

Phenotypic Alterations in MCF-7 Human Epithelial Cells Chronically Exposed to TCDD

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ABSTRACT

Some epidemiological studies suggest that dioxins or dioxin-like compounds are involved with the increased incidence of breast cancer in exposed-women. However, the *in vitro* and animal studies have provided inconclusive results, although some reports suggest that exposure duration and timing may be involved in this possible association. Since dioxins' half-lives have been calculated to be in several years, in this study we examined the effects of chronic exposure (>one year) to Tetrachlorodibenzo-*p*-dioxin (TCDD) on some biological markers of breast cancer progression in MCF-7 human breast cancer cells.

By definition, cancer is an uncontrolled cell proliferation. Previous reports indicate that short-term (16 days) exposure to TCDD inhibits post-confluent cell proliferation or foci in MCF-7 cells. However, results from this study indicate that chronically exposed MCF-7 cells (LTD_X cells) acquire resistance to TCDD, and cross-resistance to the therapeutic antiestrogen ICI 182,780. Microscopic examination indicated that estradiol (E2)-induced foci in LTD_X cells were larger and more compacted than those developed in untreated MCF-7 cells. Chronic exposure to TCDD also inhibited the development of E2-independent cord-like three-dimensional structures that were evident after short-term TCDD treatment removal. These results suggest that chronic exposure to TCDD results in acquired resistance to antiestrogens and inhibition of development of three-dimensional structures.

E-cadherin protein expression is required in epithelial tissues to maintain polarity, strong cell-cell adhesion, proper inter-cellular communication and signaling. Previous reports show that short-term exposure to TCDD down-regulates E-cadherin and up-regulates β -catenin gene expression. The results of the fluorescence immunocytochemistry study shown here indicate that short-term exposure to TCDD resulted in the internalization of E-cadherin, while chronic exposure inhibited E-cadherin protein immunolocalization. De-localization of E-cadherin was associated with the inhibition of E2-induced nuclear translocation of β -catenin. Chronic co-exposure to TCDD and E2 partially maintained E-cadherin at the cell-cell contact sites, although β -catenin was mostly localized at the cytoplasm. These results indicate that chronic exposure to TCDD alters E-cadherin and β -catenin cell localization, and suggest a possible interference with the E2-induced β -catenin signaling pathway.

Previous reports have shown evidence of an interaction between aryl hydrocarbon receptor (AhR) and estrogen receptor alpha (ER α). The results from this study indicate that exposure to TCDD inhibited E2-induced CYP1A1 protein expression. In addition, chronic exposure to TCDD induced the re-expression of approximately 30% of the initially inhibited ER α . This re-expression may be the result of the presence of two cell sub-populations that differentially express ER α in LTD_X cells. When exposed to E2, LTD_X-derived-ER α -negative clonal cells develop foci in a way similar to foci developed in the parent MCF-7 cell line. We speculate that this capacity of ER-negative cells to reproduce an ER-mediated response, such as the post-confluent cell proliferation, is consistent with previous reports suggesting the presence of stem cells in MCF-7 cell line. Further studies are necessary to address this question.

Together, these results suggest that chronic exposure to TCDD induces alterations that are consistent with those observed during the progression of breast cancer from ER-dependent to ER-independent, and the severity of these alterations is greatly influenced by the estrogenic status. Therefore, the results of this study support the hypothesis of an association, at least indirectly, between chronic exposure to TCDD and the increased incidence of breast cancer in chronically exposed women.