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Enhanced expression of Annexin IV in clear cell carcinoma of the ovary and its association with chemo-resistance

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Background: Ovarian cancer is the eighth most common cancer among women and also the eighth most common cause of cancer death in Japan, with approximately 7,700 new cases (2001) and 4,400 deaths (2005) reported yearly. Of all patients with ovarian cancer, over 20% are classified as clear cell carcinoma (CCC) in Japan. Importantly, CCC of the ovary is known to be resistant to platinum-based chemotherapy compared with both serous and endometrioid ovarian adenocarcinoma. There is thus a continuing need to identify novel protein biomarkers for the specific detection of ovarian CCC and to further our understanding of the pathogenesis of this disease, particularly with respect to chemo-resistance. Using a proteomics approach, we investigated novel biomarkers related to chemo-resistance specific to CCC of the ovary.
Materials and Methods: Proteins from human ovarian cancer cell lines – OVISE-CCC and OVSAHO – serous adenocarcinoma were separated by 2D gel electrophoresis (2D-DIGE). Proteins over-expressed in OVISE cells compared with OVSAHO cells were selected and identified by mass spectrometry and further evaluated by Western blot analysis and quantitative real-time PCR. Immunohistochemical analysis of selected proteins was performed in 88 surgically obtained ovarian cancer samples (42 CCC, 13 endometroid, 8 mucinous, 25 serous). Chemo-resistance to carboplatin in OVSAHO cells stably expressing selected proteins compared with parental non-transfected cells was analysed by MTT assay.
Results: We identified Annexin IV as a protein highly expressed in OVISE-CCC cells compared with the OVSAHO non-CCC cells by 2D-DIGE and this observation was confirmed by both Western blot analysis and real-time PCR. By immunohistochemistry, Annexin IV displayed significantly stronger staining (% positive staining cells) in patient ovarian CCC samples compared with ovarian non-CCC samples ($p < 0.0001$). An association between chemo-resistance and Annexin IV expression was observed in OVSAHO cells stably expressing Annexin IV, which displayed stronger resistance to carboplatin (IC50 = 100 uM carboplatin) than non-transfected cells (IC50 = 50 uM carboplatin).
Conclusion: Annexin IV is highly expressed in CCC of the ovary compared with other ovarian cancers. Enhanced expression of Annexin IV in ovarian CCC is associated with chemoresistance. Thus, Annexin IV may represent a novel biomarker for the detection of ovarian CCC and may also represent a novel therapeutic target of chemo-resistance in patients with this disease.

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Regulation of Nrf2-antioxidant system and glutathione transferases by 5-fluorouracil in colon cancer HT-29 cells: potential implication in drug resistance

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A primary cause of cancer treatment failure and patient relapse is an acquired or intrinsic resistance to anticancer therapies. Acquisition of drug resistance can be attributed to various factors including inhibition of apoptosis, altered expression of multidrug resistance-associated proteins, altered drug uptake or metabolism, and/or overexpression of defence system genes. Being potential inducers of defence pathways, various anticancer drugs could have a marked incidence on cancer cell resistance. Among anticancer drugs, 5-fluorouracil (5-FU) remains the most commonly used drug for the treatment of colorectal cancer despite the fact that objective response rates are as low as 20%. The aim of our study was to investigate the effects of 5-FU on cell defence systems using human colon HT29 cells. Our results demonstrate that 5-FU induced the expression of mRNAs encoding GST (GSTM3, GSTS1) and antioxidants enzymes such as NAD(P)H: quinone oxidoreductase 1 (NQO1, heme oxygenase-1 (HO-1) and γ-glutamylcysteine synthetase (γ-GCS). To further determine the mechanisms involved in 5-FU effects, we also investigated whether it activates the Nrf2/antioxidant response element (ARE) pathway which is an implicated in the regulation of several genes involved in cell defense systems. Translocation of Nrf2 into the nucleus after 5-FU exposure was demonstrated by immunolocalization and western blotting. By using an ARE driven-reporter gene (luciferase) assay, activation of the luciferase activity by 5-FU was evidenced; this effect was inhibited by co-transfecting a vector expressing a dominant negative Nrf2. Moreover, transfection of Nrf2 siRNA into HT-29 cells increased 5-FU cytotoxicity and inhibited induction of Nrf2 target genes (HO-1, γ-GCS and NQO1) but not GST (GSTM3 and GSTS1). In conclusion, these results demonstrate that 5-FU activates the Nrf2/ARE dependant pathways which in turn regulates some antioxidant enzymes, modulates the chemosensitivity of colon cancer HT29 cells and might represent a potential therapeutic target in 5-FU treatments.

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Different patterns in telomerase activity (TA) change after acquisition of resistance to tamoxifen in hormonal receptor positive breast cancer cells

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Introduction: Telomerase, a ribonucleoprotein enzyme that functions as a reverse transcriptase, is detected exclusively in immortal cells such as germ cells, stem cells and cancer cells. Telomerase activity (TA) is present in almost all human cancers. Telomerase activation is considered to be essential to maintain the integrity of the replicating tumor cell and to establish immortality. Based on this concept antiestrogen should initially regulate estrogen-stimulated telomerase, but TA was reported to be increased highly and not regulated by estrogen and antiestrogen in the previous study with tamoxifen-resistant T47D:A18 breast cancer (T47D:A18/4-OHT) cells. We performed this study to investigate the change of TA in tamoxifen-resistant MCF-7 breast cancer (MCF-7/4-OHT) cells, and the differences of the changing pattern of TA between MCF-7/4-OHT and T47D:A18/4-OHT cells.
Methods: MCF-7/4-OHT cells were established by culture in the media containing 1 μM of 4-hydroxytamoxifen (4-OHT) for 3 months. TA was detected by TRAP assay with a TRAPEZE Telomerase detection kit and the results were compared with that of T47D:A18/4-OHT cells.
Results: TA of MCF-7/4-OHT cells were regulated by estradiol and blocked by antiestrogens, 4-hydroxytamoxifen (4-OHT) and ICI 182,780. As compared with TA of parental MCF-7 cells, TA of MCF-7/4-OHT was significantly decreased to the half of parental cells. As compared with that of T47D:18/4-OHT, the changing pattern of TA in MCF-7/4-OHT cells was completely different: the basal TA of MCF-7/4-OHT was not increased and the TA regulation by estradiol was preserved on the contrary to that of T47D:A18/4-OHT cells.
Discussion: The changing patterns of TA were completely different between the MCF-7/4-OHT and T47D:A18/4-OHT breast cancer cells, and the difference might be originated from the cellular characteristics. The increased TA might not be a necessary step for acquisition of tamoxifen resistance in breast cancer cells and further study is required to explain the difference.

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Interleukin-8 signalling contributes to chemotherapy resistance in colorectal cancer cells

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Background: We have previously demonstrated that the sensitivity of androgen independent prostate cancer (AIPC) cells to chemotherapy, radiotherapy and novel biological agents is modulated by a constitutive interleukin-8 (IL-8) / CXCR2 signalling pathway. We have shown that treatment with oxaliplatin (L-OHP) induces IL-8 signaling conferring a chemoresistant phenotype via NF-κB mediated induction of IL-8, IL-8 receptors and anti-apoptotic protein expression. The aim of this study was to determine whether IL-8 signalling may modulate the sensitivity of colorectal cancer (CRC) cell lines to L-OHP reflecting the clinical relevance of L-OHP as a mainstay of treatment for patients with advanced CRC.
Materials and Methods: Initial characterisation studies were carried out in a panel of CRC cell lines. L-OHP and IL-8 treatments were evaluated using HCT116 CRC cell lines. IL-8 expression was assessed using qRT-PCR, western blotting and ELISA. IL-8 receptor expression was assessed by flow cytometry. Anti-apoptotic signalling was assessed using qRT-PCR and western blotting. NF-κB activity was assessed by electrophoretic mobility shift assay. Response to L-OHP treatment was assessed using cell count and clonogenic assays.
Results: All cell lines evaluated (i) secrete IL-8 under resting conditions, with highest secretion observed in metastatic cell lines and, (ii) express CXCR1 and CXCR2 receptors. Secretion of IL-8 and expression of IL-8 receptors is increased following treatment with L-OHP. Treatment with L-OHP induces NF-κB activation and increases the expression of anti-apoptotic proteins including Bcl-xL and survivin, in addition to promoting transcriptional regulation of IL-8 and CXCR2. Treatment with recombinant IL-8 also induces NF-κB activation and anti-apoptotic protein expression indicating that this chemokine promotes cell survival signalling. Co-administration of a pharmacological antagonist of CXCR2 with L-OHP