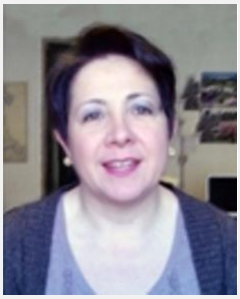
ImmunoTools *special* Award 2014



Tiziana Guarnieri, PhD
Group leader

Dept. of Biology, Geology and Environmental Sciences Alma Mater Studiorum – Università di Bologna Via Francesco Selmi, 3 40126 Bologna, Italy

Mutual interaction between aryl hydrocarbon receptor activation and IL-6-driven inflammation in non transformed and transformed breast cell lines

Description

Human exposure to dioxin-like polychlorinated biphenyls (PCB), a set of 12 coplanar PCBs, has been associated to cancer onset and progression, but a convincing and unequivocal evidence on their role in breast cancer aetiology has not been yet provided. Our interest is targeted to the set of 12 coplanar congeners that bind to the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor involved in the regulation of several genes. These congeners are often referred as dioxin-like PCBs because, similarly to the most potent AhR ligand, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are able to bind to AhR and exert a similar toxic action. The aim of this project is to get new insight on the possible link between dioxin-like PCBs exposure and breast cancer onset. Starting from Hollingshead team data (2008; doi: 10.1158/0008-5472.CAN-07-6168) in MCF7 breast cancer cells, we will focus on interleukin 6 (IL6)-driven inflammatory processes which can be evoked by aryl hydrocarbon receptor (AhR) activation in a non transformed breast cell model. In our experimental design, adherent cells and three dimensional cultures from non transformed MCF10 cell line will be exposed to selected dioxin-like PCBs molecules and compared with same specimens from the MCF10-derived tumoral cell lines sequence (MCF-10AT1, MCF10DCIS and MCF-10CA1a). We expect to obtain information on the putative inflammogenic role of dioxin-like compounds in the inflammation-driven acquisition of malignant genotypic and phenotypic features in non transformed adherent cells and three dimensional structures models. Particularly, we will also evaluate the role of the inflammation-linked transcription factor NF-kB, as a close interaction between AhR and the NF-kB subunits RelA and RelB has already been shown in breast cancer cell lines and primary breast tissues (Vogel et al., 2014; doi: 10.1074/jbc.M113.505578). This experimental design will be developed through: **Work package 1: Task 1: adherent cells cultures setting and PCBs treatments**: according to Turci (2006; PMID: 16909958) and Consonni and colleagues (2012; doi: 10.1016/j.envint.2012.01.004.), cell cultures will treated with

non-ortho dioxin-like PCBs 77, 81,126 and 169 (alone or as mixtures), employing concentrations near to levels detected in human serum of people living in the North of Italy. After acute (24hrs) and chronic (7,14,30 days) exposition regimen, phenotypic analysis will be performed (**Task 2**) employing: a) trypan blue exclusion assay, to evaluates the viability of normal/treated cells grown either in normoxia either in hypoxia. This last parameter plays a pivotal role in cancer growth, since it is related to the ability of cells to survive and growth in an hypoxic environment, as occurs when the tumour mass increases; b) chemoinvasion assay in matrigel-coated Boyden blind-well chambers, which evaluates the invasive potential of most cell types (Albini and Benelli, Nat Protoc. 2007;2(3):504-11.); c) gelatine zymography, wich evaluates the activity of metallo-proteinases 2 and 9 (MMP-2 and MMP-9), gelatine-degrading enzymes which play a pivotal role during the metastatic process (Li et al., 2009; doi: 10.1111/j.1442-2050.2008.00928.x.) d) According to Stobbe-Maicherski (2013; doi: 10.1111/febs.12571.), ELISA evaluation of secreted IL6. **Task 3**: genotypic analysis. We plan to evaluate whether exposure of normal cells to selected dioxin-like PCBs is associated to the induction of interleukin-6 (IL-6) and NF-kB genes.

Work package 2: Task 1: We will assess the expression of the following panel of genes that are known to be involved in breast cancer cells function and regulation: Carbonic anhydrase IX (CA-IX), Jagged-1, SLUG, IL-6, RelA, RelB, HIF1α, HIF-1β (ARNT), SNAIL, TWIST, ZEB-1, ZEB-2, E-Cadherin, NOTCH- 1, -2, -3, -4, ER , ERß, ER-α, CD-44, LEF-1, CK-5, CK-8, EGFr, CD-133, OCT3/4, ErbB-2, SOX-2, COX 1/2, P21, P66Shc, VEGF, KDr, MSI, c-KIT, Vimentin, BMI-1, Bcrp-1, Bcl-2, P53, PUMA, BAX, NOXA, BNIP3, BNIP3L. Task 2: Stable RNA interference in MCF-10 cells. In order to verify the role of IL-6 and NF-kB genes in the putative PCBs-mediated malignant transformation, the expression of the more represented genes from the list below will be also assessed in MCF-10 cells stably infected with retroviral vectors encoding for a shRNA directed against IL-6 and NF-kB mRNA.

CONCLUSIONS: We believe that our approach, developed in a highly standardized cellular model, could provide new insights about the possible link between dioxin-like PCBs exposure, AhR activation and inflammation as breast cancer aetiologic factors.