**250** PUBLICATION

**Cytotoxic T lymphocyte antigen-4 promoter variants in breast cancer**

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**Background and Objectives:** CTLA-4 is a co-inhibitory molecule expressed mainly on activated T lymphocytes. To test the putative involvement of CTLA-4 in inhibitory state of immunity to breast cancer, we genotyped 283 patients and 245 healthy controls for −1722 T/C, −1661 A/G and −318 C/T polymorphisms in ctla-4 promoter region.
**Methods:** −1722 T to C dimorphism was genotyped by a Polymerase Chain Reaction with Confronting Two Pairs Primers (PCR-CTPP) method. −1661 A to G polymorphism was investigated by a PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) method. −318 C to T polymorphism was genotyped by a PCR− Amplification Refractory Mutation System (ARMS) method.
**Results:** There were no significant differences in genotype, allele and haplotype frequencies in all three loci between patients and healthy controls. Moreover, the frequency of the most frequent haplotype combination (TAC/TAC, T-1722, A-1661, C-318) was only slightly higher among healthy controls than patients (68.4% vs. 64.8%, P = 0.2). However, this haplotype combination was associated with lower stages of the disease (P = 0.0007) and higher expression of Estrogen Receptor (ER) in patients (P = 0.006). Association with tumor prognostic/predictive factors was also observed in the case of certain genotypes: −1661 AA genotype was associated with less LN involvement (P = 0.017) and high ER expression (P = 0.004), and −318 CC genotype with less LN involvement (P = 0.007).
**Conclusions:** These results suggest that CTLA-4 promoter variants participate, at least partly, in the progression of breast cancer rather than its initial development.

**251** PUBLICATION

**Acute phase proteins in malignancy: role of CRP in the discrimination of infectious complications in cancer patients**

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**Background:** Acute phase response is one of the defense mechanisms that maintains organism against stimulants such as invasions of microorganisms, trauma, immunological diseases, and cancer. An increased acute phase response was found in patients with various malignant tumors and could be a sign of poor prognosis. The aim of this study was to determine the rate of acute phase response in cancer patients and comparing the response against healthy controls and patients with infectious disease(diseased control group).
**Materials and methods:** 104 cancer patients(45 male, 59 female), 25 healthy controls(14 male, 11 female) and 25 patients with infectious disease (11 male, 14 female) were included in the study. Serum levels of c-reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, haptoglobin, fibrinogen, and erythrocyte sedimentation rate (ESR) were measured.

Table 1

| | CRP (mg/l) | Haptoglobin (mg/dl) | Ferritin (ng/ml) | Fibrinogen (mg/dl) | LDH (U) | ESR (mm/h) |
|---|---|---|---|---|---|---|
| Group I (cancer) | 25.3 ±47.37 | 216.54 ±131.33 | 171.58 ±234.55 | 484.32 ±177.09 | 416.54 ±371.83 | 36.36 ±29.64 |
| Ia (metastatic) | 39.25 ±62.12 | 252.13 ±151.23 | 209.42 ±260.22 | 546.02 ±201.04 | 484.98 ±515.24 | 47.52 ±33.07 |
| Ib (non-metastatic) | 10.72 ±11.66 | 172.95 ±87.33 | 119.13 ±166.37 | 408.89 ±120.81 | 334.28 ±64.76 | 26.31 ±19.20 |
| Group II (healthy control) | 2.21 ±0.94 | 126 ±44.05 | 152 ±73.71 | 270 ±56.22 | 279.32 ±44.11 | 8.7 ±4.37 |
| Group III (infection) | 42.26 ±65.63 | 180.04 ±92.57 | 285.54 ±416.10 | 533.78 ±201.2 | 334.28 ±64.7 | 26.73 ±19.36 |

**Results:** Cancer patients were found to have higher levels of CRP, LDH, haptoglobin, and fibrinogen than healthy control group($p < 0.001$). Serum levels of acute phase proteins were found to be similar between cancer patients and patients with infectious disease for all the checked parameters. Subgroup analysis of cancer patients showed that cancer patients with metastatic disease had significantly higher CRP, fibrinogen, and ESR levels than non-metastatic group.
**Conclusion:** All types of acute phase proteins increase in cancer patients and may be a marker of tumor burden in this group of patients. Very high levels of acute phase proteins could be a sign of metastasis in cancer patients. However, measurement of serum levels of quantitative CRP cannot discriminate secondary infections in cancer patients, that may superimpose in any immunosuppressed patients.

**252** PUBLICATION

**Relationship between genes expression, proteins induction, polymorphisms and radiotherapy complications in Saudi cancer patients**

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**Background:** Patient-to-patient variations in the sensitivity of normal tissues are well recognized in clinical radiotherapy practice. Studies in exquisitely sensitive patients have identified mutations in cell cycle and DNA repair genes and suggested that polymorphic variations in multiple genes could be associated with radiation sensitivity (RS). In this study we sought to: 1. Affirm these variations in our radiotherapy patients; 2. Identify some of the genes and proteins involved; 3. Explore the association with certain single nucleotide polymorphisms (SNPs) in susceptible genes.
**Materials and methods:** Fibroblasts were established from 53 Head and Neck cancer patients treated with radiotherapy. The *in vitro* RS was measured using the clonogenic assay. Genes and proteins expression were studied in representative cell strains using 1.2 cDNA macroarrays and Western blotting, respectively. Basal genes and protein levels and induction 3 hours after a dose of 5 Gy were evaluated. SNPs were studied by heteroduplex analysis (MDE gel) and/or sequencing.
**Results:** Survival curves showed a wide range of RS. The surviving fraction at 2 Gy (SF2) ranged between 0.16 and 0.56 (mean, 0.36). There was a statistically significant difference in SF2 between the group of patients having minimal late complications (Grade 0–1 fibrosis) and the group who suffered more severe fibrosis (Grade 2–3) ($p = 0.001$). Genes' expression profile was distinct in cell strains studied and was associated with RS. Basal protein levels varied but no correlation was observed with RS. In control cells, CDKN1A, TP53 and MDM2 proteins were induced $\geqslant$1.5 fold after irradiation, and to a lesser extent DNA LIG IV, MRE11 and NBS1, but not ATM, DNA-PKcs, Ku70, Ku80, XRCC4, RAD50, BRCA1, CDKN1B and RB. MDM2, CDKN1A, and TP53 proteins induction showed tendency toward a correlation with both the *in vivo* grade of fibrosis and the *in vitro* SF2. Preliminary results with SNPs in TP53 codon 72, CDKN1A codon 31 and XRCC3 codon 241 showed no association with RS. Other SNPs in XRCC1, MDM2, ATM, LIG IV and TGFb1 are being studied.
**Conclusions:** These results suggest that our patients vary significantly in RS and potentially can benefit from a predictive test. The RS is associated with distinctive genes expression profile and with the level of induction of TP53, CDKN1A and MDM2 proteins; however, this was not associated with the SNPs studied in TP53, CDKN1A or XRCC3. Supported by KFSHRC grants 2000 031 and 2040 025.

**253** PUBLICATION

**Factor V Leiden and PT G20210A mutations in cancer patients with and without thrombosis**

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**Background:** Cancer patients have an increased risk for thromboembolism (TE). Factor V Leiden (FVL) has been identified as the most common inherited risk factor for TE. The aim of the study was to determine the prevalence of FVL and PT G20210A mutations in cancer patients who developed TE during treatment, e.g. surgery, radiotherapy and chemotherapy as compared to those who did not.
**Material and Methods:** The study consisted of 35 cancer patients who developed TE during treatment (group I), 50 cancer patients without TE (group II), 50 patients with TE without malignancy (group III), and 60 healthy controls (group IV). FVL and PT G20210A mutations were measured by the method of polymerase chain reaction-based DNA analysis.
**Results:** The prevalence of FVL was significantly greater in cancer patients with TE (10 of 35, 28.6% compared with the other groups: 2 of 50 (4%) in group II, 2 of 50 (4%) in group III, and 4 of 60 (6.7%) in group IV ($p = 0.003$). There was no significant difference in the prevalence of PT G20210A between the groups ($p > 0.05$).

**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