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Seminars in Cancer Biology xxx (xxxx) xxx-xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: http://ees.elsevier.com

Epigenetic Regulation of Cancer Stem Cells by the Aryl Hydrocarbon Receptor Pathway

Sabah Akhtar [a,1], Shireen Hourani [a,1], Lubna Therachiyil [a,b,1], Abdullah Al-Dhfyan [c], Abdelali Agouni [a], Asad Zeidan [d], Shahab Uddin [b], Hesham M. Korashy [a,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha, Qatar
[b] Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar
[c] Stem Cell & Tissue Re-Engineering, King Faisal Specialist Hospital and Research Centre, Riyadh, 11211 Saudi Arabia
[d] Department of Biomedical Sciences, College of Medicine, QU Health, Qatar University, Doha, Qatar

ARTICLE INFO

Keywords
Cancer stem cells
aryl hydrocarbon receptor
epigenesis
histone modification
DNA methylation
microRNAs
molecular mechanisms
CYP1A1
CYP1B1

ABSTRACT

Compelling evidence has demonstrated that tumor bulk comprises distinctive subset of cells generally referred as cancer stem cells (CSCs) that has been proposed as a strong sustainer and promoter of tumorigenesis and therapeutic resistance. These distinguished properties of CSCs have raised interest in understanding the molecular mechanisms that govern the maintenance of these cells. Numerous experimental and epidemiological studies have demonstrated that exposure to environmental toxins such as the polycyclic aromatic hydrocarbons (PAHs) is strongly involved in cancer initiation and progression. The PAH-induced carcinogenesis is shown to be mediated through the activation of a cytosolic receptor, aryl hydrocarbon receptor (AhR)/Cytochrome P4501A pathway, suggesting a possible direct link between AhR and CSCs. Several recent studies have investigated the role of AhR in CSCs self-renewal and maintenance, however the molecular mechanisms and particularly the epigenetic regulations of CSCs by AhR have not been reviewed before. In this review, we first summarize the crosstalk between AhR and cancer genetics, with particular emphasis on mechanisms relevant to CSCs such as Wnt/β-catenin, Notch, NF-κB, and PTEN-PI3K/Akt signaling pathways. The second part of this review discusses the recent advances and studies highlighting the epigenetic mechanisms mediated by the AhR pathway that control CSC gene expression, self-renewal, and chemoresistance in various human cancers. Furthermore, the review also sheds light on the importance of targeting the epigenetic pathways as a novel therapeutic approach against CSCs.

1. Cancer stem cells

Cancer has become the second leading cause of death worldwide after cardiovascular disease. In 2018, around 9.6 million people died of cancer and this number is expected to increase [1]. Cancer affects people of different ages and targets a broad variety of organs and cells [1]. Despite all treatment strategies, including surgery, radiation, and chemotherapy, poor prognosis and high rate of recurrence is a challenging factor of cancer. A high degree of chemoresistance and relapse is observed in almost all types of cancer which could be defined by the development of a regenerative sub-population of cancer cells with acquired stemness properties, generally known as cancer stem cells (CSCs).

CSCs are a subpopulation of cancer cells with extensive ability of tumor initiation, progression, vascularization, and metastasis [2]. It is proposed that CSCs are either generated upon mutations in the normal stem cells of same tissue in which tumor develops, or they are originated at embryonic stages and remain dormant, however, their mechanism of origination remains uncertain [2–6]. CSCs acquire the ability to compile genetic changes over long periods and escape the normal control system of the body [7]. These cells exhibit specific characteristics such as infinite proliferation potentials, self-renewing capacity within a tumor that can give rise to all other neo-

Abbreviations: 5-aza-CdR, 5-Aza-2'-deoxycytidine; α-NF, Alpha-naphthoflavone; ABC, ATP-binding cassette; AhR, Aryl hydrocarbon receptor; AhRR, Aryl hydrocarbon receptor repressor; ALDH, Aldehyde dehydrogenase; ALL, Acute lymphoblastic leukemia; BaP, Benzo[a]pyrene; BRCA1, Breast cancer gene 1; CSCs, Cancer stem cells; CXCR4, Chemokine receptor type 4; CYP1A1, Cytochrome P450 1A1; CYP1B1, Cytochrome P450 1B1; DMBA, 7,12-Dimethylbenz[a]anthracene; DNMT1, DNA methyltransferase 1; EMT, Epithelial to Mesenchymal Transition; H3K27me3, Histone 3 at lysine 27 trimethylation; HAHs, Halogenated aromatic hydrocarbons; HATs, Histone acetyltransferases; HDAC, Histone deacetylase; Hes1, Enhancer of Split homolog 1; NQO1, NAD(P)H:quinone oxidoreductase 1; LTEE, long-term estrogen exposure; NR2E3, Nuclear Receptor subfamily 2, group E, member 3; Oct4, Octamer Binding Transcription Factor 4; PAHs, Polycyclic aromatic hydrocarbons; PTEN, Phosphatase and tensin homolog; SOX, SRY-Box Transcription Factor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TNF-α, Tumor Necrosis Factor - alpha; TSA, Trichostatin A; XRE, Xenobiotic Response Element.

* Corresponding author at: College of Pharmacy, Qatar University, P. O. Box 2731, Doha, Qatar.
E-mail address: hkorashy@qu.edu.qa (H.M. Korashy)

[1] Authors contributed equally to this work and are joint first authors.

https://doi.org/10.1016/j.semcancer.2020.08.014
Received 21 July 2020; Received in revised form 20 August 2020; Accepted 23 August 2020

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Available online xxx
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plastic cells found within that tumor, enhanced chemo- and radio-resistance, and high tumorigenicity with metastasis and relapse properties [8]. In addition, CSCs are characterized by the ability to form tumor spheres [9,10] and to express high levels of ATP-binding cassette (ABC) drug transporters such as ABCG2, that pump numerous endogenous and exogenous compounds out of the cells against the concentration gradient, that leads to a side population (SP) which appears as a distinct dim 'tail' in the flow cytometry plot type [11–13]. Moreover, these cells express specific cell surface markers that include epithelial cell adhesion molecule (EPCAM), CD133, CD44, CD24 [14], aldehyde dehydrogenase I (ALDH1) [11,12], and stemness genes (Notch1, 2) [9,10]. The identification of CSCs was first defined by the combined expression of cell surface markers, $CD44^+$/CD24-, in which injection of only 200 of these cells was able to induce breast cancer lesions in nude mice, whereas 20,000 cells that did not display this phenotype failed to induce breast cancer lesions [15]. In addition, ALDH1+ breast CSCs can induce tumor formation with as few as 500 cells that are resistant to conventional chemotherapy [16]. Moreover, CSCs can also be characterized based on some transcription factors such as ctamer binding transcription factor 4 (OCT4), SRY-box transcription factor 2 (SOX2), Nanog, and Krüppel-like factor 4 (KLF4) [17].

Numerous studies have identified and characterized CSCs in many types of cancer including leukemia [4,5], breast [15], brain [18], lung [19,20], and colon [21]. The CSCs have been shown to be potentially responsible for tumor malignancy, chemoresistance, and tumor recurrence [8,22]. Accumulating reports indicate that highly refractory and aggressive tumors contain increased number of CSCs [19,20]. Evidence propose that cancer invasion and metastasis, which eventually lead to the patient's death is intervened by chemoresistant CSCs [9]. Thus, it is currently accepted that failure to eradicate CSC populations severely limits the ultimate effectiveness of many current cancer therapies, and hence elimination of CSCs is critical to improve treatment outcomes and to reduce recurrence and relapse. Therefore, in order to implement new treatment regimen, comprehensive understanding of CSC initiation, survival, and the metabolic as well as signaling pathways involved warrant further investigation.

In normal conditions, signaling pathways that regulate normal stem cells equilibrium are highly coordinated and controlled [10]. Notably in cancer, these pathways are either repressed or abnormally structured where these distinct abnormalities and variations control the self-renewal, proliferation, survival, and differentiation properties of CSCs. Research has proven that these pathways are not linear, but rather interwind together leading to an inter-pathway crosstalk. Examples of the most studied pathways that control CSCs progression, self-renewal, and chemoresistance include Wnt/β-catenin, Notch, Janus kinase (JAK)/signal transducer and activator of transcription (STAT) [10], Hedgehog [23], phosphoinositide 3-kinase (PI3K)/ Phosphatase and tensin homolog (PTEN), and nuclear factor κ-B (NF-κB) [24]. However, the development and progression of CSCs cannot be only attributed to genetic regulations, in fact, changes in CSCs including DNA methylation, chromatin remodeling, and non-coding RNA, which are known as epigenetic modifications, have been recently shown to regulate cancer gene expression and impact CSCs formation and maintenance [25,26]. Epigenetic modifications of the genome are simply defined as an alteration of the genetic code, without changes on the DNA sequences, to control cellular developmental hierarchies. These epigenetic modifications crosstalk with genetic and post-translational mechanisms in CSCs of different types of cancer to control their proliferation, self-renewal, and chemoresistance. Interestingly, it has been reported that early-life exposure to environmental pollutants and carcinogens such as polycyclic aromatic hydrocarbons (PAHs) is accompanied by epigenetic modifications, suggesting interactions between genetic, epigenetic, and environmental factors. Knowing that PAHs mediate carcinogenicity and tumorgenicity through activation of a cytosolic receptor, the aryl hydrocarbon receptor (AhR) [25,26], the crosstalk between CSCs-regulating genes and AhR pathway through epigenetic mechanisms has not been reviewed before.

2. The Aryl hydrocarbon receptor

AhR is a cytosolic DNA binding ligand activated transcriptional factor, regulating the expression of certain genes involved in xenobiotic metabolism [27–29]. AhR was primarily identified as a regulator of biological and toxicological responses to environmental toxic planar aromatic hydrocarbons such as PAHs and synthetic halogenated aromatic hydrocarbons (HAHs) which have high affinity towards AhR [30–32]. As of its role in xenobiotic metabolism, AhR has been studied extensively for many years in toxicology, pharmacology, and in the field of medicine such as, neurological diseases [33], cardiovascular diseases [34], and cancer [35].

2.1. Molecular regulation of AhR and target genes

AhR exists in the cytoplasm as a heteromeric core complex bound with distinct chaperone proteins such as heat shock proteins 90 (HSP90) and immunophilin-like protein XAP2. AhR is activated and induced by a group of environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 7,12-dimethylbenz[a]anthracene (DMBA), benzo[a]pyrene (BaP), 3-methylcholanthrene (3-MC), and β-naphthoflavone (β-NF) [30–32]. Immediately after ligand binding, AhR undergoes certain conformational changes and then translocates to the nucleus where it heterodimerizes with a nuclear translocator, aryl hydrocarbon nuclear translocator (ARNT). The AhR-ARNT complex then binds with specific DNA sequences, Xenobiotic Response Element (XRE), located in the enhancer region of certain genes, resulting in the transcriptional activation of enzymes involved in xenobiotic metabolism, such as the cytochrome P450 (CYP) enzymes 1A1 (CYP1A1), CYP1B1, CYP1A2, AhR repressor (AhRR), and the anti-oxidant genes, such as NAD(P)Quinone oxidoreductase 1 (NQO1) and glutathione s-transferase (GSTA1) [36,37]. Induction of CYP1A1 and CYP1B1 mediates the biotransformation of the environmental pollutants and pro-carcinogenic chemicals into highly carcinogenic and reactive diol-epoxide (DE) intermediates [36,37]. The resultant intermediates intercalate with DNA, forming adducts and activate cytotoxic genes which mediate cell mutation and tumor initiation (Fig. 1) [29].

CYP1A1, among other CYPs, is highly capable of bioactivating toxic and environmental contaminants, PAHs and HAHs, to carcinogenic metabolites and thus is considered a useful biomarker of exposure to environmental carcinogens [38]. The carcinogenic role of CYP1A family is supported by the fact that DMBA, a well-known AhR ligand, induces cancer in wild type, but not cyp1a1 knockout mice [39]. Although CYP1A1 is expressed at low levels in the lung and placenta [40,41], it is highly inducible in almost all tissues of most mammalian species including human, rat, mouse, and rabbit [42]. CYP1B1, on the other hand, is a tumor-related form of CYPs which is constitutively expressed in extrahepatic tissues and is markedly overexpressed in a wide variety of primary tumors [43]. In this regard, the high expression levels of CYP1B1 in tumor tissues, with lack of expression in normal tissues, was found to be partially regulated through proteasomal degradation of the enzyme [44] and by both transcriptional and post-translational mechanisms [45]. On the other hand, two AhR-regulated genes, AhRR and NQO1, have been shown to protect against the carcinogenic role of AhR. AhRR is a specific competitive repressor of AhR that competes with ARNT to form AhRR/ARNT complexes for binding to XRE. This results in in-

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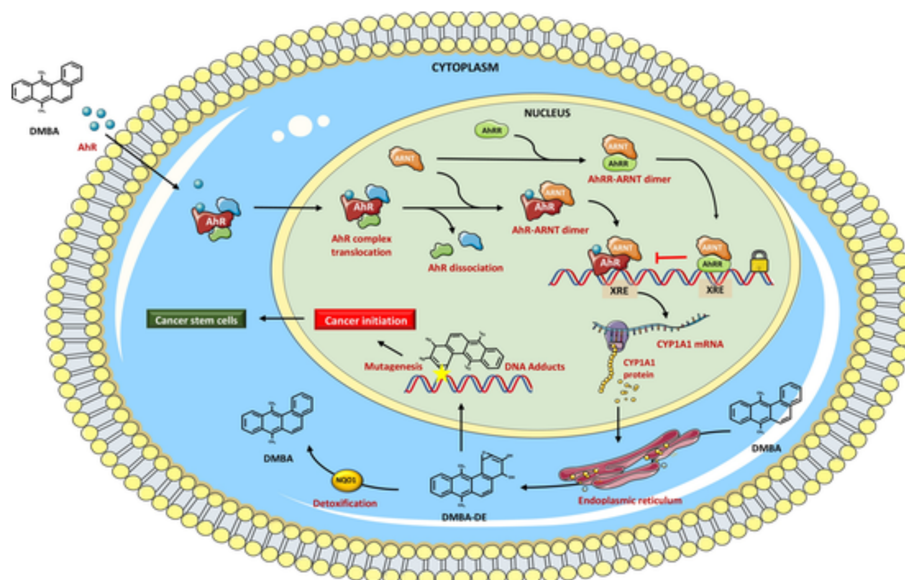

Fig. 1. Molecular pathway of AhR activation and tumor initiation.

activation of AhR and suppression of the transcriptional induction of CYP1 genes, and thus AhRR serves as a tumor suppressor gene in several types of cancer cells [46]. NQO1, on the other hand, is an anti-oxidant and detoxifying gene that protects cells against various chemical stresses and carcinogenesis through catalyzing the two-electron reduction which leads to removing the diol-epoxide group (Fig. 1) [47]. The protective effect of NQO1 is supported by the finding that NQO1 knockout mice are more susceptible to DMBA-induced cancer than their wild-type littermates [48].

The present review focuses on exploring the impact of epigenetic regulation of AhR pathways particularly CYP-regulated genes (CYP1A1 and CYP1B1), on CSCs development, self-renewal, and chemoresistance.

2.2. The regulatory effects of AhR/CYP1 pathway on CSCs' Development, Self-renewal, and Chemoresistance

The AhR/CYP1 pathway is known to have tumor activator or suppressor activities depending on the phenotype of the target cancer cells. The role of AhR/CYP1 pathway in carcinogenesis and cancer initiation as well as its potential use as a therapeutic target has been studied in breast cancer [49] glioblastoma [50], gastric cancer [50], lymphoma [51], colon cancer [52], ovarian and choriocarcinoma [9], melanoma [53], leukemia [54], multiple myeloma [55], lung cancer [56], liver cancer [57], and prostate cancers [58]. Since CSCs are known to be tumor-initiating cells and are major targets for chemical carcinogens, it is highly hypothesized that AhR plays a role in controlling CSCs. The hypothesis that AhR activation enhances CSCs self-renewal and progression is supported by several reports which showed that CSCs of different cancer types exhibit a higher expression and functional levels of AhR than corresponding differentiated non-CSCs. The activation of AhR in different human cancer cells is associated with increased CSC characteristics such as tumorigenic potential, cell proliferation, chemoresistance, $ALDH^+$ cells, and the number and size of spheres formation [59–62]. In *in-vivo* cancer tissues, AhR has been found to be constitutively active as compared to normal tissues, in which low AhR expression levels are associated with reduced tumor size and a better overall patient survival rate [63].

AhR regulates tumorigenesis through the maintenance of CSC characteristics [59]. It mediates these characteristics through controlling drug resistance and cell proliferation [59,64]. Multiple studies have shown that the knockdown of AhR in breast CSCs and subsequent treatment with chemotherapeutic agents decreased the cell viability and increased the chemosensitivity as evidenced by induced apoptosis levels [49], whereas induction of AhR using 3-MC was associated with increased $ALDH^+$ cell population in chemoresistant, but not in chemosensitive, human breast cancer MCF7 cells [65]. This was further supported by several findings where, the injection of nude mice with breast cancer cells expressing low AhR level and resistance to tamoxifen, delayed tumor formation [65]. This indicates that AhR activation is associated with drug resistance and its reduced expression increases chemosensitivity in cancer cells.

On the contrary to the prominent findings, several studies have reported inverse effect of AhR activation on CSC development and self-renewal. This hypothesis is also supported by several studies, for instance, Zhao et al., have reported that activating AhR and induction of CYP1A1 expression in MCF-7 cell lines, by β-NF, represses the mammosphere formation and the size and rate of secondary sphere formation [66]. These effects of AhR activation on CSCs were associated with a decrease in CSC markers such as expression of Notch, Bim1, β-catenin, Nanog, and $ALDH^+$ cells [66]. This reversed correlation between ALDH and AhR was reported in both human (A375 and C8161) and murine (B16-F10) melanoma cell lines, in which sequence analysis of the ALDH1A1 gene in murine melanoma cells demonstrated the presence of four XRE binding sites, suggesting a transcriptional effect [67]. In addition, it was reported that activation of AhR/CYP1B1 in human acute myeloid leukemia (AML) cell lines (MOLM-14 and MV4-11) by 6-formylindolo[3,2-b]carbazole (FICZ) decreases CSCs population and invasion while increases the apoptosis level, whereas chemical or genetic inhibition of AhR/CYP1A1 increases the CSC populations, characteristics, and tumorigenicity [68]. Similar effects were also reported in prostate cancer [69], colon [70], and liver [71]. These studies support antitumorigenic activity of AhR and shed the light on the potential of AhR agonists in the treatment of cancer. The tissue-specific variations in response to AhR agonist or antagonist could be attributed to several factors such as ligand-induced conformational changes in the receptor, recruitment of critical coactivators, corepressors, and other nu-

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clear cofactors that exhibit tissue-specific expression [72]. These discrepancies warrant further investigations to explore the molecular mechanisms mediating the effects of AhR/CYP1 in the development, maintenance, and chemoresistance of CSCs.

2.3. Molecular pathways mediating the effect of AhR/CYP1A1 on CSCs

How does the AhR modulate CSCs? What are the molecular mechanisms and pathways involved? Unfortunately, these questions have not been answered definitively. Although the effects of AhR/CYP1 activators, particularly TCDD, DMBA, 3-MC, and BaP, on the CSCs of different cancer types have been examined in several species, little is known about the molecular pathways involved. This section presents a description of the most studied molecular pathways that mediate the regulation of CSCs by AhR/CYP1A1 activators or inhibitors in several cancer types of different species. In general, five main mechanisms are proposed: 1) Wnt/β-catenin pathway, 2) Notch pathway, 3) NF-κB pathway, 4) PTEN-PI3K/Akt pathway, and 5) resistance-mediating pathways. Table 1 summarizes these mechanisms in different cancer types.

2.3.1. Wnt/β-catenin pathway

Wnt family of secreted glycolipoproteins plays major roles in cell proliferation, cell polarity, and cell fate determination during embryonic development and tissue homeostasis [73]. The Wnt signaling pathway is a signal transduction pathway that operates via passing signal from outside to the inside of the cell through cell surface receptors [74]. Activation of Wnt receptors blocks the GSK3β activity and the degradation of β-catenin, which is translocated to the nucleus where it interacts with members of the T cell factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors to activate Wnt target gene transcription [75]. Wnt signaling pathway plays an important role in CSC mediated metastasis and stemness [10]. CSCs express elevated level of Wnt signaling proteins, such as LEF1, cyclin D1, β-catenin, and TCF-4 [10]. The knockdown of the Wnt proteins has been shown to decrease the expression of stemness genes (CD44, ALDH1, and Sca-1), reduce mammosphere formation, and diminish CSC population in cancer cells [10]. Wnt signaling pathway is also involved in regulation of self-renewal of the CSCs through β-Catenin/TCF transcription factor [76]. The association between Wnt signaling and AhR in disease development has been proven by Schneider and his team [77]. The crosstalk between AhR and Wnt/β-catenin for the regulation of CSCs has been supported by several pieces of evidence. First, activation of β-catenin enhances the AhR transactivation and activity via physical interaction with its DNA responsive elements [78]. The activation of AhR through β-catenin supports the fact that β-catenin interacts with certain transcription factors and increases their transcriptional activity [79,80]. Second, induction of AhR/CYP1 causes activation and nuclear translocation of β-catenin in breast CSCs and enhances expression of its downstream target Cyclin D1 [49]. This is supported by the observations that CYP1B1 fosters cancer cell proliferation as well as metastasis via epithelial-mesenchymal transition (EMT) and Wnt pathways [81]. In inflammatory breast cancer SUM149 cells, it has been reported that deletion of AhR with CRISPR-Cas9 gene editing or CYP1B1 knockdown reduces the mRNA expression of Wnt5a/b and β-catenin, which are correlated with increased lymph node metastasis and $CD44^+$/CD24- cells [82]. Al-Dhfyan et al., and others have also reported a crosstalk between AhR and Wnt/β-catenin in CSCs of breast cancer MCF-7, Hs578 T, and SUM149 cells, in that activation of AhR/CYP1A1 by DMBA or TCDD significantly increased breast CSCs properties such as β-catenin expression, $ALDH^+$ cells, SP cells, and mammosphere formation, whereas, genetic and chemical inhibition of Wnt/β-catenin by XAV-939, blocks CSCs induction by the AhR activator, suggesting that AhR increases CSCs population through Wnt/β-catenin pathway [49].

2.3.2. Notch pathway

The Notch signaling is a transduction pathway that is essential for the regulation of embryonic development in numerous metazoan organisms [83]. In adult tissue, activation of the Notch1 pathway mediates context-specific functions, such as self-renewal and T-cell differentiation. Notch signaling is essential for stem and progenitor cell functions in several tissues [10]. The cells that exhibit higher Notch activity have increased CSC characteristics and tumor initiating properties [84]. In addition, Notch signaling pathway is associated with pro-survival genes which regulate self-renewal and proliferation of the cancer cells [84–87]. Dysregulation of Notch signaling has been observed in various cancers [88]. CSCs derived from pancreatic cancer expressed elevated levels of the Notch signaling genes, Notch1 and Notch3 and its target gene Hairy and Enhancer of Split homolog 1 (Hes1) [10]. Upon knockdown of the Hes1 gene in pancreatic cancer primary xenografts, the CSC mammosphere formation decreased, whereas treating the CSCs with Notch agonists showed an increase in mammosphere formation [10]. Accumulating evidence indicates that Notch reduces the expression of PTEN, a tumor suppressor gene, and enhances the expression of c-Myc oncogene [89]. Evidence supporting the crosstalk between AhR and Notch was reported by Alam et. al., who demonstrated that activation of Notch signaling pathway in lung cancer cells induces AhR by enhancing the secretion of endogenous ligands which subsequently stimulates interleukin-22 (IL-22) secretion from $CD4^+$ T cells [90]. IL-22 is specifically overexpressed in serum and tissue of recurrent non-small cell lung cancer (NSCLC) and promotes cancer cell proliferation and migration which are characteristics of CSCs [91]. Moreover, IL-22 also induces cell invasion in lung adenocarcinoma cell line A549 which is a fundamental feature of CSCs [91]. On the other hand, Al-Dhfyan et al., have shown that activation of the AhR/CYP1A1 pathway in human breast cancer MCF-7 cells by TCDD did not significantly alter the basal expression and translocation of ICN-1, a Notch transcription factor, and the chemical inhibition of the Notch pathway by FLi-06 did not reverse the effect of AhR activator DMBA on the induction of CSCs markers [49]. In liver cancer, it has been reported that human primary hepatocellular carcinoma tissues and cell lines express high levels of Notch1 which is associated with overexpression of AhR compared to normal hepatic cells [92]. Furthermore, activation of AhR in rat hepatic stem cells with TCDD increased cell proliferation and formation of stem cell colonies [93]. Taken together, these results clearly suggest that AhR differentially regulates the function and activity of Notch pathway in CSCs.

2.3.3. NF-κB pathway

NF-κB signaling pathway regulates radioresistance and cancer progression through IκB kinase α (IKKα). It causes expansion and self-renewal of tumor-initiating cells and metastasis in prostate and breast cancer [94,95]. Moreover, IKKα regulates the expression of stemness-related genes and enhances CSCs development and chemoresistance through AhR-dependent mechanism [28]. In CSCs, it has been shown that IKKα co-localizes with AhR in the nucleus and interacts with the promoter of stemness-related genes. Additionally, this enrichment of IKKα at the promoters of stemness-related genes is dependent on AhR activation as the depletion of AhR reduces the concentration of IKKα at their promoters. This indicates that AhR together with IKKα promote CSC characteristics through colocalization in nucleus [28]. In addition to IKKα, the NF-κB subunit, RelB, plays an important role with AhR in the anti-apoptotic re-

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inhibition by α-naphthoflavone (α-NF) restored these effects [9,49]. sponse in breast cancer through the regulation of IL-8. In that, it has

Table 1
Mechanisms involved in the modulation of CSCs by AhR/CYP1 pathways.

Pathways	Cancer type	Species	AhR/CYP1 Modulation	Mechanism of AhR modulation	Effects on cancer/CSCs	Ref.
Notch pathway	Breast cancer	Human MCF-7 cells	AhR/CYP1 activation by TCDD	No change in Notch/ICN-1 level	↔ No effect	[49]
			Inhibition of NOTCH by FLi-06	No change in the AhR activation by DMBA	↔ No effect	
	Lung cancer	Human cancer tissues	Overexpression of AhR activation	↑ Notch activate	↑ IL-2 secretion from CD4 $^{+}$ T cells & ↑ cell invasion	[243]
		Human A549 cells	Overexpression of NOTCH	↑ AhR activation		[91]
	Liver cancer	Human primary HCC tissues and HepG2, HUH-7 cells	Overexpression of AhR activation	↑ NOTCH1 levels in 19 out of 25 samples	↑ cancer progression	[92]
Wnt/β-catenin pathway	Breast cancer	Human MCF-7, Hs578 T, SUM149 cells	AhR/CYP1 activation by TCDD and DMBA	↑ β-catenin activity and nuclear translocation and ↑ cyclin D1 expression of in breast CSCs.	↑ ALDH+, SP, and spheroid formation	[9,49]
		Mice BALB/c	AhR/CYP1 activation by DMBA	↑ β-catenin expression by IHC	↑ ALDH1/2 cytoplasmic and nuclear expression	[49]
		Human MCF-7 cells	AhR knockdown or inhibition by α-NF	↓ Wnt/ β-catenin activation	↓ ALDH $^{+}$, SP, spheroid formation	[49]
			AhR/CYP1 activation by TCDD and DMBA	↓ Wnt/β-catenin by XAV-939	↓ ALDH $^{+}$ and SP population	
		Human SUM149 cells	AhR deletion by Crispr-cas9	↓ Wnt5a/b and β-catenin mRNA expression	↑ lymph node metastasis and CD44 $^{+}$/CD24 $^{-}$ cells	[82]
			CYP1B1 knockdown			
	Choriocarcinoma	Human JEG-3 and BeWo cells	AhR/CYP1 activation by TCDD	↑ β-catenin protein level and nuclear translocation	↑ cell proliferation markers, cyclin D1 and c-MYC expression	[59]
		Human JEG-3 cells	AhR knockdown	↓ β-catenin nuclear localization and translocation	↓ CSC spheroid formation	[59]
			Wnt/ β-catenin inhibition by XAV-939			
	Lung cancer	Mouse primary lung fibroblast	AhR/CYP1A1 activation by TCDD	↓ Wnt5a, 5b, and 9a and ↑Went1	↑ Axin2, Lef1, c-Myc expression	[244]
	Colon cancer	Mice	AhR activation (AhR $^{+/+}$)	↓ β-catenin levels through enhancing its degradation	↓ intestinal carcinogenesis	[70]
			AhR knockout (AhR $^{-/-}$)	↑ β-catenin level	↑ carcinogenesis	

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Table 1 (*Continued*)

Pathways	Cancer type	Species	AhR/CYP1 Modulation	Mechanism of AhR modulation	Effects on cancer/CSCs	Ref.
NF-κB pathway	Lung cancer	Human A549 cells	AhR/CYP1 activation	↑ IKKα	↑ ALDH $^{+}$, ABCG2 expression and spheroid formation in resistant cells	[28]
			AhR knockdown	↓ AhR	↓ growth of resistant cells	
	Osteosarcoma	Human MG-63 cells	AhR/CYP1 activation by TCDD	↑ receptor activator NF-κB ligand (RANKL)	↑ tumor imitation, invasion, and metastasis	[97]
	Breast cancer	Human MCF-7 and MDA-MB-436 cells	AhR/CYP1 activation by TCDD	↑ NF-κB subunit, RelB	↑ IL-8 and cell proliferation through ↓ apoptosis	[96]
PTEN-PI3K/Akt pathway	Breast cancer	Human MCF-7 cells	AhR/CYP1 activation by TCDD or DMBA	↓ PTEN through c-Myc and ↑ Akt/p-Akt level	↑ CSC self-renewal, proliferation, ↑ ALDH $^{+}$, SP, spheroid formation	[49,106]
			Akt Inhibition o by LY294002	↓ AhR effect	↓ CSCs features SP	[49]
		Mice BALB/c	AhR activation by DMBA	↑ p-Akt and ↓ PTEN expression	↑ ALDH1/2 cytoplasmic and nuclear expression	[49]
	Lung Cancer	human H1975 cells	AhR/CYP1 activation	↑ Scr and Pi3K/Akt activation	↑ chemoresistance	[107]
			AhR knockdown	↓ phosphorylation of Akt, ERK, Src, but not EGFR	↓ cell proliferation and chemosensitivity	
		Nude mice	AhR knockdown by injecting mice with AhR knockout H1975 cells	↓ Akt expression	↑ chemosensitivity, remission, apoptosis level	
	Glioblastoma	Human U87 cells	AhR/CYP1 activation by ITE	↓ Akt activity	↓ OCT4 expression, the proliferation and ↑ apoptosis	[66]
	Liver cancer	Nude mice	AhR activation (liver transplanted with HCCLM3 cells)	↓ Akt activity	↓ the tumor size compared to control and ↓ OCT4 expression	[66]
		Murine Hepatoma LA1 cells	AhR deletion by knockout as AhR- deficient Hepa1c1c7	↓ levels and activation of PI3K/Akt	↑ Apoptosis	[108]
Drug resistance proteins	Lung cancer	Human A549 cells	AhR Inhibition	↓ ABCG2 expression	↓ ALDH1A1. KLF4, CXCR4, and c-Myc	[28]
	Choriocarcinoma	Human JEG-3 and BeWo cells	AhR/CYP1 activation by TCDD	↑ ABCG2 mRNA and protein of in spheroid cells	↑ cell proliferation and spheroid formation	[59]
			AhR knockdown / CYP1A1 inhibition		↑ chemosensitivity, ↓ cell proliferation and spheroid formation	
		Nude mice Xenograft Balb/c nude mice	AhR knockdown by transfecting the shAhR stably transfected JEG-3 cells	↓ ABCG2 level by IHC	↓ tumor size compared to control	

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Table 1 (*Continued*)

Pathways	Cancer type	Species	AhR/CYP1 Modulation	Mechanism of AhR modulation	Effects on cancer/CSCs	Ref.
	Breast cancer	Human MCF-7 cells	AhR/CYP1 activation	↑ ABCG2 and CXCR4 expression	↑ SP, ALDH $^{+}$ cells, chemoresistance, tumorigenesis	[65]
		TNBC Hs578 T and IBC SUM149	AhR/CYP1 activation	↑ Sox2 expression and nuclear translocation through direct AhR-Sox2 binding	↑ ALDH $^{+}$ cells and chemoresistance	[9]
		Nude mice Xenograft	Mice were injected with SUM149 cells, stably transduced with shAhr with ALDH ^{low}cells	↓ SOX2 mRNA expression	↓ tumor growth rate and size	
	Melanoma	Murine B16-F10 cells	Silencing Sox2	↑ AhR protein level and AhR nuclear translocation	↑ cells entry into dormancy and proliferation arrest and ↑ cell cycle inhibitor proteins p27 and p21	[114]
			AhR knockdown	↑ Aldh1a1 expression, activity, and ALDH $^{+}$ cells	↑ cancer proliferation	[67]
		Mice C57BL6 albino	Depletion of AhR	↑ Aldh1a1 activity	↑ primary tumorigenesis and metastasis	[67]
			Depletion of AhR and ALDH	↓ Aldh1a1 activity and SOX	↓ cell migration, tumorigenesis, metastasis, ↓CD133 $^{+}$ CD29 $^{+}$/CD44 $^{+}$cells and mlanosphere size	

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been shown that activation of AhR through TCDD in human breast cancer cells, MCF-7 and MDA-MB-436, induced IL-8 expression and RelB resulting in decreased cell apoptosis and increased cell proliferation. This effect was further supported by activation of apoptosis in ERα-negative cell lines by knockdown of AhR or RelB [96]. In human osteosarcoma MG-63 cell line, the activation of AhR through TCDD was associated with increased protein and mRNA levels of receptor activator of NF-κB ligand (RANKL) [97]. The RANK/RANKL permits development of cancer cells and plays a role in the formation of primary and secondary tumors in leukemia, breast cancer, bone cancer, and prostate cancer [98]. Whereas, the inhibition of RANKL in mouse models resulted in reduced mammary tumorigenesis and pulmonary metastasis [99]. This suggests that AhR participates in the development of cancer through the activation of RANKL which plays a role in CSC invasion and metastasis.

2.3.4. PTEN-PI3K/Akt pathway

The PTEN-PI3K/AKT is an intracellular signaling pathway that plays an important role in regulating the cell cycle and thus it is directly related to cellular quiescence, proliferation, cancer, and cellular longevity. PI3K activation phosphorylates and activates Akt, characteristics, whereas, activation increases the EMT, a feature of CSCs, in human breast cancer MCF-7 and MDA-MB-231 cells through induction of miR-21 [104]. The involvement of the AhR/CYP1 pathway in regulating PI3K/Akt in CSCs has been demonstrated in several types of cancer. In breast cancer cells, it has been reported that activation of AhR/CYP1A1 result in increased expression of Akt which induces CSC features such as ALDH^{+} cells, SP cells, and mammosphere formation [49,105]. In addition, inhibition of PI3K/Akt pathway in breast cancer MCF-7 cells by LY294002 blocked the AhR-induced CSCs population [49]. Furthermore, activation of AhR in breast CSCs reduced the expression of PTEN, a tumor suppressor gene and a negative regulator of PI3K/Akt, which permits CSCs self-renewal, proliferation, and undifferentiation through regulation of c-Myc [49,106]. In lung cancer, activation of AhR in NSCLC H1975 cells increases Akt phosphorylation which is associated with increased chemoresistance to tyrosine kinase inhibitor, Afatinib, through activation of Src and MEK/ERK signaling pathway, whereas the knockdown of AhR inhibits cell proliferation and enhances the chemosensitivity [107]. Similar observations were reported in H1975 AhR shRNA xenograft tumors characterized by complete remission and increased apoptosis level [107]. In liver cancer, murine

which in turn regulates the expression of carcinogenesis-related genes such as β-catenin, p21, p27, Mdm2, and Forkhead transcription factors [100,101]. PI3K/Akt pathway is associated with conferring chemoresistance, cell proliferation, and survival [89,102,103]. Inactivation of PI3K/Akt pathway is associated with decreased CSCs hepatoma Hepa1c1c7 lacking AhR (LA1) exhibits lower level of PI3K/Akt activity than wild-type Hepap1c1c7 cells, which leads to increased susceptibility to apoptosis [108]. On the contrary, Zhao et al., and colleagues have reported that activation of the AhR by 2-(1' H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE),

an AhR agonist, in glioblastoma U87 cell lines leads to impaired Akt activity, increased apoptosis function, and decreased the CSC marker, OCT4 expression, in U87 spheroids [66]. Similar observations were also reported in nude mice transplanted with liver cancer HCCLM3 cells [66]. These findings indicate a correlation between AhR and PTEN-PI3K/Akt pathway for maintenance of CSCs.

2.3.5. Drug Resistance-mediating pathways

During tumor progression, chemoresistance is the leading cause of treatment failure and disease relapse in cancer patients [109]. It is well-reported that most of the chemoresistant cells express high CSC characteristics and features, such as self-renewal and tumorgenicity [109]. AhR has been shown to mediate chemoresistance in CSCs by regulating the expression of several genes, such as ABCG2. ABCG2 is a member of the ABC family G, known also as breast cancer resistant protein (BCRP), that protects cells and tissues against xenobiotics. Overexpression of ABCG2 contributes to multidrug resistance in cancer and CSCs by its capacity to efflux many chemotherapeutic agents [59,65]. The link between ABCG2 and chemoresistance in CSCs has been demonstrated by Dubrovska et al., who showed that drug resistant MCF-7 breast cancer cells, which express higher levels of ABCG2, exhibit increased CSC markers such as SP cells and ALDH enzymatic activity [65]. The AhR crosstalk with ABCG2 has been established in the stem cells of several cancer types. ABCG2 has been reported to be transcriptionally activated by the AhR/CYP1 pathways through binding of AhR to the XRE sequence on ABCG2 promoter region [59,110–112]. Interestingly, the inhibition of AhR in radio-resistant lung adenocarcinoma A549 cell line, reduced expression of ABCG2 which is associated with reduction of many stemness marker genes such as ALDH1A1, KLF4, chemokine receptor type 4 (CXCR4), c-Myc, and Lgr6 which indicates the association of AhR with a number of tumor inducing genes [28]. Furthermore, overexpression of ABCG2 in tamoxifen resistant MCF-7 xenograft tumors which was associated with increased CSCs markers, SP cells, and ALDH activity, was blocked by inhibition of CXCR4 [65], a chemokine receptor that is known to be induced by AhR activation by TCDD [97]. In addition, choriocarcinoma spheroid cells (JEG-3 and BeWo) with CSC properties exhibited elevated expression of AhR, ABCG2, and stemness markers Sox2, Oct4, Nanog, and CD44/CD133 [59]. Therefore, these findings reveal that activation of AhR/CYP1A1 pathway increases ABCG2-mediated chemoresistance.

Another gene that is known to mediate AhR-induced CSCs population is SOX2. SOX2 is a transcription factor that plays a role in maintaining stemness of embryonic stem cells and hence its dysregulation has impact on cancer cell proliferation, invasion, self-renewal, and chemoresistance [113]. Stanford et al., have demonstrated that AhR activation in human TNBC Hs578 T and inflammatory breast cancer SUM149 cells increases SOX2 expression and nuclear translocation through direct AhR-SOX2 binding, leading to increased $ALDH^{+}$ cells and chemoresistance [9]. Similar results were reported in *in-vivo* nude mice model, in which injection of SUM149 cells, stably transduced with shAhR with $ALDH^{low}$cells, inhibited SOX2 mRNA expression and reduced tumor formation [9]. On the other hand, silencing of SOX2 by shSOX2 in murine melanoma B16-F1 cells significantly activated AhR expression and nuclear translocation and induced these cells into dormancy and proliferation arrest through upregulation of cell cycle inhibitor proteins (p27 and p21), whereas complete deletion of SOX2 by knockout caused cells to exit indicate that AhR is a central player in maintenance of CSCs through modulation of ABCG2 and SOX2.

3. Epigenetic regulation of cancer and CSCs

The accumulation of genetic mutations and disruption of cellular functions are the main reasons behind cancer induction and progression [115]. These modifications further lead to changes at the histone and DNA levels, also known as the epigenome level which further contributes to tumor initiation [115]. Acquired or genetic epigenetic modifications, which could occur to the genome regardless of the DNA sequence, involve the interaction with various enzymes and molecules [116]. Epigenetic regulation of chromatin plays an essential role in the control of gene expression through DNA methylation and demethylation, histone modification, chromatin post-translational modifications (PTM), and non-coding RNAs regulations [117]. Since epigenetic regulators are reversible and affected by external factors, they are becoming a promising chemotherapeutic target for various therapy-resistant cancers [116].

3.1. DNA Methylation

DNA methylation is one of the main pathways in epigenetics that controls gene regulation [118]. It indicates the inclusion of a methyl group to carbon number five in the pyrimidine ring in the CpG dinucleotide islands of the DNA [118]. Silencing of tumor suppressor genes in many types of human cancers occur via promoter methylation. This process is mediated by the activation or repression of certain enzymes specifically DNA methyltransferase and demethylase. DNA methylation and demethylation induce different effects at the transcriptional level, where hypermethylation of the enhancer or promoter region of DNA leads to gene silencing while hypomethylation results in upregulated gene expression [118]. In cancer, hypermethylation of the promoter region is the corner stone for inactivating genes responsible for regulating tumor suppression, cell apoptosis, and DNA repair [119]. Methylation due to cancer is known as de novo methylation because it either inhibits genes that are formerly active in the tissue or prevents the activation of already repressed genes [120].

Several studies have examined the impact of DNA methylation of certain genes on cancer and CSCs progression, proliferation, and chemoresistance. One of these genes is CD271, a tumor necrosis factor receptor (TNF) that has an essential role in programmed cell death, cell proliferation, and survival [121]. Cells with high CD271 expression have been proven to exhibit CSC-like properties with high chemoresistant, tumorigenic, and metastatic abilities in human melanoma tissues [121]. It has also been shown that CSC $CD271^{+}$ cells (spheroids) are more resistant to cancer therapy compared to CD271- cells [121]. Several pieces of evidence suggest that CD271 expression is negatively controlled by DNA methylation. In that, the inhibition of DNA methyltransferase I by 5-Aza-2'-deoxycytidine (5-aza-CdR) increased CD271 expression [121], suggesting that CSC features and chemoresistance are regulated epigenetically. Similarly, Wang et al., have shown that the chemoresistance of liver cancer cells to anti-cancer agents is attributed to increased Oct4 gene linked to epigenetic demethylation of its CpG site [109]. Moreover, overexpression of $CD133^{+}$ cell surface marker in human endometrial cancer tissues has shown to play a role in chemoresistance through epigenetic modification of its CpG sites. In that, the CD133 promoter

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dormancy and resume proliferation [114]. Furthermore, it is reported that depletion of both AhR and ALDH1 in *in vitro* melanoma cell lines and *in vivo* mice model reduced tumor progression, melanosphere size, tumorigenesis, and organ metastasis via reducing the expression of SOX2 [67]. Taken together, these studies clearly

CpG is shown to be hypomethylated in the malignant tumor tissues compared to non-malignant [122]. Hypomethylation of CD133 is associated with an increase in its mRNA and protein expression and localization in cancerous compared to benign tissues. Demethylating agent 5-aza-CdR has shown to increase CD133 expression [122]. Furthermore, methylation of the promoter region was significantly

reduced in the malignant tumor expressing CD133 marker compared to normal endometrium cells, which proves that CSCs are epigenetically controlled by hypomethylation of the stemness markers [122]. In Head and Neck Squamous Cell Carcinoma (HNSCC) cell lines, Furusawa et al., have reported that overexpression of CD44^{high} subpopulation, which express higher levels of CSC markers, is attributed to hypomethylation of several genes that play a role in tumor initiation and progression [123].

3.2. Histone Modification

Histone modification occurs in the chromatin material that is mainly composed of chromosomal DNA and histone octamers of four core histone proteins, H3, H4, H2A, and H2B [124,125]. Alteration in the chromatin structure is caused by two main mechanisms, either through adjustment of N-terminal ends of histones by chromatin modifying enzymes at the post-transitional level, or through altering the interaction between DNA and histones by ATP-dependent chromatin remodeling complexes [124]. Some critical modifications at the N-terminal are acetylation through histone acetyltransferases (HATs) and histone deacetylases (HDACs) on the lysine residue of the ε-amino group. The methylation through H3K4 methyltransferases controls genes of the developmental stage, ubiquitination, and phosphorylation, which drastically affects the gene stability and chromosomal segregation [124,126]. This abnormal histone modification initiates cancer through differential gene expression and oncogene regulation [124].

Yan et al., studied the effect of epigenetic factors on chemoresistant AML cells characterized by $CD123^{+}/CD47^{+}$ and demonstrated that inhibition of HDAC by Romidepsin results in induction of apoptosis and cell cycle arrest of chemoresistant cells *in vitro* and xenograft mouse model of AML [127]. Moreover, HDAC inhibitors showed a synergistic effect when given as a combination with chemotherapy drug Ara-C [127]. In breast cancer, Darvin et al., have shown that breast CSCs of MCF-7 and BT-549 express high level of PD-L1 which is mainly mediated through hypomethylation of its promoter region and active histone markers, including H3K9me3 and H3K27me3 [128]. These results were further confirmed by the over-expression of TET3 and downregulation of DNMTs, the enzymes responsible for active demethylation [128]. Furthermore, it has been reported that several breast cancer cells (T47D, MDA-MB-231, MDA-MB-468, and MCF-7) express high levels of HDAC-3 which is positively correlated with advanced breast cancer TNM stage [129]. On the other hand, cancer associated fibroblasts (CAF), a mediator of tumor progression and metastasis, express higher levels of HDAC-6, causing immunosuppression and chemoresistance [130]. In ovarian cancer, it was reported that metformin inhibits ovarian adenocarcinoma cell (SKOV3) and ovarian clear cell carcinoma cells (ES2) proliferation and induces apoptosis through decreasing histone H3 lysine 27 trimethylation H3K27me3 [131]. Moreover, some studies on ovarian cancer have shown that histone modification is responsible for the loss of tumor necrosis factor members such as FAS and hence cause chemoresistance. In that, the level of acetylated histone H3 associated with FAS promoter was drastically decreased in the chemoresistant A2780-AD cells compared to normal ovarian cells, whereas, HDAC-1 was elevated in A2780-AD cells [132]. Inhibition of the HDAC-1 caused a significant genic miRNA in colon CSCs [133]. These studies suggest that targeting HDAC could be a potential target for chemoprevention and therapy.

3.3. MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNA regions, localized in the nucleus and cytoplasm of the cell [134,135]. Once developed, the miRNAs translate into RNA-induced silencing complexes (RISC) controlling the gene expression at the post-transcriptional level by binding to the 3'-untranslated region (3'-UTR) of the mRNA [134,135]. These miRNAs control critical cell pathways such as cell maturation, growth, division, and survival, and thus they are considered to be a potential therapeutic target [135]. The dysregulation of miRNAs has been associated with the pathogenesis of human diseases including cancer. In cancer, the epigenetic regulation of miRNA occurs through DNA methylation, histone modification, and gene silencing [134]. Studies have shown that miRNA genes are most silenced after DNA methylation in the CpG islands of the promoter region, whereas distant CpG islands located away from miRNA, function as enhancers to regulate the miRNA gene expression [136]. Moreover, the hydroxy-methylated cytosine formed during the active demethylation of the CpG transcripts acts as transcription enhancers of some miRNA [136]. Furthermore, the miRNA expression is either enhanced or inhibited by post-transcription histone modifications depending on whether the N-terminal was methylated or acetylated [136].

The involvement of miRNA dysregulation in CSCs and chemoresistance has not been thoroughly investigated. Breast CSCs expressing elevated levels of stem cell surface markers $CD44^{+}$/CD24- and SP cells have been shown to express reduced levels of miR-34a, a tumor suppressor miRNA that targets NOTCH pathway [137]. The silencing effect of miR-34 on Notch pathway and CSCs was supported by the observations that restoration of the miR-34a level by transfecting breast cancer resistant cells with miR-34a mimics, reduces mammosphere formation, $CD44^{+}$/CD24- population, Notch expression levels, and CSC self-renewal capacity [137]. This overexpression of miR-34a was associated with a decrease in tumor formation and increase in sensitivity to chemotherapeutic agents in nude mice cancer model [137]. These results suggest that miR-34a suppresses CSC self-renewal capacity through targeting Notch pathway. The miRNAs 451, 144, and 139-5p have been reported to negatively modulate CSC features in colorectal cancer. In that, colorectal CSCs characterized by elevated levels of markers such as, $CD44^{+}/CD133^{+}$, EpCam, CD166, and CD24 expressed low levels of miR-451 [138] or miR-139-5p [139] compared to adherent colorectal cancer cells. The downregulation of miR-451 or miR-139-5p is correlated to tumor aggressiveness, chemoresistance, and higher risk of relapse [138,139]. Furthermore, overexpression of miR-451 inhibits spheres production and colony formation abilities, and increases sensitivity to several chemotherapeutic agents through downregulation of drug-efflux genes, ABCB1 [138] and NOTCH1 expression [139]. In pancreatic cancer, Cioffi et al., have demonstrated that cancer recurrence and chemoresistance are controlled by epigenetic regulators in which, pancreatic CSC spheres show downregulation of the miR-17-92, miR-513a-5p, and miR-513b [140]. This tumor suppressing function of these miRNAs is evidenced by the fact

increase of FAS expression in A2780-AD cells [132], indicating that epigenetic changes regulated by histone modification contributed to chemoresistance due to the loss of tumor suppressor genes [132]. Kim and co-workers have shown that the anticancer effect of β-carotene against colorectal cancer cells is mediated by increasing the histone acetylation of H3 and H4 through downregulating tumori-

that knockdown of miR17-92 resulted in upregulation of CD133 CSC surface marker, ABC transporters, and the ability of cancer cells self-renewal, as evidenced by sphere formation [140]. Similarly, bladder CSCs with high $ALDH^+$ and tumorigenic capacity were inversely governed by the low expression levels of a long-noncoding RNA lnc-LBCS [141].

4. Epigenetic regulation of AhR expression and function in cancer

Activation of the AhR causes a crosstalk with coactivator complexes inside the nucleus to induce epigenetic modifications at the promoter and enhancer regions of target genes, including CYP1A1, CYP1B1, and CYP1A2. In this section, we review the effects of DNA methylation, histone modification, and miRNA on the expression, function, and activity of AhR, CYP1A1, CYP1B1, and CYP1A2 genes in different tissues and cell lines.

4.1. AhR

DNA methylation of the promoter region of genes permit gene silencing by preventing the recruitment of transcription factors to the consensus sequence [142]. The studies involving isolation and characterization of AhR gene demonstrate that the promoter of AhR gene is a GC rich region which lacks CCAAT and TATA box and has a minimum of four functional specificity protein 1 (SP1)-like binding sites [143]. These CpG islands are easily methylated which leads to gene suppression. AhR is found to be hypermethylated in about 33% of primary human acute lymphoblastic leukemia (ALL) which suggests that the tumor suppression role of this receptor is disrupted in these cancers [144]. The suppression of AhR through methylation in the promoter region has been observed in different cancer cell lines such as chronic myeloid leukemia (K562) and acute lymphoblastic leukemia (REH) cells [144]. In that, treatment of AhR^{low} expressing ALL cells (REH) with DNA methylation inhibitor, 5-aza-CdR, significantly increased the mRNA and protein expression of AhR. This inhibitory effect of DNA methylation on AhR expression is attributed to the impaired binding of sp1 transcription factor to AhR promotr [144]. On the other hand, in mouse liver, AhR activation by TCDD caused an AhR-dependent CpG demethylation and recruitment of DNA glycosylase at CYP1A1 promotr [145].

Histone acetylation plays a role in the transcriptional activity of AhR gene promoter. The constitutive expression of AhR is increased by the HDAC inhibitors, butyrate or trichostatin A (TSA), in wild-type and AhR-deficient mouse hepatoma Hepa1 cells, whereas the activity of AhR is decreased upon the treatment with HDAC [143]. In addition, it has been demonstrated that increased expression of AhR in human breast cancer MCF-7 cells is attributed to decreased histone trimethylation, but not DNA methylation [146]. In human hepatocellular carcinoma HepG2 cells, it has been reported that inhibition of histone deacetylation by TSA increases the AhR-XRE luciferase activity [147]. Additionally, AhR activation is also associated with reduced expression of certain miRNAs that are believed to play a role in cancer. AhR is implicated in reducing the levels of miR-96 which plays a role in tumor progression through the suppression of FOXO3a, a tumor suppressor transcription factor that is frequently inactivated in cancer [148–150].

4.2. CYP1A1

Epigenetic regulation of CYP1A1 has been shown to alter the function and expression of several genes and transcription factors that are known to play a crucial role in the development of diseases. treatment in mouse liver causes AhR dependent demethylation in two CpGs in CYP1A1 proximal promoter which suggests that AhR agonists transcriptionally activates CYP1A1 in mouse liver [145]. These studies collectively suggest a negative impact of the DNA methylation on the AhR/CYP1A1 and XRE function.

Histone modifications at the CYP1A1 promoter region has also been shown to permit the induction of CYP1A1 expression. Chromatin immunoprecipitation experiments revealed that the human prostate cells PWR-1E and RWPE-1 exhibit trimethyl histone H3 lysine 4 which is a marker of active CYP1A1 gene, whereas the cancerous LNCaP cells lack this histone modification which indicates the importance of histone modification in the expression of CYP1A1 [153]. In addition, the activation of the AhR by TCDD enhances the trimethylation of H4Ac and H3K4 in the promoter region of CYP1A1 in mouse liver, whereas decreases repressive marker, H4K20me3 [145]. Specifically, it was reported that inhibition of HDAC1 either genetically or by using chemical inhibitor TSA, increased the expression of CYP1A1 and AhR function in human neuroblastoma [155], breast MCF-7, and cervical HeLa [154], HepG2 [147], and mouse hepatoma Hepa1 [156] cells. Using chromatin immunoprecipitation (ChIP) assay, Jin and co-workers have demonstrated that treatment of Caco-2 cells with butyrate, a HDAC inhibitor, recruited AhR and the polymerase II and enhanced H3K47Ac and H3K9Ac on CYP1A1 promotr [157]. Altogether, these studies reveal the importance of histone modifications in the expression of CYP1A1 gene.

The posttranslational expression of the CYP1A1 was shown to be regulated by miRNAs, particularly miR-125b-2, miR-488, miR-657, miR-892a, miR-511, and miR-626 [158,159]. For example, overexpression of miR-892a in human breast cancer MCF-7 cells caused CYP1A1 protein inhibition, which is in agreement with the observations that BaP, a potent CYP1A1 inducer, decreased the miR-892a expression [159]. In addition, Rieger et al., have also shown that miR-21, miR-132 and miR-142-3p negatively regulated the expression of CYP1A1 in human liver tissues [160] but not in human hepatocyte HepaRG cells [161]. Interestingly, the binding sites of these miRNA have been identified on CYP1A1 mRNA [162,163], suggesting a regulatory role for the miRNA in CYP1A1 regulation.

4.3. CYP1B1

The CYP1B1 expression has been found to be lower in hepatic cancer, HepG2 cell line owing to the CYP1B1 promoter methylation. In human liver cancer HepG2 cells [164] and colorectal cancers [165], it was reported that the CpG dinucleotides within the CpG island encompassing CYP1B1 promotr are fully methylated, whereas partially methylated in the enhancer region. This hypomethylation of the CYP1B1 in the enhancer allows XRE/ARNT dimerization with subsequent CYP1B1 induction [164]. CYP1B1 promoter also contains sequence for the Sp1 transcription factor binding that participates in the regulation of CYP1B1. Therefore, the changes in DNA methylation in the CpG motif affect the binding and activity of these transcription factors which subsequently affect CYP1B1 expression [166]. Similar to CYP1A1, the CYP1B1 inducibility in HepG2 cells is induced by the demethylating agent 5-aza-CdR in human hepatoma HepG2 [164] and colorectal SW48 and Caco-2 [165] cells. In human prostate cancer, it has been shown that induction of CYP1B1

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In cancer, it has been shown that DNA methylation of CYP1A1 enhancer region blocked the AhR activation and CYP1A1 induction by the AhR inducer 3-MC in rabbit normal lung R9ab [151] and mouse hepatoma [152] cell lines. Whereas, inhibition of DNA methylation using 5-aza-CdR significantly increased the induction of CYP1A1 expression in cancerous, but not in noncancerous, human prostate LNCaP cells [153], human hepatoma HepG2 [147], human breast MCF-7 and cervical adenocarcinoma cells [154]. Additionally, TCDD protein and mRNA is regulated by the CpG hypomethylation of the promoter region [58], indicating that hypomethylation of CYP1B1 promoter plays a significant role in increasing the CYP1B1 expression in cancer. On the contrary, upregulation of CYP1A1 mRNA in long-term estrogen exposed MCF-7 cells was resulted from CpG methylation of the CYP1B1 promoter [146].

Beedanagari and co-workers found through ChIP analysis that induction of CYP1B1 expression by the trimethylation of histone H3 at

lysine 4 (me3H3K4), acetylation of histone H4 (AcH4), and acetylation of histone H3 at lysine 9 and 14 (AcH3K9 and AcH3K14) were lesser in HepG2 cells than MCF-7 cells both before and after dioxin treatment. Thus, dioxin induces histone modifications at CYP1A1 and CYP1B1 promoter regions in MCF-7 cells leading to increased their expression [164]. It was reported that HDAC inhibitors, TSA or valproic acid, decreased the mRNA expression of CYP1B1 in neuroblastoma UKF-NB-3 and -4 cell lines [151], whereas increased the AhR-mediated induction of the CYP1B1 and AhRR in Caco-2 cells [153], suggesting a cell-specific mechanism. Tsuchiya et al., have shown that the posttranscriptional regulations of CYP1B1 in MCF-7 [167] and HepG2 [168] cells were negatively controlled by miR-27b, where its recognition element (MRE27b) was identified in CYP1B1 mRNA. Furthermore, silencing of miR-27 successfully restored the enzymatic activity and protein expression of CYP1B1 [167]. This suggests that decreased expression of miR-27 could be one of the mechanisms responsible for the higher expression of CYP1B1 in cancer cells.

4.4. CYP1A2

Expression of CYP1A2 is affected epigenetically in a tissue-specific manner. CpG site on the promotor region of CYP1A2 was hypomethylated in murine liver compared to the lung and kidney [169]. CpG hypermethylation of the putative GC box on CYP1A2 promoter in human liver HepG2 cells and tissues showed significant inverse regulation of its mRNA expression [170,171], whereas the enzymatic activity showed inter-individual variation [170]. In that, the inhibition of DNA methyltransferases by 5-aza-CdR induced CYP1A2 transcript levels in human embryonic stem cell-derived hepatocytes, hESC-Hep [172]. In hepatoma Hepa1c1c7 cells, non-expression of CYP1A2 is attributed to the hypermethylation of its CpG site, however, in mouse primary hepatocyte culture, demethylation with 5-aza-CdR did not induce CYP1A2 [169]. Histone modification has been reported to regulate CYP1A2 expression in different cells. In this context, it was demonstrated that inhibition of HDAC by TSA or valproic acid significantly induced CYP1A2 expression and promoter activity in hepatocellular carcinoma Hep3B [173], human breast cancer MCF-7 and cervical cancer HeLa cells [154].

In addition to genetic polymorphisms and transcription factors, the expression of CYP1A2 is influenced by the post-transcriptional regulation of miRNAs. Patients with steroid-induced avascular necrosis of femoral head (SANFH) characterized by low expression levels of miR-320 exhibit high CYP1A2 expression and enzymatic activity, suggesting a negative regulation [174]. In non-cancer liver tissues, the expression of hsa-miR-221-5p and hsa-miR-132-5p were inversely correlated with the expression of CYP1A2 which indicate a possible anti-cancer potential of these miRNAs [175–177]. Moreover, hsa-miR-132-5p interacts with CYP1A2 within the transcript of CYP1A2 3'-UTR through its cognate target and is considered as CYP1A2 regulator [177]. Additionally, the transfection of hsa-miR-132-5p in hepatic stem cells HepaRG and human hepatoma cell lines, Huh-7 and HepG2, significantly reduced basal and lansoprazole-induced CYP1A2 mRNA and protein expression [177], suggesting that targeting hsa-miR-132-5p could be a novel drug target for tone modifications, and miRNAs are believed to play a role in the maintenance of CSCs properties [179,180]. Although the epigenetic regulation of CSCs has been reviewed before [181], the current review will be the first to address the role and impact of the epigenetic regulation of AhR/CYP1 on CSCs. In general, epigenetic regulations of the AhR and downstream genes, particularly CYP1A1 and CYP1B1, have been shown to modulate the proliferation of cancer cells and the development and chemoresistance of CSCs through the modulation of several transcription factors and regulatory genes. In this section, we highlight the most common mechanisms of epigenetic regulation of CSCs by AhR/CYP1 pathways (Fig. 2) in several cancer types which are summarized in Table 2.

5.1. Breast Cancer

Breast cancer is the most extensively studied type of cancer for the investigation of the epigenetic regulations of CSCs by AhR/CYP1 pathway. In general, AhR/CYP1A1 can regulate breast CSCs epigenetically through modulating the expression and activity of four major genes: breast cancer gene 1 (BRCA1), p53, SOX, and β-catenin.

Breast cancer gene 1 (BRCA1) is a tumor suppressor gene that is known to play a vital role in repairing DNA damage and hence the genomic stability. BRCA genes are mainly expressed in the breast tissues and hence, abnormal regulation of BRCA genes either epigenetically or mutationally is known to increase the risk of breast cancer. In that, BRCA1 down-regulation in breast cancer cells is observed to increase CSC populations and characteristics, whereas its up-regulation suppresses CSC features such as colonogenic potential, CD44 expression, and ALDH1A1 activity [182]. Recent studies on breast cancer have reported the impact of epigenesis on the interaction between BRCA genes and AhR [183,184]. In that, BRCA1 gene is subjected to epigenetic modifications and silencing by AhR and regulated genes which caused gene suppression in sporadic breast tumor [185]. The constitutively high AhR expression in human TNBC tumor tissue is associated with BRCA1 promoter CpG methylation, which is higher in TNBC than luminal A, luminal B, and HER2 positive breast cancer, suggesting that AhR/BRCA genes expression could be a molecular marker for TNBC [183]. Additionally, the introduction of AhR inducer, DMBA, in rat mammary tumor increased BRCA1 CpG methylation, CYP1A1 and CYP1B1 expression, and cell proliferation markers Ccnd1 and Cdk4, whereas treatment of estrogen receptor α-negative human sporadic breast cancer UACC-3199 cells with α-NF, an AhR antagonist, was reported to partially rescue BRCA1 expression [183]. At the inducible level, activation of AhR by TCDD in human breast cancer MCF-7 cells permits the association of DNA methyltransferase-1 (DNMT1), mono-methylated-H3K9, and methyl-binding domain protein-2 (MBD-2) with BRCA1 promoter [185]. This effect of AhR on BRCA1 hypermethylation was reversed by AhR inhibition either using siRNA or chemical inhibitor, resveratrol [185]. Although resveratrol has multiple targets other than AhR, the siRNA confirms the involvement of AhR in BRCA1 hypermethylation. Exposure of rats to TCDD, a strong AhR activator and CYP1A1 inducer, *in utero*, revealed an association between hypermethylation of BRCA1 gene promoter in breast tissues and incidence of breast cancer in adulthood. In MCF-7 breast cancer cells, the BRCA1

cell expressing CYP1A2-induced chemotherapy resistance.

5. Mechanisms of the epigenetic regulations of CSCs by the AhR and regulated genes

Epigenetic dysregulation in various types of cancer has been shown to play critical roles in producing CSCs and tumorigenesis via modulating the expression of tumor suppressor and differentiation genes [178]. The silencing of these genes contribute to the formation of CSCs in a tumor population, therefore, DNA methylation, histone modification, and noncoding RNAs have been implicated in the development of CSCs. In breast cancer, AhR has been shown to regulate the epigenetic silencing of BRCA1 gene. BRCA1 repression also results from increased BRCA1 gene occupancy by AhR and HDAC1 and reduced association of histone acetyltransferase (HAT) p300 [186], AcH3K9, acetylated H4 (H4Ac) [185], and SRC-1 with BRCA1. In addition, AhR also confers other epigenetic alterations that lead to BRCA1 repression such as deacetylation of H3K9; elevated levels of DNMT1, DNMT3a, DNMT3b, H3K9me3, and MBD-2; and CpG hypermethylation [185,187]. Therefore, the activation of AhR induces epigenetic silencing of tumor suppressor, BRCA1 gene, which in turn increases CSCs population and markers.

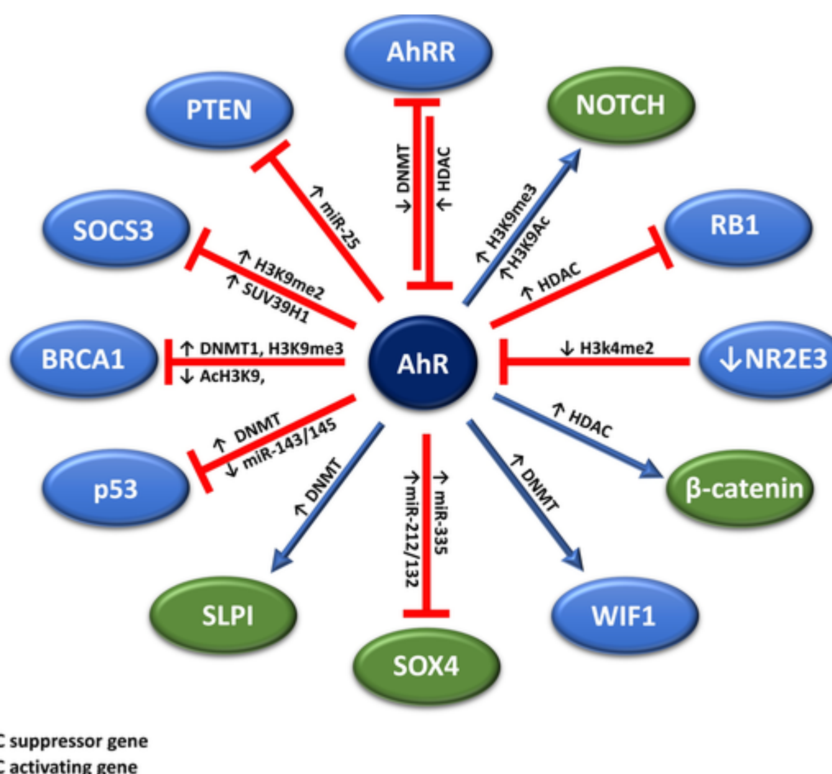

Fig. 2. Schematic diagram summarizing the molecular mechanisms of epigenetic regulation of the CSCs by AhR and the target genes.

Several studies have reported that BRCA1 selectively coactivates p53, a tumor suppressor gene, towards DNA repair and cell cycle arrest in breast cancer [188]. Impaired p53 expression and function in different breast cancer models has been reported to increase CSC expansion and chemoresistance through induction of multidrug-resistant genes [189,190]. Activation of p53 by CP-31398 and PRIMA in breast cancer cell lines (MDA-MB-231, SUM149) inhibited the ALDH^{+} cells and the sphere formation ability [190,191]. The epigenetic regulation of p53 by AhR in breast cancer has not been well studied and warrant further investigation. Locke and his team have demonstrated that induction of carcinogenesis in mammary epithelial cells by AhR activation was associated with increased hypermethylation of p53 binding sites in *EPHB3* and *TRIM6* resulting in repression of the tumor suppressor hsa-miR-143/145 cluster and deregulation of p53 target genes, MDM2 and CDKN1A [192]. Similarly, AhR activation and CYP1 induction by TCDD in human keratinocyte has shown to increase p53 methylation resulting in gene silencing [193]. This AhR-induced hypermethylation of p53 target genes resulted in loss of cell cycle control and breast carcinogenesis [192,194].

SRY-box transcription factor 4 (SOX4) is a pro-metastatic mediator that is highly expressed in different types of cancer, including breast cancer. Overexpression of SOX4 is associated with increased mal breast cells harbour more miR-335 compared to sporadic breast cancer cells, whereas in TNBC tissues, the expression of miR-335 is reduced compared to its adjacent tissues, which increases the chemoresistance of these cells to doxorubicin, paclitaxel, and cisplatin [197]. Furthermore, it was reported that treatment of breast cancer MDA-MB-231 and T47D cells with AhR/CYP1A1 activator such as TCDD suppresses SOX4 expression through an AhR-mediated transcriptional activation of miR-212/132 cluster [198]. On the other hand, it has been shown that inhibition of AhR/CYP1A1 by resveratrol, a well-known AhR antagonist, induced hypomethylation and activation of SOX17, a tumor suppressor gene, in TNBC MDA-MB-231 cells [199].

Wnt/β-catenin is an important regulator of cancer development and initiation. Activation of AhR and CYP1A1 by DMBA has been reported to increase the expression and activity of β-catenin in human breast cancer MCF-7 cells, which was associated with increased CSC properties [200]. Epigenetic study investigating the mechanism of β-catenin activation by AhR inducer BaP has shown an increase in the expression of HDAC-6 in TNBC MDA-MB-231 cells, which in turn contributes to the nuclear formation of β-catenin-LEF1/TCF4 transcriptional complex that subsequently participates in transcriptional activation of its target gene, c-Myc [201]. Moreover, in breast epithelial MCF10A cells, overexpression of AhR caused increased CSCs

breast CSC features such as EMT and $CD44^+/CD24^-$ population [195]. The AhR-mediated epigenetic regulation of SOX4 occurs through the modulation of several miRNAs, such as miR-335 and miR-*212/132. In that, activation of AhR/CYP1A1 pathway by TCDD or 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) in human breast cancer BT474* and MDA-MB-231 cells caused inhibition of the mRNA and protein expression of SOX4 through the induction of miR-335, an effect that was blocked by AhR silencing, suggesting an interaction between AhR and miR-335 for the regulation of SOX4 [196]. The normal breast cells treated with TCDD showed increased proliferation due to methylation of CpG of Wnt inhibitory factor 1 (WIF-1), which encodes inhibitor of Wnt pathway, resulting in activation of Wnt/β-catenin pathway [202]. Another mechanism of AhR epigenetic regulation of c-Myc expression is through the modulation of miR-494 in estrogen receptor MCF-7 cells [203]. Activation of β-catenin and c-Myc is associated with increased mammosphere formation, percentage of SP, and $ALDH^+$ cells [200]. To explore the impact of epigenesis in the crosstalk between AhR and estrogen receptor, Englert and co-workers have demonstrated that long-term estrogen exposed (LTEE) MCF-7 cells increased the expression of AhR

Table 2
Summary of the epigenetic regulations of CSCs by AhR/CYP1 pathway.

Epigenetic Mechanism	Cancer type	Species		Epigenetic modifications by AhR /CYP1 pathway	Genes affected	Effect on cancer/CSCs	Ref
DNA methylation	**Breast**	Human	TNBC patient tissues	High constitutive expression of AhR → hypermethylation of BRCA1	↓ BRCA1	↑ CSC features.	[183,182]
			MDA-MB-231 cells	AhR/CYP1A1 inhibition → hypomethylation of SOX17	↑ SOX17	↑ tumor suppression & ↓ CSC formation.	[199]
			MCF-7 cells	AhR/CYP1A1 activation → ↑ DNMT1,3, H3K9me3, and MBD-2 → hypermethylation of BRCA1 promoter	↓ BRCA1	↑ CSC features.	[185,182]
			MCF-10A cells	AhR/CYP1A1 activation → hypermethylation of WIF1 Promoter	↑ WIF1	↑ Wnt/β-catenin pathway & CSC survival.	[202]
			Mammary epithelial cells	AhR/CYP1A1 activation → hypermethylation of p53 binding sites	↓ p53	↑ cell proliferation through deregulation of MDM2 and CDKN1A	[192]
		Rats	DMBA induced Mammary tumor in Sprague-Dawley rats	AhR/CYP1A1 activation → hypermethylation of BRCA1 promoter	↓ BRCA1	↑ cell proliferation markers (CCnd1 and Cdk4) & promotes CSCs characteristics.	[183]
	Ovarian	Human	Cancer tissues	AhR/CYP1A1 activation → ↑ DNMT1,3, MBD2 → hypermethylation of BRCA1 promoter	↓ BRCA1	↑ CSC features.	[208,209]
			Cancer tissues	AhR/CYP1A1 activation → hypermethylation of AhRR	↓ AhRR	↑ CSC properties	[218]
		Rats	Daily exposure of immature rats to AhR inducers	AhR/CYP1A1 activation → hypermethylation of DMR of Igf2/H19	↓ Igf2 and H19	↑ premature ovarian failure of the offspring rats and ↑ incidence of cancer	[214]
	Colorectal	Human	Cancer tissues	AhR/CYP1A1 activation → hypermethylation of AhRR	↓ AhRR	↑ CSC properties	[218]
			Cancer cells (HCT	AhR/CYP1A1 activation →	↑ WIF-1	↑ AXIN2, ↑ SFRP-1, ↑	[239,240]

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			116, SW 480, CACO2, RKO, DLD1, HT 29, LOVO)	hypermethylation of Wnt pathway members		DKK1, and ↑ CSC differentiation and progression	

Table 2 (*Continued*)

Epigenetic Mechanism	Cancer type	Species		Epigenetic modifications by AhR /CYP1 pathway	Genes affected	Effect on cancer/CSCs	Ref
			Cancer cell lines SW48 and Caco-2 cells and primary colorectal cancers	Hypermethylation of CYP1B1 promoter region DNMT inhibitor 5-aza-CdR → hypomethylation of CYP1B1	↓ CYP1B1	↓ tumor promotion and CSC properties	[165]
					↑ CYP1B1	↑ cell cancer proliferation	
	Liver	Mice	C3H mice	Activation of AhR/ARNT binding in spontaneous liver tumor → hypermethylation of SLPI promoter	↑ SLPI	↑ tumorigenesis in mice hepatic tissues	[222]
	Lung	Human	Smoker subjects	Smokers → ↑ hypomethylation of AhRR	↓ AhRR	↑ risk of lung carcinogenesis	[231]
			Lung cancer tissues	Smokers → ↑ Hypermethylation of CYP1A1	↑ CYP1A1	↑ risk of lung carcinogenesis	[232]
			Blood	Exposure to $PM_{2.5}$ → hypomethylation of AhRR at cg05575921	↓ AhRR	↑ AhR and cancer progression	[230]
			A549 cells	DNMT inhibitors 5-aza-CdR and TSA → hypomethylation of AhRR	↓ AhRR	↑ risk and development of lung cancer	[46,218]
	leukemia	Human	Low AhR expressing REH cells	DNMT inhibitor → hypomethylation of AhR	↑ AhR	↓ tumor and cell cycle progression	[144]
			Primary ALL patients	Hypermethylation of AhR promoter in 33% of ALL	↓ AhR	ALL tumor	
	Prostate	Human	LNCaP cells	DNMT inhibitor → hypomethylation of CYP1A1	↑ CYP1A1	↑ Prostate cancer initiation & progression	[58]
			PC-3 cells	AhR/CYP1B1 activation → hypomethylation of CYP1B1	↑ CYP1B1	↑ cancer initiation rather than progression	[58]
Histone modification	**Breast**	Human	MCF-7 cells	↑ HDAC1 → deacetylation of H3K9 and ↑ expression of H3K9me3	↓ BRCA1	↑ CSC features.	[186] [185,187]
				↓ HAT → AcH3K9, H4Ac			
				AhR/CYP1A1 activation by LTEE → ↓	↑ AhR	↑ breast carcinogenesis	[146]

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				trimethylation of histone 3 at lysine 27			
				↑ association of mono-methylated-H3K9	↓ BRCA1	↑ CSC features.	[185,182]
			MDA-MB-231 cells	AhR/CYP1 activation → ↑ HDAC6	↑ β-catenin-LEF1/TCF4 transcriptional complex	↑ transcriptional activation of CSC regulating genes, c-Myc	[201]

Table 2 (*Continued*)

Epigenetic Mechanism	Cancer type	Species		Epigenetic modifications by AhR /CYP1 pathway	Genes affected	Effect on cancer/CSCs	Ref
	Ovarian	Human	IGROV-1 cells	AhR/CYP1 activation by 5F203 → phosphorylation of histone H2AX	↑γH2AX	↑ DSB and affect DNA damage response in CSCs	[216]
				AhR inhibition by α-NF → ↓ phosphorylation of H2AX	↓γH2AX	↑ CSC features	[216]
		Rats	Immature rats	AhR/CYP1 activation by 3-MC → ↑ trimethylation of H3K4me3 and ↑ acetylation H3K9Ac	↑ Notch2, ↑ Hes1, ↑ Cyclin D, and ↑ Akt expression	↑ CSC features	[215]
	Liver	Mice	Hepa1c1c7 cells	AhR/CYP1 activation → ↑ HDAC-1,-2, -3, -4, -7, -8,-9, and -10, of RB1	↓ RB1	↑ tumor progression and promotes CSC expansion, ↑ mammospheres formation, SP, and ALDH $^{+}$ cells	[219,221]
			In vivo Rd7 mice	NR2E3 depletion → ↑ HDAC, H3K4me2	↓ AhR and CYP1A1 expression	↓ tumor promotion and CSC progression	[225]
		Human	HepG2 cells	NR2E3 depletion → ↑ HDAC, H3K4me2	↓ AhR and CYP1A1 expression	↓ tumor promotion and CSC progression	[225]
				Curcumin administration → ↑ HDAC	↓ CYP1A1	↓ cell proliferation	[147]
				HDAC inhibitor TSA	↑ AhR-XRE	↑ cell proliferation	
	Lung	Human	BEAS-2B cells	AhR/CYP1A1 activation by BaP and As → ↑ SUV39H1 and H3K9me2	↓ SOCS3	↑ CSC sphere formation, Akt/ERK expression, ALDH $^{+}$, and tumorigenicity	[233]
		Mice	xenograft tumors in nude mice	Nude mice injected with BaP + As treated BEAS-2B cells → ↑ SUV39H1 and H3K9me2	↑ p-Akt	↑ lung tumor size and formation	
MicroRNAs	**Breast**	Human	BT474 cells	AhR/CYP1 activation → ↑ miR-335	↓ SOX4	↓ cancer invasion and chemoresistance to chemotherapy	[196] [197]
				AhR/CYP1 activation → ↑ miR-212/132	↓ SOX4	↓ CSC formation, ↓metastasis, ↓ migration and ↓	[198]

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						invasion properties	
			MDA-MB-231 cells	AhR/CYP1 activation → ↑ miR-335	↓ SOX4	↓ cancer invasion and chemoresistance to chemotherapy	[196] [197]

Table 2 (*Continued*)

Epigenetic Mechanism	Cancer type	Species		Epigenetic modifications by AhR /CYP1 pathway	Genes affected	Effect on cancer/CSCs	Ref
				AhR/CYP1 activation → ↑ miR-212/132	↓ SOX4	↓ CSC formation, ↓metastasis, ↓ migration and ↓ invasion properties	[198]
			MCF-7 cells	AhR/CYP1 activation → ↓ miR-494	↑ c-Myc	↑ mammospheres formation, percentage of SP and ALDH $^+$ cells.	[203]
			Mammary epithelial cells	AhR/CYP1 activation → ↓ hsa-miR-143/145	↓ p53	↑ cell proliferation of cancer cells through deregulation of MDM2 and CDKN1A	[192]
	Liver	Human	HepG2 cells	AhR/CYP1 activation → ↑ miR-25	↓ PTEN	↑ CSC features and ↓ sensitivity of liver CSCs to TRAIL-induced apoptosis	[224]
	Prostate	human	PC-3 and DU145	AhR/CYP1 activation → ↑ miR-150-5p	↑ AhR ↓ MAP3K12	↓ cancer cell proliferation and invasion	[69]
	Leukemia	Human	Patient samples	Transcriptional activation of the AhR/CYP1 pathway regulates miR-129 expression	miR-129	↑ tumor progression	[54]
	Multiple Myeloma	Human	MM1.s	AhR/CYP1 activation → ↑ miR-25, miR-15a, miR-16, miR-92, miR-125b, miR-141, and miR-200a	↓ P53	↑ tumor progression	[55]
	Neuro blastoma	Human	SK-N-SH	AhR is negatively controlled by miR-124	miR-124	↑ tumor progression	[242]
	lung	Mice	AhR $^{-/-}$ mice	AhR $^{-/-}$ mice exposed to cigarette smoke	↑ miR-96	↑ cancer invasion and metastasis	[149]

through histone modification. Moreover, decreased trimethylation of histone 3 at lysine 27 was particularly seen at the proximal promoter of AhR in LTEE MCF-7 cells. The promoter of AhR in LTEE MCF-7 cells is not associated with the markers of epigenetic silencing which caused increased expression of AhR contributing to breast carcino-[206,207]. In that, silencing of BRCA1 gene by hypermethylation of its promoter was demonstrated in primary ovarian carcinoma. BRCA1 promoter hypermethylation has been confirmed as a factor for BRCA1 gene inactivation in ovarian cancer patients and several ovarian cancer cell lines [208,209]. AhR is found to be an epige-

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genesis [146]. These studies demonstrate the differential role of AhR in breast cancer as it participates in various epigenetic modifications.

5.2. Endometrial and Ovarian Cancer

Ovarian cancer is the most lethal out of all gynecological malignancies and stands fourth in terms of cancer related deaths globally. Ovarian cancer is characterized by nonspecific and vague symptoms that make two-third of patients diagnosed only at late-stages leading to poor prognosis (II-IV) [204]. Small tumor initiating stem cells has been discovered in borderline ovarian carcinoma that formed tumor like spheroids *in vitro* with specific CSC like properties [205]. In ovarian cancer, an increased methylation of tumor suppressor genes is observed, among which BRCA1 is a major mutated gene netic disruptor of BRCA-1 gene which has a major role in tumor initiation and progression in ovarian cancer. For example, AhR activation and CYP1 induction by TCDD causes epigenetic modifications in the BRCA1 gene through CpG hypermethylation and hence silencing of BRCA1 expression [187]. This effect is attributed to the presence of several XREs at the proximal promoter region of BRCA-1 that are responsible for endogenous AhR functioning [187]. In addition, it was reported that TCDD enriched the crosstalk between AhR and DNA methyl transferases-1, -3a, 3b; MBD-2, trimethylated H3K9 (H3K9me3), and the BRCA1 promoter. The observations from this study provides evidence on the role of AhR agonists in BRCA1 promoter hypermethylation. A transient relationship between AhR expression and DNA methylation status during embryonic development has also been established, illustrating a role of AhR in configuring a

repressive chromatin structure regulating the stem cell differentiation [210].

AhR is known to be expressed in different ovarian cells, including follicles and oocytes in almost all species. Although, PAHs, which are potent AhR activators, are also known potent ovotoxicants, the role of the AhR/CYP1 pathway has not been extensively investigated in endometrial and ovarian cancer. Exposure to TCDD, a well-known AhR agonist, was found to stimulate pubertal malformations and ovarian disease in 4 months old F3 generation rats [211]. TCDD exposure of the fetus during gonadal sex determination was also found to alter the epigenetic programming thereby transferring the altered epigenome to the next generations promoting disease occurrence [212]. *In vivo* animal study involving the investigation of epigenetic transgenerational activity of TCDD has shown a higher incidence of poly cystic ovarian disease (PCOD) in the F1 and F3 generations [213]. Having known that PCOD is a predisposing factor for ovarian cancer, this finding can be clearly correlated with the involvement of AhR in epigenetic modification of the ovaries thereby acting as a predisposing factor for the ovarian cancer. A study monitoring the DNA methylation analysis of rats exposed to TCDD identified an increased hypermethylation in high dose TCDD induced rats. This study also speculates that TCDD exposure to mother might lead to premature ovarian failure of the offspring rats [214]. In addition, it has been shown that daily exposure of immature rats to 3MC, a potent AhR activator, increases ovarian cancer stemness through AhR binding to promoter regions of CYP1A1 and genes involved in cancer stemness and proliferation such as Notch2, Hes1, Cyclin D, and Akt, an effect which was blocked by α-NF, an AhR antagonist, indicating an AhR-dependent mechanism [215]. Induction of these stemness genes was shown to be attributed to 3MC-induced histone modification, particularly trimethylation of H3 lysine4 (H3K4me3) and acetylation of histones H3 (H3K9Ac), leading to ovarian diseases [215]. Furthermore, activation of AhR/CYP1A1 pathway using 5F203 in human ovarian cancer IGROV-1 cells induced DNA double strand break which mediated phosphorylation of histone H2AX, a marker for cancer progression [216], producing γH2AX at the double strand break site, which was blocked by the AhR antagonist, α-NF [217], confirming that AhR activation is needed to induce histone modification in ovarian cancer. Another possible role of epigenesis on the effect of AhR in ovarian cancer, is the modulation of AhR repressor, AhRR. The involvement of AhRR, as a potential tumor suppressor, has been reported in different cancer types. It has been reported that AhRR is downregulated in cancer through hypermethylation of its promoter region. Specifically, almost 100% of ovarian cancer tissues exhibited AhRR hypermethylation, whereas no methylation was found in normal tissues [218]. Therefore, the absence of AhRR expression in cancer cells decreases its competition with ARNT for binding to AhR and XRE, resulting in activation and induction of its tumor activating genes, CYP1A1 and CYP1B1.

quences are also illustrated in HCC [220]. Differential expression of HDAC levels in CYP1A1 gene is also found in human and mice hepatoma cells accounting for the differential expression of these genes in both cells [221]. In addition to HDAC-6, the induction of AhR in liver cancer cells also increases the expression of HDAC-8 which represses tumor suppressor gene RB1, and thus promotes cell proliferation [219]. In an *in vivo* study on C3H mice with spontaneous liver tumor, it was reported that the differentially methylated regions of secretory leukocyte peptidase inhibitor gene (SLPI), a serine protease inhibitor that is overexpressed in various types of cancer, including the core sequence of XRE, and that hypermethylation of CpG in the XRE core sequence in SLPI may be involved in SLPI expression through AhR activation [222].

The differential expression of miR-25 has been observed in a number of solid tumors [223]. In liver CSCs, miR-25 is overexpressed compared to non-CSCs through modulation of PTEN expression in HepG2 cells. In that, knockdown of miR-25 increases expression of PTEN and the sensitivity of liver CSCs to TRAIL-induced apoptosis [224]. Therefore, miR-25 is considered as a potential oncogenic miRNA which is activated through AhR and that overexpression of this miRNA in liver CSCs reduces their sensitivity to TRAIL-induced apoptosis and its expression in these cells is also inversely related to PTEN expression. In addition to the correlation of miR-25 and AhR, a crosstalk between NR2E3 and AhR has also been reported, in that AhR and CYP1A1 expressions in human liver cancer HepG2 cell line has been found to be positively regulated by NR2E3, an orphan nuclear receptor and oxidative stress-responsive epigenetic regulator that is overexpressed in HCC. Loss of NR2E3 expression in *in vivo* mice (Rd7) and in *in vitro* HepG2 cells enhanced histone demethylation of histone 3 lysine 4 di-methylation (H3K4me2), to the AhR gene promoter region, resulting in repression of AhR function and CYP1A expression and enzymatic activities [225]. These results indicate that AhR-mediated liver cancer occurs through epigenetic histone modification by NR2E3.

5.4. Lung Cancer

Lung cancer is the leading cause of death globally in both sexes [226]. Exposure to environmental pollutants such as PAHs has been well linked to increase the risk of lung cancer [56]. High inducibility of AhR/CYP1A1 in smokers of tobacco, which contains numerous PAHs, is known as a risk factor for lung cancer. Hypomethylation of AhR regulated gene, AhRR, has been reported in smokers [227]. A detailed study on the smoking associated methylation of AhRR was done earlier which found a positive correlation with the time since quitting of smoking, whereas showed a negative correlation with the number of cigarettes smoked per day and the urinary concentration of cotinine [228]. Another recent study conducted on non-smokers aimed at assessing the association between exposure to high concen-

5.3. Liver Cancer

In hepatocellular carcinoma (HCC), AhR promotes tumorigenesis in association with a proto-oncogene, intestine-specific homeobox (ISX) [57]. In HCC, AhR is found to regulate the epigenetic histone acetylation as well as deacetylation by HAT and HDAC, respectively, where imbalance of these two enzymes is associated with cancer. AhR activation and HDAC overexpression is highly correlated in HCC development, in that a concomitant expression of HDAC enzymes HDAC-1, -2, -3, -4, -7, -8, -9, and -10 was observed with higher expression of AhR [219]. Furthermore, activation of AhR with TCDD in hepatoma cells leads to the activation of HDAC expression, which in turn binds to and then suppresses the tumor suppressor gene, RB1. DNA methylation at the cytosines of CpG concentrations of environmental particulate matter $PM_{2.5}$, which is also positively related to lung cancer, and blood AhRR methylation at cg05575921 showed inverse relationship [229]. Indeed, subjects who lived in areas with higher $PM_{2.5}$ concentrations exhibited low blood AhRR methylation levels. This is explained by the fact that areas with higher $PM_{2.5}$ are associated with higher PAHs levels which are potential activators of AhR [230]. This is in agreement with other studies which showed that hypomethylation of AhRR using demethylating agent, 5-aza-CdR or TSA increases its expression and the risk and development of lung cancer [46,218]. In contrast to the above mentioned information, Chen et al., illustrated that inactivation of the AhR pathway rather than hypomethylation of the repressor AHRR, is more important and possibly leads to smoking-mediated lung carcinogenesis [231].

DNA methylation of CYP1A1-mediated PAH metabolism was found to be affected by smoking. Higher levels of hydrophobic DNA adduct due to methylation of CYP1A1 were observed in lung tumor samples when compared with corresponding histologically normal lung from lung cancer patients [232]. In that, quantitative analysis of the DNA methylation levels at multiple CpG sites of CYP1A1, which carries three functional XREs, showed hypermethylation at the enhancer region of *CYP1A1* in lung cancer [232]. On the other hand, it has been reported that AhRR causes histone deacetylation of XRE-containing gene promotor and hence abrogation of the transcriptional activation of AhR leading to reduced risk of lung carcinogenesis [46]. Co-exposure to AhR/CYP1 inducer, BaP, and heavy metal, Arsenic (As), has been shown to synergistically induce lung cancer CSCs, sphere formation ability, ALDH expression in *in vitro* human bronchial epithelial BEAS-2B cells and increase lung tumorigenicity in *in vivo* mice model [233]. This synergistic effect is attributed to the induction of AhR and CYP1A1 expression causing epigenetic upregulation of histone H3 lysine 9 methyltransferase SUV39H1 and H3 lysine 9 dimethylation (H3K9me2), which further decreases the expression of tumor suppressive SOCS3 leading to activation of Akt/ERK, CSCs properties, and tumorigenicity [233]. Furthermore, exposure of $AhR^{-/-}$ mice to cigarette smoke, which is known to contain many PAHs such as BaP, significantly increases the expression of miR-96, which promotes cancer invasions and metastasis [149].

5.5. Prostate Cancer

Incidence of prostate cancer is known to be positively correlated to exposure to environmental pollutants, such as PAHs, that mediate AhR activation [234]. AhR is established to modulate several signaling pathways in prostate cancer, in that AhR and its regulated genes, CYP1A1 and CYP1B1, were found to be overexpressed in prostate cancer tissues and cell lines, whereas they were entirely absent in benign prostate tissues and non-cancerous cell lines [58,235,236]. The effect of cancer related epigenetic mechanisms on suppressing PAH-induced activation of CYP1A1 in prostate cancer was analyzed by Okino and co-workers who reported that DNA methylation inhibitor, 5-aza-CdR, increases TCDD-induced CYP1A1 mRNA expression in cancerous LNCaP, but not in non-cancerous PWR1E and RWPE-1 prostate cells [153]. An increased enhancer methylation was also observed in LNCaP cancer cells that directly inhibit XRE function, but this was not observed in non-cancerous prostate cells RWPE-1 cells, suggesting the role of AhR hypermethylation in prostate cancer progression. A lack of histone modification especially histone acetylation of H3 and H4, which are important markers of active genes, was also observed in normal prostate cells than cancer cells upon TCDD treatment [153]. This could be attributed to the recruitment the expression of miR-150-5p via mitogen-activated protein 3 kinase 12 (MAP3K12)-mediated mechanism [69]. This antiproliferative effect of TCDD is reversed by using either miR-150-5p inhibitor or AhR knockdown [69].

5.6. Colorectal Cancer

Epigenesis of the colorectal cancer has been recently reviewed by Goel and Roland [238]. AhR and its ligands; both endogenous and exogenous, are found to have a major role in the colon carcinogenesis. A study carried out in seven colorectal cancer cell lines as well as 40 primary colorectal cancer tissues, to investigate the epigenetic mechanisms for the expression of both CYP1A1 and CYP1B1 genes, showed a hypermethylation in the promoter region of CYP1B1, but not in CYP1A1. Interestingly, the CpG islands within the 5' region of both genes were methylated in cancer cells but not in primary cancer tissue [165], suggesting a tissue-specific epigenetic regulation in colorectal cancer. In colon cancer, enhanced promoter methylation thereby activation of Wnt/β-catenin pathway as well as silencing of Wnt inhibitors genes such as WIF-1, AXIN2, SFRP1, and Dickkopf-related protein-1 (DKK1) were observed [239]. For instance, AhR ligands were reported to significantly influence colonic stem cell homeostasis, gene expression, and regulate their reaction to Wnt/β-catenin pathway initiating stem cell differentiation as well as renewal in colorectal cancer [240]. This finding suggests that AhR through its E3 ubiquitin ligase activity, facilitates β-catenin accumulation via transcriptional regulation of the Wnt/β-catenin pathway inhibitors [240].

5.7. Leukemia, Multiple myeloma, and Neuroblastoma

Although the epigenetic control of AhR pathway has not been thoroughly studied in leukemia, Rager and his colleagues have demonstrated that AhR expression and activity are controlled by multiple miRNAs, such as miR-125b, miR-126, miR-142-3p, miR-155, miR-223, miR-29a, and miR-29b, which are known to play role in leukemogenesis [241]. In addition, Scoville et al., have reported that transcriptional activation of the AhR/CYP1 pathway regulates miR-129 expression in natural killer (NK) cells leading to impairment of NK function and hence tumorigenesis, which is reversed by AhR antagonist, CH223191 [54]. Activation of AhR with BaP and TCDD in human multiple myeloma cells (MM1.s) activate the expression of several p53-targeting miRNAs, such as miR-25, miR-15a, miR-16, miR-92, miR-125b, miR-141, and miR-200a that interact with the 3'-UTR of p53 gene leading to repression of p53 tumor suppression effect [55]. Huang and colleagues have shown that AhR activation promotes neuroblastoma cell growth. Furthermore, increased AhR expression and activity was reported to be negatively controlled by miR-124 in 13 patients with neuroblastoma and also in

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of proteins with histone acetyl transferase activity by AhR [237]. In addition, it has been reported that induction of CYP1B1, but not AhR, by TCDD in human prostate adenocarcinoma PC-3 cells and prostate cancer tissues was potentiated by aberrant CpG promoter/ enhancer hypomethylation as evidenced by treatment with a DNA methyltransferase inhibitor, 5-aza-CdR [58]. These findings could be explained by the observations that AhR/ARNT complex could not bind to methylated XRE [151] as in the case of benign prostate samples, indicating that epigenetic hypomethylation of CYP1B1 and XRE have a crucial role in prostate cancer initiation rather than progression [58].

The miR-150-5p and 3p are known tumor suppressers in prostate cancer and their downregulation is linked to poor prognosis. On contrary to the carcinogenic role of AhR, Yu and colleagues have shown that activation of AhR by TCDD suppresses the proliferation and invasion of prostate cancer (PC-3 and DU145) cells through enhancing neuroblastoma (SK-N-SH) cell lines, in which silencing of miR-124 enhanced AhR-induced neuroblastoma cell proliferation [242].

6. Summary, Remarks, and Future directions

The emergence of CSCs in solid as well as hematological malignancies have been a threat to treatment strategies. Several signaling cascades have been found relevant in imparting chemoresistance in CSCs in several malignancies thereby contributing to poor prognosis and higher recurrence and relapse. Since CSCs are known to be tumor-initiating cells and are major targets for chemical carcinogens, it is highly possible that these cells are regulated by the AhR. The role of AhR/CYP1 pathway in carcinogenesis and cancer initiation as well as its potential use as a therapeutic target has been studied in all cancer types. The function of AhR in CSCs has recently gained attention due to the severe impact of CSCs on chemoresistance, disease recurrence, and poor patient survival. Data available on the exact

role of AhR in CSC regulation and progression in different tumor types is still very controversial and requires regular evaluation and summarization of recent insights in the field. The AhR/CYP1 pathway is known to have tumor activator or suppressor activities depending on the phenotype of the target cancer cells. The hypothesis that AhR activation enhances CSCs self-renewal and progression, is supported by several reports which showed that CSCs of different cancer types exhibit a higher expression and functional levels of AhR than corresponding differentiated non-CSCs. The activation of AhR induces chemoresistance through the expression of the CSC markers such as ALDH and SP. Moreover, it facilitates the growth, maintenance, and production of long-lived secondary mammospheres, from primary progenitor cells, through the activation of AKT/Wnt/β-catenin signaling pathways. In addition, AhR supports the proliferation, invasion, metastasis, and survival of the CSCs in choriocarcinoma, hepatocellular carcinoma, oral squamous carcinoma, and breast cancers leading to therapy failure and tumor recurrence. On the other hand, the anti-tumor effect of AhR is also supported by several studies which showed that activation of AhR/CYP1A in several cancers represses spheres formation and expression of Notch, β-catenin, Nanog, and $ALDH^{+}$ cells [66]. The presence of AhR ligands such as TCDD, DMBA, and BaP ubiquitously in environment promotes cancer invasion and permits cancer progression through epigenetic modifications. Activation of the AhR/CYP1A1 pathway is known to induce epigenetic repression of many tumor suppressor genes such as BRCA1, p53, AhRR and/or activation of many tumor activating genes, such as WIF-1, β-catenin, and NOTCH, though modulation of their DNA methylation, histone acetylation/deacetylation, and the expression of several miRNAs. This makes AhR an important target for development of anti-cancer therapies. In cancers, where the treatment options are limited, the potential of AhR can be exploited for the development of new class of anti-cancer drugs. Overall, there is an essential need to further understand the molecular mechanisms of epigenetic regulation of CSCs by AhR for optimum cancer treatment, patient survival, and prevention of chemoresistance.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors are very grateful to the Office Research Support at Qatar University (grants no. IRCC-2019-006, QUCG-CPH-20/21-1, QUST-1-CPH-2020-6) and the Qatar Foundation – Qatar National Re-

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search Fund (grant no. UREP24-163-3-049) to HMK for funding this work.

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