

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 immediately after removal and cut into two parts: one half was paraffin embedded to perform pathohistological evaluation and the other was mechanically disrupted 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 staining of IFN γ was done according to standard BD procedure.

Results: We show that NK cells isolated from histopathologically 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 benign 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 the 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 inhibitory 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 aberrant 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 analyzed 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 etiology 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 cytotoxic 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-fluorouracil) 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 etiology 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 therapeutic approach could be more useful for destruction of tumour cells: renewal of the cellular pathways that lead directly to apoptosis. Traditional anti-cancer therapies have limited effects, therefore the cytotoxic 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- α , - β , LKB1, TSC2, mTOR and S6K1 by cytotoxic drugs (such as 5-fluorouracil and cisplatin) 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 compounds) 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

response was evaluated by means of frequency analysis ($p < 0.05$). A reactivity index (RI) was calculated as intensity (I) x percentage of positive area (A); RI values varied from 0–12; low (0–6) and high (7–12). Intensity, percentage of positive area, immunohistochemical pattern and RI were analyzed employing Chi-Square test and ANOVA ($p < 0.05$), after data standardization.

Results: Primary malignant samples expressed MUC2 in 52% of cases; MUC1, 94%; MUC5AC, 14%; CEA, 93%; beta-catenin, 98%; Lex, 74%; sLex, 66%; Ley, 91%; sLea, 90% and Tn, 41%. N+ expressed MUC2, 30%; MUC1, 89%; MUC5AC, 19%; CEA, 80%; beta-catenin, 83%; Lex, 69%; sLex, 72%; Ley, 75%; sLea, 86% while Tn, 44%. In general, CCR showed a higher RI than control samples. In patients with N+ with a low sLex RI presented a longer SFD than patients with a high sLex RI (40 vs 14 months) ($p = 0.07$). Although a significant difference was not found, patients which showed a primary tumour low Lex RI presented a longer SFD than patients with a high RI, (44 vs 26 months). A long OS was found in patients which primary tumours showed a low Lex RI (18 months). In N+ patients, Lex was also studied in lymphatic nodes, a low Lex RI correlated with a longer SFD than patients with a high Lex RI (24 vs. 18 months).

Conclusions: This study showed that, in CCR patients, carbohydrate antigenic expression may indicate a difference in SFD and OS.

313 Immunity to phytohemagglutinin in some NHL patients

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Background: Non-Hodgkin lymphoma (NHL) represents heterogeneous group of diseases either B-, or T-cell origin. Immunity to food antigens is in the attention of modern medicine. Many articles reported data about immune reactions to proteins from strawberries, peanuts, elevated levels of IgA and IgG antibodies to native gluten sequences or to cow's milk proteins. In order to assess whether food antigens contribute to the imbalance of immune response in patients with lymphoma, the aim of this work was screening the sera of patients with (mostly) B cell NHL as well as of healthy people for their immunoreactivity to food constituent phytohemagglutinin (PHA).

Patients Material and Methods: Fifty-five patients with NHL (before therapy) and 25 healthy individuals were included in the study. Levels of serum IgA, IgG and IgM antibodies to PHA, isolated from *Phaseolus vulgaris* (red kidney bean) were determined by ELISA test.

Results: Data obtained by ELISA tests show that the existence of the enhanced immunoreactivity to PHA is not so frequently found in tested NHL patients. Higher than cut off anti-PHA IgA immunoreactivity was found in 14/52 NHL patients. Elevated anti-PHA IgG immunoreactivity was found in 2/55 NHL patients and enhanced anti-PHA IgM immunoreactivity was not found in examined 52 NHL patients.

Conclusion: These results call for the new research are there any clinical importance of elevated humoral immunity to PHA, for the small group of immunoreactive NHL patients, for the course of this very severe hematological disease.

314 The role of pre and post treatment evaluation of Immunoglobulin A in hypopharyngeal malignancies in southern India

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Background: Head and neck is one of the most complicated regions of the body, and its cancers present a challenge in multidisciplinary and multimodality management.

Methods: Objectives of the study are to:

1. Compare Immunoglobulin A (IgA) levels pre- and post treatment and recognize its relationship to stage of disease, histopathological grading and determine if it could be used as a reliable indicator to assess the prognosis of the disease;
2. Assess if IgA levels have any relationship to total serum protein levels and haemoglobin.

A prospective study was conducted from 2006–2008. 27 cases of hypopharyngeal cancers were randomly selected. 27 controls were also randomly selected. All cases staged as per TNM system and were histologically proven cases. Cases were grouped according to age, sex, risk factors, total serum proteins, hemoglobin levels, subsite of tumour, stage of disease, histopathological grading and treatment modality. Pre-treatment blood sample was collected 1 week prior to treatment and post-treatment sample 6 months after treatment. The samples were assessed for IgA, Total Protein, albumin, globulin and Hb%. A special questionnaire was prepared to evaluate the cases. The patients were subjected to 3 different treatment modalities: Surgery, RT and combined modality, and their results were also compared. ANOVA test was used to assess 'p' value.

Results: Mean age group of cases was 56.8 yrs. 59% cases were in 41–60 yrs group. Male:Female 4:1. 85% had history of risk factors; of which smoking was the commonest seen in 70% cases. A significant increase in serum IgA was observed in patients with low total serum protein and hemoglobin levels ($p < 0.05$). Serum IgA level was evaluated in both cases and controls, and a statistically significant increase in IgA level was found among cases (64%, $p < 0.01$). As stage of disease progressed there was progressive rise in levels of IgA being highest for stage IV disease. A comparison of treatment modality in stage III and stage IV showed that combined modality is a better option to radiotherapy alone.

Conclusions: In India, hypopharyngeal cancers are common in male due to the associated risk factors. Age groups commonly affected is above 40. A significantly high level of IgA was observed in these patients; which proves it's a definite but delicate indicator in the evaluation of hypopharyngeal cancers. Increased levels of IgA in anemic and malnourished patients indicates that presence of anemia and malnutrition can affect the treatment and prognosis of patients. Significantly increased levels of IgA are seen in patients with stage IV disease and poorly differentiated type of squamous cell carcinomas. The difference in the increase in the serum immunoglobulin level as the stage and differentiation of the tumour progresses can be used as a prognostic tool. Persistently high serum IgA levels even after treatment indicate residual or recurrence of tumour hence needs a close follow up. Comparison of pre- and post-treatment IgA levels suggests that combined modality of treatment is ideal for stage III&IV disease.