UC San Diego

UC San Diego Previously Published Works

Title

Advances in the role of the aryl hydrocarbon receptor to regulate early hematopoietic development.

Permalink

<https://escholarship.org/uc/item/74b530cr>

Journal

Current opinion in hematology, 25(4)

ISSN

1065-6251

Authors

Angelos, Mathew G
Kaufman, Dan S

Publication Date

2018-07-01

DOI

10.1097/moh.0000000000000432

Peer reviewed

Advances in the role of the aryl hydrocarbon receptor to regulate early hematopoietic development

Mathew G. Angelos^{a,b,c} and Dan S. Kaufman^d

Purpose of review

We summarize current advances to define the role the aryl hydrocarbon receptor (AHR) plays in mammalian hematopoiesis. We emphasize approaches to modulate AHR throughout human hematopoietic development *in vitro* to support the production of clinically relevant blood products suitable for patient care.

Recent findings

Initial data demonstrate that both pharmacologic AHR inhibition and genetic deletion from human pluripotent stem cells provide useful strategies to enhance the yield of hematopoietic stem and progenitor cells. AHR hyperactivation following the induction of CD34⁺ megakaryocyte-erythroid progenitors skews development toward erythroid lineages, whereas AHR inhibition supports platelet production. At the level of lymphoid specification, AHR inhibition enhances the proliferation and differentiation of functional human natural killer cells, whereas hyperactivation leads to production of Group 3 innate lymphoid cells and provides a novel platform for studying human innate lymphoid cell development.

Summary

Modulation of AHR in human hematopoietic cells *in vitro* is a promising tool to mediate development of terminal hematopoietic cell populations with significant clinical implications to generate cells suitable for antitumor immunotherapy and bone marrow transplantation.

Keywords

aryl hydrocarbon receptor, hematopoietic stem cells, innate immune cells, natural killer cells

INTRODUCTION

Hematopoiesis consists of temporally and spatially ordered developmental phases during the course of embryogenesis. The first phase, termed the ‘primitive wave,’ begins with the differentiation of mesodermal cells into progenitor cells that are bipotent for hematopoietic and endothelial fate [1–4]. These cells ultimately give rise to immature erythroid and myeloid cells within the extraembryonic yolk sac and transiently provide an oxygen reservoir to support embryogenesis [4]. By day 28 of human embryogenesis, a second phase, termed the ‘definitive wave,’ initiates from a specialized vascular precursor, referred to as hemogenic endothelium, that is primarily localized to the ventral wall of the aortagonad-mesonephros region [5–7]. It is at this point and time where bona fide hematopoietic stem cells (HSCs) are formed, characterized by their ability to differentiate into every terminal hematopoietic cell lineages (i.e., erythroid, myeloid, and lymphoid cells) and mediate long-term self-renewal and permanent hematopoietic cell engraftment.

A growing body of studies has identified the aryl hydrocarbon receptor (AHR) as an important transcriptional regulator in several pathways of definitive hematopoietic development. AHR is a member of the Per/Arnt/Sim family of environment-sensing, basic helix-loop-helix transcriptional regulators and is activated by a variety of small molecules provided by the diet and host metabolism (e.g., indole-based derivatives generated from the metabolism of *Brassica* spp. vegetables, metabolism of tryptophan via the kynurenine pathway or photoactivation with

^aDivision of Hematology, Oncology, and Transplantation, Department of Medicine, ^bStem Cell Institute, ^cMedical Scientist Training Program, University of Minnesota, Minneapolis, Minnesota and ^dDivision of Regenerative Medicine, Department of Medicine, University of California-San Diego, La Jolla, California, USA

Correspondence to Dan S. Kaufman, MD, PhD, 9500 Gilman Drive, MC 0695, La Jolla, CA, 92093–0695, USA. Tel: +858 822 1777; e-mail: dskaufman@ucsd.edu

Curr Opin Hematol 2018, 25:273–278

DOI:10.1097/MOH.0000000000000432

KEY POINTS

- New developments of the AHR demonstrate important functionality at nearly all stages of hematopoietic cell differentiation.
- Inhibition of AHR function via small-molecule antagonists and/or genetic deletion may be a useful tool to generate clinically useful blood products.

ultraviolet light, and serotonin degradation) [8–13], the microbiota (e.g., bacterial tryptophanase digestion of tryptophan into indole derivatives and certain virulence factors derived from bacterial infections) [14–16], and xenobiotic compounds found in the environment (e.g., dioxin-like compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin) [17–19]. Comprehensive reviews detailing both known exogenous and physiological AHR ligands have been published elsewhere [20,21]. In brief, mechanistically, the AHR is a cytoplasmic receptor normally stabilized by cochaperone protein heat shock protein 90 (HSP90), X-associated protein 2, and p23 [22–24]. Following ligand binding to AHR, the HSP90: X-associated protein 2:p23 complex is removed and becomes stabilized by aryl hydrocarbon nuclear translocator/hypoxia-inducible factor 1 β (ARNT/HIF1 β), forming a functional heterodimer. This heterodimer subsequently binds to the 5'-GCGTG-3' consensus within a xenobiotic response element that is located immediately upstream of several AHR target genes, such as *CYP1A1*, *CYP1B1*, *AHR* repressor gene, *IL-17*, and *IL-22*.

AHR-mediated signaling has been previously shown to play an important role in definitive hematopoiesis and maintenance of HSCs in mammalian systems. Genetic profiling studies and computational analyses from mouse models reveal AHR is expressed in HSCs [19,25] and highest in immature hematopoietic cells (including immature B-cells, T-cells, and bone-marrow derived Sca⁺ HSCs) relative to terminally differentiated phagocytes, dendritic cells, erythroid cells, T-cells, and megakaryocytes [25]. AHR activation is indispensable for the development of Th₁₇ cells, regulatory T-cells (T_{reg}), and Group 3 innate lymphoid cells (ILC3) [18,26–28]. StemRegenin-1, a potent AHR antagonist, was identified based on its ability to dramatically expand the number of engraftable human CD34⁺ hematopoietic cells *ex vivo* [29]. Interestingly, murine HSCs are not expanded by StemRegenin-1, suggesting key species differences. This result has since been translated as a Phase I and II clinical trials that demonstrated StemRegenin-1 conditioned CD34⁺ cells derived from umbilical cord blood robustly expanded over 300-fold and

conferred faster time to engraftment of neutrophils and platelets relative to patients treated with non-manipulated units [30,31]. Together, these important basic, translational and clinical research studies demonstrate that AHR is a crucial mediator of HSC growth and differentiation and has since stimulated further study on its role in hematopoietic lineage commitment and potential exploitation for additional clinical applications.

In this brief review, we highlight significant recent advancements on the function of AHR in early hematopoiesis. We also comment on the utility of AHR modulation to develop blood products and other cell-based clinical therapies.

NEW ROLES OF ARYL HYDROCARBON RECEPTOR IN HEMATOPOIETIC STEM AND PROGENITOR CELL DIFFERENTIATION

Our lab recently identified a previously unknown role of AHR to initiate human early hematopoiesis at the level of hematopoietic stem and progenitor cells (HSPCs). These studies demonstrated pharmacological inhibition of AHR with StemRegenin-1 and clustered regularly interspaced short palindromic repeats/Cas9 deletion of AHR in both human embryonic and induced pluripotent stem cells (hESCs/hiPSCs) significantly enhanced the efficiency of HSPC differentiation *in vitro* from hemogenic endothelial cells [32²²]. Induction of hematopoietic lineage commitment, as defined by the expression of a *RUNX1c* reporter protein in hematopoietic cells, was increased almost five-fold in differentiating *AHR*^{-/-} hESCs as compared with wild-type hESCs. Furthermore, the number of functional hematopoietic progenitor cells as quantified by hematopoietic colony forming unit assays was also significantly enhanced by similar proportions in *AHR*^{-/-} hESCs and StemRegenin-1 treated hESCs, a finding that has since been corroborated by others [33]. Collectively, these data demonstrate that AHR is a critical mediator at the level of the endothelial-to-hematopoietic transition and functional HSPC differentiation.

Jackson *et al.* [34] mechanistically proposed that conditions that promote the ARNT/HIF-1 α heterodimer, such as hypoxia-mimicking culture conditions with the small molecule dimethyloxaloylglycine and StemRegenin-1 treatment, would also promote the expansion of both primitive and definitive HSCs, also referred to as long-term HSCs (LT-HSCs). CD34⁺ cells derived from umbilical cord blood and treated with both dimethyloxaloylglycine and StemRegenin-1 resulted in a higher percentage of HSPCs in addition to a lower cell dose to confer long-term engraftment in irradiated nod-scid-gamma mice. These findings

suggest that synergistic targeting of the AHR/HIF-1 α axis may be beneficial to expand a specific CD34⁺ hematopoietic subpopulation from a limited umbilical cord blood volume that also has high potential to engraft.

Using *Ahr* exon 3 knock-out mice, Unnisa *et al.* report a hematopoietic phenotype that is similar to prior studies dependent on *Ahr* exon 2 knock-out mice [35]. *Ahr* exon 3 knock-out mice yielded increased numbers of functional hematopoietic progenitor cell colonies, increased cell number, and enhanced proliferation of Lin⁻Scal1⁺ckit⁺ hematopoietic progenitors [35]. These findings correlate to our studies using hESC/hiPSC-derived hematopoietic cells with an *AHR* deletion in exon 1 [32²²]. Intriguingly, the authors also preliminarily demonstrated that *Ahr* deletion significantly modulates the bone marrow microenvironment, resulting in a three-fold increase in the percentage of mesenchymal stem/stromal cells. This supports the idea that AHR may also play a role, in part, in regulating extrinsic factors that may also affect HSPC senescence, proliferation, and differentiation. Future studies focused on the mechanistic contributions of the bone marrow microenvironment in *AHR*-deficient models, such as vascular/endosteal/mesenchymal physical interactions and consequences of their paracrine signaling with HSPCs would be helpful to further understand what role, if any, AHR plays to modulate hematopoietic proliferation and conferring engraftment potential.

NEW ROLES OF ARYL HYDROCARBON RECEPTOR IN THE ERYTHRO-MEGAKARYOCYTIC AXIS

AHR has previously been identified as an important intracellular component to regulate differentiation along the erythro-megakaryocytic axis following the development of megakaryocyte-erythrocyte progenitor cells (MEP) [36–38]. *Ahr*^{-/-} mice generally possess a megakaryocyte biased lineage commitment within the erythro-myeloid compartment, yielding significantly increased numbers of low-ploidy megakaryocytes in the bone marrow and significantly decreased hematocrit values within the peripheral blood [39]. This result has since been exploited to modulate erythroid and megakaryocyte differentiation from hiPSCs using chemically defined and serum-free conditions. Smith *et al.* [19] elegantly demonstrated that following seven days of hiPSC specification into hematopoietic progenitors and then subsequent long-term culture in hematopoietic growth media conditioned with 6-formylindolo[3,2-b]carbazole, a potent AHR agonist, hematopoietic cells progressively downregulated CD71, an immunophenotypic marker expressed by

MEPs, and gradually gained CD235a, a marker found on mature erythrocytes. hiPSC-derived erythrocytes were functional in that they generated fetal hemoglobin and phenotypically resembled maturing erythroblasts when exposed to hypoxic environments. Reciprocally, hiPSCs that were transduced with a constitutively expressed AHR repressor gene and carried through toward MEP differentiation yielded a significantly higher ratio of CD41⁺ megakaryocytes to CD235a⁺ erythroid cells, providing novel insight that AHR, in part, regulates the bipotentiality of MEPs.

Strassel *et al.* [40²²] recently expanded on these findings to assess whether AHR antagonism with StemRegenin-1 could be used both as a single agent to promote proplatelet-bearing megakaryocytes and platelet differentiation from peripheral blood derived CD34⁺CD41^{low} MEPs. Like preceding studies, StemRegenin-1 treatment for up to 10 days significantly preserved the total number of CD34⁺ hematopoietic cells, but these studies advanced previous work to demonstrate enhanced development of CD34⁺CD41^{low} immature megakaryocytes and increased the proportion of differentiated megakaryocytes as compared with untreated controls. Continuous long-term culture with StemRegenin-1 further resulted in a 6.8-fold enhancement of platelet-like elements. The authors further went on to identify that the AHR pathway is an overriding driver of megakaryopoiesis, as culture of CD34⁺CD41^{low} cells in the presence of formylindolo[3,2-b]carbazole and mesenchymal stem cells (MSCs) abrogated the enhancement of platelet-like elements that was observed when cells were cocultured with MSCs alone [40²²]. Although the authors did not report a combinatorial approach to enhance megakaryopoiesis using both StemRegenin-1 and MSC coculture, these data nonetheless provide important mechanistic insights on enhancing in-vitro platelet formation from a single-donor source.

NEW ROLES OF ARYL HYDROCARBON RECEPTOR IN LYMPHOPOIESIS AND IMMUNE CELL FUNCTION

With the growing efficacy of chimeric antigen receptor (CAR)-T against tumor antigen targets and potentially CAR natural killer (NK) cells in the near future, there is a pressing need to better understand the mechanisms underlying terminal lymphocyte development. This is particularly crucial to support use of a patient's own autologous CD34⁺ peripheral blood cells and/or somatic cells that can be reprogrammed into hiPSCs to be utilized as a vehicle for genetic modulation to introduce CARs prior to differentiation of functional hematopoietic lineages [41].

Murine studies over the past decade have characterized AHR as an important mediator of adaptive immune cell development and function [21]. These studies demonstrate synergism between AHR hyperactivation and *Foxp3* transcription that serves as a master regulator in T_{reg} differentiation [27]. AHR functions both in direct activation of *Foxp3* expression, as well as altering the epigenetic landscape of the *Foxp3* locus [26,42,43]. AHR has also been implicated in the development of type-1 T_{reg} s specifically, which are genetically and functionally distinct as *Foxp3*^{low/-} cells with high interleukin (IL)-10 secretion that helps maintain immune tolerance [44–46]. AHR has also been identified as an important molecular driver of Th_{17} differentiation and IL-17A secretion in an $ROR\gamma t$ -dependent process, as well as Th_{22} differentiation and IL-22 secretion [28]. Intriguingly, AHR hyperactivation has a detrimental effect on classical $CD4^+$ and $CD8^+$ T-cell quantity and functionality [47–49].

An exciting recent focus has been how AHR influences differentiation and function of human NK cells and other innate lymphoid cells. A specific interest revolves around NK cell expansion from either autologous or allogeneic sources to use as adoptive NK cell transfer following HSC transplant may serve as an adjunct therapy to prevent relapse [50–52]. Roeven *et al.* utilized apheresed peripheral blood and bone-marrow derived $CD34^+$ HSPCs and induced NK-cell differentiation using chemically defined conditions and with the addition of StemRegenin-1 [53]. AHR inhibition for 5 weeks of culture significantly increased the mean NK-cell yield 235-fold for mobilized peripheral blood and 129-fold for bone-marrow derived $CD34^+$ HSPCs with a NK-cell homogeneity of approximately 80% for both cell types [53]. StemRegenin-1 expanded NK-cells expressed classical, matured NK transcription factors, secreted IFN- γ , and killed various target cell populations similar to non-StemRegenin-1 treated controls. Our lab has

FIGURE 1. Impact of AHR modulation on mammalian hematopoietic development. The relative expression of AHR can influence both the proliferation (curved arrow) and differentiation of many hematopoietic lineages. AHR hyperactivation (grey protein) may be accomplished with both natural and xenogenic ligands such that AHR expression exceeds that of the endogenous hematopoietic cell. Reciprocally, AHR inhibition (grey protein with 'X') accomplished with pharmacological or gene knockout methods, can also significantly affect the resulting hematopoietic progeny. Grey arrow: AHR modification enhances differentiation; Grey bar: AHR modification inhibits differentiation. CLP, Common lymphoid progenitor; CMP, Common myeloid progenitor; cNK, conventional natural killer cell; GMP, Granulocyte-monocyte progenitor; HE, Hemogenic endothelium; ILC3, Group 3 innate lymphoid cell; LT-HSC, Long-term hematopoietic stem cell; Mega, Megakaryocyte; MEP, Megakaryocyte-erythroid progenitor; Mono, Monocyte; MPheg, Macrophage; MPP, Multipotent progenitor cell; NKP, natural killer progenitor cell; RBC, red blood cell; ST-HSC, Short-term hematopoietic stem cell.

shown comparable data by using both hESCs and hiPSC as a starting population for NK-cell differentiation. We reported that StemRegenin-1 treatment in NK-cell specific growth conditions supported the development of CD56⁺CD45⁺ NK cells enriched for NK-specific transcriptional regulators, whereas AHR hyperactivation with 2,3,7,8-tetrachlorodibenzo-p-dioxin decreased NK-cell phenotype and gene expression [32²²]. Both modifications to AHR expression did not significantly alter the cytolytic antitumor activity of the NK cells.

Notably, we also established for the first time that the differentiation of ILC3 from hESC/hiPSCs is dependent on AHR hyperactivation [32²²]. Treatment of differentiating hemato-lymphoid cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin produced a significant increase in the proportion of phenotypic ILC3 (CD94⁺CD117⁺CD56⁺LFA⁻) as compared with both controls and StemRegenin-1-treated conditions. These ILC3 cells demonstrated a hallmark of ILC3 function by expression of significantly large quantities of IL-22 mRNA [32²²]. Li *et al.* [54²³] also provided some recent mechanistic insight that ILC3 development is directly dependent on Ikaros. Ikaros is well known as a critical mediator of lymphoid progenitor cell development. Using proximity ligation assays and immunoprecipitation, these studies demonstrate that Ikaros and AHR were associated with each other and a critical mediator of the AHR-ARNT heterodimerization, specifically within gut phenotypic ILC3 [54²³]. Furthermore, in the absence of Ikaros using a mouse possessing germline targeted deletion of *Ikzf1* and *Ahr*, phenotypic ILC3 were completely abolished. These findings are provocative for future studies aimed at understanding whether Ikaros can be exploited within a human context and be used to better promote the development of lymphoid subsets.

CONCLUSION

AHR has emerged as an important transcriptional regulator of nearly all aspects of hematopoietic development. In many ways, AHR functions as a 'molecular rheostat' within hematopoietic progenitor cells to modulate lineage commitment into terminal hematopoietic phenotypes (Fig. 1). Equally important are studies that demonstrate AHR is also a critical component to maintain the proliferative potential of HSPCs and NK cells, and possibly additional hematopoietic populations that have yet to be investigated.

One obvious clinical potential, as already discussed, is the notion that AHR inhibition can support a significant expansion of CD34⁺ HSPCs (as already being utilized in clinical trials for umbilical

cord blood expansion) and other hematopoietic subsets, such as NK cells. Homogenous expansion of functional NK cells is of growing clinical importance since their progenitors may be genetically modified to express tumor-specific CARs, expanded via AHR inhibition, and transplanted back to a patient in an autologous manner. Another potential concept involves the use of NK cells coinjected with an inhibitor of kynurenine metabolism. As kynurenine is a potent agonist of AHR, inhibition of either kynurenine or its metabolizer indoleamine 2,3-dioxygenase pharmacologically may be usefully in downregulating AHR and promoting the total number of localized delivered NK cells at the tumor site. In turn, this would enhance the local prevalence of NK cells and improve their overall antitumor activity.

Moving forward, an emphasis must be placed not only on the hematopoietic phenotypes generated from AHR modification but also a more comprehensive understanding of the transcriptional, genetic, and epigenetic mechanisms that AHR cooperates with to produce functional hematopoietic cells. These studies will pave the way for future research focused on using additional pharmacological targets to further enhance the production of clinically useful blood products in an efficient and homogenous manner.

Acknowledgements

M.G.A. performed the literature review and wrote the manuscript. D.S.K. wrote and edited the manuscript. Figure was produced in part using Servier Medical Art.

Financial support and sponsorship

Support for studies in the Kaufman lab include the following National Institutes of Health grants: National Cancer Institute R01 CA203348 and U01 CA217885 (D.S.K.), National Institute of Diabetes and Digestive and Kidney Diseases F30 DK107071 (M.G.A.), and the California Institute for Regenerative Medicine (D.S.K.). D.S.K. serves as a consultant and receives research funding from Fate Therapeutics.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Doulatov S, Notta F, Lauretti E, Dick JE. Hematopoiesis: a human perspective. *Cell Stem Cell* 2012; 10:120–136.
2. Palis J, Yoder MC. Yolk-sac hematopoiesis: the first blood cells of mouse and man. *Exp Hematol* 2001; 29:927–936.

3. Palis J. Primitive and definitive erythropoiesis in mammals. *Front Physiol* 2014; 5:3.
 4. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* 2008; 132:631–644.
 5. Rowe RG, Mandelbaum J, Zon LI, Daley GQ. Engineering hematopoietic stem cells: lessons from development. *Cell Stem Cell* 2016; 18:707–720.
 6. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell* 1996; 86:897–906.
 7. de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. *EMBO J* 2000; 19:2465–2474.
 8. Wirthgen E, Hoefflich A, Rebl A, Günther J. Kynurenic acid: the Janus-faced role of an immunomodulatory tryptophan metabolite and its link to pathological conditions. *Front Immunol* 2017; 8:1957.
 9. Rothhammer V, Mascarfoni ID, Bunse L, *et al.* Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med* 2016; 22:586–597.
 10. Dudek SM, Chiang ET, Camp SM, *et al.* Abl tyrosine kinase phosphorylates nonmuscle Myosin light chain kinase to regulate endothelial barrier function. *Mol Biol Cell* 2010; 21:4042–4056.
 11. Bjeldanes LF, Kim JY, Grose KR, *et al.* Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* 1991; 88:9543–9547.
 12. Dietrich C. Antioxidant functions of the aryl hydrocarbon receptor. *Stem Cells Int* 2016; 2016:7943495.
 13. Nguyen NT, Nakahama T, Le DH, *et al.* Aryl hydrocarbon receptor and kynurenine: recent advances in autoimmune disease research. *Front Immunol* 2014; 5:551.
 14. Schroeder JC, DiNatale BC, Murray IA, *et al.* The uremic toxin 3-indoxyl sulfate is a potent endogenous agonist for the human aryl hydrocarbon receptor. *Biochemistry* 2010; 49:393–400.
 15. Moura-Alves P, Faé K, Houthuys E, *et al.* AhR sensing of bacterial pigments regulates antibacterial defence. *Nature* 2014; 512:387–392.
 16. Jin UH, Lee SO, Sridharan G, *et al.* Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities. *Mol Pharmacol* 2014; 85:777–788.
 17. Hahn ME. Aryl hydrocarbon receptors: diversity and evolution. *Chem Biol Interact* 2002; 141:131–160.
 18. Juliard W, Fechner JH, Mezrich JD. The aryl hydrocarbon receptor meets immunology: friend or foe? A little of both. *Front Immunol* 2014; 5:458.
 19. Smith BW, Rozelle SS, Leung A, *et al.* The aryl hydrocarbon receptor directs hematopoietic progenitor cell expansion and differentiation. *Blood* 2013; 122:376–385.
 20. Nguyen LP, Bradfield Ca. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* 2008; 21:102–116.
 21. Gutierrez-Vazquez C, Quintana FJ. Regulation of the immune response by the aryl hydrocarbon receptor. *Immunity* 2018; 48:19–33.
 22. Murray IA, Patterson AD, Perdew GH. Aryl hydrocarbon receptor ligands in cancer: friend and foe. *Nat Rev Cancer* 2014; 14:801–814.
 23. Tsuji N, Fukuda K, Nagata Y, *et al.* The activation mechanism of the aryl hydrocarbon receptor (AhR) by molecular chaperone HSP90. *FEBS Open Bio* 2014; 4:796–803.
 24. Cox MB, Miller Ca. Cooperation of heat shock protein 90 and p23 in aryl hydrocarbon receptor signaling. *Cell Stress Chaperones* 2004; 9:4–20.
 25. Frericks M, Meissner M, Esser C. Microarray analysis of the AHR system: tissue-specific flexibility in signal and target genes. *Toxicol Appl Pharmacol* 2007; 220:320–332.
 26. Mezrich JD, Fechner JH, Zhang X, *et al.* An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* 2010; 185:3190–3198.
 27. Quintana FJ, Basso AS, Iglesias AH, *et al.* Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 2008; 453:65–71.
 28. Veldhoen M, Hirota K, Westendorf AM, *et al.* The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 2008; 453:106–109.
 29. Boitano AE, Wang J, Romeo R, *et al.* Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 2010; 329:1345–1348.
 30. Wagner JE, Brunstein CG, Boitano AE, *et al.* Phase I/II trial of stemregenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. *Cell Stem Cell* 2015; 18:144–155.
 31. Wagner JE, Brunstein CG, DeFor TE, *et al.* Phase 2 trials with mgta-456, single cord blood units (CBU) expanded with an aryl hydrocarbon receptor (AHR) antagonist, demonstrate uniform engraftment and rapid hematopoietic recovery in patients following myeloablative or non-myeloablative conditioning. *Blood* 2017; 130(Suppl 1):662.
 32. Angelos MG, Ruh PN, Webber BR, *et al.* Aryl hydrocarbon receptor inhibition promotes hematolymphoid development from human pluripotent stem cells. *Blood* 2017; 129:3428–3439.
- Established AHR as a critical mediator of the endothelial-to-hematopoietic transition, in addition to regulating differentiation into NK-cell and ILC3 lineages.
33. Tao L, Togarrati P, Choi K, Sukuntha K. StemRegenin 1 selectively promotes expansion of multipotent hematopoietic progenitors derived from human embryonic stem cells. *J Stem Cells Regen Med* 2017; 13:75–79.
 34. Jackson CS, Durandt C, Janse van Rensburg I, *et al.* Targeting the aryl hydrocarbon receptor nuclear translocator complex with DMOG and StemRegenin 1 improves primitive hematopoietic stem cell expansion. *Stem Cell Res* 2017; 21:124–131.
 35. Unnisa Z, Singh KP, Henry EC, *et al.* Aryl hydrocarbon receptor deficiency in an exon 3 deletion mouse model promotes hematopoietic stem cell proliferation and impacts endosteal niche cells. *Stem Cells Int* 2016; 2016:453187.
 36. Vargiolu M, Fusco D, Kurelac I, *et al.* The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. *J Clin Endocrinol Metab* 2009; 94:2571–2578.
 37. Lindsey S, Papoutsakis ET. The aryl hydrocarbon receptor (AHR) transcription factor regulates megakaryocytic polyploidization. *Br J Haematol* 2011; 152:469–484.
 38. Motohashi H, Kimura M, Fujita R, *et al.* NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. *Blood* 2010; 115:677–686.
 39. Singh KP, Garrett RW, Casado FL, Gasiewicz T a. Aryl hydrocarbon receptor-null allele mice have hematopoietic stem/progenitor cells with abnormal characteristics and functions. *Stem Cells Dev* 2011; 20:769–784.
 40. Strassel C, Brouard N, Mallo L, *et al.* Aryl hydrocarbon receptor-dependent enrichment of a megakaryocytic precursor with a high potential to produce platelets. *Blood* 2016; 127:2231–2240.
- Established AHR in human cells supported functional megakaryocyte and platelet-product differentiation *in vitro*.
41. Angelos MG, Kaufman DS. Pluripotent stem cell applications for regenerative medicine. *Curr Opin Organ Transplant* 2015; 20:663–670.
 42. Goettel JA, Gandhi R, Kenison JE, *et al.* AHR activation is protective against colitis driven by T cells in humanized mice. *Cell Rep* 2016; 17:1318–1329.
 43. Singh NP, Singh UP, Singh B, *et al.* Activation of Aryl hydrocarbon receptor (AhR) leads to reciprocal epigenetic regulation of Foxp3 and IL-17 expression and amelioration of experimental colitis. *PLoS One* 2011; 6:e23522.
 44. Apetoh L, Quintana FJ, Pot C, *et al.* The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol* 2010; 11:854–861.
 45. Gandhi R, Kumar D, Burns EJ, *et al.* Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell–like and Foxp3+ regulatory T cells. *Nat Immunol* 2010; 11:846–853.
 46. Mascarfoni ID, Takenaka MC, Yeste A, *et al.* Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1- α . *Nat Med* 2015; 21:638–646.
 47. Winans B, Nagari A, Chae M, *et al.* Linking the aryl hydrocarbon receptor with altered DNA methylation patterns and developmentally induced aberrant antiviral CD8 + T cell responses. *J Immunol* 2015; 194:4446–4457.
 48. Boule LA, Winans B, Lawrence BP. Effects of developmental activation of the AhR on CD4+ T-cell responses to influenza virus infection in adult mice. *Environ Health Perspect* 2014; 122:1201–1208.
 49. Schulz VJ, Smit JJ, Bol-Schoenmakers M, *et al.* Activation of the aryl hydrocarbon receptor reduces the number of precursor and effector T cells, but preserves thymic CD4+CD25+Foxp3+ regulatory T cells. *Toxicol Lett* 2012; 215:100–109.
 50. Miller JS, Soignier Y, Panoskaltis-Mortari A, *et al.* Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 2005; 105:3051–3057.
 51. Rubnitz JE, Inaba H, Ribeiro RC, *et al.* NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *J Clin Oncol* 2010; 28:955–959.
 52. Curti A, Ruggeri L, Addio AD, *et al.* Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells. *Blood* 2011; 118:3273–3279.
 53. Roeven MW, Thordardottir S, Kohela A, *et al.* The aryl hydrocarbon receptor antagonist stemregenin1 improves in vitro generation of highly functional natural killer cells from CD34 + hematopoietic stem and progenitor cells. *Stem Cells Dev* 2015; 24:2886–2898.
 54. Li S, Heller JJ, Bostick JW, *et al.* Ikaros inhibits group 3 innate lymphoid cell development and function by suppressing the aryl hydrocarbon receptor pathway. *Immunity* 2016; 45:185–197.
- Provides novel mechanistic insight on how AHR functions in concert with Ikaros in regulation ILC3 differentiation and function.