Unravelling the Adipose Tissue-BPA interaction in triple negative breast cancer progression: the role of the tumour microenvironment

Anna CITARELLA – *University of Rome La Sapienza*

Adipose tissue (AT) is a complex endocrine organ with a fundamental role in the organism's homeostasis. AT is not only a player in physiological functions but also a prevalent component of tumor microenvironment (TME), which plays a pivotal role in cancer growth. Thus, a dysfunctional AT could be related to cancer initiation, progression, and metastatization. What if the chemicals that we are exposed to daily can affect AT balance and function? In this context, Bisphenol A (BPA) is an environmental pollutant and one of the most ubiquitous endocrine disruptors, known to regulate adipogenesis. BPA is found in many products of daily use and was found to be significantly accumulated in breast adipose tissue of breast cancer patients compared to healthy subjects. Of note, dysfunctional AT promotes chronic low-grade inflammation which is directly linked to increased risk of breast tumor development. Triple-negative breast cancer (TNBC) is the most aggressive type of breast cancer, prone to metastasis and recurrence. Cytotoxic chemotherapy is the mainstay treatment but patients often face relapse, caused by the selection of resistant clones and the mis-activation of aggressiveness-related pathways. Thus, understanding the mechanisms related to AT dysfunction in TME caused by BPA could be useful for the identification of new molecular targets for TNBC treatment. Our work aimed to study the effect of BPA in adipose cells and investigate how, in turn, BPA-treated adipose cells modulate TNBC behaviour, thereby identifying the molecular interactors between cancer cells and AT. Experiments were performed using the pre-adipocyte cell line 3T3-L1 and TNBC cell lines MDA-MB-231 and HCC1395. 3T3-L1 cells were treated with 20nM BPA (which represents the environmentally relevant concentration) during differentiation, at the end of which cells were characterized by Oil Red-O staining, Western Blot, and Real Time qPCR. To identify the putative interactors of AT-TNBC crosstalk we analyzed cytokines secreted in the culture medium through a multiplex cytokines array. Moreover, TNBC cell lines were cultured both in 2D and in 3D conditions with the conditioned medium (C.M.) of BPA-treated and differentiated 3T3-L1 to analyze TNBC growth and migration capacity.

BPA treatment in 3T3-L1 resulted in an impairment in lipid accumulation, in a decrease of expression levels of mature adipocytes markers FABP4, LPL, PPAR-γ and CEBP-α and in an increase of protein levels of the fibroblast marker Vimentin protein. Moreover, the BPA induced the modulation of 68 cytokines with a significant increase in the secretion of VEGF, TWEAK receptor, Stromal cell derived factor-1 (SDF-1), MMP2, GAS1, and MIP-3a. These results demonstrate the putative role of BPA in disrupting the balance of differentiation processes, creating a dysfunctional AT with a "fibroblast-like phenotype" able to secret cytokines that could forage TNBC progression. To further explore this aspect, a transwell migration assay was performed to evaluate the migration ability of TNBC cells exposed to the 3T3-L1 C.M. in both HCC1395 and MDA-MB-231 cell lines,registering an increase of cancer cell migration and invasiveness. Moreover, TNBC 3D organoids grew better with 3T3-L1-BPA C.M. These data support the role of BPA in sustaining a dysfunctional AT, thus contributing to TNBC aggressiveness by regulating the migration capacity of cancer cells and laying the foundation for further investigation of the role of tumor microenvironment in cancer development.