(3):1515-1524.

Staversky RJ, Byun DK, Georger MA, Zaffuto BJ, Goodman A, Becker MW, Calvi LM & Frisch BJ (2018a) The Chemokine CCL3 Regulates Myeloid Differentiation and Hematopoietic Stem Cell Numbers. *Sci. Rep.* 8(1):14691.

Staversky RJ, Byun DK, Georger MA, Zaffuto BJ, Goodman A, Becker MW, Calvi LM & Frisch BJ (2018b) The Chemokine CCL3 Regulates Myeloid Differentiation and Hematopoietic Stem Cell Numbers. *Scientific reports* 8(1):14691.

Stier S, Cheng T, Dombkowski D, Carlesso N & Scadden DT (2002) Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. *Blood* 99(7):2369-2378.

Stier S, Ko Y, Forkert R, Lutz C, Neuhaus T, Grunewald E, Cheng T, Dombkowski D, Calvi LM, Rittling SR & Scadden DT (2005) Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. *J. Exp. Med.* 201(11):1781-1791.

Sugimura R, He XC, Venkatraman A, Arai F, Box A, Semerad C, Haug JS, Peng L, Zhong XB, Suda T & Li L (2012) Noncanonical Wnt signaling maintains hematopoietic stem cells in the niche. *Cell* 150(2):351-365.

Taichman R, Reilly M & Emerson S (1996) Human osteoblasts support human hematopoietic progenitor cells in vitro bone marrow cultures. *Blood* 87(2):518-524.

Taichman R, Reilly M, Verma R, Ehrenman K & Emerson S (2001) Hepatocyte growth factor is secreted by osteoblasts and cooperatively permits the survival of haematopoietic progenitors. *Br. J. Haematol.* 112(2):438-448.

Takada R, Satomi Y, Kurata T, Ueno N, Norioka S, Kondoh H, Takao T & Takada S (2006) Monounsaturated fatty acid modification of Wnt protein: its role in Wnt secretion. *Dev. Cell* 11(6):791-801.

Takizawa H, Fritsch K, Kovtonyuk LV, Saito Y, Yakkala C, Jacobs K, Ahuja AK, Lopes M, Hausmann A, Hardt WD, Gomariz A, Nombela-Arrieta C & Manz MG (2017) Pathogen-Induced TLR4-TRIF Innate Immune Signaling in Hematopoietic Stem Cells Promotes Proliferation but Reduces Competitive Fitness. *Cell Stem Cell* 21(2):225-240 e225.

Takizawa H, Saito Y, Kovtonyuk LV, Frisch K & Manz MG (2014) Direct Sensing of Lipopolysaccharide Limits Hematopoietic Stem Cell Selfrenewal Via TLR4-TRIF-ROS-p38 Pathway. *Blood* 124(21):604.

Tamura M, Hattori K, Nomura H, Oheda M, Kubota N, Imazeki I, Ono M, Ueyama Y, Nagata S, Shirafuji N & et al. (1987) Induction of neutrophilic granulocytosis in mice by administration of purified human native granulocyte colony-stimulating factor (G-CSF). *Biochem. Biophys. Res. Commun.* 142(2):454-460.

Tan SH, Senarath K, Chung MT, Longaker MT, Wu JY & Nusse R (2014) Wnts produced by Osterix-expressing osteolineage cells regulate their proliferation and differentiation. *Proceedings of the National Academy of Sciences* 111(49):E5262.

Tirosh O, Artan A, Aharoni-Simon M, Ramadori G & Madar Z (2010) Impaired liver glucose production in a murine model of steatosis and endotoxemia: protection by inducible nitric oxide synthase. *Antioxid. Redox Signal.* 13(1):13-26.

Tokuhara M, Hirai M, Atomi Y, Terada M & Katoh M (1998) Molecular cloning of human Frizzled-6. *Biochem. Biophys. Res. Commun.* 243(2):622-627.

Tomchuck SL, Zwezdaryk KJ, Coffelt SB, Waterman RS, Danka ES & Scandurro AB (2008) Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. *Stem Cells* 26(1):99-107.

Tran HT, Sekkali B, Van Imschoot G, Janssens S & Vleminckx K (2010) Wnt/beta-catenin signaling is involved in the induction and maintenance of primitive hematopoiesis in the vertebrate embryo. *Proc. Natl. Acad. Sci. U. S. A.* 107(37):16160-16165.

Trowbridge JJ, Xenocostas A, Moon RT & Bhatia M (2006) Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. *Nat. Med.* 12(1):89-98.

Valencia J, Hernandez-Lopez C, Martinez VG, Hidalgo L, Zapata AG, Vicente A, Varas A & Sacedon R (2011) Wnt5a skews dendritic cell differentiation to an unconventional phenotype with tolerogenic features. *J. Immunol.* 187(8):4129-4139.

Valtieri M & Sorrentino A (2008) The mesenchymal stromal cell contribution to homeostasis. *J. Cell. Physiol.* 217(2):296-300.

Van Den Berg DJ, Sharma AK, Bruno E & Hoffman R (1998) Role of members of the Wnt gene family in human hematopoiesis. *Blood* 92(9):3189-3202.

van der Sar AM, Spaink HP, Zakrzewska A, Bitter W & Meijer AH (2009) Specificity of the zebrafish host transcriptome response to acute and chronic mycobacterial infection and the role of innate and adaptive immune components. *Mol. Immunol.* 46(11-12):2317-2332.

Vaure C & Liu Y (2014) A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Front. Immunol.* 5:316-316.

Verfaillie CM, Catanzarro PM & Li WN (1994) Macrophage inflammatory protein 1 alpha, interleukin 3 and diffusible marrow stromal factors maintain human hematopoietic stem cells for at least eight weeks in vitro. *J. Exp. Med.* 179(2):643-649.

Vijayaragavan K, Szabo E, Bosse M, Ramos-Mejia V, Moon RT & Bhatia M (2009) Noncanonical Wnt signaling orchestrates early developmental events toward hematopoietic cell fate from human embryonic stem cells. *Cell Stem Cell* 4(3):248-262.

Villar J, Cabrera-Benitez NE, Ramos-Nuez A, Flores C, Garcia-Hernandez S, Valladares F, Lopez-Aguilar J, Blanch L & Slutsky AS (2014) Early activation of pro-fibrotic WNT5A in sepsis-induced acute lung injury. *Crit. Care* 18(5):568.

Villeval JL, Gearing A & Metcalf D (1990) Changes in hemopoietic and regulator levels in mice during fatal or nonfatal malarial infections. II. Nonerythroid populations. *Exp. Parasitol.* 71(4):375-385.

Walker F, Zhang HH, Matthews V, Weinstock J, Nice EC, Ernst M, Rose-John S & Burgess AW (2008) IL6/sIL6R complex contributes to emergency granulopoietic responses in G-CSF- and GM-CSF-deficient mice. *Blood* 111(8):3978-3985.

Wang Y, Chang H & Nathans J (2010) When whorls collide: the development of hair patterns in frizzled 6 mutant mice. *Development* 137(23):4091-4099.

Wang Y, Guo N & Nathans J (2006) The Role of Frizzled3 and Frizzle6 in Neural Tube Closure and in the Planar Polarity of Inner-Ear Sensory Hair Cells. *The Journal of Neuroscience* 26(8):2147.

Weber GF, Chousterman BG, He S, Fenn AM, Nairz M, Anzai A, Brenner T, Uhle F, Iwamoto Y, Robbins CS, Noiret L, Maier SL, Zonnchen T, Rahbari NN, Scholch S, Klotzsche-von Ameln A, Chavakis T, Weitz J, Hofer S, Weigand MA, Nahrendorf M, Weissleder R & Swirski FK (2015) Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis. *Science* 347(6227):1260-1265.

Willert K & Nusse R (2012) Wnt Proteins. *Cold Spring Harb. Perspect. Biol.* 4(9):a007864.

Wilson, Hansen CD, Azkur D, Kocabas CN, Metin A, Coskun Z, Schwartz ME, Hull PR, McLean WH & Smith FJ (2013) Recessive mutations in the gene encoding frizzled 6 cause twenty nail dystrophy-expanding the differential diagnosis for pachyonychia congenita. *J. Dermatol. Sci.* 70(1):58-60.

Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E, Lio P, Macdonald HR & Trumpp A (2008) Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* 135(6):1118-1129.

Winkler IG, Barbier V, Nowlan B, Jacobsen RN, Forristal CE, Patton JT, Magnani JL & Levesque JP (2012) Vascular niche E-selectin regulates hematopoietic stem cell dormancy, self renewal and chemoresistance. *Nat. Med.* 18(11):1651-1657.

Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, Poulton IJ, van Rooijen N, Alexander KA, Raggatt LJ & Lévesque J (2010) Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. *Blood* 116(23):4815.

Wright DE, Wagers AJ, Gulati AP, Johnson FL & Weissman IL (2001) Physiological migration of hematopoietic stem and progenitor cells. *Science* 294(5548):1933-1936.

Wu Q-L, Zierold C & Ranheim EA (2009) Dysregulation of Frizzled 6 is a critical component of B-cell leukemogenesis in a mouse model of chronic lymphocytic leukemia. *Blood* 113(13):3031.

Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, Ema H & Nakauchi H (2013) Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell* 154(5):1112-1126.

Yan J, Liu T, Zhou X, Dang Y, Yin C & Zhang G (2016) FZD6, targeted by miR-21, represses gastric cancer cell proliferation and migration via activating non-canonical wnt pathway. *American Journal of Translational Research* 8(5):2354-2364.

Yang YC, Ciarletta AB, Temple PA, Chung MP, Kovacic S, Witek-Giannotti JS, Leary AC, Kriz R, Donahue RE, Wong GG & et al. (1986) Human IL-3 (multi-CSF): identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. *Cell* 47(1):3-10.

Yokota T, Oritani K, Garrett KP, Kouro T, Nishida M, Takahashi I, Ichii M, Satoh Y, Kincade PW & Kanakura Y (2008) Soluble Frizzled-Related Protein 1 Is Estrogen Inducible in Bone Marrow Stromal Cells and Suppresses the Earliest Events in Lymphopoiesis. *The Journal of Immunology* 181(9):6061.

Yoshihara H, Arai F, Hosokawa K, Hagiwara T, Takubo K, Nakamura Y, Gomei Y, Iwasaki H, Matsuoka S, Miyamoto K, Miyazaki H, Takahashi T & Suda T (2007) Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell* 1(6):685-697.

Zhan & Cheers C (2000) Haemopoiesis in mice genetically lacking granulocyte-macrophage colony stimulating factor during chronic infection with Mycobacterium avium. *Immunol. Cell Biol.* 78(2):118-123.

Zhan, Lieschke GJ, Grail D, Dunn AR & Cheers C (1998) Essential roles for granulocyte-macrophage colony-stimulating factor (GM-CSF) and G-CSF in the sustained hematopoietic response of Listeria monocytogenes-infected mice. *Blood* 91(3):863-869.

Zhan T, Rindtorff N & Boutros M (2016) Wnt signaling in cancer. *Oncogene* 36:1461.

Zhang & Li L (2008) Stem Cell Niche: Microenvironment and Beyond. *J. Biol. Chem.* 283(15):9499-9503.

Zhang, Rodriguez S, Wang L, Wang S, Serezani H, Kapur R, Cardoso AA & Carlesso N (2016) Sepsis Induces Hematopoietic Stem Cell Exhaustion and Myelosuppression through Distinct Contributions of TRIF and MYD88. *Stem Cell Reports* 6(6):940-956.

Zhang, Zhang D, Tu C, Zhou P, Zheng Y, Peng Z, Feng Y, Xiao S & Li Z (2015) Wnt5a is involved in the pathogenesis of cutaneous lichen planus. *Clin. Exp. Dermatol.* 40(6):659-664.

Zhao, Blum J, Chen A, Kwon HY, Jung SH, Cook JM, Lagoo A & Reya T (2007) Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell* 12(6):528-541.

Zhao, Ma C, O'Connell RM, Mehta A, DiLoreto R, Heath JR & Baltimore D (2014a) Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. *Cell Stem Cell* 14(4):445-459.

Zhao, Perry JM, Marshall H, Venkatraman A, Qian P, He XC, Ahamed J & Li L (2014b) Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. *Nat. Med.* 20(11):1321-1326.

Zhu H, Kwak HJ, Liu P, Bajrami B, Xu Y, Park SY, Nombela-Arrieta C, Mondal S, Kambara H, Yu H, Chai L, Silberstein LE, Cheng T & Luo HR (2017) Reactive Oxygen Species-Producing Myeloid Cells Act as a Bone Marrow Niche for Sterile Inflammation-Induced Reactive Granulopoiesis. *J. Immunol.* 198(7):2854-2864.

ANNEX

Fwd: Stem Cell Reports Submission Confirmation

Heinonen, Krista

Sent: Thursday, December 13, 2018 8:46 AM

To: Nguyen, Hai Trieu

Krista Heinonen, Ph.D.

Professeur agrégée/

Associate professor

INRS-Institut Armand-Frappier

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Objet: Stem Cell Reports Submission Confirmation

Répondre à: Stem Cell Reports Editorial stemcellreports@isscr.org

Dear Dr. Heinonen,

Stem Cell Reports has received your submission. An email will be sent to you with a manuscript number once an editor has been assigned.

All the best,

The Stem Cell Reports Editorial Team

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