**1123** POSTER

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

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**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.

**1124** POSTER

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

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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-$\alpha$ (tumour necrosis factor-alpha) and IL-1$\beta$ (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 (NF$\kappa$B), 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-$\alpha$, 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 $\beta$-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**

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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 $\gamma$H2AX and 53BP1, as well as growth arrest, as assessed by p21 and apoptosis by $\gamma$H2 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**

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**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 $\mu$g/L) and 28% had levels above 800 $\mu$g/L. Besides FIIa, FVIIa and FXa, we found TF-levels of 247.9$\pm$210.7 $\mu$g/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.