AhR, inflammation and breast cancer

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Contributor: Tiziana Guarnieri

AhR, an environmentally sensitive transcription factor, is one of the more evolutionary conserved molecules in living cells.

Aryl Hydrocarbon Receptor | Cytochrome P450 Family | TCDD

1. History

Since 550 million years ago, it has constantly been expressed in animal cells, but its exact physiological role remains elusive [1]. AhR was initially identified as a xenobiotic sensor and a key regulator of xenobiotics metabolism and persistent chemicals of concern, such as halogenated aromatic hydrocarbons (HAHs) and polycyclic aromatic hydrocarbons (PAHs). These compounds degrade very slowly in the environment, bio-accumulate in the food chain and are lipid soluble. Typically, they may be detected in human blood, adipose and breast tissue, where dichlorodiphenyldichloroethylene (DDE), the major metabolite of DDT (dichloro-diphenyl-trichloroethane), and PCBs (polychlorinated biphenyls) are the most prevalent contaminants. In this scenario, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most infamous member of this class of environmental pollutants and one of exogenous best agonists of AhR. Considering the evolutionary conservation, it makes sense to hypothesize that over time, biosensor functions have been added to its physiological role, which to this day remains elusive. As Mulero-Navarro and Fernandez Salguero recently pointed out, the role of AhR in systems homeostasis preceded its participation in xenobiotic sensing, as this function is lacking in invertebrates, where AhR homolog is a pivotal gene in the development of the nervous system, antennae, eyes and legs.

2. Structure, Physiology and Target Genes

In the cytoplasm, AhR forms a macromolecular inactivating complex with two heat shock proteins 90 (HSPs90), one XAP2 (hepatitis B virus X-Associated Protein 2 or AIP, Immunophilin-like Ah Receptor-interacting protein), one HSP90 co-chaperone protein named p23 and one pp60 src (Figure 1). After ligand binding, the AhR-inactivating complex is remodeled. XAP2 is released and, soon after, the complex moves to the nuclear compartment. Here, the two HSPs90 proteins, together with p23 and pp60 src, are replaced by AhR Nuclear Translocator (ARNT)/ hypoxia-induced factor β (HIF-1β). This heterodimer is the active form of AhR. It regulates the transcription of target genes through binding to the xenobiotic responsive elements (XREs) in their promoter containing the sequences 5′-GCGTG-3′ or 5′-ACGTG-3′. Once the transcription starts, AhR separates from XRE and is exported out of the nucleus by Exportin 1 (XPO1), also known as chromosome region maintenance 1 (CRM1). In the cytoplasm, it is inactivated by 26S ubiquitin–proteasome. Intriguingly, AhR self-regulates its nuclear activity as it

activates the gene encoding for Aryl hydrocarbon Receptor Repressor (AhRR). This repressor competes with AhR for the binding with ARNT and forms the inactive heterodimer AhRR/ARNT. In this way, AhR lacks its partner that makes it transcriptionally active and its nuclear activity is hindered. Another target of AhR is the TCDD-inducible Poly ADP-Ribose Polymerase (*TIPARP*) gene, which negatively regulates *AR* expression and thus AhR levels . These negative feedback mechanisms, together with the nuclear exportation and ubiquitin-proteasome-mediated degradation of AhR, regulate the transcription of AhR-responsive genes. Back in 1983, Israel and Whitlock showed that cytochrome P_1-450 genes (*CYP450*, class 1A (1 and 2)*)*, encoding enzymatic metalloproteins involved in endogenous and exogenous substrates transformation, are targeted from “the TCDD receptor”, later identified as the AhR. In the year 2000, Nebert group described four additional AhR target genes: 1. *ALDH3A1* (Aldehyde dehydrogenase family 3, subfamily 1); 2. *GSTA1* (Glutathione S-transferase, alpha 1); 3. *NQO1*, (NAD(P)H dehydrogenase quinone 1); 4. *UGT1A6* (UDP glucuronosyltransferase family 1 member A6). Together with *CYP1A1 AND CYP1A2* genes, they form the so-called “AhR gene battery”, which controls the cell cycle and the initiation of the apoptotic cascade. Later, other groups confirmed and extended the list of AhR-responsive genes which are involved in different steps of cell lifecycle and metabolism. These include also Phase III transporters, as the Proteins 2 and 3 associated to multidrug resistance (*MRP2-MRP3*), the organic anionic and cationic transport proteins (*OATP and OCTP*), the breast cancer resistance proteins (*BCRP*) [1]. More recently, it has been suggested that AhR participates in pluripotency and stemness regulation through a possible link with transposable elements. Alternative, “unorthodox” signaling has been described in the last few years, as it has been observed that some AhR-responsive genes do not contain the XREs. This is the case for plasminogen activator inhibitor-1 (*PAI-1*), encoding a fibrinolysis inhibitor which has been connected to inflammatory endothelial fibrosis. The *PAI-1* promoter is not a classical XRE, but is responsive to TCDD, one of the more potent exogenous agonists of AhR binding. In fact, Wright group described a nonconsensus XRE (NC-XRE) in *PAI-1* promoter which interacts with AhR alone when it is associated to Kruppel-like factor 6 (KLF6). AhR dimerizes with the KLF family member KLF6 and binds to a novel nonconsensus XRE (NC-XRE) in the promoter of target genes after TCDD exposition. NC-XRE and XRE have no sequence homology and interact with different proteins, this suggesting that AhR also has different targets. In particular, they demonstrated that the complex AhR/KLF6 is of pivotal importance in the control of cell cycle, as it regulates the expression of p21Cip1, or, cyclin-dependent kinase inhibitor 1A (CDKN1A), which inhibits the cell cycle and regulates cell metastasis by switching between invasion and proliferation. Considering these data, it is reasonable to hypothesize that originally AhR had a checkpoint role in the cellular metabolism and that, over time, AhR has acquired the ability to bind to a multitude of molecules, both exogenous and endogenous. Over time, it has been demonstrated that, due to its promiscuous binding site, AhR is responsive not only to exogenous, but also to a variety of natural molecules, among which some derivatives of arachidonic acid as prostaglandins and leukotrienes, lipotoxin A 4 and 7-ketocholesterol , the hemoglobin catabolites bilirubin and biliverdin, the tryptophan products kynurenine (Kyn), tryptamine and 6-formylindolo[3,2-b] carbazole (FICZ), some indole metabolites derived from diet and from host bacteria metabolism, such as Indole-3-acetic acid (IAA) and Indol [3,2-b]carbazole (ICZ) (Figure 2). The list of possible AhR ligands is constantly growing and some of these compounds are now included into the category of selective AhR modulators (SAhRMs), that bind AhR with low to medium affinity. Interestingly, they can behave as an agonist, antagonist or a mixed agonist/antagonist, depending on the metabolic set-up of the organism with which they come into contact. Most information about the

role of AhR in organ and system physiology comes from multiple systemic abnormalities described in AhR^{-}/AhR^{-} mice. Obviously, these rodents are not sensitive to TCDD and are also smaller in size than AhR^{+}/AhR^{+} mice. They exhibit a spectrum of anomalies that includes reduced fertility, portal tract fibrosis, hepatocyte microvesicular fatty metamorphosis, cardiac hypertrophy, epidermal hyperplasia, T cell deficiency in the spleen and altered circadian rhythms[2].

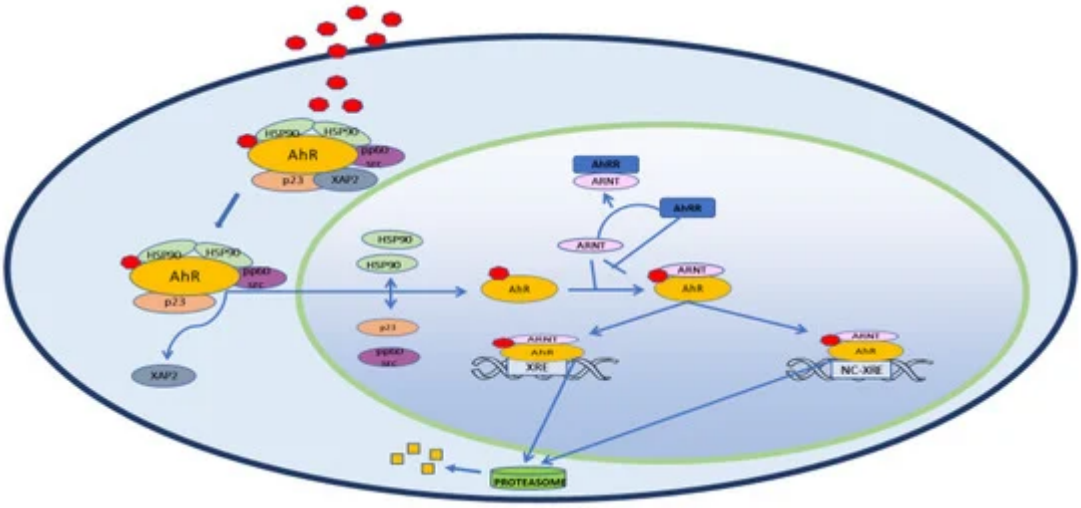

Figure 1. The Aryl hydrocarbon receptor (AhR) pathway. This transcription factor usually resides in the cytoplasm, where it associates with some inactivating molecules: two HSPs90s, one XAP2 or AIP, one HSP90 co-chaperone protein named p23, one pp60(c-src). Once it binds a ligand, XAP2 leaves the complex that moves to the nucleus. Here, the two HSP-90s, p23 and pp60-src drop AhR that binds to ARNT. This new complex is now ready to interact with XRE of genes like *CYP1A1*, *CYP1A2*, *CYP1B1* and *AHRR*, usually containing the sequences 5'-GCGTG-3′ or 5′-ACGTG-3′. AhR also targets NC-XREs, which bind AhR, while not containing the canonical consensus sequence. Just before the transcription starts, AhR moves from XREs, is translated out of the nucleus by XPO1 (not shown) and is degraded in the cytoplasm by 26S ubiquitin–proteasome. AhRR = Aryl Hydrocarbon Receptor Repressor; AIP = Immunophilin-like Ah Receptor-interacting protein); ARNT = Aryl Hydrocarbon Nuclear Translocator; CYP1A1 = Cytochrome P450 Family 1 Subfamily A Member 1; CYP1A2 = Cytochrome P450 Family 1 Subfamily A Member 2; CYP1B1 = Cytochrome P450 Family 1 Subfamily B Member 1; HSPs90 = Heat Shock Proteins 90; NC-XRE = Nonconsensus Response Element; p23 = Proteolytically Resistant 23-kDa protein; pp60(c-src) = Proto-oncogene tyrosine-protein kinase 60 (Sarcoma); XAP2 = Hepatitis B virus X-Associated Protein 2; XPO1 = Exportin 1; XRE = Xenobiotic Response Element.

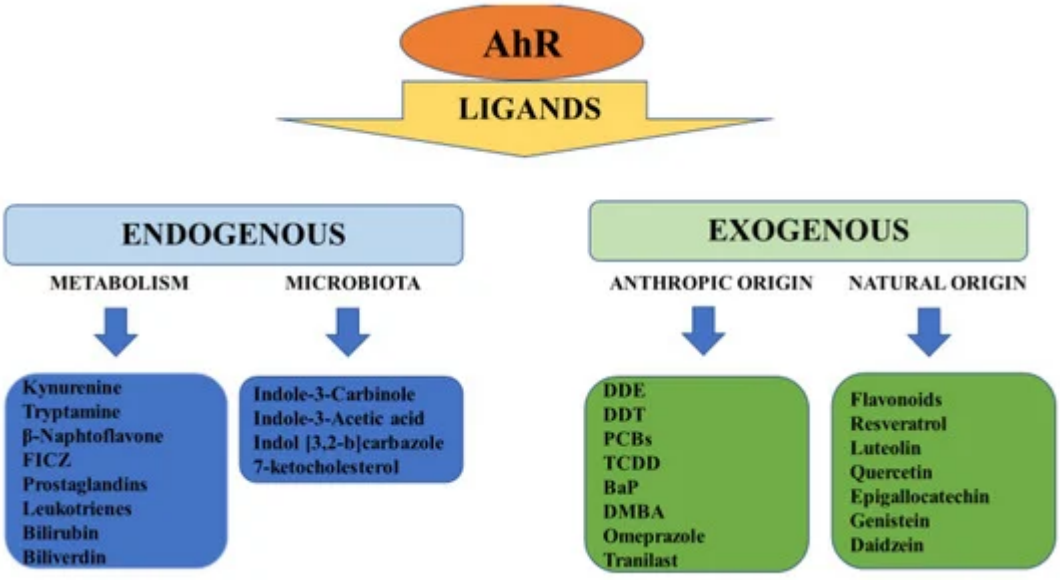

Figure 2. Endogenous and exogenous ligands of AhR. Endogenous ligands come from anabolism (kynurenine, tryptamine, β-Naphtoflavone, FICZ, prostaglandins, leukotrienes) and catabolism (bilirubin, biliverdin) of individuals and their commensal flora (microbiota: Indole-3-Carbinole; Indole-3-Acetic acid; Indol [3,2-b]carbazole; 7-ketocholesterol). Exogenous ligands can be manmade products which have a heterogeneous nature and uses (DDE, DDT, PCBs, TCDD, BaP, DMBA, Omeprazole Tranilast). Several exogenous ligands of natural origin are contained in plants and vegetable foods (Flavonoids, Resveratrol, Luteolin, Quercetin, Epigallocatechin, Genistein, Daidzein). DDE = dichlorodiphenyldichloroethylene; DDT = dichloro-diphenyl-trichloroethane; PCBs = polychlorinated biphenyls; TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin; BaP = Benzo [a] pyrene; DMBA = 7,12-Dymethylbenz-[α]-anthracene.

3. AhR and Inflammation

From 2000 onwards, various groups have reported a connection between AhR and inflammatory phenomena in different experimental models. In 2002, Hennig group showed that in endothelial cells, the PCB-mediated activation of AhR promotes inflammatory atherosclerotic phenomena, passing through the transcriptional activation of NF-B and the increase in IL6 production. NF-kB/AhR interactions in inflammation were also described in detail by Tian and colleagues and, in the same period, an interesting review by Dalton was focused on the connection between AhR, inflammatory signaling and oxidative stress. Over the years, AhR's pathway has been associated with various inflammatory markers, including cyclooxygenase-2 (COX2), tumor necrosis factor apha (TNFalpha), matrix metalloproteinases (MMPs), early growth response 1 (EGR1), prostaglandin E 2 (PGE2), microsomal PGE2 synthase (mPGE2S), NF-kB component RelB, RelA, inducible nitric oxide synthase (iNOs), interleukin 8 (IL8)[3] .

4. AhR and Breast Cancer

More generally, inflammation is an ascertained risk factor for the onset of a plethora of pathologies, among which cancer. Here, a frequently emerging role in the modulation of the inflammatory state is emerging for AhR[4].The connection between inflamed phenotype and neoplasia is now evident in BC. BC is the most common invasive malignancy among women in the industrialized world. It is a complex, multifactorial and extremely heterogeneous disease. Genetic factors account for up to 10% of all BCs. Among inherited mutations, BRCA1 e BRCA2 (BReast CAncer) suppressor genes are the most involved in BC susceptibility. BRCA1 interacts both at transcriptional and post-transcriptional level with estrogens pathway, in order to limit their positive eects on proliferation of mammary tissues.

The lack of this control is a well-known risk factor for TNBC occurrence and this can occur due to BRCA1 epigenetic mutations, among which AhR-instigated hypermethylation[5].This type of BC aects women in all age groups. It is a highly aggressive neoplasia whose cells, do not express ER, PR and HER2. Therefore, it has fewer options for targeted treatment, as hormone therapy and HER2 drugs cannot be used. For this reason, its outcome is not favorable. Over the last 10 years, the urgency to identify alternative therapeutic options for TNBC has fueled the selection and the screening of selected molecular targets, some of them belonging to the AhR pathway. Goode and colleagues [6]proposed that in TNBC tumors and cell lines, AhR expression is directly proportional to tumoral aggressive behavior, both in vivo and in vitro. In MDA-MB231, a TNBC cell line, they observed the attenuation of the malignant phenotype after AHR knockdown. Considering these premises, AhR inhibition could be a promising therapeutic target in TNBC. This result can be obtained in vivo by the administration of selected antagonists, among which genistein. The dietary intake of this isoflavone had been shown to nhibit AhR interaction with BRCA1 exon 1 in mice mammary tissue and AhR-driven hypermethylation of CpG in BRCA1 gene. These results are consistent with those obtained by the administration of the synthetic alpha-naphthoflavone and the natural flavonoid Galangin in MCF7 cells. The antiproliferative properties of these molecules have long been known and are supported by data identifying them as AhR modulators in TNBC. Interestingly, galangin and the natural isoflavone genistein are also known for their anti-inflammatory properties[7].

5. Conclusions

In conclusion, it appears evident that there is a clear link between AhR pathway, inflammation and BC, particularly triple negative (Figure 3). Since inflammation is one of the factors that favors and supports tumor transformation through several factors, among which AhR, it is reasonable to include AhR among the targets of a combined anti-inflammatory/antitumoral therapy.

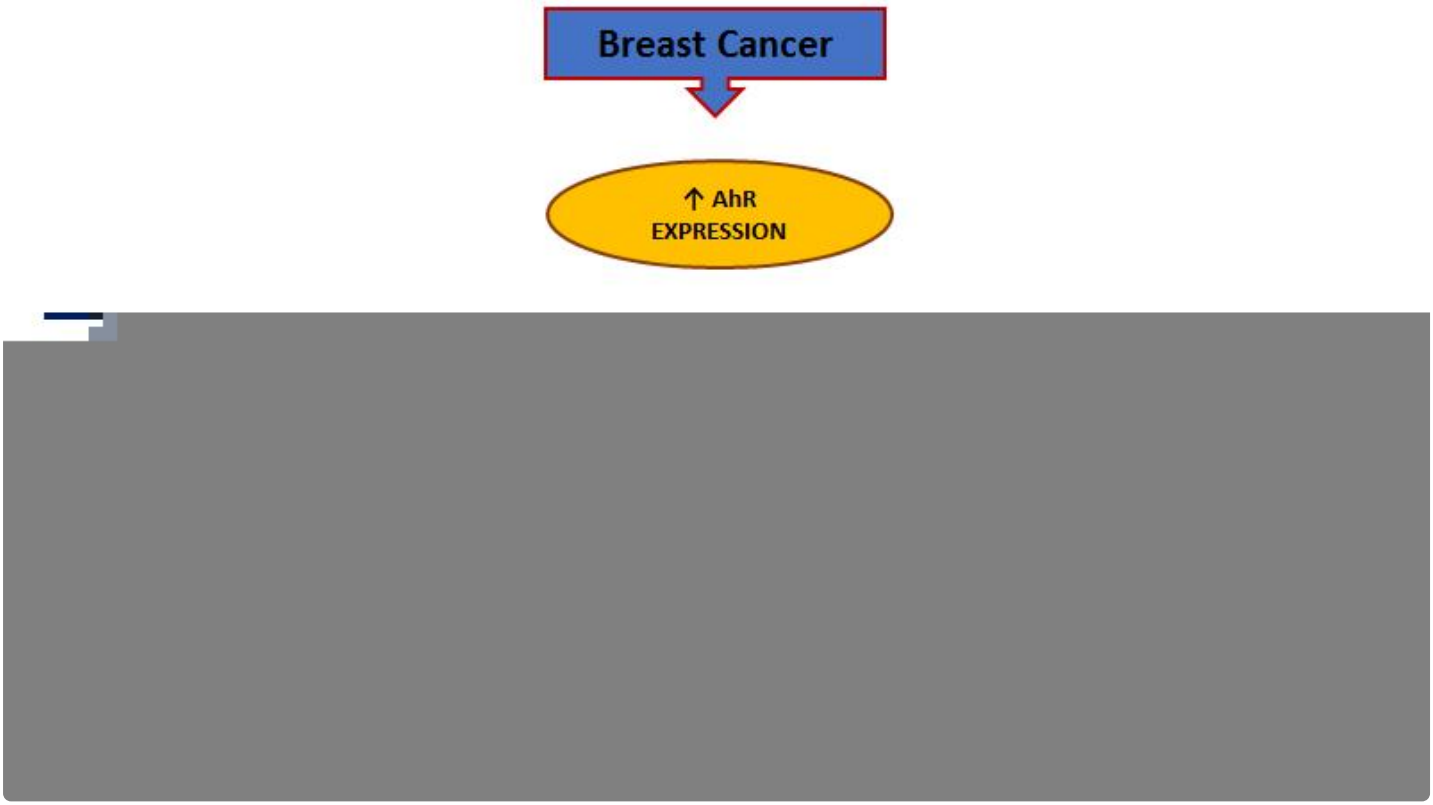

Fig. 3. AhR pathway interactions in cancer breast cells. AhR is usually overexpressed in cancer breast cells. Here, it correlates with several markers of: 1. proliferation (NF-κB, IL6, c-Myc, BRCA1); 2. DNA repair (BRCA genes); 3. cell migration (kynurenine) 4. metastatic behavior (MMPs and PLAU); 5. immune system (Th17 and Treg cells); 6. inflammation (NF-κB, IL6, Kyn). BRCA = BReast CAncer gene; Kyn = Kynurenine; c-Myc = Avian myelocytomatosis virus oncogene cellular homolog; IL-6 = interleukin 6;MMP = matrix metalloproteinase; NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells; PLAU = Plasminogen Activator, Urokinase *(gene);* ↑ = increase; ↓ = decrease

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