1123 POSTER

Enoxaparin Down Regulates Inflammatory and Thrombotic Mediators in Cancer Patients as Studied Using Protein and Biochip Array Approaches

D. Hoppensteadt[1], E. Litinas[1], H. Khan[1], J. Cunanan[1], I.M. Thethi[1], J. Fareed[1]. [1]*Loyola University Medical Center, Pathology, Maywood, USA*

Introduction: The pathogenesis of cancer is known to upregulate inflammatory and thrombotic processes which contribute to the increased mortality. We hypothesized that the baseline inflammatory and thrombotic mediators are upregulated in cancer and treatment with LMWHs such as enoxaparin may downregulate them.
Methods: To test this, plasma samples were retrospectively analyzed from an open label multi dose active comparator parallel design study in which all patients (n = 110) were initially treated with enoxaparin (1−1.5 mg/kg sc) for 5 days. These patients were subdivided into two groups. Group A continued to receive enoxaparin whereas Group B received warfarin. Baseline blood samples, 5 days and 12 weeks post treatment samples were analyzed using bio chip arrays (Randox analyzer) and protein chip array using surface enhanced laser desorption ionization (SELDI) technique.
Results: In the cerebral biochip array analysis, levels of CRP, TNFRI, D DIMER, NGAL and TM were elevated at baseline which reduced after treatment with enoxaparin at three months except for NSE and TNFRI. In the cytokine biochip array, IL2, IL4, IL6, IL8, IL10, VEGF, IFNG, TNFA, IL1A, IL1B, MCP1 and EGF showed marked upregulation at baseline with enoxaparin treatment resulting in a decrease of IL6 alone.
The baseline plasma samples from the patients recruited with multiple cancers in the Oncenox study showed a greater prevalence of the 11.6 kDa biomarker (76%) with average amplitude of 23.6. The samples collected after 3 Months of enoxaparin treatment revealed markedly reduced prevalence (38%) and average amplitude of 5.4.
Conclusions: These results confirm that inflammatory and thrombotic mediators are downregulated by treatment with enoxaparin. The biochip and protein arrays provide unique tools to profile the known mediators and identify newer biomarkers.

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Loss of NKX3.1 by Inflammatory Microenvironment Resulted in Uncontrolled Proliferation in Prostate Cells

B. Debelec-Butuner[1], C. Alapinar[1], K.S. Korkmaz[2]. [1]*Ege University Institute of Science, Biotechnology, Izmir,* [2]*Ege University Faculty of Engineering, Bioengineering, Izmir, Turkey*

This report aims to define the relationship between inflammation; generation of reactive oxygen species (ROS), and uncontrolled prostate cell proliferation due to the loss of Androgen Receptor (AR), and androgen responsive factors such as NKX3.1. Inflammation in prostate tissue is associated with prostate cancer and recently reported that inflammatory cytokines, TNF-α (tumour necrosis factor-alpha) and IL-1β (interleukin-1beta) accelerate the protein loss of NKX3.1, which is found similar to observations in pre-invasive cancer of prostate. Therefore, an inflammation model of prostate using androgen responsive LNCaP cells was established to investigate the proliferative inflammatory atrophy (PIA) and subsequent molecular alterations in cancer development. In our model, U937 monocyte cell line was used for cytokine secretion, and further, conditioned media was used to feed LNCaP cells to achieve inflammatory prostatitis microenvironment. A decrease in the protein level of AR and its transcriptional target NKX3.1 is showed with this model and the loss of AR and NKX3.1 causes prolonged activation of a redox-sensitive transcription factor, nuclear factor kappa B (NFκB), that initiates and amplifies an inflammatory cascade within the prostate and results in sustained oxidative damage, which has to be scavenge by NKX3.1 related mechanisms. At certain doses of TNF-α, DNA damage increased and AR regulated apoptosis is down regulated. The inflammatory cascade is proposed to link with uncontrolled proliferation through up-regulated Wnt signaling showed with increased Akt phosphorylation and abnormal β-catenin accumulation. As a conclusion, Loss of NKX3.1 in inflammation conditions leaded the transition of prostate cells from PIA to cancer.

1125 POSTER

DNA Damage Response of Epithelial and Mesenchymal Cell Lineages in the Clinical Setting of Radiotherapy

I. Turesson[1], F. Qvarnström[1], M. Simonsson[1], U. Thunberg[1], I. Hermansson[2], M. Book[1], K.A. Johansson[3], J. Nyman[2]. [1]*Department of Radiology Oncology and Radiation Sciences, Section of Oncology, Uppsala,* [2]*Sahlgrenska University Hospital, Department of Oncology, Göteborg,* [3]*Sahlgrenska University Hospital, Department of Radiophysics, Göteborg, Sweden*

Cellular DNA-damage response (DDR) is a prerequisite for the prevention of normal tissue damage and cancer, and is highly relevant for most of the side-effects caused by radiotherapy (RT) in cancer treatments. The accessibility and structure of normal skin provides an excellent clinical model for studying radiation-induced DDR in various cell populations. Our collection of over 2000 skin biopsies allows us to establish detailed dose-response relationships, during and after RT. We use immunohistochemistry, imaging techniques and qRT-PCR to quantify key events in the DDR process. Low-dose hypersensitivity was evident for all investigated endpoints in keratinocytes and endothelial cells. This was observed as a non-linear dose response, in terms of effect per dose unit, established for 0.05 to 2 Gy per fraction over 5 to 7 weeks of RT. Investigated endpoints included the DSB-markers γH2AX and 53BP1, as well as growth arrest, as assessed by p21 and apoptosis by γH2 AX. A uniform up-regulation of mir-34a and p21 was observed in epidermal and dermal cell populations during RT. Importantly, both markers persisted in dermis but declined in epidermis within the 5 weeks after completion of RT. Epidermal accumulation of DSBs, persistent checkpoint activation and mitotic suppression was observed throughout the RT course. Pre-mitotic apoptosis was observed towards the end of RT and accelerated repopulation of keratinocytes did not emerge until a couple of weeks after the end of treatment. Furthermore, DSB foci kinetics revealed individual sensitivity and displayed differences between keratinocytes and endothelium. The cell reduction of basal keratinocytes during RT was dose-dependent and displayed hyperradiosensitivity to low dose fractions, while no reduction in endothelial cells was observed over the treatment course. Interfollicular keratinocyte stem cells, identified by Bmi-1, were more radioresistent than the progenitor cells, and did not change in absolute number during RT. In summary, a preserved low-dose hypersensitivity was observed for key events in the DDR of normal skin during RT. This has direct implications for techniques such as IMRT, where large tissue volumes are exposed to sub-therapeutic dose fractions. Contrary to classical radiobiology, our results also highlight a lack of keratinocyte repopulation during RT and a pronounced pre-mitotic apoptosis towards the end of treatment. Also, the observed tissue-specific differences in DDR could provide important clues to the further understanding of clinical observations such as early and late effects.

1126 POSTER

Microparticle-associated Tissue Factor as Central Activator of Coagulation in Patients With Malignant Effusions

F. Gieseler[1], B. Stelck[2], S. Stoelting[1], T. Bartscht[1], H. Ungefroren[1]. [1]*University Hospital of Schleswig-Holstein, First Department of Medicine, Luebeck,* [2]*University Hospital of Schleswig-Holstein, First Department of Medicine, Kiel, Germany*

Background: The permanent activation of the coagulation system is a clinical problem for tumour patients. Thrombosis and pulmonary embolism (PE) are frequent causes of life quality reduction or death even if the tumour is under control. Through the expression of protease activated receptors (PAR) tumour cells may profit from the activated serine proteases.
Material and Methods: We used malignant effusions after puncture from patients with disseminated malignant tumours as a model to examine the interaction of tumour cells, bystander cells and the surrounding fluid. As previously described, serine proteases such as FIIa, FVIIa, FXa can be found activated in these effusions. In addition tumour cells frequently express PARs (see literature).
Results: Permanently elevated d-dimer levels indicate systemic activation of the coagulation system in tumour patients. In a cohort of 80 patients with advanced tumours without any signs of thrombosis 77% had elevated d-dimers (>300 μg/L) and 28% had levels above 800 μg/L. Besides FIIa, FVIIa and FXa, we found TF-levels of 247.9$\pm$210.7 μg/mL (ELISA) in the effusion fluids of 60 tumour patients. These results are surprising as the effusions have been cell free filtered before examinations. Microscopical examination after centrifugation and quinacrine staining as well as immunoblots and co-immunoprecipitation revealed that

1. TF as well as PDI (protein disulfate isomerase) and TFPI (TF pathway inhibitor) are detectable in the effusion fluids. PDI acts as a regulatory protein for TF by allosteric inhibition and thereby switches TF from coagulatory to signalling activity.

2. PDI and TF are co-located (shown by co-immunoprecipitaion)
3. TF is located on microparticles
4. No truncated TF can be found
5. TF in malignant effusions is coagulatory active (shortening time)

We found a loose inverse correlation between tissue factor activity and PDI levels.

Conclusions: These insight and the development of new, more specific coagulation inhibitors such as FXa-inhibitors will help to treat hypercoagulabilitiy with all negative consequences for cancer patients.

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1127 POSTER

Expression of HER-2 and Its Relation With Pathological and Clinical Features in Differentiated Thyroid Cancers

W. Madah[1], R. Mzalbat[1], P. Gilbey[1], W. Basher[1], A. Sharabi[1], J. Zidan[1]. *[1]Ziv Medical Center, Oncology, Zefat, Israel*

Background: Human epidermal growth factor receptor2 (HER-2) is a well recognized prognostic and predictive factor in breast cancer. Its overexpression in other human cancers may have prognostic significance. The role of HER-2 in thyroid cancer is controversial. The aim of this study is to evaluate HER-2 expression in a large retrospective series of non-metastatic differentiated thyroid cancers (DTC) and to compare it with other clinical and pathological features of the patients.
Methods: We have studied 69 patients with DTC; 58 papillary and 11 follicular carcinomas. HER-2 was detected by immunohistochemistry (IHC) test on sections from formalin-fixed, paraffin-embedded tumour tissues. Dako test was used and results were scaled by Hercept test criteria. Tumours with HER-2 +2 were retested with chromogenic in situ hybridization (CISH) test. All clinical and pathological data was summarized from the hospital files of the patients.
Results: HER-2 overexpression was found in 4 (6.9%) of 58 patients with papillary carcinoma. There was no HER-2 overexpression in 11 cases of follicular carcinoma. No association of HER-2 expression was found with tumour size, pathological grade, age, gender and cervical lymph node metastases.
Conclusion: There were no HER-2 positive cases of follicular carcinoma. The incidence of HER-2 overexpression in papillary carcinoma is very low. HER-2 cannot be used routinely as a prognostic or predictive factor in DTC. The expression of other epidermal growth factor receptors in DTC merits further future studies.

1128 POSTER

Mutations in CHEK2 and TP53 Genes in High-Risk Hereditary Breast and Ovarian Cancer Patients in the Czech Republic

I. Tichá[1], J. Stribrna[1], J. Soukupova[1], P. Pohlreich[1]. *[1]Charles University in Prague First Faculty of Medicine, Institute of Biochemistry and Experimental Oncology, Prague, Czech Republic*

Background: Germ-line mutations in *BRCA1* and *BRCA2* genes account for only 40% of inherited breast or ovarian cancer cases. Thus, mutations in additional susceptibility genes also influence the risk of cancer. In this study we focused on the role of mutations in *CHEK2* and *TP53* genes in families at high risk of breast and ovarian cancer.
Material and Methods: Mutation analysis was performed in a series of 626 unrelated patients previously tested negative for *BRCA1/2* mutations. The complete coding region of *TP53* gene was analyzed by sequencing of cDNA; multiplex ligation-dependent probe amplification (MLPA) was used for the detection of the two most frequent Czech alterations in the *CHEK2* gene: c.1100delC and genomic deletion of 5395 bp comprising exons 8−9 that is probably of Slavic origin. All identified gene alterations were confirmed and characterized by direct DNA sequencing.
Results: In our cohort, 10 (1.6%) patients carried pathogenic mutations in *CHEK2* (5 carriers for each tested mutations) and 4 (0.6%) patients carried mutation in *TP53.* The two *TP53* mutations (c.818G>A and c.815T>G) have been repeatedly identified in sporadic breast tumours and seem to be pathogenic. The clinical importance of the third sequence variant (c.760A>G) which was found in two patients is not known. One of these mutations was detected in a woman with a familial breast cancer that also carried large deletion in the *CHEK2* gene. The 2 pathogenic *TP53* mutations were identified among hereditary cancer cases (2/296; 0.7%), whereas the majority of CHEK2 mutations was found in non-familial cancer cases (7/330, 2.1%).
Conclusions: Pathogenic mutations in *CHEK2* and *TP53* genes were much less frequent than mutations described in major predisposition genes *BRCA1/2*. However, our results indicate that testing for locally prevalent recurrent mutations in *CHEK2* gene may be of an important clinical relevance in our population. On the other hand, families with mutation in *TP53* gene were rare and the role of this gene in breast tumorigenesis is limited. Two pathogenic mutations were detected in cases of breast cancer prior to age 28 years. Analysis of *TP53* may be restricted to cases of early onset breast cancer.
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1129 POSTER

DNA Repair Enzyme, O6-methylguanine DNa Methyltransferase, Modulates Therapeutic Efficacy of Platinum Drugs With Radiation and Its Clinical Significance

S.H. Chen[1], Y.N. Yang[1], C.F. Li[2], H.Y. Huang[3], J.F. Liu[1], C.C. Kuo[1], J.Y. Chang[1]. *[1]National Health Research Institutes, National Institute of Cancer Research, Tainan City, [2]Chi-Mei Medical Center, Department of Pathology, Tainan City, [3]Chang Gung Memorial Hospital-Kaohsiung Medical Center, Department of Pathology, Kaohsiung City, Taiwan*

Background: In this study, we were aiming to evaluate the role of DNA repair enzyme, O^6-methylguanine–DNA methyltransferase (MGMT) in regulating the therapeutic efficacy of platinum drugs and radiation, and also investigate its clinical significance.
Materials and Methods: Tetracycline-regulated Tet-On system and RNA interference method were used to investigate the correlations between MGMT expression and platinums/radiation-induced DNA damage and cytotoxicity in cultured cells. Furthermore, 83 NPC patients received cisplatin (CDDP)-based concurrent chemoradiotherapy (CCRT) were analyzed the relationship of MGMT expression and survival.
Results: CHO-derived Tet-On-inducible cells (S12+) showed MGMT overexpression and statistically significant more resistance to CDDP, carboplatin and oxaliplatin than parental CHO cells. Knockdown of MGMT expression with small interfering RNA in HONE-1 cells conferred increased sensitivity to those platinum drugs as compared with scrambled control. Further study showed that the amount of CDDP-DNA adduct and double strand DNA breaks after CDDP exposure were significantly lower in MGMT-proficient cells than that of MGMT-deficient cells in both Tet-On and RNAi system. Host reactivation assay revealed that protection of CDDP-induced DNA damage and cell death by MGMT is through enhanced global DNA repair capacity. Otherwise, Resistance to X-ray irradiation was observed in MGMT-proficient cells, and *vice versa* in MGMT-deficient cells. The result from clinical specimens revealed that the NPC patients, who received CDDP-based CCRT, with lower level of MGMT expression had a better disease-free survival (DSS) ($P = 0.015$) and local recurrent-free survival (LRFS) ($p < 0.05$) than patients with high expression of MGMT. Multivariate analyses indicated that high expression of MGMT is an independent predictor for poor survival, with a risk ratio of 2.14 for DSS (95% CI=1.14–4.02), and 3.62 for LRFS (95% CI=1.33–9.88).
Conclusion: Our results suggested that MGMT plays an important role in determining the therapeutic efficacy of platinum drugs and radiation, and may have a relevance to clinical use of CCRT.

1130 POSTER

Spectrum of Mutations in BRCA1 and BRCA2 Genes in Families at High Risk of Breast and Ovarian Cancer in the Czech Republic

P. Pohlreich[1], J. Stribrna[1], I. Ticha[1], J. Soukupova[1], J. Kotlas[2], A. Panczak[2]. *[1]Charles University in Prague First Faculty of Medicine, Institute of Biochemistry and Exp. Oncology, Prague, [2]Charles University in Prague First Faculty of Medicine, Institute of Biology and Medical Genetics, Prague, Czech Republic*

Introduction: *BRCA1* and *BRCA2* are major genes related to hereditary breast and ovarian cancer. The purpose of our study was to estimate the incidence and spectrum of inherited mutations in these genes in a large series of Czech patients.
Materials and Methods: We evaluated DNA and RNA samples from 820 high-risk breast or ovarian cancer patients for germline mutations in *BRCA1* and *BRCA2* genes. A complete sequence analysis of *BRCA1* and *BRCA2* coding sequence was performed by protein truncation test (PTT) and direct DNA sequencing of PCR products. A total of 640 patients tested negative for point mutations and small deletions or insertions were screened for large genomic deletions and rearrangements (LGRs) at *BRCA1/2* loci by multiplex ligation-dependent probe amplification (MLPA), long range PCR and genomic sequencing. The chromosome 17-specific aCGH was used to locate deletion breakpoints in regions flanking the *BRCA1* gene.
Results: Of the 820 analyzed individuals, PTT and sequencing identified 132 (16.1%) and 48 (5.8%) mutations in *BRCA1* and *BRCA2* genes,