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CONTEMPORARY REVIEW

Converging Roles of the Aryl Hydrocarbon Receptor in Early Embryonic Development, Maintenance of Stemness, and Tissue Repair

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ABSTRACT

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor well-known for its adaptive role as a sensor of environmental toxicants and mediator of the metabolic detoxification of xenobiotic ligands. In addition, a growing body of experimental data has provided indisputable evidence that the AHR regulates critical functions of cell physiology and embryonic development. Recent studies have shown that the naïve AHR—that is, unliganded to xenobiotics but activated endogenously—has a crucial role in maintenance of embryonic stem cell pluripotency, tissue repair, and regulation of cancer stem cell stemness. Depending on the cellular context, AHR silences the expression of pluripotency genes *Oct4* and *Nanog* and potentiates differentiation, whereas curtailing cellular plasticity and stemness. In these processes, AHR-mediated contextual responses and outcomes are dictated by changes of interacting partners in signaling pathways, gene networks, and cell-type-specific genomic structures. In this review, we focus on AHR-mediated changes of genomic architecture as an emerging mechanism for the AHR to regulate gene expression at the transcriptional level. Collective evidence places this receptor as a physiological hub connecting multiple biological processes whose disruption impacts on embryonic development, tissue repair, and maintenance or loss of stemness.

Key words: Ah receptor; embryonic development; stemness; tissue repair.

Environmental sensing and integration of external cues to mount appropriate responses are critical for adaptation and survival of the organism. The aryl hydrocarbon receptor (AHR), a member of the large bHLH/PAS protein family of biosensors (Bersten *et al.*, 2013), is a ligand-activated transcription factor known to sense pollutants present in the environment and coordinate adaptive responses for their detoxification. This receptor has long been studied for its role in mediating the toxicity of environmentally persistent pollutants such as polycyclic aromatic hydrocarbons, halogenated aromatic hydrocarbons, and coplanar polychlorinated biphenyls (Nebert *et al.*, 2004). Furthermore, studies in *Ahr*-null mice or using endogenous ligands have suggested a number of regulatory functions in embryonic development and cellular homeostasis (Safe *et al.*, 2018). In addition to *in utero* embryonic resorption and neonatal death, deletion of the *Ahr* causes multiorgan defects including reproductive, hepatic, cardiovascular, and immune systems (Fernandez-Salguero *et al.*, 1997). Consistent with these observations, activation of the AHR by exposure to environmental ligands during early embryogenesis disrupts developmental trajectories and causes pathological conditions leading to defective phenotypes. Examples include cleft palate, cardiac insufficiency, hydronephrosis, and craniofacial deformities that are found in dioxin-exposed mice (Yoshioka and Tohyama, 2019).

These developmental defects point at the involvement of the Ah receptor in proper ontogenetic processes, suggesting that disruption of AHR signaling derails developmental trajectories during embryogenesis resulting in organ dysfunction.

Recent studies in our laboratory have shown that timely and coordinated expression of the AHR is required to prevent pluripotency loss and cause premature differentiation of mouse embryonic stem (ES) cells. ES cells are resident cells of the embryonic inner cell mass that are capable of self-renewal and with the ability to differentiate into the three germ layers of the embryo proper (ectoderm, endoderm, and mesoderm); termed pluripotency. AHR expression, which under physiological circumstances is actively repressed by pluripotency factors OCT4, NANOG, and SOX2 (Ko *et al.*, 2014), is quickly upregulated during ES cell differentiation, suggesting that the AHR has a regulatory function in the commitment of the differentiating cells to their cellular lineages (Ko and Puga, 2017). If untimely derepressed, however, AHR causes OCT4 and SOX2 downregulation and loss of pluripotency (Ko *et al.*, 2016). This interaction between AHR and pluripotency factors may play a decisive role in the differentiation of preimplantation embryonic cells and be critical for the repair process of injured tissues, as shown in *Ahr*-null tissues, which express higher levels of SOX2, OCT4, and NANOG and regenerate faster after chemical injury than their wild-type counterparts (Morales-Hernández *et al.*, 2017; Moreno-Marín *et al.*, 2017). This is because AHR expression is required for the adult/somatic stem cells to decide whether to proliferate or differentiate and thus controls the rate of tissue repair (Di Giaimo *et al.*, 2018; Metidji *et al.*, 2018). In cancer stem cells (CSCs) and myeloblastic leukemia cells, AHR drives differentiation by repressing OCT4, NANOG, and SOX2 (Bunaciu and Yen, 2011; Cheng *et al.*, 2015). In contrast, subsequent to AHR activation, the upregulated expression of stem-like genes promotes the expansion and tumorigenicity of breast CSCs and CS-like cells (Al-Dhfyan *et al.*, 2017; Stanford *et al.*, 2016). Variations in the tumor microenvironment, including the types of oncometabolites, epigenetic landscape, and signaling pathways, collectively contribute to diversify the role of the AHR in different CSCs. It is likely that the unique cell-specific interaction patterns established between ligands and the ligand-binding domain of the Ah receptor may dictate its differential functionality in regulating tissue homeostasis and cancer stemness (Giani Tagliabue *et al.*, 2019).

In this short review, we summarize recent findings and discuss the involvement of the AHR in (1) preimplantation embryogenesis, (2) tissue repair, and (3) maintenance of stemness in cancer stem-like cells through the regulation of pluripotency factors. We highlight AHR-modulated chromatin interactions as an emerging mechanism of AHR function.

CROSSTALK BETWEEN THE AHR AND PLURIPOTENCY FACTORS REGULATES MAINTENANCE OF PLURIPOTENCY IN EARLY EMBRYOGENESIS

During differentiation, successive lineage choices orchestrated by lineage-specific transcription factors gradually restrict cellular plasticity (Kalkan and Smith, 2014). In preimplantation embryos, the high levels of *Ahr* expression found in fertilized eggs become heterogeneous among blastomeres by the 4-cell stage embryos. Although undetectable during cleavage, low levels of *Ahr* expression are found in early blastocysts and thereafter spread in organ primordia (Ko and Puga, 2017). In parallel with these *in vivo* observations, *Ahr* expression is repressed in mouse pluripotent ES cells by direct binding of core pluripotency factors OCT4, NANOG, and SOX2 on the promoter distal silencer domain of the *Ahr* gene (Ko *et al.*, 2014) (Figure 1A). Release from the repressive mechanism mediated by the Polycomb Group proteins and induction of a paused RNA polymerase II complex, quickly upregulate the expression of the *Ahr* during differentiation, in strong support of the importance of the AHR in embryonic development (Figure 1B). On the other hand, untimely AHR expression in ES cells lengthens mitotic progression and leads to loss of the pluripotent state, suggesting that repression of the *Ahr* is required to prevent premature loss of pluripotency and entry in differentiation (Ko *et al.*, 2016). Although *Ahr* repression in mouse ES cells may be required to maintain the pluripotent state, a recent study has shown that paracrine signaling mediated by a kynurenine/AHR complex is essential to sustain the undifferentiated state of human ES cells, to the extent that the concentration of kynurenine can serve as a marker of the undifferentiated state (Yamamoto *et al.*, 2019). Kynurenine, a metabolite generated from the action of indoleamine 2,3-dioxygenase (IDO1) in the tryptophan catabolic pathway, is also an endogenous AHR ligand secreted by human pluripotent stem cells (PSCs). The kynurenine/AHR complex selectively binds to promoters of self-renewal genes, together with *IDO1*, and *AHR*, forming a paracrine signal loop that leads to sustained maintenance of the undifferentiated state (Yamamoto *et al.*, 2019). In contrast, the tryptophan metabolite 2-aminoadipic acid (2-AAA), generated through the kynurenine catabolic pathway by kynurenine aminotransferase 2, has been proposed as a marker for ectodermal differentiation. Upon ectodermal commitment, human PSCs take up less tryptophan and secrete 2-AAA into the media (Yamamoto *et al.*, 2019). Taken together, these results suggest that, AHR may fine-tune the decision between maintenance of pluripotency and differentiation of PSCs. Furthermore, upregulation of IDO1 by the AHR may be responsible for the maintenance of pluripotency at the metabolic level, as IDO1 has been shown to suppress mitochondrial activity and promote glycolysis through increase of the NAD^+/NADH ratio (Liu *et al.*, 2019).

The AHR controls pluripotency by regulating, even silencing altogether, the expression of the pluripotency factors. Studies in human embryonic carcinoma cells (ECCs) conducted in the Fernandez-Salguero laboratory have shown that during differentiation of human ECCs, *Alu*-retrotransposons located in the NANOG and OCT4 promoter domains bind AHR and are transcribed by RNA polymerase-III, repressing NANOG and OCT4 in differentiated cells by a mechanism likely to involve processing of the *Alu*-derived transcripts by non-coding microRNAs (Morales-Hernández *et al.*, 2016). In addition, an interaction between AHR and CTCF was found to form a regional heterochromatin loop anchored at *NANOG*-flanking *Alu* elements that silenced *NANOG* expression (González-Rico *et al.*, 2020; Morales-Hernández *et al.*, 2016) (Figure 1C). Thus, AHR may regulate the function of pluripotency factors and differentiation through more than one mechanism.

In mouse ES cells, an interaction between AHR and the chromatin remodeling NuRD-Sall4 complex upregulates *Cdx2* while downregulating *Sox17* expression, promoting trophectoderm differentiation and inhibiting primitive endoderm differentiation (Gialitakis *et al.*, 2017). The AHR-NuRD-SALL4 complex alters the expression of differentiation-specific genes that control the appearance of the first embryonic lineage occurring in 8- to 16-cell-stage mouse embryos. It appears that depending on the partner proteins with which it interacts, the AHR complex has a dual function in controlling the balance between maintenance of pluripotency and differentiation. On the one hand, the partnership with SALL4 stabilizes the pluripotent state in ES

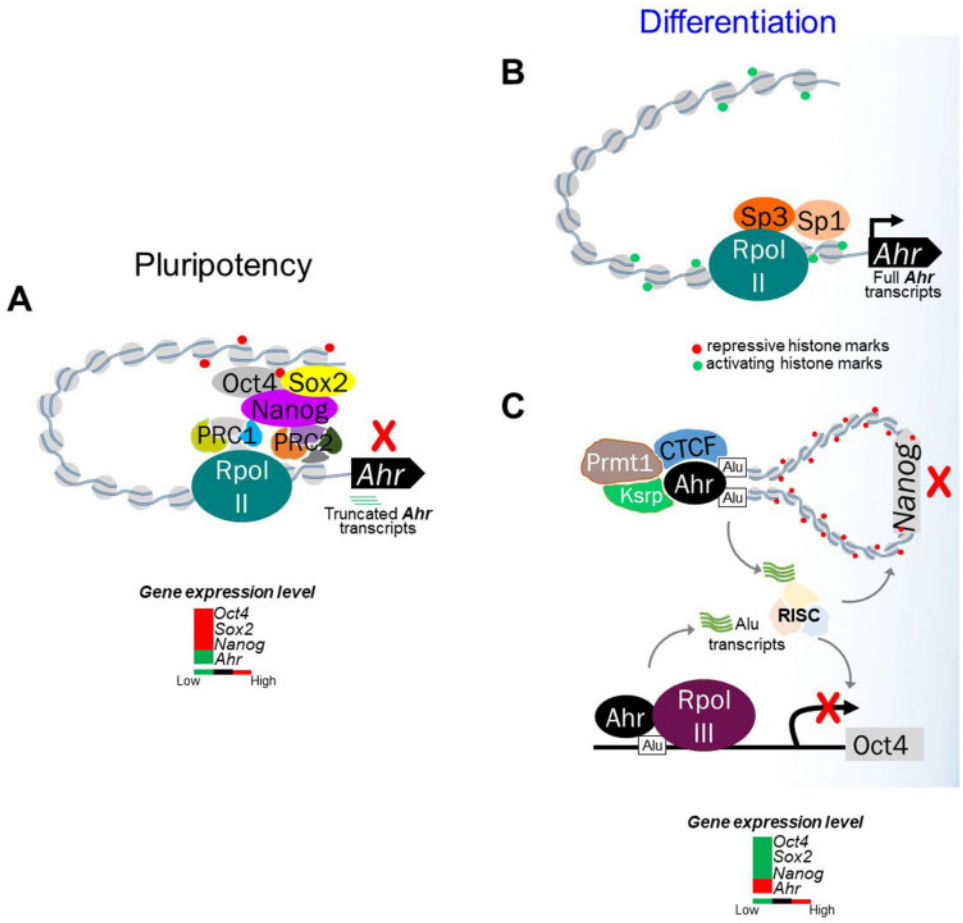

Figure 1. Crosstalk between the AHR and pluripotency factor in pluripotent state and during differentiation. A, In pluripotent ES cells, complexes of OCT4, NANOG, and SOX2 cooperate with Polycomb Group Repressive Complexes PRC1/2 to bind to a distal silencer domain in the *Ahr* upstream region and actively repress AHR expression. Unproductive RNA polymerase II is paused at the *Ahr* transcription start site and drives the synthesis of short abortive transcripts. B, During differentiation, AHR expression is derepressed by reversal of repressive marks in the *Ahr* promoter chromatin, release of pluripotency factors and PcG proteins, binding of Sp factors, establishment of histone marks of open chromatin, and engagement of active RNAPII to drive full-length RNA transcript elongation. C, In human embryonal carcinoma cells, AHR suppresses OCT4 and NANOG expression by binding to flanking *Alu* elements. This generates short noncoding RNA transcripts that target the degradation of OCT4 and NANOG mRNA through the RISC complex. At the NANOG locus, AHR cooperatively binds with CTCF, thus initiating chromatin looping and heterochromatinization around the *NANOG* gene leading to silencing of *NANOG* expression to allow differentiation to proceed.

cells by repressing the expression of trophectodermal and neural genes (Miller *et al.*, 2016; Yuri *et al.*, 2009). On the other hand, the trophectoderm transcriptional program driven by ARID3A may maintain trophoblast self-renewal, including maintenance of *Cdx2* expression, and promote further trophoblast differentiation through epigenetic HDAC1/2-dependent acetylation/deacetylation-mediated repression of *Oct4* and *Nanog* (Rhee *et al.*, 2014). Given the function of the NuRD complex in the control of lineage commitment and regulation of chromatin accessibility (Bornelöv *et al.*, 2018; Reynolds *et al.*, 2012), it is possible that the AHR could play a central role in cell fate decisions driven by cofactor-mediated epigenetic changes during early embryonic development.

INTERPLAY BETWEEN AHR AND PLURIPOTENCY FACTORS REGULATES TISSUE REPAIR AND REGENERATION

The adult stem cell population expresses the pluripotency factors OCT4, SOX2, and NANOG to maintain multi- or unipotency and self-renewal (Driessens and Blanpain, 2011; Tsai *et al.*, 2012). A timely and well-orchestrated replenishment of differentiated cells and stroma together with the dedifferentiation of terminally differentiated cells are the main mechanisms of tissue repair and regeneration after pathological injury (Galliot *et al.*, 2017; Merrell and Stanger, 2016). Analogous to its function in ES cells, AHR signaling appears to be involved in the maintenance of the somatic stem cell population. In several types of somatic stem cells and/or progenitors, including basal bronchiolar stem cells, intestinal stem cells, neural progenitor cells, ependymal cell, and hematopoietic stem cells (HSCs), the Ah receptor is required to restrict proliferation and maintain the cell population in a quiescent state (Boitano *et al.*, 2010; Han *et al.*, 2020; Metidji *et al.*, 2018; Morales-Hernández *et al.*, 2017; Singh *et al.*, 2011). It is therefore not surprising that both AHR activation by TCDD and *Ahr* deletion disrupt proliferation, gene expression, and the repopulation capacity of HSCs (Laiosa *et al.*, 2016; Singh *et al.*, 2009). Indeed, chemical damage in mouse liver and lung is promptly and efficiently repaired in the absence of the AHR (Jackson *et al.*, 2014; Mitchell *et al.*, 2006; Morales-Hernández *et al.*, 2017; Moreno-Marín *et al.*, 2017), whereas AHR activation negatively regulates wound repair and fin regeneration in zebrafish (Carvajal-Gonzalez *et al.*, 2009; Mathew *et al.*,

2009), and liver regeneration after hepatectomy in mice (Jackson *et al.*, 2014). In good agreement with these observations, conditional *Ahr* knockout in a brain ischemic model in mice has been recently shown to increase proliferation of progenitor cells while reducing inflammatory gliosis, therefore favoring restorative neurogenesis (Chen *et al.*, 2019).

The AHR is expressed in several barrier tissues, including lung, skin, and gut epithelia (Esser and Rannug, 2015), and by regulating the expression of pluripotency factors, it may also modulate tissue repair and regeneration in these tissues. In agreement with this idea, high levels of *Sox2*, *Oct4*, and *Nanog* expression have been found in tissues of *Ahr*-null mice, including brain, liver, and lung, suggesting that lack of the AHR may expand the population of progenitor or stem-like cells and prime tissues for repair after a pathological insult (Chen *et al.*, 2019; Morales-Hernández *et al.*, 2017; Moreno-Marín *et al.*, 2017) (Figure 2). Expansion of the stem cell population after diethylnitrosamine treatment has been shown to promote tumor development and progression in $Ahr^{-/-}$ livers (Moreno-Marín *et al.*, 2017). This is consistent with findings that *Ahr* ablation favors proliferation of several types of stem cells and leads to malignant transformation of intestinal stem cells (Han *et al.*, 2020; Metidji *et al.*, 2018) and to loss of barrier and inefficient repair in intestine after microbial infection (Metidji *et al.*, 2018). Therefore, although lack of the receptor allows prompt tissue repair, its presence is required to curtail uncontrolled stem cell proliferation. Consistently, initial downregulation of the *Ahr* followed by its upregulation has been observed during the repair process of injured zebrafish brain, allowing proliferation of ependymal cells that subsequently transdifferentiate into postmitotic neurons (Di Giaimo *et al.*, 2018). Similarly, mice exposed to naphthalene showed facilitated lung regeneration in the absence of AHR (Morales-Hernández *et al.*, 2017). Conversely, activation of the AHR after TCDD exposure has been shown to delay cell cycle progression by upregulating the G_1-phase cyclin-dependent kinase inhibitor $p27^{Kip1}$ in the regenerating mouse liver (Jackson *et al.*, 2014) (Figure 2). Hence, the AHR may function as a restorative chronometer to control the speed of tissue repair after injury. Modulation of AHR expression may provide a unique opportunity for therapeutic tissue regeneration.

Besides its interplay with pluripotency factors, the AHR crosstalks with WNT signaling to coordinate responses after tissue damage. AHR activation by TCDD blocks caudal regeneration of zebrafish by upregulating WNT signaling and inducing the expression of R-Spondin1, a novel ligand of the WNT coreceptor (Mathew *et al.*, 2008). Likewise, dietary derivatives that activate *Ahr* reduce proliferation and tumorigenesis of intestinal stem cells through downregulation of WNT target genes and upregulation of WNT inhibitor proteins (Metidji *et al.*, 2018). Specifically, AHR upregulation suppresses FOXM1, a downstream member of WNT signaling that modulates the nuclear localization of β-catenin (Zhang *et al.*, 2011), leading to depletion of the colon stem cell pool (Han *et al.*, 2020). Considering the involvement of WNT/β-catenin signaling in the mobilization of the stem cell population during regeneration events (Bastakoty and Young, 2016), AHR-mediated downregulation of WNT signaling may represent yet another therapeutic target of tissue regeneration. Repression of AHR signaling may facilitate tissue repair and regeneration by regulating the size of the adult stem cell pool.

AHR REGULATES STEMNESS IN CANCER STEM CELLS IN A CONTEXT-DEPENDENT MANNER

CSCs are a tumorigenic and often chemo-resistant subpopulation of cancer cells responsible for cancer relapse. Multiple lines

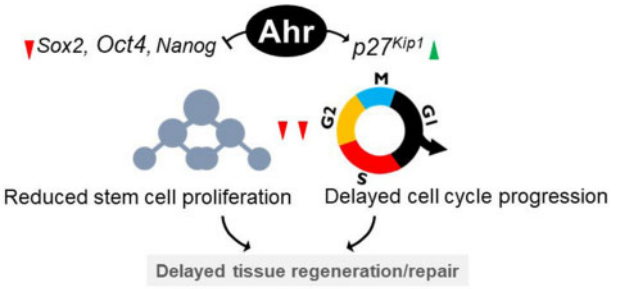

Figure 2. Ahr regulates tissue repair and regeneration. The activated AHR is known to induce the expression of the G1-phase cyclin-dependent kinase inhibitor $p27^{Kip1}$ thus delaying the cell cycle. In addition, the pluripotency factors OCT4, SOX2, and NANOG are upregulated in the absence of AHR. These changes likely bring about stasis in cell proliferation and depletion of the stem cell pool. Thus, tissue repair proceeds swiftly in the absence of AHR, and lags upon AHR activation.

of evidence suggest that similar to its role in embryonic and adult stem cells, the Ah receptor regulates stemness in CSCs, but unlike in ESCs, the AHR-mediated regulation of stemness in CSCs is either suppressive or promotive depending on the cellular context. The specificity of the AHR function in CSCs depends on the cell type, on the level and type of oncometabolites present in the tumor microenvironment, and on the signaling pathways that the AHR interacts with (Safe *et al.*, 2018).

The AHR functions as a tumor suppressor by inducing the expression of differentiation markers and reducing the expression of pluripotency factors and other CSC markers. Among these, AHR has been shown to induce the differentiation of stem-like glioblastoma cancer cells by repressing *OCT4* through direct binding of the AHR to the *OCT4* promoter (Cheng *et al.*, 2015). In addition, AHR represses *OCT4* and *NANOG* and other CSC markers, including *CD133*, *ALDH1*, *BMI1*, and *MUSASHI-1* and either impairs clonogenic potential or induces differentiation of various CSC subtypes (Bunaciu and Yen, 2011; Prud'Homme *et al.*, 2010; Zhao *et al.*, 2012). Similarly, *AHR* ablation in melanoma cells upregulates the expression of stemness genes *ALDH1A1*, *CD133*, and *CD44*, and leads to melanosphere formation (Contador-Troca *et al.*, 2013; 2015). The Ah receptor has also been shown to reduce the intranuclear levels of OCT4 in human leukemia cells, leading to loss of stemness and triggering differentiation (Bunaciu and Yen, 2011). To regulate the nuclear translocation of OCT4, the AHR may be responsible for blocking its phosphorylation on threonine residue 235 (Oct4-pT235), a post-translational modification responsible for nuclear translocation of OCT4 (Lin *et al.*, 2012). Indeed, activation of the AHR by 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE, a tryptophan metabolite and AHR agonist) has been shown to reduce phosphorylation at OCT4-pT235, resulting in inhibition of proliferation and limitation of tumorigenesis in xenografted human hepatocellular carcinoma cells and in human glioblastoma neurospheres (Zhao *et al.*, 2015).

Collectively, these results indicate that the Ah receptor may function as a tumor suppressor by inhibiting the expression of pluripotency factors and markers of CSCs. Yet, it may also behave as a tumor promoter by controlling the WNT-PTEN/PI3K/AKT circuit in CSCs. A strong correlation between AHR overexpression and high levels of WNT5a and β-catenin has been observed in inflammatory CSCs from breast cancer patients (Mohamed *et al.*, 2019). In cells from these patients, the AHR-promoted tumor expansion by activating AKT signaling and upregulating the expression of β-catenin and downstream genes of the WNT pathway

while suppressing PTEN (Al-Dhfyan *et al.*, 2017). Similarly, in stem-cell-like choriocarcinoma cells, activation of AHR by TCDD was found to increase their chemoresistance and tumorigenic potential by activating β-catenin (Wu *et al.*, 2018). Thus, upregulation of WNT signaling appears to be required to increase the expression of pluripotency factors OCT4, NANOG, and SOX2 and maintain the CSC population (Lien and Fuchs, 2014), and ALDH1A1 (Cojoc *et al.*, 2015; de Sousa e Melo and Vermeulen, 2016), whereas loss of PTEN activates the AKT pathway to induce expression of ABCG2 (Wang *et al.*, 2019). In addition, stabilization of the AHR by deubiquitylation in human lung cancer cells has been shown to result in its nuclear interaction with IKKα and gene activation of *KLF4*, *c-MYC*, *ABCG2*, and *ALDH1* (Ouyang *et al.*, 2020) and increased the expression of *ABCG2*, *c-MYC*, *KLF4*, *ALDH1A1*, and *LGR6* in radioresistant stem-like lung cancer cells by directly binding to their promoter regions (Yan *et al.*, 2018). Apart from the interaction with signaling pathways, activation of the AHR has been shown to increase the nuclear localization of SOX2 and to drive the expansion of human breast cancer cells. By direct binding to the promoter region of *SOX2*, the AHR activates expression of ALDH1 and pluripotency factors and promotes the trans-differentiation of breast cancer cells (Stanford *et al.*, 2016). Considering that expression of these genes may be activated by direct binding of the AHR to their promoter domains, the tumor-promoting function of the AHR can be the result either of a direct action on the transcription of these genes or of an indirect action through integration with the WNT and PTEN/PI3K/AKT signaling pathways.

GENOMIC ORGANIZATION AS AN EMERGING EPIGENETIC MECHANISM REGULATED BY THE AHR

A growing body of recent experimental evidence supports the contention that the activated AHR, like other ligand-activated nuclear receptors, directs a highly specific genome-wide mechanism of chromatin reorganization. By this mechanism, the AHR establishes local nucleosomal remodeling events to facilitate access to transcription factor binding sites on the promoter domain of its classical target, *Cyp1a1* and other genes in the AHR battery (Fujii-Kuriyama and Mimura, 2005). Other long-range conformational changes induced by the activated AHR in the genome have been observed that result in looping of enhancer-promoter chromatin domains at the *Cyp1a1* promoter, first observed by the Whitlock group 30 years ago (Elferink and Whitlock, 1990). In agreement, the AHR was shown to regulate chromatin accessibility in innate lymphoid cells at selected enhancer domains, including those in its own locus, through a positive feedback mechanism (Li *et al.*, 2018). Evidence of the involvement of the receptor in chromatin remodeling is further strengthened by a recent study investigating the implication of smooth muscle cell-to-chondromyocyte transition in atherosclerotic tissues. The data support a model whereby the AHR plays a protective role in the maintenance of lesion cap integrity by increasing global chromatin accessibility around chondrocyte marker genes (Kim *et al.*, 2020).

Mechanistically, the AHR partners with the ATP-dependent chromatin remodeling complexes SWI/SNF and NuRD to activate genes responsible for xenobiotic metabolism and embryonic development (Gialitakis *et al.*, 2017; Hankinson, 2005). Furthermore, benzo-a-pyrene-activated AHR binds to the Lymphoid-Specific Helicase (LSH, also known as SMARCA6) promoter, inducing its expression in non-small-cell lung carcinoma (Mao *et al.*, 2018). LSH is also a chromatin remodeling protein that belongs to SNF family, that not only directs DNA methylation (Zhu *et al.*, 2006), but also orchestrates genome-wide nucleosome occupancy changes around putative enhancers and binding sites of lineage specification factors (Ren *et al.*, 2019). It is also important to mention that recent studies conducted in differentiating multipotent bone marrow stromal cells have identified the AHR as a target regulated by super-enhancers. During differentiation of bone marrow stromal cells, 4 super-enhancer clusters on the *AHR* locus coordinate the downregulation of the AHR, which results in upregulation of adipocyte and osteoblast-specific differentiation genes (Gérard *et al.*, 2019). Lastly, the AHR has also been shown to be the target of active ILC3s and Th17s cell-specific super-enhancers, which control differentiation of IL22-producing cells and their lineage specification (Koues *et al.*, 2016).

In both ES and differentiated cells, AHR binds to genomic loci found at varying distances away from promoter regions of known target genes, suggesting that AHR may direct long-range chromatin interactions upon activation. In an AHR ChIP-seq assay using TCDD-treated MCF7 cells, peaks of AHR binding were predominantly found in intragenic and intergenic areas (Figs. 3A and 3B), indicating that to regulate expression of target genes AHR may preferentially bind to distal regulatory elements (Dere *et al.*, 2011; Fu *et al.*, 2019; Lo and Matthews, 2012; Yang *et al.*, 2018). Furthermore, an exploratory analysis of an enhancer sequencing dataset using MCF7 cells showed that many of the AHR-bound enhancer regions were linked by both the CCCTC-binding factor (CTCF) and the CTCF complex cohesin component, RAD21 (Figure 3C). Bearing in mind the essential function of CTCF in chromatin organization (Merkenschlager and Nora, 2016), it is plausible that the AHR orchestrates global but highly specific enhancer-promoter interactions to facilitate appropriate gene expression changes in response to environmental and developmental stimuli. It is noteworthy that the Mediator complex, which interacts with the CTCF complex to coordinate chromatin looping, is an AHR coactivator that only binds the *Cyp1a1* enhancer, but not the promoter (Kagey *et al.*, 2010; Wang *et al.*, 2004). Consistently, other studies have shown an insulator function for the AHR, with SLUG and CTCF cobound to a B1 short interspersed nuclear element retrotransposon (B1-X35S) to demarcate genomic boundaries (Román *et al.*, 2011). Furthermore, during differentiation of human ECCs, AHR and CTCF have been shown to coimmunoprecipitate, cooccupying two *NANOG*-flanking *Alu* elements, initiating heterochromatinization and silencing NANOG expression (González-Rico *et al.*, 2020). Studies examining genome-wide binding sites of the AHR showed that, in fact, CTCF was called among the top 10 motifs in AHR ChIP-seq peaks, suggesting AHR-CTCF cooccupancy or potential synergy between AHR and CTCF by AHR-directed recruitment of CTCF to is binding sites (Lo and Matthews, 2012; Yang *et al.*, 2018). Hence, it is possible that AHR directs long-range chromatin contacts through CTCF to influence expression of target genes.

CONCLUSIONS AND FUTURE DIRECTIONS

It is crucial to explore the precise mechanisms through which the AHR regulates the expression of target genes during differentiation of early embryonic cells, during the regenerative events regulating the tissue repair processes, and during the maintenance versus loss of tumorigenic potential of CSCs. As shown in earlier studies, most of AHR-bound chromatin domains lack canonical AHR binding motifs, implying that AHR interacts with DNA though other protein complexes or by chromatin reorganization (Dere *et al.*, 2011). Therefore, a precise AHR interactome map is needed that integrates both enhancer activity assays and

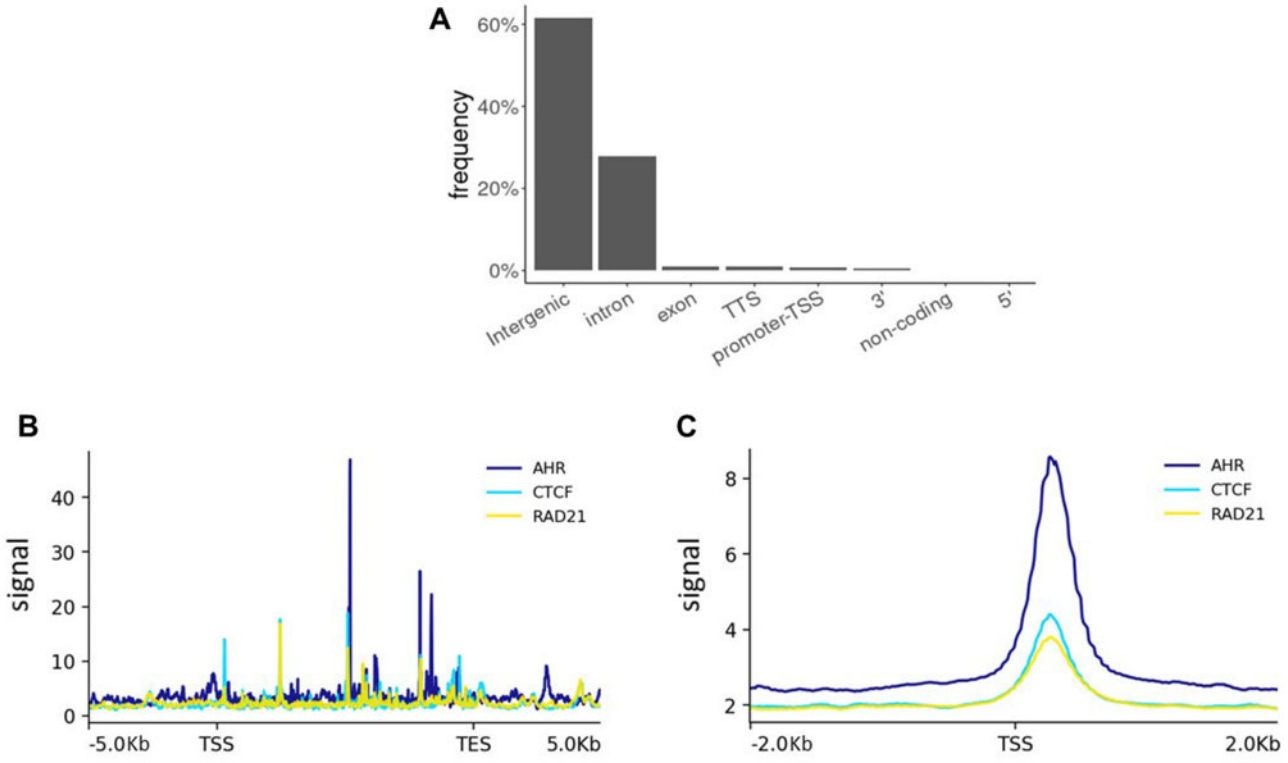

Figure 3. Cooccupancy of AHR-binding sites by complexes of AHR and CTCF. A, Statistics summarizing locations of AHR binding sites in TCDD-treated MCF7 cells. Public sequencing data was downloaded from Geo Omnibus where it is available with the accession number GSE90550 (Yang *et al.* 2018). B, AHR, CTCF, and RAD21 binding profile across 47 dioxin-responsive genes in TCDD-treated MCF7 cells (Tomblin *et al.* 2016). Strongest AHR binding was observed in gene bodies, which were cooccupied by CTCF and RAD21. SRA database files SRR5057971, SRR10096218, and SRR10096220 were downloaded from http://ncbi.github.io/sra-tools/ and used to generate bam files. DeepTools was used to generate the 1× RPGC-normalized bigwig files from the bam files and to calculate signal values. C, AHR, CTCF, and RAD21 binding across candidate enhancer locations in MCF7 cells. AHR cooperatively binds the enhancers with CTCF and RAD21. MCF7 STARR-seq databases obtained from ENCODE (ENCFF356ZLC) and were analyzed the same way as in Figure 2B. TSS: transcription start site, TTS: transcription termination site, TES: transcription end site.

genome-wide conformation data under a variety of metabolizable and nonmetabolizable ligands. Correlation between the AHR interactome map with transcriptional and chromatin changes will allow us to identify key signaling pathways directly regulated by the AHR. Furthermore, 3-dimensional chromatin conformation studies are also necessary to determine AHR-directed enhancer-promoter interactions and to delineate AHR-regulated transcriptional hubs during embryonic development, tissue repair, and tumorigenesis. This mechanistic approach will give us the needed information that will allow us to understand the role of this receptor in health and disease and open avenues of access to intervention. Another important question revolves around the role of endogenous ligands on AHR function. It remains to be known which specific ligand(s) drive AHR action during early embryonic development in tissue homeostasis and cancer biology, as well as their nature and sources. When well characterized, endogenous ligands will present an excellent therapeutic opportunity to modulate AHR function.

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