EGF-INDUCED EMT REGULATES CELL CYCLE PROGRESSION THROUGH p53-INDEPENDENT p21 UPREGULATION IN OVARIAN CANCER CELLS

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Ovarian cancer (OvCa) is the most lethal gynecologic cancer and currently ranks fifth in causing cancer-related deaths among women. The poor survival of patients diagnosed with OvCa is attributed to chemotherapy resistance and to diagnosis at advanced stage, when the tumor has metastasized. During cancer progression, the metastatic cascade comprises several steps, ultimately leading to the emergence of secondary tumors at distant sites from the primary lesions. One process contributing to the first phase of metastasis is the epithelial-mesenchymal transition (EMT), which is characterized by the loss of the cell-cell contacts so typical of epithelial cells, and the acquisition of migratory and motile properties. However, these changes do not fully occur in ovarian carcinoma, and are even reversed in tumor cells present in malignant peritoneal and pleural effusions. In this study, we evaluated if epidermal growth factor (EGF) could induce EMT in Caov-3 human ovarian cancer cell line. EMT markers were evaluated by immunofluorescence microscopy, qPCR and western blotting. Quantitative proteomic analysis using SILAC was performed by mixing Caov-3 and Caov-3/EGF cells extracts with Caov-3 cells labeled with heavy lysine. Data was collected using high-resolution mass spectrometry coupled to liquid chromatography (LC-MS/MS) in a Orbitrap Elite instrument. Regulated proteins were validated using flow citometry and western blotting. After 96 hours, EGF induced a dramatic morphological change in Caov-3 cells. Western blotting and immunofluorescence analysis showed high levels of vimentin and decrease of E-cadherin, in association with upregulation of fibronectin and N-cadherin. Quantitative proteomic analysis based on SILAC method together with bioinformatics tools for the data functional interpretation showed clear regulation of proteins relevant to cell cycle progression - ribosomal proteins and elongation factors - by EGF-induced EMT in Caov-3 cells. After specific validation our data suggest
that EGF-induced EMT regulates G1/S phase arrest besides upregulation of p21 independent of p53.

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