PS2-20 / Epigenetic changes induced by pesticide exposure reactivate LINE-1 retrotransposon in breast cancer and mammary epithelial cells

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Expression of long interspersed nuclear element-1 (LINE-1) is reactivated during breast cancer initiation and progression. Strong ligands of aryl hydrocarbon receptor (AhR) activate LINE-1 through the transforming growth factor-β1 (TGF-β1)/Smad pathway. Studies have linked breast cancer risk with pesticide exposure, including hexachlorobenzene (HCB) and chlorpyrifos (CPF), both weak AhR ligands which promote alterations in mammary gland and tumor growth in animal models. We examined the pesticides action on LINE-1 reactivation in MDA-MB-231 breast cancer cells and NMuMG epithelial breast cells, and we evaluated the role of TGF-β1 and AhR. Results show that 0.5 μM CPF and 0.005 μM HCB reduced the methylation of the 5´-UTR of LINE-1 and increased LINE-1 mRNA expression via Smad and AhR signaling in MDA-MB-231. Besides, 5 μM CPF and 0.005 μM HCB heighten ORF1p nuclear import, the protein encoded by LINE-1, through TGF-β1/Smad and stimulate DNA double-strand breaks. Disturbingly, 5 μM CPF and 0.005 μM HCB also enhanced LINE-1 mRNA levels in NMuMG cells. CPF effect was through AhR and TGF-β1, while HCB action depends only of AhR. In addition, both pesticides increased ORF1p expression and nuclear localization. In conclusion, HCB and CPF induce LINE-1 reactivation, not only in breast cancer cells but also in epithelial mammary cells, supporting the idea that pesticide exposure could promote epigenetic changes, contributing to cell transformation and tumorigenesis in breast cancer.

PS2-21 / Norcantharidine treatment inhibits *in vitro* parameters associated with tumor progression in triple negative breast cancer cell lines

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Triple negative breast cancer (TNBC) is a subgroup of very aggressive mammary cancer that do not express estrogen or progesterone receptors and neither overexpress the HER2 receptor. Norcantharidin (NCTD) inhibits the progression of several types of cancer however, the effect on breast cancer has not been studied yet. So, we have evaluated the effect of NCTD on human (HS578T) and murine (4T1) TNBC cell lines. NCTD induced an important antiproliferative effect, with an IC50 of 56 uM for HS578T and 35 uM for 4T1 cell lines. This antiproliferative effect was accompanied with the reduction in ERK activated levels (p-ERK) as well as an increase in the Sub-G0 cell cycle fraction, compatible with the presence of apoptotic cells. In both cell lines, NCTD reduced adhesive and migratory capacities ($p < 0.05$, Anova test) also displaying an important reduction in MMP-9 secreted activity. Although these parameters could have a direct implication in the malignant progression, clonogenic and *in vivo* assays showed an inverse behavior. In this regard, the pretreatment of 4T1 cells with NCTD induced an increase in the number of *in vitro* colonies and no effect could be detected in the amount of experimental lung metastatic nodes. Even though some results obtained are encouraging, we must seek the appropriated therapeutic strategy, probably combining with another drug, in order to allow and effective use of NCTD for the treatment of triple negative breast cancer.

PS2-22 / Dual galectin-8 and ALCAM silencing delays triple negative breast cancer progression

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Triple-negative breast cancer (TNBC) comprises 10-15% of breast tumors, and lacks targeted therapy. Galectin-8 (Gal-8) is a tandem-repeat type galectin involved in cell adhesion and migration, angiogenesis and tumor progression. Here, we studied the tumorigenic properties of Gal-8 and its ligand ALCAM/CD166 in TNBC. We silenced both Gal-8 and ALCAM in MDA-MB-231 cells with specific (MDA-shGal8 and MDA-shALCAM, respectively) or scrambled shRNA lentiviral particles (MDA-shControl). Interestingly, both MDA-shALCAM ($p < 0.01$) and double silenced MDA-shALCAM-shGal8 ($p < 0.001$) cells showed decreased proliferation and Bcl-2 down-regulation ($p < 0.001$) compared to control cells. Moreover, ALCAM-silenced cells showed impaired ability ($p < 0.001$) to form anchorage-dependent colonies and tumor spheres. Silencing of ALCAM decreased cell adhesion and migration onto Gal-8-coated surfaces in a glycan-dependent fashion. Remarkably, either Gal-8 or ALCAM silencing significantly disrupted ($p < 0.05$) cell-cell adhesion. *In vivo*, in a TNBC experimental model, at 56 days post-inoculum (pi), MDA-shGal8 ($p < 0.05$) and ALCAM-silenced ($p < 0.01$) cells generated smaller tumors than control cells. Notably, at day 98 pi, tumors generated by MDA-shALCAM-shGal8 cells were even smaller ($p < 0.05$) than those generated by MDA-shALCAM cells. In summary, dual knock-down of Gal-8 and ALCAM induced a pronounced delay on tumor