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Review

The aryl hydrocarbon receptor has a normal function in the regulation of hematopoietic and other stem/progenitor cell populations

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ABSTRACT

The aryl hydrocarbon receptor (AhR) is known mainly as the mediator for the toxicity of certain xenobiotics. However, there is also much information to indicate that this transcription factor has important biological functions. Here we review the evidence that the AhR has a significant role in the regulation of hematopoietic stem cells (HSCs). Data to support this come from studies with xenobiotic AhR ligands, phenotypic analyses of mice lacking AhR, examining the presence and regulation of the AhR within HSCs, knowledge of genes and signaling pathways regulated by the AhR, and investigations of hematopoietic disorders. Based on this information, we hypothesize that AhR expression is necessary for the proper maintenance of quiescence in HSCs, and that AhR down-regulation is essential for “escape” from quiescence and subsequent proliferation of these cells. This implicates the AhR as a negative regulator of hematopoiesis with a function of curbing excessive or unnecessary proliferation. This provides an important advantage by preventing the premature exhaustion of HSCs and sensitivity to genetic alterations, thus preserving HSC function and long-term multi-lineage generation over the lifespan of the organism. This also implicates a role of the AhR in aging processes. AhR dysregulation may result in the altered ability of HSCs to sense appropriate signals in the bone marrow microenvironment leading to hematopoietic disease. It is also reasonable to hypothesize that this protein has an important function in the regulation of other tissue stem cell populations. Suggestive evidence is consistent with a role in skin and neural stem cells.

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Abbreviations: 5-FU, 5-fluorouracil; AhR, aryl hydrocarbon receptor; AhRE, aryl hydrocarbon receptor response element; BrdU, bromodeoxyuridine; CFU-G, colony-forming unit granulocyte; CFU-GM, CFU-granulocyte/megakaryocyte; CFU-M, CFU-macrophage; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GNP, granule neuron progenitor; HPCs, hematopoietic progenitor cells; HPP-CFC, high proliferative potential-colony forming cells; HSCs, hematopoietic stem cells; LSK, HSC-enriched lineage-negative, cKit-positive, Sca-1-positive cells; LT-HSC, long-term repopulating HSCs; MPP, multipotent progenitors; NSC, neuroepithelial stem cell; ST-HSCs, short-term repopulating HSCs; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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Contents

1. Introduction	578
2. HSCs as models of stem cell biology	578
3. Evidence that the AhR has a role in HSC regulation	579
3.1. Xenobiotic ligands affect HSC numbers and function	579
3.2. Characteristics of HSCs from AhR-KO mice	581
3.3. Presence and regulation of AhR in HSCs	581
3.4. Known molecular targets of the AhR signaling pathway support a role in HSC regulation	581
3.5. Evidence suggests a relationship between AhR dysregulation and particular hematopoietic disorders	582
4. Hypothesis and implications	582
5. Possible role of the AhR in other stem cell populations	583
5.1. A possible role of the AhR in skin stem cells	583
5.2. Evidence for an important function of the AhR in neuronal development	584
6. Summary: significance and prospects for future work	584
Acknowledgements	584
References	584

1. Introduction

For the past 30 years, the aryl hydrocarbon receptor (AhR) has been largely known for its ability to mediate the toxicity of a wide variety of environmental pollutants including the dioxins and certain polychlorinated biphenyls. That this occurs through the ability of a ligand-activated AhR to bind to specific DNA enhancer sequences, known as AhR responsive elements (AhREs; also called dioxin responsive elements (DREs) or xenobiotic responsive elements (XREs)), to regulate a diverse set of genes has attracted the interest of biological scientists to study its mechanisms, function, and possible role in human disease. Despite much research, there are many critical questions that remain unresolved. Although the toxic and biological effects of dioxins in both animals and humans have been well characterized [1], and many biochemical effects and AhR-responsive genes have been identified [2], direct relationships between gene activation and functional consequences in specific cells and tissues have yet to be defined. The search for *bona fide* endogenous ligands that regulate AhR transcriptional activity under physiological conditions has had limited success, although several indoles, as well as related tryptophan derivatives and photoproducts, and leukotriene metabolites, have been shown to activate AhR-mediated transcription [3,4]. Finally, data showing that the AhR is conserved throughout evolution [5], and that mice lacking the AhR exhibit abnormal phenotypes [6], all strongly suggest that this protein has some important physiological function in the development and maintenance of mammalian tissues. Nevertheless, this function remains elusive.

Immune system toxicity and dysfunction are some of the most consistent features observed in all animal species following exposure to dioxins and related chemicals [7]. Mice lacking AhR or possessing a constitutively active AhR also show abnormalities in immune system development and function [8–11]. Together these data are consistent with the AhR having a significant role in the regulation of the immune system, possibly at multiple levels. Here, we review the evidence indicating that the AhR is a critical component in the regulation of hematopoietic stem (HSCs) and/or progenitor

(HPCs) cells. In addition, we offer a testable hypothesis that the AhR is important for regulating the balance between HSC quiescence and proliferation through the ability to modulate critical genes that are important for these cells to sense signals in the bone marrow microenvironment. Given a possible role of the AhR in HSCs, we also consider available data suggesting that the AhR has similar functions in other tissue stem cell populations.

2. HSCs as models of stem cell biology

A common and defining characteristic of stem cells is the ability to supply tissues with progenitors that differentiate into mature lineages while maintaining pools, through self-renewal, to satisfy the demands during the lifetime of the organism. While there are intrinsic controls over these processes, both differentiation and self-renewal are also greatly influenced by the microenvironment in which those cells exist.

Stem cells responsible for hematopoiesis in the murine bone marrow were first described more than 40 years ago. As a result, there is a great amount of information regarding HSCs and the process of blood and immune system cell formation. Today, it is possible to distinguish and isolate different populations within the bone marrow using phenotypic characterization based on flow cytometry and functional assays. The different assays developed to retrospectively study HSCs make them an excellent model to study stem cell biology [12]. The functionality of HSCs is determined by their ability to repopulate cell lineages following their injection into lethally irradiated animals, or to sustain repopulation of recipients after repetitive serial bone marrow transplantation. Since experimental isolation causes the HSCs to lose many of their properties, in most cases, only the progeny can be analyzed [13].

Through the process of multi-lineage differentiation, HSCs develop into progenitor cells, lineage committed cells and all the mature phenotypes of blood and immune tissues. It has been estimated that HSCs represent about 1 in 30,000 cells in

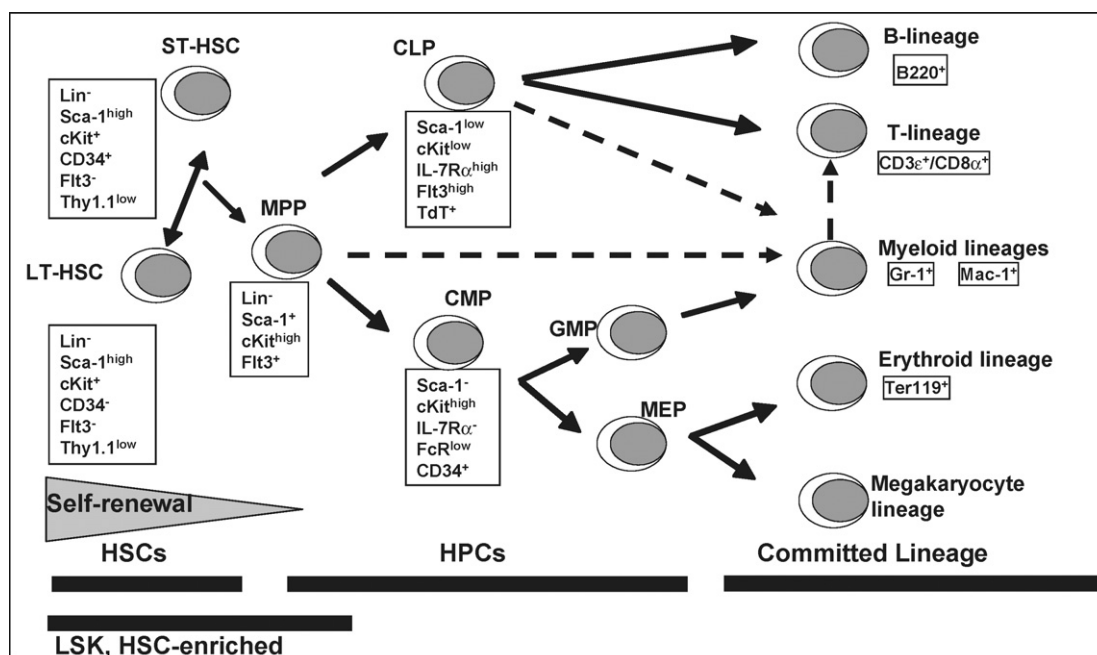


Fig. 1 – Simplified classic schematic of hematopoiesis indicating the cell surface markers expressed at different stages of differentiation. Broken lines indicate the recently proposed revision to this classical model where a myeloid based model of hematopoiesis has been suggested [97].

bone marrow [14]. The Lineage negative, stem-cell antigen 1 (Sca-1) positive and c-kit (CD117) positive (LSK) population is a phenotype enriched for HSC in mice (Fig. 1). LSK cells lack the expression of surface proteins characteristic of lineage-committed cells (i.e. CD45R/B220, CD3 ϵ , Gr-1, Ter-119, Mac-1/CD11b). Realizing that our understanding of the relationships between the expression of cell-surface markers and actual cell functions are likely oversimplified [12], the LSK population can be further phenotypically and functionally defined into both long-term (LT-HSC) and short-term (ST-HSC) repopulating HSCs as well as multipotent progenitor (MPP) populations that also have differential (decreasing) ability for self-renewal (Fig. 1).

Self-renewal and differentiation of HSCs are driven intrinsically by changes in gene expression AND extrinsically by molecules, e.g. cytokines and growth factors, present within the microenvironment. Stem cells are contained within physical and chemical environments known as microenvironments or niches that have been extensively described in the small intestine, the hair follicle, and the bone marrow for HSCs [15]. Depending on the stage of development of the organism, the location of hematopoietic niches may vary. In adult mammals, the preferential site of hematopoiesis is the bone marrow. Available data suggest the existence of both osteoblastic [16] and vascular [17] niches for HSCs. The osteoblastic niche is located at the endosteum of the bone marrow where the bone forming osteoblasts and/or other components of the microenvironment and HSCs are in close physical contact. However, the interactions of HSCs with the osteoblast niche do not appear to account for processes necessary for the mobilization and migration of HSC from and to the circulation and others sites of hematopoiesis such as

liver and spleen. It has been shown that mobilized HSCs are associated with sinusoidal endothelium in the red pulp of the spleen as well as with the endosteum of bone [17]. Future research is needed to determine if there is a physiologically driven compartmentalization or if both niches are in such a close physical proximity that they act as a functional unit to support hematopoiesis.

3. Evidence that the AhR has a role in HSC regulation

Several different types of data support the postulate that the AhR has an important role in HSC regulation and function. These include results from studies with xenobiotic AhR ligands, phenotypic analyses of mice lacking AhR, examining the presence and regulation of the AhR within HSCs, knowledge of genes directly regulated by the AhR in conjunction with those known to regulate HSC function, and investigations of hematopoietic disorders.

3.1. Xenobiotic ligands affect HSC numbers and function

While examining the mechanism of TCDD-induced thymic atrophy, we previously observed that thymic seeding by progenitors isolated from bone marrow of TCDD-treated mice was substantially reduced. This was consistent with a significant reduction in mRNA for lymphoid-specific terminal deoxynucleotidyl transferase (TdT) and recombinase-activating gene 1 (RAG-1) in these cells [18,19]. That the effect was not specific for just the T-lymphoid precursor population was suggested by studies showing that numbers of cells in the

immature B cell compartment also decreased following TCDD exposure [20]. However, the further differentiation of committed, but immature, B cells to more mature cells was not affected by the direct exposure of these cells to TCDD under culture conditions [21]. These studies suggested that either more immature progenitors or stem cells were directly affected by TCDD or that TCDD was targeting cell populations in the microenvironment *in vivo* that are, in part, responsible for directing B cell differentiation and which may not be active or present under culture conditions. To address the latter issue, wild-type and AhR null-allele (KO) mice were used to produce radiation chimeric animals in which AhR was present or absent in hematopoietic or non-hematopoietic cell populations. These studies demonstrated that AhR presence in hematopoietic cells and NOT cells in the supporting bone marrow microenvironment is necessary for these effects to occur [22,23], suggesting that TCDD was acting directly on AhR present in HSCs and/or immature progenitor populations. Notably, a persistent several-fold increase in the number of HSC-enriched LSK cells was also observed following TCDD treatment [24], AND that this was also dependent on AhR presence in hematopoietic cells [22]. Further analysis indicated that TCDD treatment predominantly affected numbers of phenotypically defined LT- and ST-HSCs but not MPPs, CLPs or CMPs contained within the LSK subset [25].

The studies summarized above suggested that an effect of TCDD on HSCs and/or very immature progenitor cells might be responsible for the observed effect on the lymphoid populations. A more detailed analysis of other lineage-committed cells and functional lineage-restricted progenitors suggested

that *in vivo* TCDD-treatment causes a skewing of differentiation away from the lymphoid lineage in favor of granulocyte/macrophage myeloid lineage cells [25]. This was consistent with the finding that the number of functional CFU-preB progenitors was decreased following TCDD treatment [25]. A previous report indicated that cultured mobilized human progenitor CD34⁺ cells have a skewing towards myeloid cells in response to the polyaromatic hydrocarbon benzo(a)pyrene, another AhR ligand [26]. However, treatment of these cells with TCDD failed to produce this same response, suggesting that the effects were due to metabolites of benzo(a)pyrene and not solely due to AhR activation.

When the overall function of HSCs was evaluated in a competitive repopulation assay (Fig. 2), TCDD treatment to donor mice led to a substantially decreased short-term (6 wk) and long-term (40 wk) reconstitution activity of either LSK or LSK/CD34⁺ cells [25]. These data were consistent with a previous report observing an almost total loss in the ability of LSK or LSK/CD34⁺ cells from TCDD-treated mice to reconstitute white cells in peripheral blood of irradiated recipient mice [27]. They are also consistent with a recent finding from our lab that marrow taken from TCDD-treated mice exhibited a time-dependent decrease in HPP-CFC (unpublished observations). HPP-CFCs are among the most immature hematopoietic progenitors grown under culture conditions and their assessment has been used as a short-term assay of stem cell functional potential.

Together these data strongly suggest that TCDD may inhibit HSC functions through an ability to alter genes critical to the homing and/or trafficking of HSCs within the bone marrow microenvironment. Notably, it was reported that

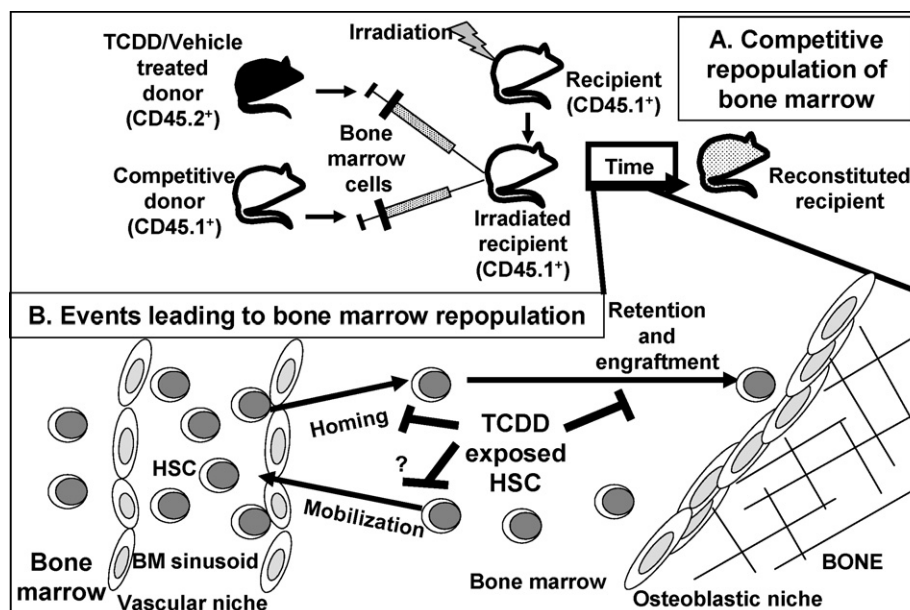


Fig. 2 – TCDD inhibits steps leading to repopulation of the bone marrow (BM) after transplantation. (A) Competitive repopulation of recipients evaluates the ability of HSCs from TCDD- or vehicle-treated CD45.2⁺ donor mice to reconstitute the BM of irradiated recipient CD45.1⁺ animals in the presence of competitive donor (CD45.1⁺) HSCs. The percentages and phenotypic composition of CD45.2⁺ cells in the reconstituted recipient are analyzed. **(B)** TCDD treatment alters HSCs to prevent successful repopulation of the BM. Repopulation requires HSC to migrate from the circulation through endothelial cells of the BM sinusoids into the BM. Once in the BM, HSC must be retained and engrafted or they will be mobilized back into circulation to find other niches to populate.

TCDD exposure down-regulates the expression of mRNAs for the G-protein-coupled receptor CXCR4 and its chemokine ligand CXCL12 (SDF-1), in MCF-7 breast cancer cells [28]. These molecules are critically important for HSC homing and movement within the marrow niche [29]. Since these molecules are also necessary for lymphoid cell development [30], we further postulate that TCDD-elicited alterations in this pathway may be responsible for the observed effects on B-cell development and decreased thymic seeding. Lacking sufficient signals for differentiation towards the lymphoid lineage, increased numbers of progenitors might then proceed along a default myeloid lineage [12,31], as we observed in response to TCDD. The possible significance of these pathways in HSCs exposed to TCDD still needs to be examined and verified. It should be pointed out that the whole-animal dosages (10–30 µg TCDD/kg) used in these investigations are somewhat higher than those observed to cause significant effects on other endpoints in the immune system [7]. However, at these dosages the bone marrow cell concentrations of TCDD are much less than 1 nM [19], indicating the high sensitivity of these cells to this chemical. Nevertheless, all of the above information is consistent with the hypothesis that dysregulation of the AhR by TCDD results in the inappropriate regulation of critical genes in HSCs that impairs their detection of signals within the marrow microenvironment to result in altered HSC function.

3.2. Characteristics of HSCs from AhR-KO mice

If TCDD exposure of HSCs results in altered HSC characteristics and function, one might suspect that lack of AhR would also affect HSCs. Young adult AhR-KO mice have increased numbers of LSK and LSK/CD34⁺ cells in bone marrow compared to either wild-type or heterozygote animals (unpublished observations). Consistent with this, but opposite to that observed in wild-type mice treated with TCDD [25], analysis of bone marrow from KO mice demonstrated increased numbers of HPP-CFCs (unpublished observations). LSK cells from young adult KO mice also have very high rates of cell division, determined by the *in vivo* incorporation of BrdU, compared to cells from wild-type animals. Surprisingly, the BrdU incorporation was found to be nearly identical to cells from wild-type animals treated with 5-FU that kills very rapidly dividing cells and stimulates HSCs into division. Treatment of KO mice with 5-FU did not further increase BrdU incorporation into LSK cells, suggesting that these cells are already dividing at a maximal rate as if under increased stimulatory conditions. Consistent with this, we observed a greater percentage of KO LSK cells to be in G₁/S cell cycle phases compared to that from wild-type or heterozygote animals (unpublished observations). That progenitor cells from KO mice also have increased growth rates in culture compared to cells from wild-type mice (unpublished observations), supported the notion that high proliferation rates of KO cells *in vivo* is a property inherent to these cells and not due to a possible increased level of stimulatory signals present *in vivo*. Together these data further support a hypothesis that the AhR has a normal function in the regulation of HSCs, and more specifically as a regulator of the balance between quiescence and proliferation.

3.3. Presence and regulation of AhR in HSCs

Data from our lab and from other groups have shown the presence of both AhR protein and mRNA in phenotypically defined HSCs [25,26,32]. Furthermore, TCDD treatment alters gene expression in hematopoietic precursors [32,33]. These data indicate the presence of a functional AhR protein and associated machinery necessary for an active AhR signaling pathway in HSCs. Of particular interest, however, are other data suggesting that the *Ahr* gene is actively regulated during different cell cycle phases in HSCs; cells actively cycling or stimulated to cycle following 5-FU treatment to animals have significantly less *Ahr* mRNA than HSCs in quiescence (G₀) [34,35]. In addition, we have shown that treatment of mice with growth factors and cytokines (IL-6 + G-CSF or IL-11 + G-CSF) known to stimulate hematopoiesis resulted in a nearly 60% loss of *Ahr* mRNA expression in hematopoietic progenitors [25]. Data indicating that the *Ahr* is regulated in HSCs with cell cycle phases is also consistent with a number of studies suggesting that the AhR has a normal functional role in cell cycling [36].

That *Ahr* expression in HSCs may be regulated under conditions of quiescence/proliferation has several implications. HSC susceptibility to the actions of xenobiotic or endogenous ligands may depend on the marrow environment. Conditions in which HSCs are stimulated to proliferate may actually protect these cells from toxic AhR ligands. This also might be a protective mechanism against chemicals that can be metabolized to mutagenic intermediates by the cytochrome P450 isozymes regulated in part by the AhR. To test the former possibility, mice were exposed to 5-FU 2 days prior to treatment with a dose of TCDD previously found to produce significant effects on bone marrow cell populations. Under these conditions and up to 16 days after TCDD treatment, absolutely no effects of TCDD on bone marrow were observed compared to the vehicle-treated group also treated with 5-FU [25]. Thus, it appears that the down-regulation of *Ahr* in HSCs occurring following treatment with 5-FU renders these cells less susceptible to the effects of xenobiotic ligands such as TCDD.

3.4. Known molecular targets of the AhR signaling pathway support a role in HSC regulation

Under normal homeostatic conditions, HSCs exhibit a low metabolic rate as indicated by a high percentage (70–85%) of cells in quiescence (G₀) [37]. As such, the entrance into and exit from cell cycle critically regulates HSC quiescence, proliferation and self-renewal. For example, in the absence of the G₁ checkpoint regulator CDK inhibitor p21^{cip1/waf1}, both the proliferation and numbers of HSCs are increased. However, self-renewal of HSCs deficient in p21 is blocked following transplantation, leading to hematopoietic failure [38]. In addition to regulating the expression of p21 and the CDK2 inhibitor p27^{kip1} [2,39,40], the AhR interacts with the tumor suppressor protein pRb to modulate its function as a G₁ checkpoint regulator [36]. Other intrinsic genes and pathways known to regulate HSC characteristics and function include *c-Myc*, *C/EBP*, *HES1*, among others [41,42]. *HES1* and *c-Myc* appear to be directly regulated by the AhR [2,43,44], and *C/EBP*

expression is altered by AhR agonists in several cell types [45]. Finally, as indicated above, it is becoming appreciated that microenvironment-dependent signaling by soluble cytokines and contact with stromal cells in the bone marrow niche are both critical for maintaining the balance among HSC quiescence, division, differentiation and trafficking to other environments. In addition to altering the expression of CXCR4 and CXCL12 [28], AhR agonists are known to modulate the expression of a number of cell surface proteins and adhesion molecules [46,47] that are also important for the ability of HSCs to sense and respond to their environment. Clearly, it remains to be determined whether the AhR critically regulates these or other pathways that play a role HSC function. Nevertheless, these findings further implicate an important role of the AhR in HSC regulation.

3.5. Evidence suggests a relationship between AhR dysregulation and particular hematopoietic disorders

Increased incidence of leukemia and lymphoma has been reported in humans accidentally exposed to TCDD [48–52]. AhR ligands are major components of tobacco smoke [53], and smoking during pregnancy increases the risk of certain cancers including leukemia in the offspring [54,55]. A recent study found that the AhR promoter is silenced by hypermethylation in human acute lymphoblastic leukemia cells [56]. Notably, the authors postulated that the AhR could be a cell-specific negative regulator of cell proliferation. AhR activation results in resistance to apoptosis in human lymphoma cell lines, and the pathogenesis of AhR-mediated lymphoma in animal models is associated with induced COX-2 by AhR ligands [57]. Finally, the AhR signaling pathway has been implicated in animal models of benzene-induced leukemia [58]. Together these studies suggest that AhR dysregulation may play an extremely important role in the etiology and/or progression of certain hematopoietic diseases.

4. Hypothesis and implications

Together the available data are consistent with a compelling argument for a function of the AhR in HSC regulation. More specifically, the data suggest that the AhR has an important role in HSCs for regulating the balance between quiescence and proliferation. Consistent with this postulate are data indicating a role of the AhR in cell cycle [36], demonstrating hyperproliferation of HSCs from AhR-KO mice (Section 3.2), and showing differential regulation of AhR expression in HSCs during cycles of quiescence and proliferation (Section 3.3). These data would further suggest that AhR expression is necessary for the proper maintenance of quiescence in HSCs, and that AhR down-regulation allows HSCs to “escape” from quiescence and subsequently proliferate. This implicates the AhR acting specifically as a negative regulator of hematopoiesis with a function of curbing excessive or unnecessary proliferation of HSCs. Clearly, more work is needed to define these relationships. Furthermore, the specific genes and signaling pathways controlled by the AhR that may regulate HSC quiescence/proliferation need to be identified, although as indicated in Section 3.4 there are several “likely suspects”. Notably, both

CXCR4 and CXCL12 genes contain putative AhREs in the upstream promoter regions [28], and this signaling pathway is important in the maintenance of the quiescent HSC pool [59]. In addition, the AhR has been shown to regulate the expression of c-myc [44,60], and c-myc regulates the balance between HSC self-renewal and differentiation through the expression of adhesion molecules [61]. Ultimately, it appears likely that AhR may regulate critical genes within HSCs that allows them to differentially respond to signals in their microenvironment. This is consistent with a hypothesis for an overall function of proteins like the AhR within the PAS superfamily as “sensors of environmental and developmental signals” [62]. In this case, the presence and functional activity of the AhR may be to provide an important advantage to organisms by preventing the premature exhaustion of HSCs and sensitivity to genetic alterations, thus preserving HSC function and long-term multi-lineage generation.

There are several important implications for a primary role of the AhR as a negative regulator of HSC proliferation. This may provide a plausible mechanism for reported relationships between dysregulation of AhR function and the etiology and/or progression of certain hematopoietic diseases such as leukemia (see Section 3.5). In the case of inadvertent exposure to xenobiotics such as the dioxins, the inappropriate and persistent AhR activation may result in HSCs dysfunction, as seen in mice exposed to TCDD, by altering the ability of HSCs to traffic and sense the appropriate signals within the bone marrow microenvironment (see Fig. 3). This may provide a permissive environment for altered hematopoietic development and the selection and expansion of tumor cell clones. Loss of AhR function, through an epigenetic control of AhR expression by, for example, hypermethylation of the AhR gene [56], could have the same net effect through uncontrolled cell growth and proliferation (Fig. 3).

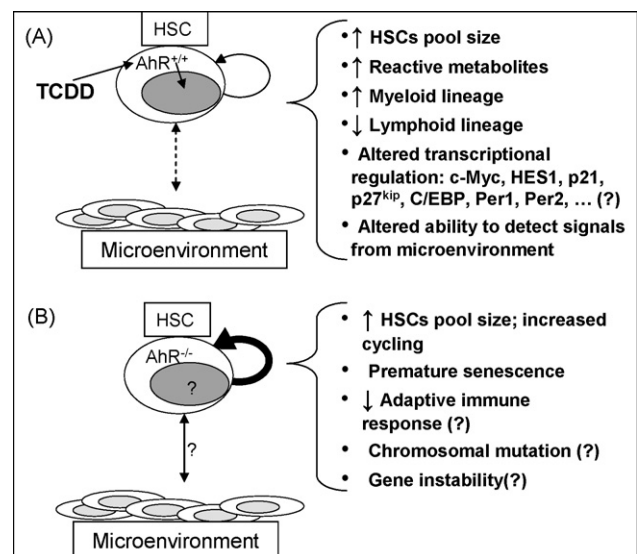


Fig. 3 – Simplified model of AhR regulation of HSCs. (A) The AhR-ligand TCDD binds to AhR which translocates to the nucleus and mediates genetic/epigenetic regulation, HSC-niche interactions, and alterations of different end-points. (B) In AhR^{-/-} mice, the HSCs are constitutively in a higher cycling status that corresponds with a larger HSC pool size.

Notably, there is much evidence of a causal relationship between low to moderate cycling rates of HSCs and hematopoietic longevity [63,64]. Likewise, since adult stem cells are incapable of sustaining an indefinite line of generations, there is a general expansion of the HSC pool from youth to maturity, but this declines in old age. Many investigations have indicated genetic regulation of stem cell exhaustion in mice and a role of this exhaustion in the aging process [64–67]. In fact, there is an excellent negative correlation between the mean lifespan of various mouse strains and the replication rates of HSCs within these strains [64,68]. This is consistent with Hayflick's proposal for a mitotic clock in which cells have an upper limit to replication, and once this is reached, proliferation decreases and the cells ultimately senesce [69]. Thus, a higher proliferation rate of stem cells throughout the lifetime of an organism decreases the time to reach this limit. However, this may also increase the rate at which potential fatal DNA damage accumulates. Not surprisingly then, there is also evidence to indicate that the increased proportion of actively cycling stem cells correlates with increased incidence of leukemia, lymphoma, and myelodysplastic syndrome in old age [66,67]. Importantly, a grouping of mouse strains correlated with differences in longevity [64] also corresponds to different *Ahr* polymorphisms present in these animals. For example, the C57Bl/6 and 129/Sv strains are characterized by low HSC cycling rates and long lifespan, and also possess a high affinity *Ahr* (i.e. high affinity for TCDD). Other strains, DBA/2 and AKR, that are characterized by high HSC cycling rates and short lifespan possess a low affinity *Ahr* form. The association between the *Ahr* and longevity was actually made years earlier when it was found that there was a correlation between *Ahr* expression levels and responsiveness with lifespan [70,71]. Whether these relationships are causal remains to be determined.

These relationships would also suggest that, due to a lifetime of high cycling rates, the HSCs in aging *Ahr*-KO mice might undergo premature senescence. Notably, some lesions appear only with aging in the *Ahr*-KO mice, and there has been a report of decreased lifespan in these animals [9]. In preliminary investigations, we have noted the following characteristics in aging *Ahr*-KO animals: (1) Compared with young adult KO mice or old (10 month) wild-type C57Bl/6 mice (the parental strain), old KO mice have approximately 50 percent fewer LSK cells. (2) An analysis of bone marrow from 1-year old KO mice showed significantly increased BFU-E, CFU-E, CFU-G, and CFU-M, but a profound decrease in CFU-preB progenitors. There is also splenic myeloid hyperplasia in aging KO mice [9]. It is known that as bone marrow senescence occurs in mice, there is a skewing of lineages towards myeloid at the expense of lymphoid populations [63,64,66,67,72]. (3) HSCs from old KO mice have decreased proliferation in culture, as determined by cell number and incorporation of ³H-thymidine, compared to cells from young adult KO animals or cells from old age-matched wild-type mice. All of these data are consistent with premature senescence occurring in the HSC/progenitor compartment during aging in KO mice, and further suggest a role of the *Ahr* in the process of aging.

Data showing that HSCs from *Ahr*-KO mice are hyperproliferative *in vivo* and *ex vivo* suggest that the *Ahr* signaling pathway is important in the regulation of quiescence/proliferation in these cells since compensatory changes in

KO-HSCs have not occurred to correct for *Ahr* loss. In this sense, it will be important to determine what actually controls *Ahr* gene expression in HSCs, especially during loss of quiescence. The promoter region of the murine *Ahr* contains several GC and Sp1 sites. Other transcription factor sites include those for HNFs, DLX3, NKX3, PIT1, LHX3, STAT6, BRN3, TCF/Lef, AP-1, and E-box for a c-myc binding site, a CAGA-box that may be regulated by TGF β , CRE, and potential binding sites for several hormone receptors [2,73,74]. Cytokines that operate through STAT-binding sites and those regulated by c-myc may be good candidates for factors regulating *Ahr* expression in bone marrow.

5. Possible role of the *Ahr* in other stem cell populations

Given a likely role of the *Ahr* in regulating HSCs, it is not unreasonable to hypothesize that this transcription factor may also have an important function in the regulation of other tissue stem cell populations. To date, however, data supporting or refuting such a hypothesis are very limited, simply because few studies have examined for a function of the *Ahr* in any stem cells. There have been numerous studies examining the effects of TCDD on the differentiation of a variety of precursor cells (e.g. [75–78]), and in most cases TCDD exposure affects differentiation and/or proliferation in an *Ahr*-dependent manner. However, the type of effect observed is often very cell type-specific. For example, in some cases the *Ahr* appears to act as a negative regulator of proliferation, while in other cases it affects proliferation in a positive manner [79,80]. This likely is dependent on the cellular context as well as the microenvironment in which these cells exist. TCDD has been shown to alter gene expression and characteristics of a number of stem or “stem-like” cells, including embryonic stem cells, liver stem cells, and human mesenchymal stem cells when exposure occurs under conditions in culture [81–84]. Although, these data suggest that the *Ahr* is functional in these cells and that they may be sensitive targets for dysregulation by xenobiotic *Ahr* ligands, it is difficult to know whether they might respond differently or at all under conditions *in vivo* within the particular microenvironment in which they are functional.

5.1. A possible role of the *Ahr* in skin stem cells

A recent publication proposed that TCDD-elicited activation of the *Ahr* in skin stem cells and a shift in differentiation of their progeny is the mechanism for the ability of this chemical to produce chloracne in both humans and animals [85]. They noted that the pathogenesis of the chloracne indicated alterations in several skin stem cell characteristics including (1) stimulation of stem cell into cycling, (2) increased stem cell self-renewal, and (3) a skewing of differentiation towards the epidermal pathway at the expense of hair follicle and sebaceous gland cells. Some of these features are similar to what appears to occur in HSCs in response to TCDD. The authors also noted some, but not a complete, similarity between the skin phenotype induced by TCDD and that in transgenic mice overexpressing c-Myc [86]. Based on this, they

further postulated a link between the c-myc and AhR signaling pathways in the regulation of skin stem cells.

5.2. Evidence for an important function of the AhR in neuronal development

There are many parallels between the regulation of neuroepithelial stem cells (NSCs) and HSCs during the processes of neurogenesis and hematopoiesis [87]. For example, the NSCs have the capacity to self-renew, and also are multipotent as they give rise to the three major cell types in the central nervous system: neurons, astrocytes, and oligodendrocytes [88]. The generation of differentiated cells also appears to be dependent on the intermediated differentiation of lineage restricted progenitor cells. As with HSCs, NSC expansion and differentiation is governed by various transcription factors that regulate the expression of genes associated with the generation of neurons and glia. Recent studies have reported that there are common genes expressed in both HSCs and NSCs, suggesting that there may be a conserved group of molecules that govern stem cell behavior [89,90].

Previous reports have revealed that both *Ahr* and *Arnt* genes are expressed in the embryonic neuroepithelium [91,92]. We recently confirmed that NSC isolated from the developing forebrain expresses robust levels of AhR protein (unpublished observations). Moreover, granule neuron progenitors (GNPs) express transcriptionally active AhR during a critical period of neurogenesis [93]. Both *in vivo* and *in vitro* data support the contention that TCDD disrupts the balance between proliferation, differentiation, and apoptosis in GNPs during the early postnatal period, and that this ultimately results in diminished cell numbers in the cerebellum [93,94]. Other studies in *C. elegans* and *Drosophila* suggest that the AhR participates in normal nervous system development [95,96]. These observations suggest an ancestral role for the AhR family. Since the AhR is present and coordinately regulated with *Arnt* in the embryonic mouse neuroepithelium during prenatal neurogenesis [91,92], it is conceivable that AhR mediates similar developmental events in mammalian systems.

Studies in the AhR-KO mice are beginning to provide information regarding potential roles for the AhR in brain development [93]. Initial observations in KO mice indicate that cell number is diminished in the developing and adult cerebellum [94]. Moreover, GABAA α 6 receptor expression, which is normally restricted to mature granule neurons, was markedly reduced [94], suggesting that AhR is important for development and/or maintenance of this neuronal population. More recently, we determined that NSC cell birth in the adult hippocampus was reduced by approximately 60% in KO mice (unpublished observations). Together, these studies are consistent with the argument that AhR participates in regulating neurogenesis through an important role in NSC and neuronal precursor cell regulation.

6. Summary: significance and prospects for future work

Further defining a precise role of the AhR in HSCs or other tissue stem cells may lead to the identification of previously

undefined functions of this transcription factor in particular human diseases. This could have important implications for the diagnosis and treatment of these diseases. Genetically or epigenetically defined AhR dysregulation may have a certain pattern of disease progression that may respond better to a focused treatment strategy. Given that the AhR is ligand activated also offers the opportunity to develop selective and therapeutic AhR modulators. Some of these might be useful for the maintenance of stem cells and their subsequent use in tissue regeneration, for example in the treatment of degenerative diseases. The exposure of human stem cells to a variety of AhR xenobiotic ligands, e.g. dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and ligands in tobacco smoke, may also contribute to dysfunction of the AhR, and either directly or in conjunction with some other permissive condition, lead to abnormal development and disease. The implication that tissue stem cells may be targets for xenobiotic AhR ligands is itself a novel hypothesis that needs to be further assessed considering that (1) developing tissues, in particular, are extremely sensitive to these chemicals, (2) the precise cellular targets have not been clearly identified, and (3) AhR activation may result in increased levels of metabolizing enzymes in these stem cells (e.g. [26]) and subsequent bioactivation of chemical carcinogens or other toxic chemicals. Finally, it should be emphasized again that the normal physiological function of the AhR is unknown and *bona fide* endogenous ligands have not been identified. Research on a possible role in stem cells will undoubtedly break new ground on the regulation of stem cells and, in particular, a role of the AhR in these cells.

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