Conclusions: The present study suggested that cancer patients with TE should be evaluated for FVL but PT G20210A was not contributing factor to be development of TE during cancer treatment.

254 PUBLICATION

The RBBP6 expression in oesophageal tumours

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The RbBP6 is a 36 kb gene and containing 18 exons. It is transcribed to three mRNA transcripts, 1.1 kb and 6.1 kb that have a splice variant missing exon 16 splice. Deletion or mutation of the 1.1 mRNA variant in CHO cells have been found to render cells resistant to apoptosis induced by chemical inducers such as staurosporine and this directly links RbBp6 gene to apoptosis.. The l.1 mRNA is translated into a 13 kDa protein (isoform 1) containing the DWNN domain only whereas the 6.1 mRNA is translated to 200 kDa proteins isoform 2 and 3 having the RING Finger, Rb and p53 binding domains linked to the DWNN domain.
The aim of the study was to determine the expression pattern and tissue distribution of RbBp6 gene products in oesophageal tumours. We have studied poorly, well and moderately differentiated human squamous oesohageal tumours. We have also compared the levels of expression and apoptosis in these tissues.
Using both in situ hybridization and immunocytochemistry, we have found that RbBP6, is found upregulated and we also found that it accumulates in the cytoplasm like the mutated p53. In normal tissues RbBp6 was found to be localizing mostly in nuclei and rarely in the cytoplasm. We have found that RbBp6 is upregulated around islands of tumours in well differentiated squamous tumours where the apoptosis is high and very much involved in fighting the invading tumours and found none or little RbBP6 localization in the islands of tumours where apoptosis had completely halted. The RbBP6 expression level correlated with apoptosis and was found to be inversely proportional to proliferation as it was shown by TUNEL and Ki67 respectively. We have also used real time quantitative RT-PCR using Roche LightCycler and have confirmed that the RbBP6 expression levels are increased in oesophageal tumours as compared to normal oesophageal tissues.
The RbBP6 200 cDNA had previously been cloned by detecting its interaction with tumour suppressor proteins p53 and Rb, which have a major role in apoptosis and cancer development. Accumulation of these proteins, p53 and RbBP6, suggests that RbBP6 may be involved in a p53 dependant apoptotic pathway.

255 PUBLICATION

Prediction of the response to radiotherapy by comet assay: preliminary results in cervical tumors

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Background: Intrinsic cellular radiosensitivity (RS) is a genetic factor involved in the interindividual variability of the response to radiotherapy (RT). A major research goal is the establishment of a predictive assay based on individual radiobiologic characteristics. Comet Assay might be a reliable assay to be used in the prediction of clinical RS.
Purpose: The specific aim of our prospective study is to correlate the *in vitro* RS of tumor cells with the clinical response after curative RT.
Material and method: Twelve patients with locoregionally advanced cervical carcinoma were included so far. Tumor cells obtained from short time primary cultures of tumor tissue prelevated by biopsy were irradiated *in vitro* and analysed by Comet Assay. For each tumor two parameters of the degree of DNA lesions (Lesion Score-LS and Tail Factor-TF) were scored before, immediately after and at two hours after irradiation. Tumor response was clinically assessed at the completion of the treatment as follows: stationary disease (SD), partial response (PR) and complete response (CR). *In vitro* parameters of RS were correlated with the clinical results.
Results: According to the degree of DNA lesions, 3 biological parameters were evaluated: the background level (B), the magnitude of induction by irradiation (I), and the repair of the radioinduced lesions (R). Differences in B reflect the interindividual variability of the sensitivity to ionizing radiations. Treatment results (clinical responses at the end of RT) correlated with I and R. All 5 cases with CR were characterized by high or moderate I and /or defficient R; 2 cases with good PR had low I and lack of R. The 5 cases with SD showed low I and good R.
Conclusions: Comet assay is a modern, quick and reproducible method which seems to be a promising tool for the prediction of the clinical response to RT. Our preliminary results are encouraging but a larger number of patients must be included in order to draw reliable conclusions.

256 PUBLICATION

Flowcytometric accurate determination of ABC-transporters' activity regulating anthracycline intracellular compartmentalization in multidrug resistance cells

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Background: Intracellular compartmentalization is the major determinant of classical multidrug resistant mechanism (MDR) associated with ABC-transporters' function activity. The purpose of the study is to develop an accurate approach for separate determination of of ABC-transporters' activity regulating nuclear and cytoplasmic accumulation of MDR-drugs.
Material and methods: ABC-transporters' functional activity (MDR-phenotype) was determined as the change in doxorubicin (Dox) intracellular accumulation (ICA) under action of specific inhibitors of Pgp and MRP by flowcytometry. The following new data are the result of more than 100 biopsy sample investigation (breast, colon and cervix carcinoma).
Results:
1. Analyzing the results of MDR-phenotype study it has been revealed an interesting phenomenon: increase in Dox ICA under inhibitor action is accompanied by two opposite changes in Dox intracellular fluorescence (ICF): increase or decrease of the index.
2. Under the same inhibitor concentration the change in Dox ICF depends on tumor cell investigated.
3. The direction of the change in Dox ICF in the same cells depends on inhibitor concentration: increase in Dox ICA is accompanied by decrease in Dox ICF under action of higher inhibitor concentration but index increases in lower inhibitor concentration.

Conclusions: 1. Well-known phenomenon of Dox fluorescence quenching as a result of anthracycline binding to DNA let us conclude that decrease in Dox ICF means main increase in nuclear Dox accumulation and binding to DNA under inhibition of ABC-transporters regulating Dox accumulation in the nucleus. On the contrary, increase in Dox ICF results from main increase in cytoplasmic Dox accumulation under inhibition of ABC-transporters regulating cytoplasmic Dox accumulation. 2. Nuclear ABC-transporters are more resistant to inhibitors' action. 3. So, investigation of Dox ICF changes under ABC-transporters' inhibition by flowcytometry make it possible determination of anthracycline intracellular distribution and separate estimation activity of ABC-transporters regulating nuclear and cytoplasmic accumulation of the drug. The latter is the most important index of MDR-phenotype for prognosis of resistance to chemotherapy in cancer patients.
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Breast Cancer

Oral presentations (Thu, 3 Nov, 8.30–10.35)

Molecular characterization of breast cancer and its clinical implications

257 ORAL

Combination of two biological gene expression signatures in predicting outcome in breast cancer as an alternative for supervised classification

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Introduction: Gene expression profiling has been used to identify specific subgroups of breast carcinomas that differ with respect to clinical and pathological features, including outcome. We have previously identified 3