Meta-Analysis of Transcriptome Data Detected New Potential Players in Response to Dioxin Exposure in Humans

Evgeniya Oshchepkova 1,*, Yana Sizentsova 1,†, Daniil Wiebe 1,‡, Victoria Mironova 1,2,§ and Nikolay Kolchanov 1

1 Institute of Cytology and Genetics, 630090 Novosibirsk, Russia; sizentsova@bionet.nsc.ru (Y.S.); daniil.wiebe@gmail.com (D.W.); kviki@bionet.nsc.ru (V.M.); kol@bionet.nsc.ru (N.K.)
2 Faculty of Natural Sciences, Novosibirsk State University, 630090 Novosibirsk, Russia
* Correspondence: nzhenia@bionet.nsc.ru
† These authors contributed equally to this work.

Abstract: Dioxins are one of the most potent anthropogenic poisons, causing systemic disorders in embryonic development and pathologies in adults. The mechanism of dioxin action requires an aryl hydrocarbon receptor (AhR), but the downstream mechanisms are not yet precisely clear. Here, we performed a meta-analysis of all available transcriptome datasets taken from human cell cultures exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Differentially expressed genes from different experiments overlapped partially, but there were a number of those genes that were systematically affected by TCDD. Some of them have been linked to toxic dioxin effects, but we also identified other attractive targets. Among the genes that were affected by TCDD, there are functionally related gene groups that suggest an interplay between retinoic acid, AhR, and Wnt signaling pathways. Next, we analyzed the upstream regions of differentially expressed genes and identified potential transcription factor (TF) binding sites overrepresented in the genes responding to TCDD. Intriguingly, the dioxin-responsive element (DRE), the binding site of AhR, was not overrepresented as much as other cis-elements were. Bioinformatics analysis of the AhR binding profile unveils potential cooperation of AhR with E2F2, CTCFL, and ZBT14 TFs in the dioxin response. We discuss the potential implication of these predictions for further dioxin studies.

Keywords: dioxin; aryl hydrocarbon receptor; meta-analysis; transcription factor binding sites

1. Introduction

Environmental pollution by industrial emissions, waste incineration, and rocket fuel contributes to xenobiotics accumulation in the environment; these xenobiotics include a group of dioxin compounds, of which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic representative [1–4]. Dioxins are one of the most potent anthropogenic poisons; in terms of overall toxicity, they exceed the most potent chemical poisons. Exposure to dioxins can damage the immune system, developing nervous system, endocrine system, and reproductive functions [4–6]. There is evidence that the effect of dioxins on children leads to a decrease in IQ, congenital anomalies, and weight loss in newborns [7].

The generally accepted concept of the dioxin action mechanism on the cells is that it serves as a ligand for ligand-activated transcription factor (TF) aryl hydrocarbon receptor (AhR). AhR belongs to the helix-loop-helix basic domain PER-ARNT-SIM (bHLH/PAS) subfamily and regulates the expression of a large number of genes via dioxin-responsive elements (DREs), also known as xenobiotic-responsive elements (XREs), and has the consensus 5′-TNGCGTG-3′ [8]. In the inactivated state, AhR is located in the cytoplasm in a complex with the dimeric chaperone 90 kDa heat shock protein (Hsp90), as well as
with the AhR-interacting protein (AIP) and with the p23 co-chaperone [9,10]. After binding to dioxin, the AhR/Hsp90 complex translocates to the cell nucleus, where it decomposes, and AhR (with c p23) forms an active transcriptional complex with the aryl hydrocarbon receptor nuclear translocator (ARNT) [11]. ARNT also belongs to the bHLH family and directly binds to DRE [12]. In vivo DNA footprinting showed that AhR binds to the 5′-CACGCNA/Γ-3′, and ARNT with 5′-GTG-3′ [13]. The molecular mechanisms underlying the action of dioxin on the cell are still poorly understood. The key transcription factor mediating the effects of dioxin is known, but the exact mechanisms at the cellular level are not yet clear and require further research.

To study the cellular response mechanisms to dioxin compounds, we performed a meta-analysis of all available RNA-seq and microarray human datasets obtained under the action of TCDD. The aim of the study was to identify the TCDD targets that were systematically affected over multiple datasets. This helped us to predict novel or poorly studied mediators of the dioxin response.

2. Results

2.1. Identification of TCDD Targets in Humans

We analyzed all available whole-genome datasets obtained under the action of TCDD on different human cell lines. The individual microarray and RNA-Seq datasets were preprocessed according to similar protocols to obtain the lists of differentially expressed genes (DEGs) (see Sections 4.1 and 4.2). As TCDD mildly affected transcription of the genes, we set a mild criterion for false discovery rate (FDR < 0.2). Twenty datasets that under this criterion yielded more than 100 up- and down-regulated DEGs were taken for further meta-analysis (Table 1). PCA analysis of these datasets showed a nice clustering of the transcriptional responses detected in different studies, with a few very specific responses (Figure S1). Gene ontology (GO) annotation of all individual datasets showed that they are informative enough with relevant GO terms significantly overrepresented (e.g., “immune system process”, “inflammatory response”, “anatomical structure morphogenesis”, etc.).

The total number of genes, which were differentially expressed in response to TCDD in at least one of the datasets, was 8813 of up-regulated and 8575 of down-regulated genes (Figure 1). As the experimental design significantly varied between the experiments, the DEG lists overlapped partially. However, many genes changed their expression in response to TCDD systematically. For example, CYP1A1 and CYP1B1, known as coding enzymes involved in the metabolism of xenobiotics and associated with the TCDD response [23], were activated in 15 and 16 of the 20 analyzed datasets, respectively (Table 2).

2.2. Robust TCDD Targets: Known Knowns and Known Unknowns

First, we looked at the genes that responded to TCDD in the most robust way in half of the tested datasets (10 out of 20). There were 19 such genes, and some of them are known to be associated with the dioxin response or the AhR pathway (Table 2); for the remaining genes, no associations were found in the literature (Table 3). TCDD inducible poly(ADP-ribose) polymerase (TIPARP), encoding a negative regulator of AhR [25], was induced the most robustly—in 17 out of 20 datasets. AhR repressor (AhRR) is another robust TCDD target that provides for negative feedback—in 15 out of 20 datasets [27].
one of the datasets, was 8813 of up-regulated and 8575 of down-regulated genes (Figure 1). As the respectively (Table 2).

example, partially. However, many genes changed their expression in response to TCDD systematically. For experimental design significantly varied between the experiments, the DEG lists overlapped

Figure 1. An overview of the numbers of TCDD-responsive genes over 20 analyzed transcriptomes on human cell lines: number of up-regulated genes (pink rectangles) and number of down-regulated genes (blue rectangles).

Table 1. Summary on the datasets for the studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) action on the human cell cultures used in the meta-analysis. Only datasets that passed the quality control are presented. GEO: Gene Expression Omnibus.

| № | GEO Accession Number | Library Strategy | Platform | Time and Concentration of TCDD Treatment | Cell Culture | Replicates | References |
|---|----------------------|------------------|----------|-----------------------------------------|--------------|------------|------------|
| 1 | GSE46874 Microarray | Affymetrix Human Gene 1.0 ST Array | 30 h, 25 nM | HepaRG cells | 3 | [14] |
| 2 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 1 nM | MCF-7 breast cancer cells | 3 | [15] |
| 3 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 10 nM | MCF-7 breast cancer cells | 3 |
| 4 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 100 nM | MCF-7 breast cancer cells | 3 |
| 5 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 1 nM | Ishikawa cells | 3 | [15] |
| 6 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 10 nM | Ishikawa cells | 3 |
| 7 | GSE69845 Microarray | Affymetrix Human Genome 219U Array | 6 h, 100 nM | Ishikawa cells | 3 |
| 8 | GSE69850 Microarray | Affymetrix Human Genome 219U Array | 6 h, 100 nM | HepaRG | 3 | [15] |
| 9 | GSE122518 Microarray | Illumina HumanHT-12 v4.0 expression beadchip | 24 h, 1 nM | HepaRG cells | 4 | [16] |
| 10 | GSE63935 RNA-Seq | Illumina HiSeq2500 | 48 h, 3 nM | The mixture of cells: combined neural progenitor cells, endothelial cells, mesenchymal stem cells, and microglia/macrophage precursor | 2 | [17] |
| 11 | GSE63935 RNA-Seq | Illumina HiSeq2500 | 144 h, 3 nM | The mixture of cells: combined neural progenitor cells, endothelial cells, mesenchymal stem cells, and microglia/macrophage precursor | 2 |
| 12 | GSE69851 RNA-Seq | Illumina HiSeq1500 | 6 h, 10 nM | MCF-7 breast cancer cells | 4 | [18] |
| 13 | GSE69851 RNA-Seq | Illumina HiSeq1500 | 6 h, 100 nM | MCF-7 breast cancer cells | 4 |
| 14 | GSE122002 RNA-Seq | Illumina NextSeq 500 | 96 h, 2 nM | Mel1 embryonic stem cells (before differentiation) | 2 | [19] |
| 15 | GSE122002 RNA-Seq | Illumina NextSeq 500 | 96 h, 2 nM | Mel1 embryonic stem cells (2-days after differentiation) | 2 |
| 16 | GSE83886 RNA-Seq | Illumina HiSeq2500 | 504 h, 2 nM | BEAS-2B bronchial epithelial cell | 2 | [20] |
| 17 | GSE83886 RNA-Seq | Illumina HiSeq2500 | 504 h, 10 nM | BEAS-2B bronchial epithelial cell | 2 |
| 18 | GSE114552 RNA-Seq | Illumina Genome Analyzer II | 24 h, 10 nM | CRL-4003 decidual stromal cells | 3 | . |
| 19 | GSE114552 RNA-Seq | Illumina Genome Analyzer II | 144 h, 10 nM | CRL-4003 decidual stromal cells | 2 |
| 20 | GSE141711 RNA-Seq | Illumina HiSeq2500 | 24 h, 1 nM | HepG2 liver hepatocellular carcinoma cell | 5 | [21] |
| 21 | GSE90550 ChIP-Seq | Illumina HiSeq2500 | 24 h, 10 nM | MCF-7 breast cancer cells | 1 | [22] |
Table 2. The most robustly affected genes in response to TCDD that are known to be associated with either the dioxin response or the AhR pathway. The gene function annotations were taken from the UniProtKB [24].

| Gene Name | Full Name | Functions | TCDD Response in 20 Datasets | Association with AhR Pathway |
|-----------|-----------|-----------|----------------------------|------------------------------|
| TIPARP    | TCDD inducible poly(ADP-ribose) polymerase | Acts as a negative regulator of AhR by mediating mono-ADP-ribosylation of AhR | 17 | 0 | 25 |
| CYP1B1    | Cytochrome P450 family 1 subfamily B member 1 | Oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, retinoid and xenobiotics | 16 | 0 | 23 |
| ALDH1A3   | Aldehyde dehydrogenase 1 family member A3 | Required for the biosynthesis of retinoic acid in the embryonic ocular and nasal regions | 15 | 0 | 26 |
| CYP1A1    | Cytochrome P450 family 1 subfamily A member 1 | Oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics | 15 | 0 | 23 |
| AhRR      | Aryl-hydrocarbon receptor repressor | Mediates dioxin toxicity and is involved in regulation of cell growth and differentiation. Represses the transcription activity of AhR | 15 | 0 | 27 |
| NFE2L2 (NRF2) | Nuclear factor erythroid 2-related factor 2 | Transcription factor that plays a key role in the response to oxidative stress: binds to antioxidant response elements present in the promoter region of many cytoprotective genes, and promotes their expression, thereby neutralizing reactive electrophiles | 15 | 0 | 28 |
| RUNX2     | Runt related transcription factor 2 | Transcription factor involved in osteoblastic differentiation and skeletal morphogenesis | 14 | 1 | 29 |
| SLC7A5 (LAT1) | Large neutral amino acids transporter small subunit 1; L-type amino acid transporter 1 | The heterodimer with SLC3A2 functions as a sodium-independent, high-affinity transporter that mediates uptake of large neutral amino acids such as phenylalanine, tyrosine, L-DOPA, leucine, histidine, methionine, and tryptophan | 14 | 1 | 18 |
| DKK1      | Dickkopf-1 | Locally inhibits Wnt-regulated developmental processes such as limb development, somitogenesis, and eye formation. In adults, Dkk1 is implicated in bone formation and bone disease, cancer, and Alzheimer disease | 1 | 11 | 30 |

Functional annotation of the DEGs frequently encountered in different datasets (at least in 4 out of 20 datasets) detected many functionally related groups, highlighting the complexity of the dioxin response in humans (Figure 2; Tables S1 and S2). We discuss the most representative functional groups and involved genes below.
The most robustly affected genes in response to TCDD that were not previously known to be associated with the AhR pathway. The gene function annotations were taken from the UniProtKB [24]. All genes are robustly up-regulated.

| Gene Name | Full Name | Functions | TCDD Response in 20 Datasets |
|-----------|-----------|-----------|-----------------------------|
|           |           |           | Up-Regulation | Down-Regulation |
| IER3      | Radiation-inducible immediate-early gene IEX-1 | May play a role in the ERK signaling pathway by inhibiting the dephosphorylation of ERK. Acts also as an ERK downstream effector mediating survival | 13 | 0 |
| SECTM1    | Secreted and transmembrane protein 1 | May be involved in thymocyte signaling | 12 | 0 |
| PHLDA1    | Pleckstrin homology-like domain family A member 1 | Seems to be involved in the regulation of apoptosis | 12 | 0 |
| SLC7A11 (xCT) | Cystine/glutamate transporter | Sodium-independent, high-affinity exchange of anionic amino acids with high specificity for an anionic form of cysteine and glutamate | 12 | 1 |
| RUNX1     | Runt-related transcription factor 1 | Forms the heterodimeric complex core-binding factor (CBF) with CBFβ. The heterodimers bind to the core site of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, etc. Essential for hematopoiesis | 11 | 1 |
| EDC3      | Enhancer of mRNA-decapping protein 3 | Binds single-stranded RNA. Involved in the process of mRNA degradation and in the positive regulation of mRNA decapping | 11 | 0 |
| TPRA1     | Transmembrane protein adipocyte-associated 1 | Regulates early mouse embryogenesis [31] | 11 | 0 |
| TTC39C    | Tetratricopeptide repeat protein 39C | Appears to be necessary for proper MAP Kinase and Hedgehog signal transduction in developing muscle cells, as well as muscle cell differentiation [32] | 11 | 0 |
| VIPR1     | Vasoactive intestinal polypeptide receptor 1 | A receptor for VIP. The activity of this receptor is mediated by G proteins that activate adenyl cyclase | 10 | 0 |
| GADI      | Glutamate decarboxylase 1 | Catalyzes the production of GABA | 10 | 1 |

Skeletal morphogenesis. There are many TCDD targets associated with skeletal morphogenesis. DKK1, coding a negative regulator of bone development, is the only down-regulated gene among the most robust TCDD targets (down-regulated in 11 of 20 tested datasets) (Table 2). Another robust TCDD target RUNX2 encodes the transcription factor that regulates bone development. Other genes systematically induced by TCDD and implicated in skeletal morphogenesis are FOSL2, SALL4, MSX2, ADAM12, BST2, and FAM20C (Table S1). Previously, they were almost never discussed in relation to dioxin.

Apoptosis, cell proliferation, and cancerogenesis. There is a large set of genes involved in essential cell processes, such as cell proliferation and apoptosis, that are either up-regulated (e.g., FZD7, MYC, CSK, ABCG2, GDF15, CABLES1) or down-regulated (e.g., CPA4, PHLDA1, ANKRD1, CRIP2, FNI) (Table S1). Robust TCDD target BMF (up-regulated in 8 of 20 analyzed RNA-seq datasets), is involved in apoptosis induction [33]. RUNX1, encoding the transcription factor that is involved in hematopoietic stem cell proliferation [34], is robustly activated by TCDD (in 11 of 20 datasets, Table 3). Both proliferation and apoptosis programs are largely affected by TCDD, this may explain the association of dioxin with
cancerogenesis processes. The relationship between dioxin compounds and cancerogenesis is debated in the Discussion.

Figure 2. Representative gene ontology (GO) terms associated with TCDD response in human cell lines: up-regulated genes (a) and down-regulated genes (b).
Immune system regulators and inflammation response. TCDD robustly activates many regulators of the immune system associated with particular host defense processes. The most robust responses detected by the meta-analysis were for \textit{SECTM1}, \textit{IL1R1}, \textit{CEBPd}, \textit{LACCl}, \textit{GADD45A}, \textit{OAS1}, \textit{C1S}, \textit{COLEC12}, \textit{GBP2}, \textit{IER3}, and \textit{GALNT10}.

Retinoic acid. Three of the most robust TCDD targets participate in the metabolism of all-trans-retinoic acid (RA), a vitamin A derivative and an essential morphogen involved in embryonic development [35]: \textit{ALDH1A3}, \textit{CYP1A1}, \textit{CYP1B1} (Table 2) [36–38]. Functional annotation analysis (Figure 2) highlights that there are many other TCDD targets from this metabolic pathway, e.g., \textit{LRAT} and \textit{DHRS3} (Table S1). We consider in more detail the crosstalk between RA and TCDD in the Discussion.

2.3. Search for Cis-Elements Associated with Dioxin Response

To identify possible mediators of the dioxin response in addition to AhR/ARNT, we studied if there are any other DNA motifs systematically overrepresented in the upstream regions of the TCDD-regulated genes. We performed both unsupervised and supervised searches.

2.3.1. Unsupervised Search: No Predetermined Gene Lists and TF Binding Sites

For the unsupervised search, we applied MetaRE [39] software packages to all datasets on TCDD exposure (see Materials and Methods). In each dataset, we looked for the k-mers that are overrepresented in the upstream regions of induced or repressed gene sets. The analysis was done with different lengths of upstream regions, but here we discuss the results taken for (–1500; +1), as the most representative. MetaRE detected hundreds of motifs frequently encountered in dioxin-regulated promoters under the stringent threshold for \( p \) value \( < 1 \times 10^{-16} \) (Figure S2). Known dioxin-responsive elements (DRE; consensus GCGTG) were not the most overrepresented among identified k-mers. For hepta- and octamers, the motifs containing GCGTG were significant only under a very mild threshold, behind hundreds of more significant motifs (the rank of GCGTG/CACGC in the list of detected potential cis-elements are shown in Table S3). DRE was noticeable under a stringent threshold as a pentamer for activated or repressed by TCDD genes and as a hexamer only for TCDD-repressed genes (Table S3). The most significantly overrepresented k-mers for those both induced and repressed by TCDD was the group of GC-rich motifs that did not match classic DRE consensus (Table S4). All octamers were GC-rich, with only 9% containing one A/T. We used the TOMTOM tool to analyze the overrepresented octamers and found significant matches with known TF binding sites. Most of the GC-rich octamers were recognized as the potential binding sites for specificity protein (Sp1) and Krüppel-like factor 12 (KLF12) (Table S4, Figure 3).

Previously, an alternative AhR-binding site was shown, which the reporting authors called a non-consensus XRE (NC-XRE). This site contains repeats of the 5′-GGGA-3′ tetramer [41]. AhR was shown to recruit Krüppel-like factor 6 (KLF6), form the heterodimeric complex AhR/KLF6, and bind to NC-XRE [42]. We also found four octamers that harbor a 5′-GGGA-3′ tetramer. TOMTOM identified them as potential binding sites of Sp1, KLF12, Zinc finger protein 281 (ZN281), and CCCTC-binding factor (CTCFL) (Table S5).

2.3.2. Supervised Search: Robust Dioxin-Responsive Genes and Known TF Binding Sites

In an alternative search, we analyzed promoters of dioxin targets to identify overrepresented known TF binding sites. For that, we applied the HOMER tool with the Hocomoco database of TF binding sites [40] as the reference. HOMER recognized (FDR < 0.05) binding sites of 134 and 126 TFs overrepresented in upstream regions of dioxin-activated or dioxin-repressed genes, respectively (Table S6). Among them, there are many GC-rich sites, including the binding sites for nine members of the KLF family, Sp1–Sp4 group, Zinc finger and BTB domain-containing protein 14 (ZBT14), ZNF281, and others (Figure 3, Table S6). HOMER detected non-GC-rich sites as well, particularly a set of TFs from the E2F family (E2F1–E2F7) (Figure 3). It is noteworthy that the binding sites of known dioxin response regulators were again not
highly represented, with AhR sites ranked 60th and 113th and ARNT binding sites ranked 64th and 91st in up- and down-regulated by dioxin genes, respectively.

Figure 3. The binding sites of known (a,b) and predicted by MetaRE or HOMER (c–h) transcriptional regulators of the transcriptional response to dioxin. The logos were generated in the Hocomoco database [40].

Together, supervised and unsupervised searches for cis-elements overrepresented in dioxin-regulated promoters gave us a wide spectrum of potential regulators of the dioxin-response.

2.4. Composite AhR-Binding Elements Analysis

As many motifs unrelated to DRE were found to be enriched in dioxin-responsive promoters, we asked whether some of them are the binding sites for AhR partners. To test this hypothesis, we studied if AhR binding sites co-occur with the motifs of AhR’s potential interactors and form composite elements (CE) of a specific structure. For that, we applied the MCOT Toolbox [43] to the AhR binding profile [22] with the AhR-binding site as an anchor and a Hocomoco collection for the partner motif candidates. MCOT detected 43 TFs that were significantly overrepresented in AhR profile CEs, all of them with full or partial overlaps of the binding sites (Table S7). Among them, the classical CE of the dioxin response, AhR-ARNT, was detected in the maximal number of peaks (27% of peaks, p value < 1 × 10⁻¹⁵). In this CE, directly oriented AhR and ARNT binding sites overlap.
by 8nt (Figure S3). Another example, AhR-ZBT14 CEs with inverted orientation and a partially overlap by 6nt of the binding sites, was present in 1.4% of peaks (p value < 1 × 10⁻¹⁵). ZBT14 has not been considered as a potential AhR co-regulator previously, however, our study suggests it as a candidate AhR partner protein.

3. Discussion

3.1. Meta-Analysis of Transcriptomes

We performed a meta-analysis of human transcriptomes exposed to TCDD to find new candidate genes of the dioxin response. Although the designs of whole-genome experiments on TCDD treatment varied from study to study—with different cell lines, TCDD dosage, time of treatment, and even expression profiling technologies—we still were able to detect the common signatures (Figures 1 and 3). For example, TIPARP, encoding a negative regulator of the dioxin response, was induced in the majority of whole-genome studies, as were CYP1A1, CYP1B1, and ALDH1A3, well-known markers of cellular response to dioxin (Table 2). In this study, we extended the repertoire of robustly regulated by dioxin genes and highlighted the molecular trends in the dioxin response. Figures 2 and 4 summarize this information. We discuss some functionally-related dioxin-responsive groups below.

**Figure 4.** The model of dioxin action on human cells based on the present meta-analysis of transcriptome data.

Immune response. Exposure of an organism to xenobiotics, in particular dioxin, is a challenge for the immune system, therefore, it is not surprising that the biggest group of genes robustly induced by TCDD relate to the host defense processes. Of the most robust TCDD targets, SECTM1 was associated with immune system response [44], but it was not well-characterized. Some well-studied genes related to the immune system and systematically affected by TCDD include IL1RI, encoding IL-1 receptor type 1, and CEBPδ, encoding a transcription factor regulating immune and inflammatory responses, both differentially expressed in 8 of 20 tested datasets (Table S1). PTGES (differentially expressed...
in 5 of 20 datasets), a mediator of prostaglandin synthesis that takes part in inflammation response, has been shown to be activated by the AhR signaling pathway [45]. Many other genes that are associated with immune processes and systematically responded to TCDD have not yet been studied in relation to dioxin, e.g., LACC1, GADD45A, OAS1, C1S, COLEC12, GBP2, IER3, and GALNT10 (Table S1). Although a number of candidate genes responding to dioxin and related to the immune system processes were found, a meta-analysis of more specific data, e.g., on immune system cells, is needed to clarify the immune response to TCDD.

RA metabolism. Another functional gene group systematically affected by TCDD relates to RA metabolism (ALDH1A3, LRAT, CYP1A1, CYP1B1, and DHRS3). Indeed, RA metabolism is compromised under dioxin exposure [26,46], however, the crosstalk between RA and TCDD is not evident [47]. Most of the identified robust targets have been discussed in the literature before in relation to dioxin, except DHRS3. Vitamin A metabolizes in two ways: it is either esterified by LRAT and stored or is reversibly oxidized to retinaldehyde by retinol dehydrogenases (DHRS3, in particular), and further irreversibly oxidized to RA [48]. It was shown previously that RA excess induces cleft palate as does TCDD exposure, and TCDD effects depend on RA signaling [49]. Our study predicted DHRS3 and LRAT could be essential mediators of TCDD action on embryonic development via the RA pathway.

Skeletal morphogenesis. A notable group of the robust dioxin-responsive genes mediates bone development and anatomical morphogenesis (Figures 2 and 4). Among the known dioxin targets, there is DKK1, encoding an inhibitor of Wnt signaling [50], that was systematically down-regulated by TCDD (Table 2). There is evidence that RA up-regulates DKK1 and DKK2, inactivating the Wnt signaling pathway [51]. Thus, the TCDD–DKK–Wnt relationship is further evidence of the interplay of the AhR and RA pathways. Another important regulator of skeletal morphogenesis, in particular osteoblastic differentiation, is a downstream target of the Wnt signaling pathway, RUNX2 (Table 2) [52]. Note that according to the meta-analysis, RUNX2 expression was significantly increased in 14 out of 20 (and down-regulated in 1 out of 20) RNA-seq datasets. However, in a number of published works, it was shown that the expression of this gene is decreased under the TCDD action [29,53]. We assume that the contradiction in the experimental data is due to the dysregulation of the RUNX2 gene: Wnt signaling activates it, while dioxin-induced AhR signaling down-regulates Wnt signaling and causes developmental defects [54]. Other genes robustly induced by TCDD are implicated in bone tissue maturation: FOSL2, SALL4, MSX2, ADAM12, BST2, and FAM20C. Multiple TCDD targets within the same gene network may explain the TCDD effects on craniofacial skeleton development [55] and imply that TCDD affects master regulators of bone development.

Cell proliferation, apoptosis, cancerogenesis. Wnt-signaling also contributes to the regulation of cell proliferation, apoptosis, and cancerogenesis. One of the members of this regulatory chain is protein yippee-like 3 (YPEL3), which is down-regulated in 7 of 20 analyzed datasets (Table S1). YPEL3 suppresses the Wnt/β-catenin signaling and its further regulation of downstream genes [56]. Another promoter of cancerogenesis that was identified by our meta-analysis as a robust dioxin target is the transcription factor RUNX1 (Table 3). RUNX1 up-regulation has previously been shown after exposure to cytotoxic agents; upon overexpression, RUNX1 reduced proliferation, promoted apoptosis, and augmented the DNA damage response in bone marrow cells [34]. Interesting, RUNX1 also has relatively high expression in the thymus (SRP056969 in SRA). In addition, we identified other robustly down-regulated genes that participate in the regulation of proliferation, apoptosis, and cancerogenesis, including FNI, PHLD1, ANKR1, CPA4, CRIP2, MYC, FZD7, CSK, CABLES1, ABCG2, GDF15, and others (Table S1, Figure 4). Thus, our analysis of genes robustly activated by TCDD supports the idea that dioxin does not induce cancerogenesis per se, but dysregulates cell proliferation and apoptosis, as well as the immune system, which could cause cancer.

3.2. Transcriptional Regulation

Another approach we used in the study was the analysis of cis-regulatory regions of dioxin-responding genes. Surprisingly, the well-known dioxin responsive element was not overrepresented;
instead, many GC-rich motifs were detected as being highly enriched in promoters of dioxin-responsive genes. To study what these motifs are, we compared them with known TF binding sites and analyzed AhR-binding regions in more detail. In Table 4, we summarize the information derived by MetaRE, Homer, and MCOT tools for the most prominent potential regulators of the dioxin response, and we discuss some of these potential regulators below. Namely, we detected E2F2 and ZBT14 as potent AhR co-regulators in the dioxin response, with ZBT14 predicted in every search performed. In addition, Sp1, KLF12, and ZNF281 were detected by MetaRE and HOMER, but not MCOT. Thus, they still might be major regulators of the dioxin response, but probably without heterodimerization with AhR on DNA.

Table 4. Summary table for known and predicted transcriptional regulators of dioxin transcriptional response. Binding sites of these transcription factors (TFs) were found to be significantly overrepresented in promoters and AhR peaks of TCDD-responsive genes. Three tools were used in the analysis: (1) MetaRE for an unsupervised search over multiple datasets of dioxin-regulated genes (stringent criterion was applied $p < 1 \times 10^{-16}$); (2) HOMER for the search of known TF binding sites within promoters of genes that are robustly regulated by dioxin (FDR $< 0.05$); (3) MCOT for the search of potential TF partners to AhR based on the co-occurrence of binding sites ($p$ value $< 1 \times 10^{-15}$).

| TF Binding Sites | Upstream Regions of Dioxin–Regulated Genes | Composite Elements in AhR-Binding Regions |
|------------------|--------------------------------------------|------------------------------------------|
|                  | MetaRE          | HOMER                  | MCOT                      |
| AhR              | + *             | +                      | +                         |
| ARNT             | −               | +                      | +                         |
| E2F2             | −               | +                      | +                         |
| KLF12            | +               | +                      | −                         |
| CTCFL            | +               | +                      | −                         |
| Sp1              | +               | +                      | −                         |
| ZBT14            | +               | +                      | +                         |
| ZNF281           | +               | +                      | −                         |

* found as an overrepresented pentamer in up- and down-regulated genes and as overrepresented hexamers in down-regulated genes.

Sp/KLF regulators. Different members of the Sp/KLF family were among the top matches detected by all applied algorithms. The family consists of Sp1–9 and Sp1-related KLF1–18 that have highly conserved DNA binding domains recognizing GC-rich sequences (GGGGCGGGG and GGTGTGGGG) [57]. Among them, two TFs were predicted the most robustly: Sp1 and KLF12 (Table 4). Sp1 is a known mediator of the dioxin response: This TF activates AhR transcription [58], and AhR recruits Sp1 to regulate CYP1A1 [59]. Transcription factors Sp1 and Sp3 also enhance AhRR [60]. This gene encodes a protein that binds to AhR ligands and DNA binding sites and suppresses the effect of activated AhR. Thus, Sp1/Sp3 negatively regulate AhR-mediated cell response through AhRR activation. There is also a link between the dioxin response and KLF6–AhR in the non-canonical signaling pathway where it forms a heterodimeric DNA-binding complex with KLF6 for participation in cell cycle regulation [61]. We also predicted that KLF12 is involved in the dioxin response, although there is no evidence in the literature. Therefore, it can be a prospective target to study further, as KLF12 is an essential regulator of embryo development, affecting the attachment of the embryo to the endometrial epithelium through the regulation of the leukemia inhibitory factor (Lif) gene [62]. KLF12 also plays a role in cancerogenesis and cell proliferation [63,64].

ZNF281 transcription factor. ZNF281 binding sites were overrepresented in dioxin-responsive and AhR binding regions. ZNF281 is a Krüppel-type zinc-finger transcriptional regulator with elevated expression levels in the placenta, adult kidney, liver, and lymphocytes [65]. It has not been studied in relation to dioxin thus far, but its role in the regulation of molecular and physiological processes suggest ZNF281 as a prospective target. It mediates DNA reparation processes [66], cell proliferation,
migration, invasion, and metastasis of colorectal cancer by inhibiting the Wnt/β-catenin pathway [67], regulates neuronal differentiation [68], and induces the inflammatory response [69].

ZBT14 transcription factor. ZBT14 binding sites are not only enriched with dioxin-responsive genes, but also with a notable number of AhR binding peaks (AhR–ZNF281 CE) with an overlap (Table S7), suggesting that ZBT14 cooperates with AhR in the regulation of some genes. ZBT14, also known as ZFP161, ZNF478, and ZF5 in mice, is ubiquitously expressed and known to regulate many essential regulators such as c-myc, X-mental retardation 1 (FMR1) gene, Klf9, Foxp1, and others [70–74]. In a breast cancer study, it was demonstrated that a decreased ZFP161 level was associated with a poor clinical forecast [75]. It was also postulated that ZBT14 is a player in some metabolic and inflammation processes because it is involved in the regulation of Interleukin-6 (Il6) and Lif in skeletal muscle [76].

E2F2 transcription factor. E2F2 binding sites were also found to be associated with the AhR-mediated dioxin response (Table 4). E2F2 participates in the regulation of cell proliferation and post-injury tissue repairs [77]. However, it was also shown to be associated with cancerogenesis, e.g., nasopharyngeal carcinoma, colon cancer, and others [78,79]. The link between this TF with the dioxin response and AhR signaling has been shown previously [80]. E2F2 negatively regulated the AhR pathway in T-lymphocytes after TCDD exposure. Here, we suggest the mechanism of this regulation: through CE of specific structures (Table S7) for which E2F2 and AhR either compete or cooperate with each other.

Transcriptional repressor CTCFL. CTCFL (or CCCTC-binding factor or BORIS (brother of the regulator of imprinted sites)) regulates testis-specific expression in spermatogenesis and is known to be a cancer antigen [81]. Recently it was supposed that CTCFL promotes regulatory chromatin interactions and therefore associates with cancerogenesis [82]. Here, we suggest that CTCFL can compete or cooperate with AhR on CE of particular dioxin-responsive genes.

### 3.3. Future Research

In this work, we focused on the robust responses and set aside the specific responses detected under particular conditions. The datasets used for the meta-analysis were obtained at different concentrations (from 1 to 100 nM), durations of TCDD exposure (from 6 h to 3 weeks), and cell cultures (cancer, embryonic, liver-derived lines, etc.) (Table 1). Identifying the specific responses, e.g., cell-type-specific, is an important task, but it requires more whole-genome datasets being available for meta-analysis. For example, when comparing the transcriptional responses in different studies, we see that most of the transcriptomes cluster together, but there are some specific responses (Figure S1). We cannot study specific responses through meta-analysis until we have more of such cases. Although many genes were highlighted as associated with the response to dioxin, many of them may be involved not in the primary, but the secondary response to TCDD. AhR can trigger a cascade of responses, inducing the functioning of a number of other transcription factors that directly regulate the transcription of identified robust genes. In addition, as we identified many known regulators of developmental processes, cell cycle, and differentiation as TCDD-responsive genes, their response to dioxin can partially explain the harmful effects of dioxin on the developing nervous, endocrine, and reproductive systems.

Here, we also predicted a number of TFs involved in the transcriptional response to dioxin (Table 4). Identified TFs can act independently or, as was proposed in this work, through heterodimerization or competition with AhR. Table 4 suggests that there might be three modes of AhR action in the TCDD response: (1) direct from the DRE located close to the transcription start site; (2) cooperative with other TFs whose binding sites are located in the upstream regions or link the distal enhancers with the transcription start site; and (3) indirect via AhR targets or AhR-independent regulators. All these questions require further experimental studies to understand the dioxin response and the ways to prevent its harmful effects.
4. Materials and Methods

4.1. Datasets

We searched all publicly available transcriptome studies on dioxin action on humans in Gene Expression Omnibus (GEO) using keywords “dioxin” and “human” and found twelve series of microarray datasets (GSE7765, GSE14553, GSE16160, GSE24193, GSE34249, GSE35034, GSE46874, GSE69844, GSE69845, GSE69849, GSE69850, GSE122518) and seven series of RNA-Seq datasets (GSE63935, GSE80953, GSE83886, GSE98515, GSE122002, GSE114552, GSE141711). After processing the datasets (see Section 4.2) we selected 9 microarrays (from 5 series) and 11 RNA-Seq (from 6 series) datasets. The processed ChIP-Seq dataset for AhR GSE90550 was taken from GEO. Additional details are given in Table 1.

4.2. Data Processing and Identification of Dioxin-Responsive Genes

We used R software version 4.0.2 and Bioconductor version 3.11 to process transcriptomics data. In the first step, we processed each individual dataset independently. The raw expression data were normalized with RMA and TMM algorithms for microarray and RNA-seq datasets, respectively [83, 84]. To identify DEGs between control and TCDD-treated samples, we used the package limma [85] for microarray and edgeR [84] for RNA-seq datasets. The significance of expression changes in the microarray data were estimated by t-test and in the RNA-seq data by quasi-likelihood F-test. The raw p values were corrected by the Benjamini–Hochberg multiple testing procedure to adjust the FDR. FDR ≤ 0.2 was set as the criterion for DEGs irrespective of to the fold-change level. Datasets in which the number of activated or repressed DEGs were greater than 100 were included in the further meta-analysis (Table 1).

In the second step, we compared the lists of DEGs to identify the robust TCDD targets. The number of experiments in which the gene must be detected as DEG to be included in the list of systemically induced genes was equal to four experiments (p value = 0.016). The threshold was estimated from a binomial distribution with the probability of the gene being DEG by chance equal to 0.05.

4.3. Functional Annotation of Dioxin-Responsive Genes

Functional annotation was made using the topGO R package with default settings, and the Fisher exact test was used for significance estimation. Annotations between genes and GO terms were retrieved from org.Hs.eg.db R package.

4.4. Motif Discovery in Promoters and Peaks of Dioxin-Responsive Genes

R package MetaRE [39] was used to identify overrepresented cis-elements in upstream regions. Upstream regions of 19,815 Homo sapiens genes (those with expression unambiguously detected by the microarrays) were taken from GENCODE Release v27. We performed the study for (−500; +1), (1000; +1), (−1500; +1), and (−2000; +1) lengths of upstream regions. As the results were comparable with a higher overrepresentation of classic DREs in (−1500; +1) regions, here we discuss the results only for the latter.

As the foreground, we used upstream regions of genes that significantly changed their expression in response to TCDD, and as the background, the upstream regions of genes that did not change their expression. MetaRE output sequences were compared with known transcription factor binding motifs from the HOCOMOCO v11 core database (http://hocomoco11.autosome.ru/) [40] with the TOMTOM tool [86] using the euclidean distance. The hits with an E-value < 0.05 were considered as significant matches.

HOMER v4.10 known motif enrichment [87] was performed on upstream regions. The search of known motifs was performed against the HOCOMOCO v11 core motif library. Up- and down-regulated dioxin-responsive genes were analyzed separately.
4.5. The Search of Potential AhR Interacting Partners

We used the MCOT tool [43] to identify pairwise TF interactions in AhR-binding regions. As the anchor motif, we took the AhR position-weighted matrix (PWM) from the HOCOMOCO collection. PWMs for the rest of the TFs from this collection were screened for potential partner motifs that co-occurred with AhR. The spacer length for CE was taken between 0 and 29. The significance of CE enrichment was estimated by the adjusted $p$ value < $1 \times 10^{-15}$.

Supplementary Materials: Supplementary Materials can be found at http://www.mdpi.com/1422-0067/21/21/7858/s1.

Author Contributions: Conceptualization, V.M., N.K., and E.O.; methodology, V.M. and Y.S.; formal analysis, Y.S., D.W., and E.O.; investigation, E.O., D.W., and Y.S.; writing—original draft preparation, E.O.; writing—review and editing, Y.S., N.K., and V.M.; visualization, E.O. and D.W.; supervision, N.K.; project administration, V.M.; funding acquisition, N.K. and V.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by RFBR Projects 18-04-01130 (Meta-analysis pipeline development) and 18-29-13040 (MCOT application). The analysis of raw experimental data was supported by project 0324-2019-0040-C-01 from the Russian State Budget.

Acknowledgments: We thank Victor Levitsky for his advice with the MCOT tool application.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ABCG2        | ATP-binding cassette sub-family G member 2 |
| ADAM12       | Disintegrin and metalloproteinase domain-containing protein 12 |
| AhR          | Aryl hydrocarbon receptor |
| AhRR         | Aryl-hydrocarbon receptor repressor |
| AIP          | AhR-interacting protein |
| ALDH1A3      | Aldehyde dehydrogenase 1 family member A3 |
| ANKRD1       | Ankyrin repeat domain-containing protein 1 |
| ARNT         | Aryl hydrocarbon receptor nuclear translocator |
| BMF          | Bcl-2-modifying factor |
| BST2         | Bone marrow stromal antigen 2 |
| C1S          | Complement C1s subcomponent |
| CA9          | Carbonic anhydrase 9 |
| CABLES1      | CDK5 and ABL1 enzyme substrate 1 |
| CE           | Composite element |
| CEBPD        | CCAAT/enhancer-binding protein delta |
| COLEC12      | Collectin-12 |
| CPA4         | Carboxypeptidase A4 |
| CRIP2        | Cysteine-rich protein 2 |
| CSK          | Tyrosine-protein kinase CSK |
| CTCFL        | CCCTC-binding factor |
| CYP1A1       | Cytochrome P450 family 1 subfamily A member 1 |
| CYP1B1       | Cytochrome P450 family 1 subfamily B member 1 |
| DEG          | Differentially expressed genes |
| DHR53        | Short-chain dehydrogenase/reductase 3 |
| DKK1         | Dickkopf-1 |
| E2F2         | Transcription factor E2F2 |
| FAM20C       | Extracellular serine/threonine protein kinase FAM20C |
| FDR          | False discovery rate |
| FN1          | Fibronectin |
| FOSL2        | Fos-related antigen 2 |
| FZD7         | Frizzled-7 |
| GADD45A      | Growth arrest and DNA damage-inducible protein GADD45 alpha |
| GALNT10      | Polypeptide N-acetylgalactosaminyltransferase 10 |
| GBP2         | Guanylate-binding protein 2 |
GDF15  Growth/differentiation factor 15
GEO  Gene Expression Omnibus
GO  Gene Ontology
Hsp90  90 kDa heat shock protein
IER3  Radiation-inducible immediate-early gene IEX-1
IL1R1  Interleukin-1 receptor type 1
KLF  Krüppel-like factor
LACC1  Laccase domain-containing protein 1
Lif  Leukemia inhibitory factor
LRAT  Lecithin retinol acyltransferase
MSX2  Homeobox protein MSX-2
MYC  Myc proto-oncogene protein
NC-XRE  Non-consensus XRE
NDRG1  N-myc downstream-regulated gene 1 protein
OAS1  2′-5′-oligoadenylate synthase 1
PHLDA1  Pleckstrin homology-like domain family A member 1
PTGES  Prostaglandin E synthase
PWM  Position-weighted matrix
RA  All-trans-retinoic acid
RUNX1  Runt-related transcription factor 1
RUNX2  Runt related transcription factor 2
SALL4  Sal-like protein 4
SECTM1  Secreted and transmembrane protein 1
Sp1  Specificity protein 1
TCDD  2,3,7,8-tetrachlorodibenzo-p-dioxin
TIPARP  TCDD inducible poly(ADP-ribose) polymerase
TF  Transcription factor
XRE  Xenobiotic-responsive elements
YPEL3  Protein yippee-like 3
ZN281  Zinc finger protein 281
ZBT14  Zinc finger and BTB domain-containing protein 14

References

1. Ssebugere, P.; Sillanpää, M.; Matovu, H.; Mubiru, E. Human and environmental exposure to PCDD/Fs and dioxin-like PCBs in Africa: A review. *Chemosphere* 2019, 223, 483–493. [CrossRef]
2. Kreitinger, J.M.; Beamer, C.A.; Shepherd, D.M. Environmental Immunology: Lessons Learned from Exposure to a Select Panel of Immunotoxicants. *J. Immunol.* 2016, 196, 3217–3225. [CrossRef]
3. DeVito, M.J.; Birnbaum, L.S.; Farland, W.H.; Gasiewicz, T.A. Comparisons of estimated human body burdens of dioxinlike chemicals and TCDD body burdens in experimentally exposed animals. *Environ. Health Perspect.* 1995, 103, 820–831. [CrossRef]
4. Wesselink, A.; Warner, M.; Samuels, S.; Parigi, A.; Brambilla, P.; Mocarelli, P.; Eskenazi, B. Maternal dioxin exposure and pregnancy outcomes over 30 years of follow-up in Seveso. *Environ. Int.* 2014, 63, 143–148. [CrossRef] [PubMed]
5. Lundqvist, C.; Zuurbier, M.; Leijts, J.; Johansson, C.; Ceccatelli, S.; Saunders, M.; Schoeters, G.; ten Tusscher, G.; Koppe, J.G. The effects of PCBs and dioxins on child health. *Acta Paediatr. Suppl.* 2006, 95, 55–64. [CrossRef] [PubMed]
6. Swanson, H.I.; Yang, J. Mapping the protein/DNA contact sites of the Ah receptor and Ah receptor nuclear translocator. *J. Biol. Chem.* 1996, 271, 31657–31665. [CrossRef]
9. Bell, D.R.; Poland, A. Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. J. Biol. Chem. 2000, 275, 36407–36414. [CrossRef]
10. Cox, M.B.; Miller, C.A., 3rd. Cooperation of heat shock protein 90 and p23 in aryl hydrocarbon receptor signaling. Cell Stress Chaperones 2004, 9, 4–20. [CrossRef]
11. Sorg, O. AhR signalling and dioxin toxicity. Toxicol. Lett. 2014, 230, 225–233. [CrossRef]
12. Hankinson, O. The role of the aryl hydrocarbon receptor nuclear translocator protein in aryl hydrocarbon receptor action. Trends Endocrinol. Metab. 1994, 5, 240–244. [CrossRef]
13. Watson, A.J.; Hankinson, O. Dioxin-and Ah receptor-dependent protein binding to xenobiotic responsive elements and G-rich DNA studied by in vivo footprinting. J. Biol. Chem. 1992, 267, 6874–6878.
14. Ambolet-Camoit, A.; Ottolenghi, C.; Leblanc, A.; Kim, M.J.; Kim, M.J.; Letourneur, F.; Jacques, S.; Cagnard, N.; Guguen-Guilhouzou, C.; Barouki, R.; et al. Two persistent organic pollutants which act through different xenosensors (alpha-endosulfan and 2,3,7,8 tetrachlorodibenzo-p-dioxin) interact in a mixture and downregulate multiple stem genes involved in human hepatocyte lipid and glucose metabolism. Biochimie 2015, 116, 79–91. [CrossRef]
15. De Abrew, K.N.; Kainkaryam, R.M.; Shan, Y.K.; Overmann, G.J.; Settivari, R.S.; Wang, X.; Xu, J.; Adams, R.L.; Tiesman, J.P.; Carney, E.W.; et al. Grouping 34 Chemicals Based on Mode of Action Using Connectivity Mapping. Toxicol. Sci. 2016, 151, 447–461. [CrossRef]
16. Ssvodobová, J.; Procházková, J.; Kabátková, M.; Krkoška, M.; Smerdová, L.; Libalová, H.; Topinka, J.; Klémá, J.; Kozubík, A.; Machala, M.; et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Disrupts Control of Cell Proliferation and Apoptosis in a Human Model of Adult Liver Progenitors. Toxicol. Sci. 2019, 172, 368–384. [CrossRef]
17. Schwartz, M.P.; Hou, Z.; Propson, N.E.; Zhang, J.; Engstrom, C.J.; Costa, V.S.; Jiang, P.; Nguyen, B.K.; Bolin, J.M.; Daly, W.; et al. Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proc. Natl. Acad. Sci. USA 2015, 112, 12516–12521. [CrossRef]
18. Tomblin, J.K.; Arthur, S.; Primerano, D.A.; Chaudhry, A.R.; Fan, J.; Denvir, J.; Salisbury, T.B. Aryl hydrocarbon receptor (AHR) regulation of L-Type Amino Acid Transporter 1 (LAT-1) expression in MCF-7 and MDA-MB-231 breast cancer cells. Biochem. Pharmacol. 2016, 106, 94–103. [CrossRef]
19. Fu, H.; Wang, L.; Wang, J.; Bennett, B.D.; Li, J.L.; Zhao, B.; Hu, G. Dioxin and AHR impairs mesoderm gene expression and cardiac differentiation in human embryonic stem cells. Sci. Total Environ. 2019, 651, 1038–1046. [CrossRef]
20. Jagannathan, L.; Jose, C.C.; Tanwar, V.S.; Bhattacharya, S.; Cuddapah, S. Identification of a unique gene expression signature in mercury and 2,3,7,8-tetrachlorodibenzo-p-dioxin co-exposed cells. Toxicol. Res. (Camb.) 2017, 6, 312–323. [CrossRef]
21. Pham, T.H.; Lecomte, S.; Le Guevel, R.; Lardenois, A.; Evrard, B.; Chalmel, F.; Ferriere, F.; Balaguer, P.; Efstathiou, T.; Pakdel, F.; et al. Characterization of Glyceollins as Novel Aryl Hydrocarbon Receptor Ligands and Their Role in Cell Migration. Int. J. Mol. Sci. 2020, 21, 1368. [CrossRef]
22. Yang, S.Y.; Ahmed, S.; Satheesh, S.V.; Matthews, J. Genome-wide mapping and analysis of aryl hydrocarbon receptor (AHR)-and aryl hydrocarbon receptor repressor (AHRR)-binding sites in human breast cancer cells. Arch. Toxicol. 2018, 92, 225–240. [CrossRef] [PubMed]
23. Landi, M.T.; Bergen, A.W.; Baccarelli, A.; Patterson, D.G., Jr.; Grassman, J.; Ter-Minassian, M.; Mocarelli, P.; Caporaso, N.; Masten, S.A.; Pesatori, A.C.; et al. CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. Toxicology 2005, 207, 191–202. [CrossRef] [PubMed]
24. UniProt Consortium. UniProt: A worldwide hub of protein knowledge. Nucleic Acids Res. 2019, 47, D506–D515. [CrossRef]
25. Diani-Moore, S.; Ram, P.; Li, X.; Mondal, P.; Youn, D.Y.; Sauve, A.A.; Rifkind, A.B. Identification of the aryl hydrocarbon receptor target gene TiPARP as a mediator of suppression of hepatic gluconeogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin and of nicotinamide as a corrective agent for this effect. J. Biol. Chem. 2010, 285, 38801–38810. [CrossRef] [PubMed]
26. Kim, S.; Dere, E.; Burgoon, L.D.; Chang, C.C.; Zacharewski, T.R. Comparative analysis of AhR-mediated TCDD-elicited gene expression in human liver adult stem cells. Toxicol. Sci. 2009, 112, 229–244. [CrossRef]
27. Haarmann-Stemmann, T.; Abel, J. The arylhydrocarbon receptor repressor (AhRR): Structure, expression, and function. Biol. Chem. 2006, 387, 1195–1199. [CrossRef]
28. Lee, H.J.; Pyo, M.C.; Shin, H.S.; Ryu, D.; Lee, K.W. Renal toxicity through AhR, PXR, and Nrf2 signaling pathway activation of ochratoxin A-induced oxidative stress in kidney cells. *Food Chem Toxicol.* 2018, 122, 59–68. [CrossRef]

29. Korkalainen, M.; Kallio, E.; Oikku, A.; Nelo, K.; Ilvesaro, J.; Tuukkanen, J.; Mahonen, A.; Viluksela, M. Dioxins interfere with differentiation of osteoblasts and osteoclasts. *Bone* 2009, 44, 1134–1142. [CrossRef]

30. Moreno-Marin, N.; Barrasa, E.; Morales-Hernández, A.; Paniagua, B.; Blanco-Fernández, G.; Merino, J.M.; Fernández-Salgueiro, P.M. Dioxin Receptor Adjusts Liver Regeneration After Acute Toxic Injury and Protects Against Liver Carcinogenesis. *Sci. Rep.* 2017, 7, 10420. [CrossRef]

31. Aki, T.; Funakoshi, T.; Nishida-Kitayama, J.; Mizukami, Y. TPRA40/GPR175 regulates early mouse embryogenesis through functional membrane transport by Sjögren’s syndrome-associated protein NA14. *J. Cell Physiol.* 2008, 217, 194–206. [CrossRef]

32. Hayes, C.S.; Labuzan, S.A.; Menke, J.A.; Haddock, A.N.; Waddell, D.S. Ttc39c is upregulated during skeletal embryonic development. *FASEB J.* 2013, 27, 4877–4889. [CrossRef]

33. Pinon, J.D.; Labi, V.; Egle, A.; Villunger, A. Bim and Bmf in tissue homeostasis and malignant disease. *Oncogene* 2008, 27 (Suppl. S1), S41–S52. [CrossRef]

34. Speidel, D.; Wellbrock, J.; Abas, M. RUNX1 Upregulation by Cytotoxic Drugs Promotes Apoptosis. *Cancer Res.* 2017, 77, 6818–6824. [CrossRef]

35. Billings, S.E.; Pierzchalski, K.; Butler Tjaden, N.E.; Pang, X.Y.; Trainor, P.A.; Kane, M.A.; Moise, A.R. The retinaldehyde reductase DHRS3 is essential for preventing the formation of excess retinoic acid during embryonic development. *FASEB J.* 2013, 27, 4877–4889. [CrossRef]

36. Chen, H.; Howald, W.N.; Juchau, M.R. Biosynthesis of all-trans-retinoic acid from all-trans-retinol: Catalysis of all-trans-retinol oxidation by human P-450 cytochromes. *Drug Metab. Dispos.* 2000, 28, 315–322.

37. Ziegler, N.; Awwad, K.; Fisslthaler, B.; Reis, M.; Devraj, K.; Corada, M.; Minardi, S.P.; Dejana, E.; Plate, K.H.; Fleming, I.; et al. β-Catenin Is Required for Endothelial Cyp1b1 Regulation Influencing Metabolic Barrier Function. *J. Neurosci.* 2016, 36, 8921–8935. [CrossRef]

38. Moretti, A.; Li, J.; Donini, S.; Sobol, R.W.; Rizzi, M.; Garavaglia, S. Crystal structure of human aldehyde dehydrogenase 1A3 complexed with NAD+ and retinoic acid. *Sci. Rep.* 2016, 6, 35710. [CrossRef]

39. Cherenkov, P.; Novikova, D.; Omelyanchuk, N.; Levitsky, V.; Grosse, I.; Weijers, D.; Mironova, V. Diversity of cis-regulatory elements associated with auxin response in Arabidopsis thaliana. *J. Exp. Bot.* 2018, 69, 329–339. [CrossRef]

40. Kulakovskiy, I.V.; Vorontsov, I.E.; Yevshin, I.S.; Sharipov, R.N.; Fedorova, A.D.; Rumynskiy, E.I.; Medvedeva, Y.A.; Magana-Mora, A.; Bajic, V.B.; Papatserno, D.A.; et al. HOCOMOCO: Towards a complete collection of transcription factor binding models for human and mouse via large-scale ChIP-Seq analysis. *Nucleic Acids Res.* 2018, 46, D252–D259. [CrossRef]

41. Huang, G.; Elferink, C.J. A novel non-consensus xenobiotic response element capable of mediating aryl hydrocarbon receptor-dependent gene expression. *Mol. Pharmacol.* 2012, 81, 338–347. [CrossRef]

42. Wilson, S.R.; Joshi, A.D.; Elferink, C.J. The tumor suppressor Kruppel-like factor 6 is a novel aryl hydrocarbon receptor DNA binding partner. *J. Pharmacol. Exp. Ther.* 2013, 345, 419–429. [CrossRef]

43. Levitsky, V.; Zemlyanskaya, E.; Oshchepkov, D.; Podkolodnaya, O.; Ignatieva, E.; Grosse, I.; Weijers, D.; Mironova, V.; Merkulova, T. A single ChIP-seq dataset is sufficient for comprehensive analysis of motifs co-occurrence with MCOT package. *Nucleic Acids Res.* 2019, 47, e139. [CrossRef] [PubMed]

44. Huyton, T.; Göttmann, W.; Bade-Döding, C.; Paine, A.; Blaszczyk, R. The T/NK cell co-stimulatory molecule SECTM1 is an IFN “early response gene” that is negatively regulated by LPS in human monocyteic cells. *Biochim. Biophys. Acta* 2011, 1810, 1294–1301. [CrossRef] [PubMed]

45. Awji, E.G.; Chan, H.; Bruse, S.; Smith, K.R.; Colby, J.K.; Mebratu, Y.; Levy, B.D.; Tesfaigzi, Y. Wood smoke enhances cigarette smoke-induced inflammation by inducing the aryl hydrocarbon receptor repressor in airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 2015, 52, 377–386. [CrossRef]

46. Hoegberg, P.; Schmidt, C.K.; Nau, H.; Ross, A.C.; Zolfaghari, R.; Fletcher, N.; Trossvik, C.; Nilsson, C.B.; Häkansson, H. 2,3,7,8-tetrachlorodibenzo-p-dioxin induces lecithin: Retinol acyltransferase transcription in the rat kidney. *Chem. Biol. Interact.* 2003, 145, 1–16. [CrossRef]

47. Shimarak, I.O. Retinoid-xenobiotic interactions: The Ying and the Yang. *Hepatobiliary Surg. Nutr.* 2015, 4, 243–267. [CrossRef]
48. Clagett-Dame, M.; Knutson, D. Vitamin A in reproduction and development. *Nutrients* 2011, 3, 385–428. [CrossRef]

49. Jacobs, H.; Dennefeld, C.; Féret, B.; Viluksema, M.; Hákansson, H.; Mark, M.; Ghysselinck, N.B. Retinoic acid drives aryl hydrocarbon receptor expression and is instrumental to dioxin-induced toxicity during palate development. *Environ. Health Perspect.* 2011, 119, 1590–1595. [CrossRef]

50. Niehrs, C. Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* 2006, 25, 7469–7481. [CrossRef]

51. Roa, L.A.; Bloemen, M.; Carels, C.E.L.; Wagener, F.A.D.T.G.; Von den Hoff, J.W. Retinoic acid disrupts osteogenesis in pre-osteoblasts by down-regulating WNT signaling. *Int. J. Biochem. Cell Biol.* 2019, 116, 105597. [CrossRef] [PubMed]

52. Gaur, T.; Lengner, C.J.; Hovhannisyan, H.; Bhat, R.A.; Bodine, P.V.; Kommm, B.S.; Javed, A.; van Wijnen, A.J.; Stein, J.L.; Stein, G.S.; et al. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. *J. Biol. Chem.* 2005, 280, 33132–33140. [CrossRef] [PubMed]

53. Tong, Y.; Niu, M.; Du, Y.; Mei, W.; Cao, W.; Dou, Y.; Yu, H.; Du, X.; Yuan, H.; Zhao, W. Aryl hydrocarbon receptor suppresses the osteogenesis of mesenchymal stem cells in collagen-induced arthritic mice through the inhibition of β-catenin. *Exp. Cell Res.* 2017, 350, 349–357. [CrossRef] [PubMed]

54. Schneider, A.J.; Branam, A.M.; Peterson, R.E. Intersection of AHR and Wnt signaling in development, health, and disease. *Int. J. Mol. Sci.* 2014, 15, 17852–17885. [CrossRef] [PubMed]

55. Burns, F.R.; Peterson, R.E.; Heideman, W. Dioxin disrupts cranial cartilage and dermal bone development in zebrafish larvae. *Aquat. Toxicol.* 2015, 164, 52–60. [CrossRef]

56. Zhang, J.; Wen, X.; Ren, X.Y.; Li, Y.Q.; Tang, X.R.; Wang, Y.Q.; He, Q.M.; Yang, X.J.; Sun, Y.; Liu, N.; et al. YPEL3 suppresses epithelial-mesenchymal transition and metastasis of nasopharyngeal carcinoma cells through the Wnt/β-catenin signaling pathway. *J. Exp. Clin. Cancer Res.* 2016, 3, 109. [CrossRef]

57. Pei, J.; Grishin, N.V. C2H2 zinc finger proteins of the SP family in metazoans and beyond. *Gene* 2015, 573, 91–99. [CrossRef]

58. Do, M.T.; Kim, H.G.; Tran, T.T.; Khanal, T.; Choi, J.H.; Chung, Y.C.; Jeong, T.C.; Jeong, H.G. Metformin regulates ornithine decarboxylase gene expression. *FEBS J.* 2014, 280, 33101–33110. [CrossRef] [PubMed]

59. Ye, W.; Chen, R.; Ren, X.; Huang, B.; Lin, R.; Xie, X.; Chen, J.; Jiang, J.; Deng, Y.; Wen, J. AhR regulates the expression of human cytochrome P450 1A1 (CYP1A1) by recruiting Sp1. *Biochem. Biophys. Res. Commun.* 2019, 52, 39–48. [CrossRef] [PubMed]

60. Baba, T.; Mimura, J.; Grishin, N.V.; Marini, A.; Pieraccioli, M.; Malewicz, M.; Venkitaraman, A.R.; Melino, G. ZNF281 is recruited on DNA breaks to facilitate DNA repair by non-homologous end joining. *Oncogene* 2020, 39, 754–766. [CrossRef] [PubMed]

61. Wright, E.J.; De Castro, K.P.; Joshi, A.D.; Elferink, C.J. Canonical and non-canonical aryl hydrocarbon receptor signaling pathways. *Curr. Opin. Toxicol.* 2017, 2, 82–92. [CrossRef] [PubMed]

62. Huang, C.; Sun, H.; Wang, Z.; Liu, Y.; Cheng, X.; Liu, J.; Jiang, R.; Zhang, X.; Zhen, X.; Zhou, J.; et al. Increased Krüppel-like factor 12 impairs embryo attachment via downregulation of leukemia inhibitory factor in women with recurrent implantation failure. *Cell Death Discov.* 2018, 4, 23. [CrossRef] [PubMed]

63. Lam, V.C., et al. Retinoic acid disrupts Arh receptor expression and is instrumental to dioxin-induced toxicity during palate development. *Cell Physiol. Biochem.* 2019, 203, 981–989. [CrossRef] [PubMed]

64. Law, D.J.; Du, M.; Law, G.L.; Merchant, J.L. ZBP-99 defines a conserved family of transcription factors and regulates ornithine decarboxylase gene expression. *Biochem. Biophys. Res. Commun.* 1999, 262, 113–120. [CrossRef]

65. Qin, C.J.; Bu, P.L.; Zhang, Q.; Chen, J.T.; Li, Q.Y.; Liu, J.T.; Dong, H.C.; Ren, X.Q. ZNF281 Regulates Cell Proliferation, Migration and Invasion in Colorectal Cancer through Wnt/β-Catenin Signaling. *Cell Physiol. Biochem.* 2019, 52, 1503–1516. [CrossRef]
19. Pieraccioli, M.; Nicolai, S.; Pitolli, C.; Agostini, M.; Antonov, A.; Malewicz, M.; Knight, R.A.; Raschella, G.; Melino, G. ZNF281 inhibits neuronal differentiation and is a prognostic marker for neuroblastoma. Proc. Natl. Acad. Sci. USA 2018, 115, 7356–7361. [CrossRef]

20. Pierdomenico, M.; Palone, F.; Cesi, V.; Vitali, R.; Mancuso, A.B.; Cucchiara, S.; Oliva, S.; Aloi, M.; Stronati, L. Transcription Factor ZNF281: A Novel Player in Intestinal Inflammation and Fibrosis. Front. Immunol. 2018, 9, 2907. [CrossRef]

21. Numoto, M.; Niwa, O.; Kaplan, J.; Wong, K.K.; Merrell, K.; Kamiya, K.; Yanagihara, K.; Calame, K. Transcriptional repressor ZF5 identifies a new conserved domain in zinc finger proteins. Nucleic Acids Res. 1993, 21, 3767–3775. [CrossRef]

22. Lee, K.H.; Kwak, Y.D.; Kim, D.H.; Chang, M.Y.; Lee, Y.S.; Lee, Y.S. Human zinc finger protein 161, a novel transcriptional activator of the dopamine transporter. Biochem. Biophys. Res. Commun. 2004, 313, 969–976. [CrossRef]

23. Kaplan, J.; Calame, K. The Zn/PoZ domain of ZF5 is required for both transcriptional activation and repression. Nucleic Acids Res. 1997, 25, 1108–1116. [CrossRef] [PubMed]

24. Orlov, S.V.; Kuteykin-Teplyakov, K.B.; Ignatovich, I.A.; Dizhe, E.B.; Mirgorodskaya, O.A.; Grishin, A.V.; Guzhova, O.B.; Prokhortchouk, E.B.; Gukiy, P.V.; Perevozchikov, A.P. Novel repressor of the human FMR1 gene—Identification of p56 human (GCC)(n)-binding protein as a Krüppel-like transcription factor ZF5. FEBS J. 2007, 274, 4848–4862. [CrossRef] [PubMed]

25. Luisier, R.; Unterberger, E.B.; Goodman, J.I.; Schwarz, M.; Moggs, J.; Terranova, R.; van Nimwegen, E. Computational modeling identifies key gene regulatory interactions underlying phenobarbital-mediated tumor promotion. Nucleic Acids Res. 2014, 42, 4180–4195. [CrossRef] [PubMed]

26. Addou-Klouche, L.; Adélaïde, J.; Finetti, P.; Cervera, N.; Ferrari, A.; Bekhouche, I.; Sircoulomb, F.; Sotiriou, C.; Viens, P.; Moulesselh, S.; et al. Loss, mutation and deregulation of L3MBTL4 in breast cancers. Mol. Cancer 2010, 9, 213. [CrossRef]

27. Hu, Y.; Jin, G.; Li, B.; Chen, Y.; Zhong, L.; Chen, G.; Chen, X.; Zhong, J.; Liao, W.; Liao, Y.; et al. Suppression of miRNA let-7i-5p promotes cardiomyocyte proliferation and repairs heart function post injury by targeting CCND2 and E2F2. Clin. Sci. (Lond.) 2019, 133, 425–441. [CrossRef]

28. Yang, P.L.; Wang, J.S.; Cheng, X.M.; Chen, J.C.; Zhu, H.; Li, X.L.; Cao, L.; Tang, W. PPAR-γ Ligand Inhibits Nasopharyngeal Carcinoma Cell Proliferation and Metastasis by Regulating E2F2. PPAR Res. 2019, 2019, 8679271. [CrossRef]

29. Azkargorta, M.; Fullaondo, A.; Laresgoiti, U.; Aloria, K.; Infante, A.; Arizmendi, J.M.; Zubiaga, A.M. Differential proteomics analysis reveals a role for E2F2 in the regulation of the Ahr pathway in T lymphocytes. Mol. Cell Proteomics 2010, 9, 2184–2194. [CrossRef]
86. Bailey, T.L.; Boden, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Res.* 2009, 37, W202–W2028. [CrossRef]

87. Heinz, S.; Benner, C.; Spann, N.; Bertolino, E.; Lin, Y.C.; Laslo, P.; Cheng, J.X.; Murre, C.; Singh, H.; Glass, C.K. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* 2010, 38, 576–589. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).