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Review

Dioxin-induced toxicity on vascular remodeling of the placenta

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ABSTRACT

Arylhydrocarbon receptor (AhR) activated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) triggers its downstream signaling pathway to exert adverse effects on vasculature development, which can be initiated by vasculogenesis, followed by angiogenesis, or vascular remodeling, in a variety of animals including avians, piscines and mammals. The placenta, a mammalian organ rich in vasculature, consists of endothelial and trophoblast cells of fetal origin, which proliferate and differentiate under hypoxic condition in the uterine horn. Our studies demonstrated that vascular remodeling occurs prominently in the placenta of the control Holtzman rat strain during the late period of gestation, and induces changes in cell shape and elimination by apoptosis of trophoblasts. As a result, the net volumes of both maternal and fetal blood in the placenta increase to cope with the essential requirements of oxygen and nutrients in the late period of gestation. On the other hand, *in utero* exposure to TCDD markedly suppressed the development of sinusoids and trophoblast cells and the apoptosis of trophoblast cells with a concomitant increase in the incidence of fetal death under hypoxic condition. A crosstalk between the hypoxia-inducible factor (HIF)-mediated pathway and AhR-mediated pathway is considered to play an important role in this physiological process. No such changes were observed in the Sprague–Dawley rat strain that turned out to have an AhR conformation identical to that of the Holtzman rat strain. In this commentary, we will discuss a possible link of the TCDD toxicities with the AhR signaling pathway and gestation-related diseases.

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Abbreviations: AhR, arylhydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; GD, gestation day; HIF, hypoxia-inducible factor; HUVECs, human umbilical vein endothelial cells; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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1. Vascular development and dioxin toxicity

Dioxin and related compounds, which belong to a family of halogenated aryl hydrocarbons, are produced unintentionally in uncontrolled combustion processes and in various types of industrial processes [1]. Among more than 400 kinds of congeners depending on the number and position of chlorine atoms on the benzene ring, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been established as the most toxic congener on the basis of experimental studies. The fetus is one of the most sensitive targets in the life of mammals, and *in utero* and lactational exposure to dioxins has been reported to elicit a wide spectrum of biological and toxicological responses, including reproductive, neurobehavioral, and immune disturbances in the offspring, by which dams are not affected as much as their fetuses [2]. Among the wide spectrum of toxicities, vascular development is particularly sensitive to the toxic effects of TCDD compared with congenital malformations or birth defects. Previous studies demonstrated that exposure to TCDD during development results in heart size reduction in the piscine embryo [3,4], dilatation of ventricular cavity associated with thinner ventricle walls in the chicken embryo [5] and decrease in heart-to-body weight in mice [6], all of which were accompanied by the reduction in cardiomyocyte proliferation. Edema and hemorrhage were observed and considered as common features of TCDD toxicities in the vasculature irrespective of animal species. Exposure to TCDD *in utero* induced subcutaneous edema and intestinal hemorrhages in the fetuses of the rat and hamster [7], and resulted in leakage from the vasculature in the morbid avian and piscine embryos, the latter of which was confirmed by severe subcutaneous, pericardial, and peritoneal edema prior to death [5,8]. TCDD clearly has a strong impact on living organisms by causing damage to the vascular system.

The toxicity of TCDD is mediated by the binding of TCDD to the arylhydrocarbon receptor (AhR), which is then activated. The activated ligand-bound AhR translocates to the nucleus from the cytoplasm and dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT), followed by the binding of this AhR/ARNT heterodimer to the xenobiotic response element (XRE; also known as the dioxin response element, DRE) in the promoter region of a various genes [9]. If the XRE elements are functional, the AhR/ARNT heterodimer modulates the expression of those genes, including drug-metabolizing enzymes, i.e., phase I enzymes such as CYP1A1 and 1B1 and phase II enzymes such as UDP-glucuronosyl transferase, and biological and toxicological responses will emerge.

The vascular network plays a critical role at the very beginning of embryo development in order to supply oxygen and nutrients to adjacent proliferating or differentiated cells. Vasculature development in most of the organs is relatively simple and depends on two consecutive processes, vasculogenesis and angiogenesis [10] (Fig. 1A). In vasculogenesis, blood vessels form through the *in situ* differentiation between undifferentiated precursor cells, called angioblasts, to endothelial cells that assemble into a primitive vascular network, in which the adhesion of endothelial cells and periendothelial support cells is at the immature stage. The term angiogenesis was used to generally denote the growth and remodeling of the primitive network into a complex network. During this remodeling, periendothelial support cells are recruited to encase endothelial tubes, resulting in the maturation of blood vessels. In addition, some preexisting vessels send out capillary sprouts to produce new vessels. At each of these stages, growth factors and their receptors have been identified to act as modulators [10]. The vascular endothelial growth factor (VEGF) and its receptors (VEGFRs), such as fetal liver kinase-1 (Flk1) and fms-like tyrosine kinase-1 (Flt1), named the VEGF/VEGFR system, are mainly associated with vasculogenesis. Angiopoietin-1 (Ang1) and Ang2 and their receptor Tie2, named the Ang/Tie2 system, are involved mainly in angiogenesis, or vascular remodeling. Vascular development is basically stimulated under a hypoxic condition, which is dependent on transcription factors known as the hypoxia-inducible factors (HIFs). Under normal oxygen tension, HIF-1α is posttranslationally modified and subsequently degraded through the proteasome. However, under hypoxic conditions, HIF-1α can escape from degradation, and the accumulated HIF-1α binds to the oxygen-insensitive molecule known as the ARNT, also called HIF-1β. The HIF-1α/ARNT heterodimer subsequently binds to the hypoxia response element (HRE) in the promoter region of genes involved in the adaptation to hypoxia. Thus, the HIF-1α acts as a master regulator to activate the transcription of many hypoxia-response genes, including the VEGF/VEGFR or Ang/Tie2 system, and regulates the expressions of VEGF, Flt1, and Ang2 [11].

HIF-1α plays its intrinsic role in hypoxia signaling, and presumably modulates dioxin toxicities because of its ability to heterodimerize with ARNT [11]. In other words, ARNT is a common transcription factor that shares its role with AhR and HIF-1α to modulate XRE- and HRE-dependent pathways, respectively. Thus, it has been speculated that the AhR/ARNT and HIF-1α/ARNT pathways affect each other by competing for the limited quantities of ARNT molecules [12] (Fig. 1B).

Although it is considered that ARNT does not act as a limiting factor for the interaction with either AhR or HIF-1α because ARNT is abundant in cells under basal physiological condition [13], some of the experimental observations might be explained by the former hypothesis. In a study by Ichihara et al. [14], hypoxia caused by the ligation of the femoral artery was found to induce angiogenesis more significantly in AhR-null mice than in wild-type mice. In this study, electrophoretic mobility shift assay (EMSA) analysis showed that the DNA binding activity of the HIF-1α and ARNT complex is more pronounced in the AhR-null mice than in the wild-type mice under ischemic conditions. Thus, the authors suggest that the increased quantity and activity of the HIF-1α/ARNT heterodimer in ischemia-induced AhR-null mice may explain at least in part the enhancement of ischemia-induced VEGF expression and angiogenesis. In a study by Fritz et al. [15], transgenic adenocarcinoma of the mouse prostate (TRAMP) mice having AhR-null mutation develop prostate tumors with greater frequency than AhR-positive TRAMP mice. The authors showed that the stimulated development of the prostate tumor in AhR-null TRAMP mice is due to the accelerated angiogenesis resulting from the increased VEGF expression on the prostate epithelial hyperplasia, a typical malformation observed in TRAMP mice. Because the addition of vanadate, a putative inducer of the HIF-1α-VEGF pathway, resulted in VEGF induction in the organ culture experiment of the prostate obtained from AhR-null mice but not from WT mice, the authors concluded that the increased VEGF production in AhR-null TRAMP mice is due to the overstimulated HIF-1α/ARNT signaling pathway. On the other hand, exposure to TCDD or 3-methylcholanthrene decreased the VEGF expression under several experimental conditions such as in the case of coronary endothelial tube formation in chick embryos [16–18] and human umbilical vein endothelial cells (HUVECs) *in vitro* [19]. Exposure to cigarette smoke extract was shown to suppress the hypoxia-induced cellular migration and capillary-like tubule formation in HUVECs *in vitro*. In an *in vivo* experiment, blood flow perfusion in surgically induced ischemic hind limbs was significantly reduced in mice exposed to cigarette smoke. In these *in vitro* and *in vivo* experiments, the expression of HIF-1α/VEGF was downregulated [20]. These observations suggest that the AhR-dependent reduction of VEGF expression by TCDD and other AhR ligands may result in a tilted balance toward the AhR/ARNT pathway instead of the HIF-1α/ARNT pathway. It should be noted that the term 'angiogenesis' has often been used to include the

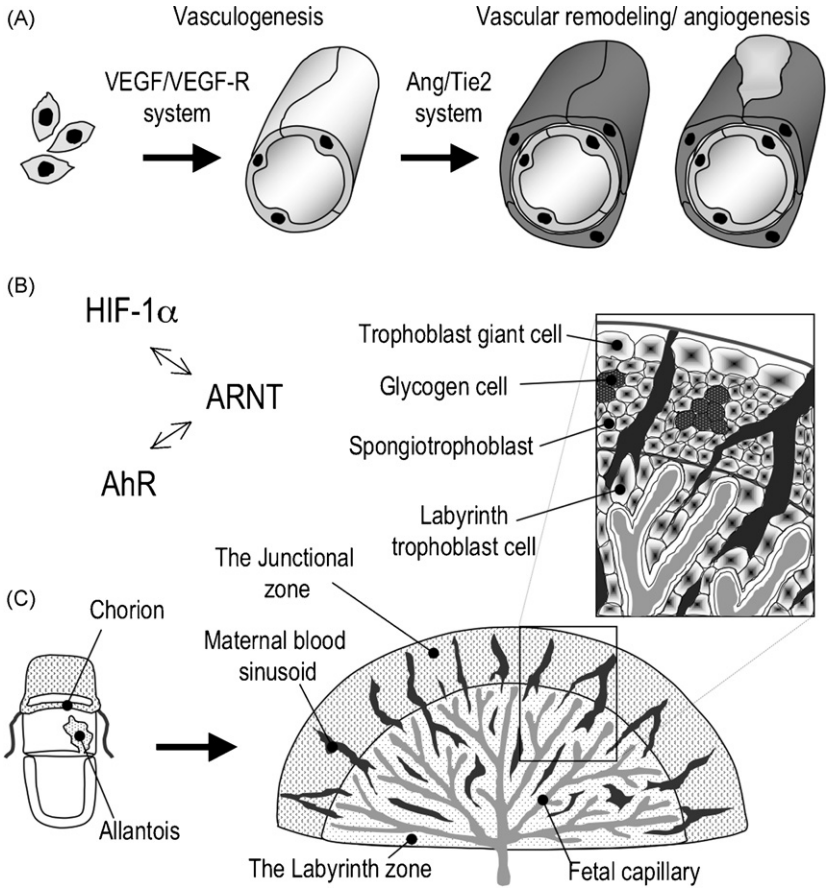

Fig. 1 – Vascular development and structure in the peripheral blood vessels and placenta. (A) In the peripheral blood vessels, a primitive vascular tube is formed during vasculogenesis, which is further processed by remodeling its structure to recruit peripheral endothelial support cells and by sending out capillary sprouts to produce new vessels. (B) Proposed model for suppressive effects of AhR-mediated signaling on HIF-1α signaling pathway by competing for limited amounts of ARNT. (C) Development of the rodent placenta. Placental development is initiated by fusing two membranes, the chorion and allantois. The mature rodent placenta is composed of the labyrinth and junctional zones as described in the text.

term 'vasculogenesis' in a number of papers, and thus, one has to pay particular attention to the blood vessel development stage described in a given paper. Generally, it is plausible to think that not only the vascular remodeling but also vasculogenesis is considered to be a target of AhR ligands.

Although it is still controversial, competition of AhR and HIF-1α with ARNT is a plausible model to work on to elucidate the inhibitory mechanisms of the AhR-mediated signaling pathway on HIF-1α signaling activity. Intriguingly, Ohtake et al. recently found a novel function of AhR [21,22]. The AhR has E3 ubiquitin-ligase activity by forming multiple protein complexes and degraded several transcription factors including the estrogen and androgen receptors. The substrates for AhR-mediated ubiquitin ligase have not been fully identified, and thus, whether HIF-1α is a target of AhR-mediated ubiquitin-ligase activity is yet unknown. In either case, the inhibitory effect of the AhR-mediated signaling pathway on the HIF-1α signaling pathway is due to the downregulation of active HIF-1α, which is consistent with the results of *in vivo* studies. Another possibility of the inhibitory effect of HIF-1α on AhR-mediated gene transcription is a competition of transcription cofactors between these nuclear receptors. Several nuclear receptor coactivators are known to interact with the AhR, including ERAP140 [23], RIP140 [24], CBP/P300 [25], BRG-1 [26], and the three members of the p160 family of coactivators: NCoA1 (SRC-1), NCoA2 (GRIP-1 and TIF-2) and NCoA3 (AIB-1, p/CIP, and ACTR) [27]. On the other hand, HIF-1α is known to interact with CBP/p300, SRC-1 and TIF2 [28] [29–32]. Thus, it is plausible that AhR and HIF-1α competes for a limited amount of CBP/p300, which may suppress transcriptional activities of these nuclear receptors. For further study, it is necessary to clarify the exact inhibitory mechanism of the AhR-mediated signaling pathway on the HIF-1α signaling pathway.

2. Vascular development in the placenta and related diseases

The placenta is an organ penetrated by maternal and fetal blood vessels, and acts as an interface between them by exchanging oxygen, nutrients and by-products. The vasculature of the placenta has been extensively studied because of its central role in pathogenesis for both maternal and fetal sides. Among mammalian species, the anatomical structures of rodents and humans are similar. The mature rodent placenta, so-called chorioallantoic placenta, is morphologically divided into two zones, the labyrinth zone and the junctional zone [33] (Fig. 1C). The junctional zone, which is devoid of fetal blood, contains three types of cells of fetal origin, i.e., the spongiotrophoblast cells, glycogen cells and trophoblast giant cells. Glycogen cells are considered to supply energy while spongiotrophoblast cells and trophoblast giant cells are known to secrete hormones, including diverse types of placental prolactin family proteins in a stage-specific manner [34]. The labyrinth zone is a place for exchanging oxygen and nutrients between maternal and fetal blood.

The most striking event during the development of the placental vasculature is the fusion of two membranes, chorion and allantois, and this event, termed as chorioallantoic fusion, begins around gestational day (GD) 10 and GD8.5 in rat and mouse, respectively [35] (Fig. 1C). After this fusion, fetal capillaries grow from the allantois, and the trophoblast cells that mostly originated from the chorion undergo extensive villous branching with its associated fetal capillaries to construct a vasculature in the labyrinth. Around the period of chorioallantoic fusion, the trophoblast giant cells invade into the maternal uterine wall, and maternal blood that leaks from ruptured uterine vessels flows into the narrow space of the labyrinthine maternal blood sinusoids that directly contact with the labyrinthine trophoblast cells. The labyrinthine vasculature is estimated to develop until around GD15 in the rat when DNA synthesis is terminated thereafter [36,37]. A crucial point raised here is that two different types of cell, endothelial and trophoblast cells, actively participate in the establishment of the complex vasculature of the placenta, whereas only endothelial cells play a major role in the development of the vasculature in other organs.

Even though vascular development in the placenta is more complicated than in other organs, knockout mouse studies showed that vascular development is strictly regulated by VEGF and HIFs in the placenta, which is similar to that in other organs. It was reported that embryos deficient in HIF-1α or ARNT are viable up to GD9.5 but could not survive beyond GD10.5, owing to severe placental defects including shallow placental invasion into the decidua and lack of vascularization of fetal vessels in the labyrinth zone because of a defect in the chorioallantoic fusion [38–41]. Furthermore, the number of spongiotrophoblast cells in the junctional zone was markedly reduced, whereas that of trophoblasts cells in the labyrinth zone was increased, suggesting that the balance of trophoblastic differentiation into each lineage was tilted [41]. These results strongly suggest that the differentiation of trophoblast cells is strictly regulated by HIF-1α/ARNT.

Impairment of placental blood circulation often results in disease conditions, such as intrauterine growth retardation of the fetus and preeclampsia, the latter of which is characterized by hypertension and proteinuria in pregnant women [42,43]. Approximately 5–7% of all pregnant women develop preeclampsia. Although the precise etiology is not known, preeclampsia is accompanied by vasospasm and endothelial injury as an end result [44]. Excessive secretion of Flt1 is considered to be responsible for endothelial injury [45]. Both disease conditions are presumably related to each other, and preeclampsia sometimes accompanies intrauterine growth restriction. In temporal aspects, these symptoms manifest during the late period of gestation, suggesting that vascular remodeling might participate in the development of this disease. Recent studies reported a possible link of preeclampsia of humans with that of rodents [35,46].

The etiologies of these diseases are complex owing to several factors including genetic as well as environmental issues [47]. As an environmental factor, cigarette smoking has been reported to impair placental vasculature and subsequent fetal growth restriction [48,49]. Microarray analysis, followed by quantitative RT-PCR analysis, of gene expression in the placentas of cigarette-smoking mothers revealed that AhR-dependent phase I enzyme genes, such as cytochrome P450 1A1 (CYP1A1) and CYP1B1, are activated, but that AhR- or Nrf2-dependent phase II genes are not. The imbalance between the

induced phase I enzymes and the noninduced phase II enzymes may result in increased oxidative stress, which could interfere with the function of the placenta and adversely affect the well-being of the fetus [50]. It is thus plausible to consider that AhR ligands act as environmental factors that affect the normal development of the placental vasculature.

3. Effects of TCDD on vascular remodeling in the placenta and the proposed mechanisms of toxicities

Because AhR ligands including dioxins and related compounds affect the early stage of organ development, it is intriguing to study how AhR-mediated signaling is involved in the development of blood vessels in the placenta. Administration of TCDD to pregnant C57BL/6 mice at a daily dose of 3 or 6 μg/kg bw from GDs 10 to 13 was found to induce histological alterations 24 h after the last administration when vasculogenesis is supposed to continue [51]. In the TCDD-exposed placenta, the vasculature that acts as a maternal-fetal barrier in the labyrinth was found to show hemorrhage of embryonic blood into the maternal circulation. This data suggests that the dose used was too high to consider subtle changes in terms of the expression of molecular markers of vasculogenesis and vascular remodeling. It has been reported that the exposure of Holtzman, Long–Evans, or Sprague–Dawley rats to TCDD before fertilization or at the early stage of gestation results in fetal death at the late, but not early, stage of gestation [7,52–54]. In these studies, no detailed analysis of the histology and molecular markers was available, and it is difficult to conclude whether and how TCDD affects chorioallantoic fusion, placental vasculogenesis, and vascular remodeling.

In the control placenta of Holtzman rats, the vascular remodeling were found to take place on GD15 even when the placental DNA synthesis was already terminated and, therefore, the vasculogenesis in the placenta presumably ceased [55]. On GD16, maternal sinusoids and fetal capillaries were narrow and the size and thickness of trophoblast cells were small, but these morphological features became reverse on GD20 with the development of the placenta (Fig. 2). In addition, the upregulation of genes involved in both the VEGF/VEGFR and Ang/Tie2 systems during this period was observed. On the other hand, administration of TCDD at 1600 ng/kg bw to Holtzman rats on GD15 to study its possible effects on the placental vasculature in the late period of gestation, the morphological features, such as maternal sinusoid, fetal capillaries and trophoblast cells, of the TCDD-exposed placenta on GD20, were very similar to the ones of the control placenta on GD16 (Fig. 2B) [55]. Lack of dilatation of both maternal blood sinusoids and fetal capillaries, existence of large size trophoblast cells, and the downregulated Tie2 mRNA level among the VEGF/VEGFR and Ang/Tie2 systems were

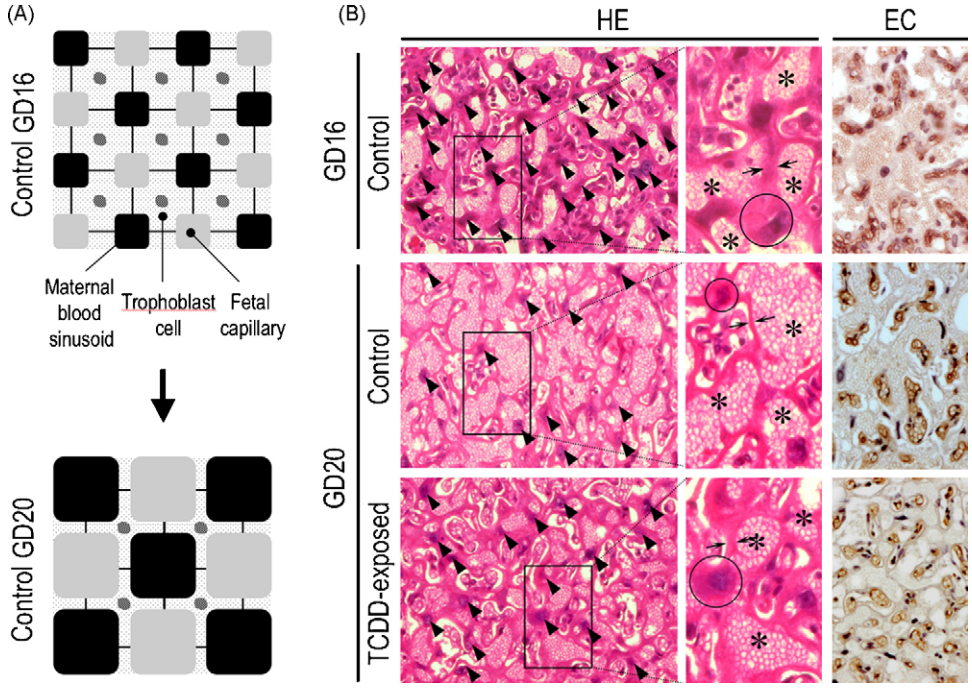

Fig. 2 – Vascular remodeling occurs in the placenta of Holtzman rats during the late period of gestation. (A) Schematic of horizontal dimension in the labyrinth zone of the placenta. Both the maternal blood sinusoids and fetal capillaries are enlarged from GDs 16 to 20 with a concomitant decrease in the number as well as thinning of trophoblast cells. (B) Suppresstion of vascular remodeling in the TCDD-exposed placenta. Horizontal sections of the placentas on GD16 and GD20 are shown. These sections were subjected to either hematoxylin and eosin (HE) staining, or endothelial cell (EC) staining using BS-1 lectin that identifies fetal capillaries. Note that the maternal blood sinusoids (asterisks) and fetal capillaries (in the EC stain) are not expanded in the TCDD-exposed placenta on GD20, which is similar to the morphology of control placenta on GD16. In addition, the number (arrow heads) and size (circle) of trophoblast cells are not decreased, and the trophoblastic interhemal membrane (between arrows) does not become thin in the TCDD-exposed placenta on GD20.

observed in TCDD-exposed placentas on GD20, suggesting that the vascular remodeling was suppressed by TCDD exposure. Furthermore, a striking effect of TCDD on apoptosis of trophoblast cells was present. Similar to the human placenta during the late period of gestation [56], a significant number of trophoblast cells (approximately 500/mm^2) were dead by apoptosis in the control placenta from GDs 16 to 20. In contrast, the number of apoptotic trophoblast cells in the TCDD-exposed placenta decreased to less than half of that in the control placenta [55]. In addition, TCDD-exposed placentas on GD20 were found to be in hypoxic condition [57], and to have an altered glucose metabolism on GD20 [58]. Under this condition, the presence of the altered glucose and glycogen metabolism was supported by the observations of decomposition products of glycogen cells in the junctional zone, increased glycogen content and upregulation of glucose transporter-2 (GLUT-2), GLUT-3, and GLUT-4 mRNAs levels [58,59].

The vascular remodeling to expand spaces for maternal blood sinusoids as well as fetal capillaries, observed in the control placentas during the late period of gestation, is considered to increase the net volume of circulating blood within the placenta. The observation is congruent with the increase in the physiological need for oxygen and nutrients of the significantly growing fetus. The apoptosis of tropohoblast cells is considered indispensable to offer spaces for the expansion of maternal blood sinusoids as well as fetal capillaries because the size of the placenta is restricted within the uterine horn and is relatively constant during the late period of gestation. Therefore, the inhibition of apoptosis by TCDD is considered to be essential for the pathogenesis of the TCDD-suppressive effect on vascular remodeling in the placenta of Holtzman rats.

The suppression of apoptosis in other types of cells following TCDD exposure has been reported in other studies. Vogel et al. [60] showed that the activation of AhR by TCDD resulted in the loss of the apoptosis response in lymphoma cell lines, which plays a key role in the development of lymphoma and leukemia, and clarified that the upregulation of cyclooxygenase-2 (COX-2), a downstream gene in the AhR signaling pathway, is associated with the suppression of apoptosis. Ray and Swanson [61] showed that TCDD exposure induces immortalization of human keratinocytes by suppressing apoptosis. Stinchcombe et al. [62] showed that the tumor promotion activated by TCDD in the rat liver is due to a decrease in apoptotic level. In this regard, the apoptotic activity of the human mammary epithelial cell line MCF10A was suppressed by TCDD exposure [63,64]. These several lines of evidence suggest that the suppression of apoptosis is considered to be a fundamental to TCDD toxicities.

How this inhibitory effect of TCDD on apoptosis is regulated? Paajarvi et al. [65] suggested that mouse double minute 2 (MDM2) which is up-regulated by AhR-mediated pathway is involved in this process. They showed that pretreatment of rats with TCDD diminished diethylnitrosamine-induced apoptosis in liver cells that is known to be p53-dependent, and that up-regulated MDM2 by AhR-mediated pathway decreased the apoptosis because MDM2 binds and degrades p53. It was reported that upregulation of MDM2 by nuclear receptor CAR is critical to suppress apoptosis in liver of mice that are exposed to pesticide contaminant 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) [66]. These results suggest that the MDM2 might play a central role in suppressing apoptosis after exposure to xenobiotics including TCDD. Further studies are necessary to assess whether MDM2 is upregulated in the placenta, especially in the trophoblast cells, after exposure to TCDD.

Chorioallantoic fusion and subsequent vasculogenesis have been proved to be regulated by the HIF-1α/ARNT signaling pathway [38–41]. The HIF-1α/ARNT pathway is possibly activated under hypoxic condition in the late period of gestation owing to an increased demand for oxygen during the fetal growth. This activation then stimulates the vascular remodeling, which results in an increase in the blood supply to overcome the hypoxic condition. In reality, these responses are highly tuned to be in a dynamic equilibrium. Our proteomics analysis data suggests that TCDD-induced placental tissues are under hypoxic status, and that the above-mentioned equilibrium is disrupted owing to the suppression of vascular remodeling in the placenta [55]. Trophoblast cells have a unique HIF-1α/ARNT signaling pathway compared with cells in other organs in order to thrive in the innate low-oxygen environment. This notion is supported by the experimental evidence showing that knockout mice of either ARNT or von Hippel-Lindau (VHL) gene, the product of which interacts with HIF-1α, has defect of blood vessel formation only in the placenta but not in yolk sac and embryos [67,68]. Further studies are required to clarify how the HIF-1α/ARNT signaling pathway regulates vascular remodeling in the placenta.

Type 1 diabetic mouse model that is induced by administration of streptozotocin exhibits intrauterine growth retardation [69]. Intriguingly, the placentas of these mice exhibit similar to those in TCDD-exposed rats including reduced blood flow [69], containing decomposition products of glycogen cells in the junctional zone [70,71], increased glycogen content [72,73], and increased GLUT3 mRNA level [74]. Moreover, fetuses in cadmium-exposed pregnant rats [75] and ethanol-fed pregnant mice [76] exhibit intrauterine growth retardation concomitant with the appearance of decomposition products of glycogen cells in the placenta. Therefore, it is conceivable that the placenta exhibits prototypical symptoms including abnormal vasculature and altered glucose kinetics regardless of the different types of insult as above. All of these insults, as well as smoking, could induce intrauterine growth retardation by disrupting the proper function of the placental vasculature in not only rodents but also humans [77]. A common process underlying the onset of these disease models is production of excessive amounts of reactive oxygen species that may cause damage to tissues, which has also been observed in TCDD-exposed placenta [76,77]. The suppression by TCDD of vascular remodeling in the placenta should be addressed on the basis of two important aspects, the involvement of the HIF-1a/ARNT pathway and the causal relationship between vascular impairment and oxidative stress.

4. Susceptibility of the fetus to TCDD toxicity and the AhR structure

Because the growing fetus requires large quantities of oxygen and nutrients particularly during the late period of gestation, a

failure in vascular remodeling is considered to increase the risk of fetal death. When Holtzman rats were administered TCDD at a dose of 0.8 and 1.6 μg/kg bw on GD15, they developed a disorder in the vasculature of the labyrinth zone, and the incidences of fetal death were 1% and 13%, respectively [58]. On the other hand, a low susceptibility of Sprague–Dawley rats to an *in utero* exposure to TCDD was found in an experiment, that is, even a six fold higher dose of TCDD at 10 μg/kg bw failed to cause any pathological alterations in the morphology of the placental vasculature and fetal death [78]. A logical explanation would be that the primary structures of the AhRs of these two rat strains differ from each other, as has been established in mouse and rat strains [9,79–81]. For example, in mouse strains, the affinity of the AhR of the C57BL/6J strain to TCDD is much higher than that of the AhR of other strains, such as DBA/2 [82]. In rat strains, Long-Evans rats have AhR that has a higher affinity to TCDD and are at least 1000-fold more sensitive (LD_{50} about 10 μg/kg) to the acute lethal effects of TCDD than Han/Wistar (kuopio; H/W) rats [83]. We thus formulated the above-mentioned hypothesis and examined the sequence of AhR. Contradictory to our hypothesis, the primary structure of AhR was found to be identical between Holtzman and Sprague–Dawley rats. We next examined the possible differences in the activity of AhR between these two rats strains by examining the TCDD-dependent expression of CYP1A1 mRNA, and confirmed that both rat strains induced CYP1A1 mRNA at an identical level in the placentas [78], suggesting that the strain difference in the TCDD toxicities on the placental vasculature and fetal death does not depend on the magnitude of AhR activities during gene transcription. Although we did not determine TCDD concentrations in the placenta, identical CYP1A1 mRNA levels suggest that TCDD was retained in this tissue presumably at a similar level.

To study the possible relationship of placental abnormalities with fetal death, we compared how the vascular structures of Sprague–Dawley and Holtzman rats develop as the gestation proceeds. In normal Sprague–Dawley rats, the vasculature in the labyrinth zone on GD16 is immature as shown by the narrow shape of both maternal blood sinusoids and fetal capillaries (unpublished data), and such morphology is very similar to that of Holtzman rats on GD16. However, the vasculature in the labyrinth zone of Sprague–Dawley rats did not change even on GD20. That is, the trophoblast cells are still large and their interhemal membrane is also thick in the labyrinth. In contrast, the vasculature in the labyrinth zone in normal Holtzman rats is altered from GDs 16 to 20, as described above. It seems likely that apoptotic elimination of trophoblast cells is decreased, or even does not occur in the placenta of Sprague–Dawley rats, suggesting that placental vascular development under the control of VEGF/VEGF-R and/or Ang/Tie2 systems of this strain might be different from that of Holtzman rats. The comparative analysis of the expression of molecular markers such as VEGF/VEGF-R and Ang/Tie2 systems during gestation could possibly unravel the mechanisms underlying vascular development and TCDD toxicity.

5. Conclusions

Vascular development, initiated by vasculogenesis angiogenesis (vascular remodeling), is regulated by an orchestration of VEGF/VEGF-R and Ang/Tie2 systems, respectively. In particular, the VEGF/VEGF-R system is upregulated by HIF-1α/ARNT under hypoxic condition in vasculogenesis, leading to the activation of the Ang/Tie2 system in vascular remodeling. It was demonstrated that *in utero* exposure to TCDD or cigarette smoke that contains AhR ligands affects vasculogenesis and vascular remodeling via AhR signaling by interacting with HIF-1α signaling depending on the vascular development stage. We found that *in utero* exposure to TCDD affects the process of vascular remodeling rather than vasculogenesis in the placenta of Holtzman rats. In this study, it was found that *in utero* exposure to TCDD markedly suppressed the development of sinusoids and trophoblast cells and the apoptosis of trophoblast cells under hypoxic condition, which results in a higher incidence of fetal death. However, no such effects were observed in Sprague–Dawley rats even if these two rat strains had identical AhR structure. The elucidation of the physiological process of vascular remodeling in these rat strains may shed light on how AhR signaling is involved in the TCDD toxicities in the placental vasculature.

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