of several NK cell receptors and early activation markers on NK cells in lymph nodes (LN) of melanoma patients.
Material and Methods: Regional (LN) were obtained from patients who underwent melanoma resection. LN were incised immideatelly after removal and cut into two parts: one half was paraffin embeded to perform pathohistological evaluation and the other was mechanically distrupted in order to obtain a single cell lymphocyte suspension. Lymphocytes were purified using Histopaque density gradient. The expression of CD16, NKG2D, CD158a, CD158b, CD25, CD69 and HLA-DR was analyzed on gated CD3-CD56+ NK cells by flow cytometer. Intracellular stainig of IFNg was done according to standard BD procedure.
Results: We show that NK cells isolated from histopathologicaly proven malignant LN have higher expression of CD16, the most important cytotoxic receptor, as well as the activating NKG2D NK cell receptor, compared to the benign LN. Regarding the expression of the inhibitory KIR receptors, malignant LN show the higher percentage of NK cells positive for CD158b, while the level of the other investigated KIR CD158a is similar to the bening LN. We also show that the presence of tumour cells in LN is associated with higher percentage of NK cells positive for CD69 activation marker, whereas the percentage of NK cells that express other investigated activation markers (CD25, HLA-DR) as well as he IFN-γ level, are similar to the benign LN.
Conclusions: Our results show that NK cells from malignant LN of melanoma patients, compared to benign LN, express higher percentage of NKG2D receptor that mediates antitumour activity by binding to stress-induced ligands on malignant cells, and also the higher level of CD158b inhibititory NK cell receptor whose ligands are MHC class I molecules that have been lost during malignant transformation. Invasion of malignant cells into LN may contribute to higher expression of CD16 cytotoxic receptor and CD69 activation marker.

309 Gene expression analysis of tumour markers associated to apoptosis and proliferation in head and neck cancer

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The development of oral and head and neck squamous cell carcinomas occurs in relation with multiple events including mainly: loss of cycle cell control, evasion from apoptosis, telomerase reactivation. Apoptosis represents a cellular "suicide" mechanism which helps keeping in normal limits the cell number in tissues and allows elimination of those cells presenting DNA mutations or having an abberant cell cycle, these cells being predisposed to malignant transformation. P53 phosphoprotein acts as a tumour suppressor and is involved in inhibiting cell proliferation when DNA damage occurs. Wildtype P53 plays a role as checkpoint protein for DNA damage during G1/S phase of cell cycle. Bcl-2 oncoprotein is a blocker of apoptotic cell death which resides on the cytoplasmic side of the mitochondrial outer membrane, endoplasmic reticulum and nuclear envelope. We have studied the gene expression of two proteins (p53 and bcl-2) related to apoptosis and proliferation of tumour cells, correlated with antigen expression by flow-cytometry and progression through cell cycle phases. Gene expression analysis was performed by real time RT-PCR on 24 tumours from patients with head and neck cancers. After total RNA extraction, cDNA synthesis and optimization of PCR reaction, data were analized by REST program in order to study the levels and variability of gene expression for the two proteins and correlations between them. In order to confirm the specificity of PCR reaction, the amplification products were examined by 2% agarose gel electrophoresis. The house-keeping gene used for normalization of C_T values was RPL 32. Progression through cell cycle phases was evaluated by PI technique and analysis, while percentages of apoptotic cells were detected by using Annexin V-FITC/PI coloration, followed by flow-cytometry. In addition, gene expression of studied molecules was correlated with antigen expression detected by flow-cytometry. Characterization of head and neck cancers by using modern methods of molecular biology and immunology might lead to a better understanding of the disease, orientation of the oncological treatment and patient's response to chemotherapy.

310 Expression of cell adhesion molecules on MDR-1 positive stimuli treated breast tumour cells

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Breast cancer represents a malignancy with high incidence and mortality throughout women, its ethiology involving many genetic, immunological and biochemical factors. Proliferation of mammalian cells is tightly regulated by multiple environmental influences, adhesion to extracellular matrix (ECM), cell-cell adhesion and soluble factors. Formation and spread of tumours are closely associated with decreased dependence on adhesion for growth and survival. Co-expression of certain cell adhesion molecules (CAMs) by tumour cells, which might be involved in cellular interactions, changes in adhesivity and cellular mobility, might influence the aggressivity and metastatic potential of a certain tumour. Malignant evolution depends on the genetic profile of the tumour that dictates its reaction to the citotoxic action exerted by drugs. Those might induce modifications of gene expression (*e.g.* glycoprotein P, known also as MDR-1) that contribute to a resistant phenotype. Therefore, the use of certain natural compounds might increase the efficacy of chemotherapy and diminish tumour resistance. The present study focused on the correlation between antigen and gene expression of several CAMs (ICAM-1, MUC-1, E-cadherin, VCAM-1) and MDR-1 associated to breast cancer cell lines in the presence or absence of drugs (doxorubicin, 5-fluorouracyl) and/or natural compounds (curcumin, quercetin). Expression of membrane associated antigens was evaluated by flow cytometry, while gene expression was detected by real-time PCR. Expression analyses of antigen *vs* gene expression showed that CAMs and MDR-1 were differentially modulated by stimuli treatment. Our results bring new information regarding the co-existence of CAM and MDR-1 associated to stimuli-treated breast tumour cells that might influence the interaction between tumour cells and host immune system. Structural or gene expression alterations are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy.

311 Effect of stimuli treatment on proliferation, apoptosis and cell signalling mechanisms associated to oral cancers

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Cancer is a disease of the cell, therefore is essential the identification of evolution stages and use of this information in prediction, prevention, early diagnosis and development of new target drugs. The main obstacle against the success of therapy in many cancers seems to be the impossibility of eradication all tumour cells. Oral cancers represent some malignancies with high incidence throughout world, their ethiology involving many genetic, immunological and biochemical factors. Oral cancer can develop in any part of the oral cavity or oropharynx. Almost all oral cancers begin in the flat cells (squamous cells) that cover the surfaces of the mouth, tongue, and lips. These cancers are called squamous cell carcinomas. These are malignant and tend to spread rapidly. A new therapeutical approach could be more useful for distruction of tumour cells: renewal of the cellular pathways that lead directly to apoptosis. Traditional anti-cancer therapies have limited effects, therefore the citotoxic action exerted by drugs on tumours might be added by natural compounds and the signalling mechanisms influenced.
The present study focused on modulation of antigen expression of AMPK-alpha, -beta, LKB1, TSC2, mTOR and S6K1 by cytotoxic drugs (such as 5-fluorouracyl and cisplatinum) in the presence or absence of natural compounds such as apigenin and curcumin. Levels of expression of phosphorylated vs non-phosphorylated proteins were evaluated by immunoblotting in oral tumour cell lines derived from squamous cell carcinomas. Protein expression was detected by Western blotting using the chemiluminescence. Our results showed a differential antigen expression of the molecules under study involved in signaling mechanisms involved in kinase activities depending on the association of stimuli and drugs. Protein expression alterations, resulting frequently from gene modifications, are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy. The knowledge of the relation between the implied molecules, of the mechanisms of regulation of the gene and protein expression, as well as their functions, of the effect of therapeutic agents (oncolytic agents, natural compunds) on proliferation, induction of apoptosis and tumour cell lysis has a potential diagnostic and prognostic value of the disease evolution, but also regarding the tumour response to immunotherapy.

312 Antigenic markers, disease progression and survival in colorectal cancer (CCR) patients

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Background: Antigenic expression in colorectal (CCR) primary tumours and metastatic lymphatic nodes (N+) may be useful for early detection of disease progression. The purpose of this research was to establish the possible relationship among antigenic expression in tumour and N+ versus survival free disease (SFD) and overall survival (OS) in CCR patients.
Materials and Methods: A total of 90 CCR patients were studied: 90 primary CCR tumour samples and 64 lymphatic nodes; 22 samples from adenomas and normal colorectal mucosa specimens were employed as controls. Antigens studied were: MUC2 mucin, MUC1, MUC5AC, CEA, beta-catenin; carbohydrate antigens such as Lewis x (Lex), sialyl Lewis x (sLex), Lewis y (Ley), sialyl Lewis a (sLea) and Tn hapten. Immunohistochemistry was performed following standard procedures with antigenic retrieval. Positive