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Dioxin-mediated regulation of expression IL-12 family cytokines in human macrophages as a factor of tumor promotion by TCDD

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Background: The environmental pollutant 2,3,7,8-tetrachloro dibenzo-p-dioxin (TCDD) is the most toxic among the dioxin xenobiotics and induces a broad spectrum of biological responses, including immunotoxicity and cancer [1]. Macrophages are very plastic cells and can acquire various functionally distinct phenotypes depending on the physiological context. They are key regulators of the innate immune response and determine the developmental thrust of the adaptive immune response. The action of TCDD on macrophages is unique in that carried out through a variety of pathways, inducing activation of a number of cytokines [2]. Our previous analysis showed that the list of such cytokines was not complete [3]. IL-12 family of cytokines is the key players in the regulation of T cell responses. Little is known of the effects that TCDD has on the expression of IL-12 family member's genes, such as IL12A, IL12B, IL27 and EBI3, which forms a critically important cytokines IL-12 and IL-27, secreted by human macrophages. In the present work, the effects of TCDD on expression of IL-12 family member's genes in human macrophages have been investigated.

Results: To examine the effects of TCDD on the functional characteristics of macrophages, experiments have been conducted on the monocyte-like cell line U937 and primary human macrophages. Obtained data demonstrate functional activity of DREs in IL12A, IL12B and IL4 gene promoters via AhR signal pathway. The mRNA expression dynamics of IL12A, IL12B, IL4, IL27 and EBI3 cytokines genes also evidence the indirect TCDD–mediated modulation of these genes via intrinsic TFs, one of which is ATF3.

Conclusion: Exposure to TCDD induces a mechanism of two-stage regulation of the expression of some IL-12 family subunits through primary activation of the aryl hydrocarbon receptor by the TCDD-containing complex followed by ATF3-dependent suppression of their expression. This may result in an abrupt reduction of the anti-tumor response mediated by O and NK cells. The observed increase in ATF3 and decrease in IL-12 following exposure to TCDD are consistent with data on the expression of these genes in tumor-associated macrophages (TAMs). Reliance upon available data that demonstrate a decrease in IL-12 alongside an increase in the expression of other cytokines typical of TAMs confirms the hypothesis that TCDD can shift the phenotype of a macrophage towards a tumor-associated direction. This mechanism may be one of the others underlying TCDD-driven tumor promotion.

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New laboratory marker of metastatic thyroid cancer

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Existing methods for the preoperative detection of metastases in thyroid cancer have low efficiency and highly invasive. Therefore, the searches for laboratory markers that will indicate the presence of metastases including hidden with high reliability in cancer of the thyroid gland are relevant.

Immune system is one of the key mechanisms for regulation of tissue proliferation. Autoreactive lymphocytes restrict the growth and proliferation of tissues. In turn autoreactive lymphocytes are controlled by idiotypic lymphocytes. Abnormalities in the regulation system can be the cause of tumor growth, such as excess activity of anti-idiotypic lymphocytes. Previously we conducted a comparative analysis of the level of autoantibodies to various antigens (thyroglobulin, thyroid stimulating hormone receptor, native DNA, anionic proteins vascular endothelium), as well as anti-idiotypic antibodies to thyroglobulin in thyroid cancer patients with metastases and without metastases. Significant differences between investigated groups were found in the level of anti-idiotypic antibodies against antibodies to thyroglobulin. Therefore, the aim of this study was to analyze the feasibility of using anti-idiotypic antibodies against antibodies to thyroglobulin as a marker of metastatic thyroid cancer.

All studied patients with thyroid cancer were hospitalized at Primushko Regional Clinical Oncology Center of the Ministry of Health of the Udmurt Republic, Izhevsk. Blood was taken from patients before surgery. The level of anti-idiotype antibodies (AIAT) against autoantibodies to thyroglobulin (Tg) and the level of autoantibodies (AUAT) to thyroglobulin were determined in the blood plasma using the test system manufacturing MRC "Immunkulus". To determine the AIAT against AUAT to Tg, we used the principle of competitive inhibition of the binding reaction with antibodies to thyroglobulin in the presence of analyzed serum. As a source of antibodies to thyroglobulin, we used serum from patients with autoimmune thyroiditis in effective dilutions for competition. Analyzed samples were previously depleted by antibodies to thyroglobulin. The level of the test antibodies was expressed in conventional unit (CU) - optical density of the reaction of antibody binding to the antigen.

We found that AIAT against AUAT to Tg were not defined in 43% of thyroid cancer patients with metastases (group I), 57%

revealed a relatively high level of AIAT against AUAT to Tg – 0.343 ± 0.027 CU (Group II). In patients with thyroid cancer without metastases, the level of AIAT against AUAT to Tg (0.185 ± 0.083 CU) was lower than that of cancer patients with metastases. Metastatic cancer cannot be differentiated from cancer without metastases in 23% of studied cases.

The level of AUAT to Tg in patients with thyroid cancer in the group I was 0.997 ± 0.084 CU and in the group II was 1.087 ± 0.174 CU. The level of AUAT to TG in cancer patients without metastasis was 0.794 ± 0.038 CU. It was found that when the level of AIAT against AUAT to Tg does not allow to distinguish metastatic thyroid cancer from cancer without metastases, it is possible to differentiate status data of thyroid cancer by using the level of autoantibodies to Tg.

Moreover, the high level of AIAT against AUAT to Tg in patients with cancer without detectable metastases may be a marker of poor prognosis. So in one of the studied patients diagnosed with papillary cancer without metastasis, the level of AIAT against AUAT to Tg was equal to 0.37 CU, thus indicating the presence of metastases. Indeed, metastases were found in this patient 4 months later.

Thus, the level of anti-idiotypic antibodies against autoantibodies to thyroglobulin may be used as a marker for metastases in the differential diagnosis of thyroid cancer.

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Multiple mechanisms of DNA minor groove binding ligand epigenetic effects

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Purpose: DNA minor groove is the main target of small molecules, which noncovalently and to a certain extent site-specifically bind to appropriate nucleotide sequences. Study of these substances can give rise to understanding the mechanistic relationship between sites of interaction and activity of appropriate enzymes with "houskeeping" function including helicases, topoisomerases, methyltransferases, demethylases and DNA/RNA-polymerases.

Results: We revealed for the first time that AT-specific minor groove binding ligands (MGBLs), in particular bisbenzimidazoles (Hoechst33258 and its derivatives), widely used in molecular and cell biology for DNA-staining, induce loss of heterozygosity at high frequency while point mutations and chromosome deletions at insignificant levels. Moreover, we demonstrated that the agents realized their genotoxic blastomogenic effects via homologous recombination mechanism exclusively. Lately the same mechanism of genotoxicity has been shown for MGBL carbazole derivative Curaxin, which is toxic for a broad range of tumor cell lines in vitro and inhibit tumor growth in different mouse models of cancer in vivo. Moreover, powerful antitumor activity has been demonstrated for Trabectedin, which binds to the DNA's minor groove and alkylate guanine residues. All this provided a framework for wide-ranging investigation of cell response to MGBLs exposure, molecular mechanisms of their recombinogenic as also their anticancer activity. A special interest is paid to epigenetic mechanisms of MGBs action.

Our study aimed to examine the epigenetic effects of recombinogenic (Hoechst33342, Hoechst33258) and non-recombinogenic (DAPI, Diminazene, Pentamidine and Netropsin) MGBLs.

After we unmasked MGBLs' recombinogenic activity, we hypothesized that their molecular mechanism of indirect DNA damage involves poly(ADP-ribose)polymerase-1 (PARP-1) activation. Surprisingly, we found that all AT-specific MGBLs preventing PARP-1 interaction with DNA inhibit its activation, and hence, the DNA-dependent pathway of PARP-1 activation function. These inhibitors effectively block PARP-1 activity in vivo, as it was demonstrated in a Drosophila experimental system and in human breast cancer-derived BT474 cell line.

Further epigenetic effects of these indirect genotoxic carcinogens were analyzed using HeLa cell population with epigenetically suppressed GFP-reporter gene as a model. All compounds had strong GFP- reactivation effect. The obtained results confirm scarce data of previous publications on the ability of DNA minor groove ligands to influence gene transcription process. Statistically significant results of changes of DNA methylation level were detected under 5-azaC and Hoechst 33258 treatment, but it was absent after Hoechst 33342 treatment. For the rest compounds significant loss of promoter region methylation was not observed. The common epigenetic marks of transcription include histone H3 trimethylation in lysine 4 (H3K4me3) and histone H3 and H4 acetylation (acH3/acH4) on promoter regions of genes. We showed that TSA, Hoechst 33342 and DAPI treatment of HeLa-TI cells lead to increased level of histone H3 trimethylation of lysines 4 (H3K4me3), but for all that the level of histone H4 acetylation remains without significant changes. On the contrary, histone H3 acetylation level was stably increased at all samples. The modifications are typical for silent genes and decrease of their amount suggests the transcriptional reactivation. Loss of H4K20me3 mark in comparison with untreated control was demonstrated for MGBLs.

Conclusion: Taking together, findings of the study will lay the fundamental groundwork for the development of novel anticancer strategies and new chemotherapy effects of small molecules. The mechanism of PARP-1 inhibition by MGBLs and its epigenetic influence on silent genes, via DNA methylation and histone modifications make reasonable further study of these compounds in three prospective directions: (1) as self-acting cytotoxic agents; (2) as a component of combined chemotherapy targeting DNA repair by PARP-1, thereby facilitating DNA damage caused by other anticancer drugs; (3) as an agents reactivating epigenetically repressed genes which silencing occurs during the earliest stages of neoplasia and accumulates with progression toward malignancy.

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