567 NOEY2 gene expression in breast tumour pathogenesis

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Background: It is known that disturbances in gene imprinting, defined as gene expression based on the gamete of origin, are implicated in oncogenesis through loss of tumour suppressor gene regulation. The inactivation of expression of NOEY2 gene, an imprinted putative tumour suppressor gene, may contribute to tumours arising in the breast.
The aim of research was to investigate the expression of NOEY2 gene in breast cancer tissue and its relation to clinical and molecular characteristics of cancer cells.
Material and Methods: We studied the biopsy material of breast cancer cases and benign breast tumours (50 samples each). All cases were not BRCA-associated. The expression of NOEY2, ER, Ki67, p53, p21 and mdr1 genes was detected by Real-Time PCR using TaqMan® gene expression assays (Relative quantification, RQ). The expression of GHPDH gene was accepted as endogenous control. The BRCA mutations (c.181T>G, c.4034delA, c.5266dupC, c.68_69delAG of BRCA1 and c.5946delT of BRCA2 gene) were detected using TaqMan® SNP custom assays.
Results: The NOEY2 mRNA was detected in 47 of 50 (94%) noncancerous tissues and in 41 of 50 (82%) breast cancer samples. In 15 of 41 (36.6%) NOEY2 mRNA-positive cancer samples the expression of NOEY2 was substantially reduced (up to 100 times).
The expression of NOEY2 gene was positively correlated with the age of disease manifestation ($r = 0.59$; $P < 0.01$). The expression of NOEY2 was not correlated with ER, Ki67, p53, p21 and mdr1 genes expression.
Conclusions: Thus, the NOEY2 expression may play an important role in abrogation of the breast cancer pathogenesis. The link between epigenetic disturbances and the spreading mechanism of breast cancer may possibly exist.
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Conflict of interest: not declared.

568 Synthesis and biological properties of some new thiosemicarbazones

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Background: Thiosemicarbazones are versatile molecules not only because of their broad profile in pharmacological activity, but also because can act as ligands in coordination chemistry in different ways. It has been demonstrated in previous publications that thiosemicarbazones afford a diverse variety of compounds with different activities. In this work new thiosemicarbazones deriving from 2′-hydroxyacetophenone or salicylaldehyde and containing rings incorporated at N(3)-position have been investigated. These compounds have been prepared and structurally characterized by means of vibrational and NMR (^{1}H and ^{13}C) spectroscopy. These compounds are of considerable interest due to their antibacterial and antitumour activities.
Material and Methods: The compounds have been identified by elementar analysis and spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, IR, MS and XRF). In vitro cytotoxicity of the thiosemicarbazones was tested by MTT assay against the target cells: human cervix carcinoma (HeLa), chronic myelogenous leukemia (K562), breast carcinoma (MDA-MB-453) and breast adenocarcinoma (MDA-MB-361). Compounds solusions were added to neoplastic cells grown in 96 flat bottomed wells, 20 h after cell seeding. Cell survival was determined 72 h after the continuous agent action. The antibacterial activity of the compounds was evaluating by broth microdilution assay using a panel which included laboratory strains obtained from American Type Culture Collection.
Results: The obtained results showed that both studied compounds expressed significant cytotoxic activity *in vitro* toward malignant HeLa, MDA-MB-361, MDA-MB-453, and K562 cell lines having IC50 values from 0.76 to 7.21 μM, pointing to their similar, or even better antiproliferative action than the action of cis-DDP on the same cell line.
Conclusions: Results obtained indicate the potential of these compounds for the antitumour action, making them particularly interesting for further *in vivo* investigations. In addition, these compounds showed a mild antibacterial activity.

569 Synergistic effect of cisplatin combination with indole-3-ethyl isothiocyanate on proliferation of human ovarian cancer cells in vitro

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Chemopreventive isothiocyanates found in cruciferous vegetables, consumption of which has been associated with reduced risk of cancer, exhibit also growth-inhibiting and apoptosis-inducing properties in cancer cell lines in vitro. Our study presents a new synthetic ITC derivate indole-3-ethyl isothiocyanate (homoITC) as an inhibitor of cellular proliferation and inducer of apoptosis with potential utility as an anticancer drug or a sensitizer to routinely used chemotherapeutic agent cisplatin (cis-Pt).
We analysed the growth inhibitory effects of homoITC in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780/CP using MTT-test and its apoptosis-inducing properties by flow cytometry.
Combination index (CI) values from Calcusyn software were used to characterize the interactions of homoITC and cis-Pt as synergistic ($CI < 1$), additive ($CI = 1$), or antagonistic ($CI > 1$).
Significant synergistic effect in growth inhibition of homoITC (5−15 μM) and cis-Pt (2.5−10 μM) on A2780 parental cell line (CI from 0.42 to 0.85) was also observed on A2780/CP resistant subline (CI from 0.18 to 0.73) for 10−50 μM cis-Pt concentrations and the same concentrations of homoITC. Synergy in growth inhibition correlated with the potential of homoITC to stimulate apoptosis induced by cis-Pt.
Conclusion: homoITC may be worth of further studies assessing its value in the ovarian cancer treatment and elucidating mechanisms of its action.

570 Expression of beta-catenin and MUC1 in malignant, benign and normal breast tissues

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Background: During cancer progression, MUC1 binds β-catenin resulting in enhanced transformation and metastasis. The purpose of this work was to study the characteristics and correlation of the immunohistochemical expression of β-catenin and MUC1 in malignant, benign and normal breast tissues.
Material and Methods: Immunohistochemical analysis was performed on 60 breast carcinoma samples, 15 benign breast diseases and 10 normal tissues. MUC1 was detected employing HMFG1 MAb and β-catenin with anti-beta-catenin 7D11 MAb (Santa Cruz, USA); Catalyzed Signal Amplification System (Dako, USA) was employed. Immunohistochemistry was performed following standard procedures without antigenic retrieval. Positive area of reaction, intensity and pattern of expression were considered. A reactivity index (RI) was calculated as intensity (I) × 100 + percentage of positive area (A). Statistical correlation analysis was performed employing Pearson correlation.
Results: Malignant samples expressed β-catenin in 51/60 (85%) samples and MUC1 in 54/60 (90%) while breast benign samples in 12/15 (80%) and 15/15 (100%), respectively; finally, normal samples expressed β-catenin in 9/10 (90%) and MUC1 in all samples. In all groups a statistical significant correlation between β-catenin and MUC1 ($p < 0.005$) was found. The pattern of β-catenin expression was different since, in malignant specimens, nuclei staining was frequently found (68%) while nuclei were not reactive in benign and normal samples. The intensity of the reaction was strong in most malignant and benign samples while normal samples showed a low reaction. MUC1 differed in the pattern of expression since malignant samples showed a mixed (cytoplasmic and membrane) non-apical staining while membrane apical pattern was predominantly found in benign and normal specimens.
Conclusions: IHC is an ancillary tool in pathological diagnosis as well as in experimental analysis; employing this methodology we found that β-catenin and MUC1increased their expression and change their pattern in malignant breast samples compared to benign and normal specimens.

571 Molecular events during cold stress induced cell-death on multidrug resistant leukemic cells

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We have previously shown that acquisition of multidrug resistant phenotype (MDR) by leukemic cells is accompanied by pleiotropic changes that result on reduced tumour capacity to survive under stress conditions such as hypothermia. Furthermore, by using selective inhibitors of individual caspases (caspase 3, 8 or 9) we have reported a reversal on cold stress-induced cell-death in the presence of any of those inhibitors, suggesting that the cell death mechanism is caspase-dependent. Next, our aim was to gain a broader insight