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PROTEOMIC IDENTIFICATION

OF NOVEL MARKERS IN BREAST AND COLON CANCER

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Abstract

Background: Discovery of new biomarker represent the greatest promise for the detection and management of cancer. Although progress in cancer biology has been rapid during the past few years, the complete understanding of molecular basis for cancer initiation, progression and efficacious treatments is still lacking. In this context, the application of proteomic strategies is now holding a focal position. The main reason is that proteins are the functional players that drive cancer phenotypes. Among cancers, breast and colon represent the most frequent forms. The evolution of these type of cancer are not easily predictable since there are several types that behave differently among patients. The biological heterogeneity is consistent with observed varied responses to therapies across patients, also. On the other hand, drug delivery is an emergent field focused on targeting drugs to a desirable group of cells, in order to minimize undesirable side-effects and maximize the therapeutic activity. Metallic nanoparticles, in particular silver nanoparticles (Ag-NPs) exhibit low toxicity to mammalian cells (Mahapatra and Karak, 2008) and are good candidate as smart therapeutics. Based on these evidences, the first part of the study was aimed to discover new potential protein biomarkers in breast and colon cancer tissues and sera, using proteomic techniques, useful as diagnostic and prognostic factors *in vivo*. The second part of the study was focused on the *in vitro* cytotoxic effects of silver nanoparticles Ag-NPs embedded on Klebsiella Oxytoca DSM29614 (KO) Exopolysaccaride (EPS), produced in aerobic *versus* anaerobic conditions.

Methods: *Diagnostic biomarkers in breast and colon cancer:* Taken advantage from previous results by the proteomic analysis performed on 13 breast cancer tissues and their matched non-tumoral adjacent tissues (Pucci-Minafra et al., 2007), we first analyze by 2D-DIGE pool of both breast and colon cancer tissues extracts compared to the matched pool of non tumoral adjacent tissues extracts. Differentially expressed proteins, identified by Maldi-TOF/TOF, were functionally clustered. We also investigate the activity levels of MMP-2 and MMP-9 in breast and colon tissues as well as in sera of the same patients.

Prognostic biomarkers in breast and colon cancer: In order to identity putative proteomic signatures for colorectal cancer (CRC) metastasis, a comparative profiling of a colon cancer tissue paired with the non tumoral adjacent mucosa and with the liver metastasis from the same patient was performed. A three-step approach (normal *versus* tumoral *versus* metastasis) was used to select unique proteins involved in liver metastasis. For breast cancer, a large proteomic investigation performed on a large sample set of breast cancer patients (Cancemi et al., 2010, 2012), pointed the important role of S100 protein members in breast cancer progression. Using on line tools, for instance GOBO and breast cancer Kaplan Meir-plotter we assessed gene expression levels and clinical correlations of S100 proteins in breast patients.

Cytotoxic effects of silver nanoparticles biosynthesized from KO (Ag-NPs-EPS) in SK-BR3 breast cancer cell line: We monitored cell proliferation inhibition rate by MTT assay, morphological changes and proteomic modulation.

Results: *Diagnostic biomarkers in breast and colon cancer:* Differentially breast and bolon proteomic profiling revealed several proteins involved in common pathways among the type of cancer. The important role of MMPs in tumorigenesis was confirmed by our observations regarding their major expressions in cancer tissues compared to the normal tissues.

Prognostic biomarkers in breast and colon cancer: Among the differentially expressed proteins between normal-tumor and liver metastasis, Cathepsin D expression was further analyzed as prognostic factor in CRC. Moreover, integrating results obtained by bioinformatics analysis performed on breast cancer gene expression dataset confirmed the important role of S100 proteins in breast cancer progression.

Cytotoxic effects of silver nanoparticles (AgNPs) biosynthesized from KO in SK-BR3 breast cancer cell line: The most important effects were obtained by aerobically AgNPs-EPS treatment, due to the major release of Ag^{+1}, as verified by voltammetry analysis. Morphological alteration were

consistent with apoptotic features. Proteomic analysis showed modulation of several proteins related to oxidative stress and apoptotic and mitochondrial pathways.

Conclusions: Conclusively, the present study contribute to the implementation of the panel of new proteomic biomarkers useful for diagnostic and prognostic applications in breast and colon cancer, providing new informations about the effects of the biosynthesized Ag-NPs-EPS on breast cancer cells.