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### Mini review

# The aryl hydrocarbon receptor has an important role in the regulation of hematopoiesis: Implications for benzene-induced hematopoietic toxicity

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### ABSTRACT

The aryl hydrocarbon receptor (AhR) belongs to the basic helix–loop–helix (bHLH) Per-Arnt-Sim (PAS) family of transcription factors. Many of these proteins are involved in regulating responses to signals in the tissue environment such as hypoxia, oxidation–reduction status, and circadian rhythms. Although the AhR is well studied as a mediator of the toxicity of certain xenobiotics, the normal physiological function remains unknown. However, accumulating data support a hypothesis that the AhR has an important function in the regulation of hematopoietic stem cells (HSCs). Persistent AhR activation by dioxin, a potent xenobiotic AhR agonist, results in altered numbers and function of HSCs in mouse bone marrow. Analysis of HSCs from AhR null-allele mice also indicates that lack of AhR expression results in altered characteristics and function of these cells. HSCs from these animals are hyperproliferative and have altered cell cycle. In addition, aging AhR-KO mice show characteristics consistent with premature bone marrow senescence and are prone to hematopoietic disease. Finally, some data suggest that the expression of the *Ahr* gene is regulated under conditions that control HSC proliferation. The presence of a normal functioning AhR may provide an important advantage to organisms by regulating the balance between quiescence and proliferation and preventing the premature exhaustion of HSCs and sensitivity to genetic alterations. This function assists in the preservation of HSC function and long-term multi-lineage generation over the lifespan of the organism. This also implicates a role for the AhR in the aging process. Furthermore, these functions may affect the sensitivity of HSCs to certain xenobiotics, including benzene. Defining the molecular mechanisms by which these events occur may lead to the identification of previously undefined roles of this transcription factor in human diseases, particularly those caused or affected by xenobiotics.

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**Abbreviations:** AhR, aryl hydrocarbon receptor; AhR-KO, AhR-null-allele; HPP-CFC, high proliferative potential colony-forming cells; HSCs, hematopoietic stem cells; LSK HSCs, Lin<sup>−</sup>Sca-1<sup>+</sup>c-kit<sup>+</sup> HSCs; PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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### 1. Introduction

The hematotoxic effects of benzene in both exposed humans and experimental animals are well known [1,2]. Most of the investigations on the mechanisms by which benzene may produce these effects have focused on the metabolism of benzene and the ability

of these metabolites to bind to DNA and protein [3,4], and/or initiate redox cycling and produce reactive oxygen species (ROS) [5]. More recent data support the hypothesis that benzene-induced hematotoxicity results from the generation of ROS by benzene metabolites, particularly those conjugated with glutathione [6], and subsequent oxidative stress.

Several years ago it was noted that *Ahr* null-allele (KO) mice failed to show hematotoxicity following exposure to benzene [7]. This same group also reported that benzene failed to induce hematotoxic effects in lethally-irradiated wild-type mice that had been transplanted with bone marrow cells from *Ahr*-KO animals [8]. These data are especially relevant given the ability of this ligand-activated transcription factor to regulate the expression of a number of enzymes responsible for the bioactivation of certain xenobiotics that can generate electrophiles and redox cycling [9]. One of the cytochrome P450 (CYP) enzymes, CYP2E1, has been shown to metabolize benzene to a number of reactive metabolites including phenol, catechol, and hydroquinone [10]. Since both the *Ahr* and CYP2E1 are present in primitive hematopoietic progenitor cells [11–14], it has been postulated [8] that the *Ahr* plays a significant role in the susceptibility to benzene through the ability to regulate, directly or indirectly, CYP2E1 expression in these cells. However, there does not appear to be a significant difference in CYP2E1 expression between wild-type and *Ahr*-KO mice [7]. Variations in the tissue levels of two *Ahr*-regulated enzymes, NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) [15,16] that are involved in the detoxification of benzene metabolites, have also been shown to modify benzene toxicity [17–19]. It is possible that the altered expression of these enzymes by the *Ahr* and/or activation of the *Ahr* by benzene metabolites could contribute to differential susceptibility to benzene. However, other studies indicate that hydroquinone or benzoquinone does not activate the *Ahr*, and that the presence or absence of the *Ahr* in mouse hepatoma cells did not affect ROS production induced by these benzene metabolites [20]. However, this study has not been performed in bone marrow cells. Thus, although it appears the *Ahr* has an important function in regulating susceptibility to benzene-induced hematotoxicity through its presence and activity in bone marrow cells, the specific role is not known.

The *Ahr* has been known primarily for its role in the regulation of several drug and xenobiotic metabolizing enzymes, as well as the mediation of the toxicity of certain xenobiotics, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In addition, accumulating evidence strongly suggests that the *Ahr* has subtle, but critical functions in the development and maintenance of a number of tissues including vasculature, liver and heart [21–25]. There is also much evidence to indicate that the *Ahr* has a role in regulating immune responses, particularly those involved in anti-viral immunity and regulatory T-cells that modulate autoimmune and allergic disease [26–30]. The *Ahr* seems to be required for optimal resistance of mice to *Listeria* infection [31]. Here we summarize recent data supporting a novel hypothesis that the *Ahr* has an important function in the regulation of hematopoietic stem cells (HSCs). Further defining this role will have important implications for understanding not only mechanisms underlying benzene-induced toxicity, but also those processes that regulate and preserve HSC function throughout the lifetime of an organism.

## 2. Exposure to xenobiotic *Ahr* ligands affects hematopoietic processes and HSC function

There have been several studies reporting an association between increased incidence of leukemia and lymphoma in human populations accidentally exposed to TCDD and other related halogenated polycyclic aromatic hydrocarbons [32–36]. Leukemia is

**Table 1**

Evidence that the xenobiotic *Ahr* ligand TCDD affects hematopoiesis in mice.

| Hematopoietic endpoint                                                                                                                                           | Refs.   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Reduced thymic seeding by bone marrow progenitors                                                                                                                | [41,42] |
| Decreased terminal deoxynucleotidyl transferase (TdT) and recombina-activating gene-1 (RAG-1) mRNA in bone marrow                                                | [41,42] |
| Required <i>Ahr</i> presence in hematopoietic cells for effects on altered thymic seeding and TdT/RAG-1                                                          | [43,44] |
| Decreased number of immature B cells in bone marrow; decreased number of CFU-pre-B progenitors                                                                   | [45]    |
| Increased number of HSC-enriched Lin <sup>−</sup> /Sca-1 <sup>+</sup> /c-kit <sup>+</sup> (LSK) cells, also being dependent on <i>Ahr</i> in hematopoietic cells | [43,46] |
| Altered circadian rhythms, percentage of cells in quiescence, and gene expression                                                                                | [47]    |
| Skewed differentiation from lymphoid lineage in favor of granulocyte/macrophage lineage                                                                          | [13]    |
| Decreased engraftment of HSCs into lethally-irradiated mice                                                                                                      | [13,48] |

also increased in human offspring exposed during pregnancy to tobacco smoke, which contains numerous *Ahr* ligands [37–40]. However, causality between exposure to these particular chemicals and the development of any hematopoietic disease mediated by the *Ahr* in humans has not been proven. Furthermore, the cellular and molecular mechanisms remain unknown; it is unclear whether these ligands are acting directly on human bone marrow cells.

On the other hand, the cumulative animal data are consistent with xenobiotic *Ahr* ligands affecting hematopoietic processes by their direct action on stem and/or progenitor populations (summarized in Table 1). In this review, only TCDD is considered since other *Ahr* ligands, such as benzo[a]pyrene and other PAHs, may elicit additional effects not solely related to *Ahr* activation because of the production of their biologically active metabolites. In addition to the actions of TCDD on specific biochemical and cellular endpoints in bone marrow, as indicated in Table 1, it is most significant that TCDD exposure to mice affects the function of HSCs as determined by their ability to engraft into lethally-irradiated animals [13,48]. This effect alone and the additional finding that this is dependent on the presence of the *Ahr* in hematopoietic cells [48], strongly suggest that TCDD is directly targeting the *Ahr* within these stem cell populations to alter their function. The finding that TCDD alters gene expression in these cells [48] is further evidence of this. However, the successful repopulation of HSCs into bone marrow is dependent on a number of events including migration from the circulation through endothelial cells of the bone marrow sinusoids, as well as retention and engraftment into the bone marrow niche. These cells must then be able to respond to signals, soluble and cellular, present in the marrow environment to differentiate and proliferate into more mature populations. Any one or several of these events could be affected by TCDD through the *Ahr*. The additional observation that TCDD exposure disrupts the cycling of HSCs to result in a decreased number of cells in quiescence [47] is relevant to this for several reasons. First, under normal 'resting' conditions the regulation of HSC division is tightly regulated with the majority (70–85%) of cells being in the quiescent state ( $G_0$  phase of cell cycle). This is critical for the maintenance of hematopoietic potential throughout the lifespan of the organism. Second, several studies noted a relationship between cycling status of HSCs and engraftability, with quiescent cells having greater ability for engraftment [49,50]. As such, it may not be surprising that HSCs from TCDD treated animals are less able to engraft into bone marrow.

Since most, if not all, of the toxicities caused by TCDD appears to be mediated through its ability to bind to the *Ahr* and alter gene

expression, it is reasonable to suspect that the persistent activation of the AhR in HSCs results in the modified expression of genes that are involved in cell cycle regulation and/or the expression of cell adhesion molecules that are important in HSC trafficking and engraftment [51]. Given the large number of genes that are directly or indirectly regulated by the AhR [29,52,53] there are several likely suspects, although the ability of TCDD to affect HSC function may depend on the modulation of a number of several signaling pathways simultaneously. Some of our preliminary data suggest that AhR dysregulation by TCDD may affect the CXCR4/CXCL12 signaling pathway (unpublished observations). This is particularly interesting since this pathway is essential to HSC trafficking and movement in the bone marrow niches and maintenance of a quiescent HSC pool [54]. Genes encoding c-Myc and HES1 are also regulated by the AhR [55,56], and these proteins are known to influence HSC characteristics and function [57,58]. HES1, in particular, has been shown to regulate the balance between cellular quiescence and proliferation [59].

For the purpose of this review, it is important to emphasize that the effects of TCDD on bone marrow may be very different than that produced by benzene. TCDD is metabolized very slowly, and most, if not all, of the toxic effects are elicited through the binding of the parent compound to the AhR and the subsequent modulation of gene transcription. With benzene, however, it seems clear that metabolites are the causative agents. In addition to any possible effects that might be due to the binding of these metabolites to the AhR, reactive benzene metabolites are likely to produce other effects related to their ability to covalently bind to DNA and protein, as well as initiate redox cycling to produce ROS.

### 3. Regulation of AhR presence and activity in HSCs

Clearly more work is necessary to determine the gene changes elicited by TCDD that are causally associated with alterations in HSC function. However, this task becomes more daunting when one considers that HSCs represent only a very small percentage of bone marrow cells. For example, although HSCs are often phenotypically defined as being Lineage<sup>−</sup>, Sca-1<sup>+</sup>, and c-kit<sup>+</sup> (LSK) as determined by flow cytometry, LSK cells are really only enriched in true hematopoietic stem cells. As such, there are likely several different populations of progenitor cells within any phenotypically-defined subset. Furthermore, these cells are likely to be at various stages of cycling. If the level and/or activity of the AhR is differentially regulated within these subpopulations or at different stages of the cell cycle, then the susceptibility to AhR ligands may likewise vary.

A number of investigations have shown that AhR levels vary in a cell- and tissue-specific manner, as well as with stage of development and age. Furthermore, within the same cell type, these levels have been found to change with cell density, state of differentiation, presence or absence of growth factors, and neoplastic transformation [52,60,61]. Several of the mechanisms controlling AhR levels and activity have been well studied (Table 2). Given that these mechanisms have been demonstrated in a number of different cell types, it would be expected that similar events occur in

HSCs. The gene encoding the AhR Repressor is expressed in HSCs and this expression is controlled by circadian rhythms [47]. Furthermore, the *Ahr* gene appears to be down-regulated during the proliferative phase of HSCs [13,69,70]. Thus, HSC susceptibility to the actions of some xenobiotic ligands may depend on the marrow environment. Conditions in which HSCs are proliferating may actually protect the cells from toxic AhR ligands or chemicals that are activated to toxic intermediates by enzymes induced by the AhR. In fact, we found that stimulating HSCs to proliferate actually protected them from the toxic effects of TCDD [13]. Likewise, if benzene hematotoxicity is dependent on the presence and activity of the AhR [7,8], the down-regulation of this transcription factor during different phases of the cell cycle and during different stages of differentiation may also render these cells less susceptible to benzene and/or its metabolites.

### 4. HSC characteristics and age-related hematopoietic patterns are altered in *Ahr* null-allele mice

Although the *Ahr* gene appears to be down-regulated during the proliferation of HSCs, it is not known if this is secondary to changes in other regulatory pathways or is a necessary event in the process of going from quiescence to proliferation. If the down-regulation were an important regulatory event controlling the balance between quiescence and proliferation, one would expect to see altered characteristics of HSCs from AhR-KO mice. We have found that AhR-KO mice have increased numbers of phenotypically-defined LSK and LSK/CD34<sup>+</sup> progenitor cells in the bone marrow compartment compared with wild-type mice (unpublished observations). Consistent with this, KO mice also have increased numbers of functionally-defined primitive progenitors (HPP-CFC). Perhaps more importantly, LSK cells in KO animals appear to have very high rates of cell division as determined by the incorporation of bromodeoxyuridine (BrdU). Notably, this rate was found to be identical to HSCs from wild-type animals treated with 5-fluoruracil (5-FU) that kills more mature rapidly dividing cells and stimulates HSCs into division. Consistent with this, there is also a significantly greater percentage of KO LSK cells in the G<sub>1</sub>/S phases of the cell cycle as compared to those cells from wild-type animals. Furthermore, progenitor cells from KO mice have increased growth rates in culture compared to cells from wild-type animals (unpublished observations), further suggesting that the high proliferation rate of KO HSCs is a property inherent to these cells and not due to changes in other signaling processes present in the bone marrow niche of the KO mice.

Together, these data support the hypothesis that the AhR has a normal role in the regulation of HSCs. More specifically, the data are consistent with the AhR being a negative regulator of the balance between quiescence and proliferation. That is, the AhR may normally serve as a “braking” mechanism to control rates of HSC proliferation. In its absence, this regulation is lost, and HSCs proliferate at a rate that would normally be seen only under conditions of extreme hematopoietic stress. Notably, a previous publication reported that the *Ahr* promoter is silenced by hypermethylation in human acute lymphoblastic leukemia cells [71]. The authors also postulated the AhR protein to be a negative regulator of growth and proliferation, and that the down-regulation of this gene by epigenetic mechanisms may significantly contribute to the development of the leukemia.

Notably, there is much data to suggest a causal relationship between low to moderate cycling rates in HSCs and hematopoietic longevity [72,73]. Furthermore, there is an excellent negative correlation between the mean lifespan of various mouse strains and cycling rates in HSCs [73,74]. Thus, animals with high rates of HSC replication tend to have shorter lifespans. Many years ago it was also observed that differences in longevity within mouse strains

**Table 2**

Mechanisms controlling AhR expression and activity.

| Mechanism                                                        | Refs.      |
|------------------------------------------------------------------|------------|
| Proteosomal degradation of AhR protein                           | [62–64]    |
| Presence and induction of the AhR Repressor                      | [65,66]    |
| Binding by agonists vs antagonists                               | [67,68]    |
| Metabolism of AhR ligands                                        | [61,69,70] |
| Transient vs sustained AhR activity                              | [61]       |
| Regulation of the <i>Ahr</i> gene by other transcription factors | [60]       |

correlated to some degree with differences in *Ahr* polymorphisms. Animals (e.g. C57BL/6 mice) possessing a high affinity receptor (i.e. high affinity for TCDD) have low HSC cycling rates and a longer lifespan, while strains (e.g. DBA/2 mice) having a low affinity receptor have high cycling rates and shorter lifespans [75,76].

If, as our data indicate, KO HSCs have high rates of proliferation, then these cells may undergo premature senescence as aging occurs. In addition to decreased longevity, some signs of premature bone marrow senescence include altered numbers of HSCs, and a skewing of lineages toward myeloid at the expense of lymphoid populations [73,77–80]. Increased cycling of HSCs also correlates with increased incidence of leukemia, lymphoma, and myelodysplastic syndrome in old age [81,82]. Our initial studies indicate that, unlike young adult mice, 10-month-old KO mice have <50% fewer LSK cells in bone marrow compared to age-matched wild-type mice. Furthermore, bone marrow from 1-year old KO mice has significantly increased numbers of lineage-restricted myeloid progenitors, while the number of B progenitors are profoundly decreased (unpublished observations). In addition, aging KO mice develop a number of lesions consistent with premature marrow senescence including an accumulation of cells of hematopoietic origin in many tissues. In particular, the splenomegaly in KO mice has been characterized as resulting from myeloid hyperplasia [23]. Many of these tissue lesions are consistent with mice in various stages of lymphoid neoplasms [83]. In preliminary studies in which splenocytes from aged KO mice were examined, we have observed alterations in the mRNA levels of several genes including *gfi-1*, *Sh2d3c*, *gata-1*, *p21*, and *c-myc* (unpublished observations). The altered expression of several of these has been associated with leukemia in mice, and/or predisposition to leukemia [84–86]. Finally, early onset of several neoplasms, including lymphomas and hepatomas, and shortened lifespan has been reported in *Ahr* deficient mice [87]. All of these data are further consistent with a normal role of the *Ahr* in the regulation of HSCs, and in particular as a negative regulator of HSC proliferation.

## 5. Conclusions

Although all data indicate a role for the *Ahr* in benzene-induced hematotoxicity, the mechanisms by which this occurs are not well understood. The *Ahr* clearly regulates the expression of several enzymes involved in the metabolism of benzene to more biologically active intermediates. However, it also seems clear from the data and information presented here that the *Ahr* has a major role in the regulation of hematopoietic stem cells, specifically in controlling the balance between quiescence and proliferation. As such, there are several possibilities by which the altered presence and/or the activity of the *Ahr* could modulate responses of hematopoietic stem/progenitor cells to benzene. (1) Altered *Ahr* activity may result in the decreased or increased presence of those enzymes responsible for the activation of benzene to toxic metabolites. (2) The regulation of *Ahr* expression and/or activity within subpopulations of stem/progenitor cells may render specific cell populations more or less susceptible to benzene. This effect might be especially profound under conditions of hematopoietic stress in which HSCs are stimulated to proliferate and *Ahr* may be down-regulated. (3) On the other hand, *Ahr* dysregulation in HSCs could exacerbate the hematotoxic effects of benzene by promoting conditions in which stem/progenitor cells undergo abnormal proliferation and differentiation. Thus, any dysregulation in *Ahr* function may promote conditions that could be additive or synergistic to the effects of benzene. For example, decreased expression of the *Ahr* gene by hypermethylation may result in uncontrolled cell growth and proliferation that may be also be permissive for the leukemogenic effects of benzene. (4) *Ahr* regulation in HSCs, and thus overall HSC function as well as benzene susceptibility, could also be very much

age dependent. (5) Although *bona fide* endogenous ligands for the *Ahr* have not been identified, one could also propose a scenario in which benzene exposure could alter the generation and/or activity of these ligands in HSCs to alter HSC function. (6) Finally, reactive metabolites of benzene may alter the expression and/or activity of the *Ahr* thus altering the balance between HSC quiescence and proliferation, further promoting a hematotoxic and or leukemogenic event.

Future research is necessary to define the precise role of the *Ahr* in HSCs especially as this may relate to HSC cycling, regulation of the balance between quiescence and proliferation, and senescence of HSCs. This information will lead to the identification of previously undefined functions of this transcription factor in particular hematopoietic diseases, especially those associated with aging and/or the exposure to xenobiotics such as benzene. Since the *Ahr* is ligand activated, there also may be the possibility of developing preventive and/or therapeutic measures to combat these diseases.

## Conflict of interest

None.

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