for early breast cancer includes a wide local excision with adjuvant radiotherapy. Clinical data suggest, that perturbations induced by surgery and the subsequent wound fluids, which are rich in cytokines and growth factors, may stimulate residual disease. Numerous studies demonstrate, that 90% of the local recurrence after surgery occur in the same quadrant as the primary cancer. It has been proposed, that cancer cells displaying the stem-like phenotype play a critical role in local recurrence, invasion and metastasis. One of the new possibilities in conservative cancer treatment is intraoperative radiotherapy (IORT). IORT delivers high dose of radiation as one single fraction at the time of surgery. It was previously reported, that IORT alters the microenvironment through the modulation of wound healing response. Thus we wondered, whether wound fluids can induce the enrichment of breast cancer stem cells phenotype in breast cancer cell lines and whether IORT plays inhibitory role in this process.

Material and Methods: Wound fluids form patients which underwent IORT (IR-WF), as well as control group without radiotherapy treatment (WF), were collected week after the surgery. Three human cancer cell lines with different molecular status (basal - MDA-MB-468, luminal - MCF7 and Her2-positive - BT-474) were then incubated with wound fluids (WF, IR-WF) in complete culture medium (10%). After four days of incubation the cancer stem-cell phenotype was established.

Results: Flow cytometry and RT-qPCR analysis revealed, that wound fluids from patients who received IORT decreased the phenotype of cancer-stem cells in the basal (MDA-MB-468) and luminal subtype (MCF7) compared to fluids harvested after surgery alone. This work was supported by NSC grant no UMO-2013/09/N/N24/02844

Electronic Poster: Radiobiology track: Normal tissue effects: pathogenesis and treatment

pinkmanidazole can be used to detect cycling hypoxia in tumours?
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Purpose or Objective: To determine the influence of two different injection schedules on the pimonidazole hypoxic fraction (pHF) in three different head and neck human squamous cell carcinoma (HNSCC) xenograft tumour models.

Material and Methods: Three different HNSCC cell lines (FaDu, UT-SCC-5, UT-SCC-14) grown as xenograft tumours in nude mice (5 per cell line) where examined with different pimonidazole injection schedules. Either one single injection 60 minutes prior to tumour excision (100 mg/kg BW i.p.) or three injections (each 33 mg/kg BW i.p.) starting 180 minutes before tumour excision with 60 minutes interval between injections. Both groups where given the perfusion marker Hoechst 33342 i.v. 1 minute prior to tumour excision. Tumours were snap frozen and consecutive central cross-sections (10µm) where stained with antibodies for pimonidazole and CD31. Using image analysis the pHF and other parameters of the microenvironment were determined.

Results: No statistically significant differences in pHF nor in visual staining patterns were observed after single versus multiple injections of pimonidazole (table and figure 1).

Table 1: Mean values of the pHF [SD] in %.

<table>
<thead>
<tr>
<th>Tumour model</th>
<th>pHF single injection</th>
<th>pHF multiple injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaDu</td>
<td>9.17 [2.32]</td>
<td>14.28 [2.73]</td>
</tr>
</tbody>
</table>

Conclusion: In the HNSCC xenograft models investigated here pimonidazole detects predominantly chronic hypoxia. Assessment of cycling hypoxia requires alternative methods. Our data suggest that cycling hypoxia occurs either at a low level in our models or that hypoxia cycles so rapid that pimonidazole cannot bind sufficiently or cycling hypoxia levels are not low enough for pimonidazole reduction.

Purpose or Objective: Radiotherapy toxicity is related to oxidative stress-mediated endothelial dysfunction. Here, we investigated on radioprotective properties of Vitamin D (Vit.D) on human endothelial cells (HUEVC).

Material and Methods: HUEVC, pre-treated with Vit.D, were exposed to ionizing radiation (IR): ROS production, cellular viability, apoptosis, senescence and western blot for protein detection were performed. The role of MAPKs pathway was investigated by using U0126 (10 µM) MEKs/ERKs-, SB203580 (2.5 µM) p38-inhibitor or by over/expressing MKK6 p38 upstream activator.

Results: Vit.D reduced IR-induced ROS production protecting proliferating and quiescent HUEVC from cellular apoptosis or senescence, respectively, by regulating MAPKs pathways. In proliferating HUEVC, Vit.D prevented IR-induced apoptosis by activating ERKs while in quiescent HUEVC counteracted IR-induced senescence by inhibiting the p38-IR-induced activation. MEK6/ERKs inhibition in proliferating or MKK6/mediated p38 activation in quiescent HUEVC,