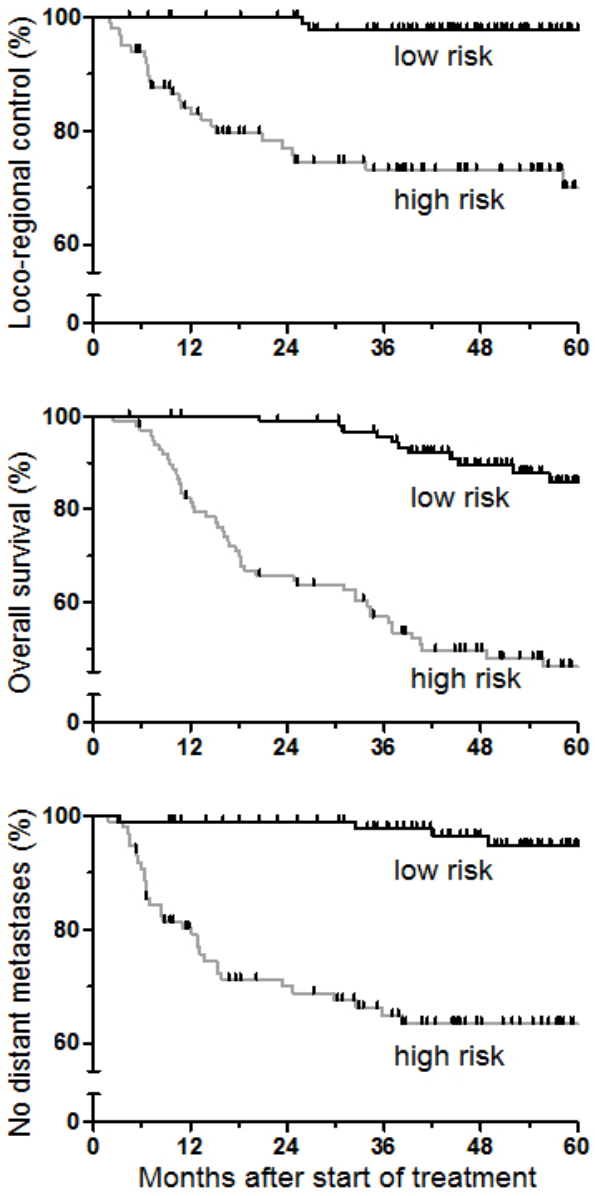

Conclusion: We determined gene signatures for the prediction of LRC, OS and FDM in a cohort of 196 HNSCC patients after postoperative radiochemotherapy. The signatures showed a good prognostic value and were validated by internal cross validation. After validation with an external dataset and in a currently ongoing multicentre prospective trial within the study group, the gene signatures may help to further stratify patients for individualised treatment de-escalation or intensification strategies.

Symposium: The tumour in 3D: the role of tumour microenvironment

SP-0583
Relevance of 3D cultures to address radiation response and novel RT combination strategies
N. Cordes[1]
[1]*OncoRay - Center for Radiation Research in Oncology, Dresden, Germany*

Novel 3D cell culture models enable cell growth in a more physiological environment than conventional 2D cell cultures. Most importantly, cells need to be embedded in a composition of extracellular matrix proteins similarly present in situ to guarantee conservation of the phenotype. As shown by comparative analyses between 2D, 3D and tumor xenografts, various processes such as signal transduction and DNA repair share great similarity in 3D and in-vivo but not 2D. Based on our long-standing experience, a large variety of endpoints can be determined and many methods can be conducted in 3D matrix-based cell cultures. While this is sometimes not as easy as in 2D and also requires a bit more financial invest, the generated data reflect cell behavior in-vivo and thus have a higher clinically relevance. Further, we are able to address specific tumor features in detail. For example, malignant tumors show great genetic/epigenetic and morphological/cell biological heterogeneity. Here, a prime example is the stiffness of a tumor. Although we know that the stiffness greatly varies in different parts of the tumor, the underlying mechanisms and prosurvival consequences on the genetic/epigenetic and morphological/cell biological level are far from being understood. 3D matrix-based cell cultures models can elegantly support our efforts to gain more knowledge in this field. Another important point is the sparing of animal experiments based on our broad knowledge that human (patho)physiology is significantly different from mice (or other species). Many decades of in-vivo research have demonstrated that only a negligible proportion of therapeutic approaches could be translated from rodents to humans. In conclusion, 3D cell cultures are powerful tools to generate more clinically relevant information. A broader implementation of this methodology is likely to underscore our efforts to better understand tumor and normal cell radiation responses and foster identification of most critical cancer targets.

SP-0584
The potential of normal tissue organoid cultures
R.P. Coppes[1,2]
[1]*University Medical Center Groningen, Department of Cell Biology, Groningen, The Netherlands*
[2]*University Medical Center Groningen, Department of Radiation Oncology, Groningen, The Netherlands*

The response of normal tissues to irradiation is mainly determined by the survival and regenerative potential of the tissue stem cells, and modulated by inflammatory processes, vasculature damage and altered neuronal innervation and fibrosis. Interestingly, transplantation of tissue specific stem cells has been shown to restores tissue homeostasis and prevent late radiation effects. Moreover, the sparing of localized stem cells was predicted to preserve salivary gland function in patients treated for head and neck cancer. Interestingly, mounting evidence indicates that cancer stem cells might contribute to the poor prospects. Recently, we and others have developed methods to culture patient specific organ and tumour stem cell containing organoids (tissue resembling structures). These organoids contain all the tissue/tumor lineages and the tissue/tumor stem cells, as indicated by their secondary organoids self-renewal potential and regeneration/regrowth potential and offer the opportunity to investigate tissue and patient specific assessment of the response of stem cells to (chemo-) radiotherapy. Stem cell survival curves and DNA DSB repair kinetics indicate that the response of organoids to different radiation qualities may differ from tissue to tissue, especially in the low dose regions typically delivered to the normal tissue outside the planning target volume. Therefore, organoids cultures could be used to investigate the mechanism of differences in response of normal and tumour stem cells to irradiation and exploit these for personalized optimisation of (chemo-) radiation treatment and prediction of treatment response.

SP-0585
The impact of a novel 3D cell culture model of glioblastoma on radiation and drug-radiation responses
N. Gomez-Roman[1], A. Chalmers[2]
[1]*Inst. of Cancer Sciences-Univ. Glasgow The Beatson West of Scotland Cancer Cente, Glasgow, United Kingdom*
[2]*Inst. of Cancer Sciences-Univ. Glasgow The Beatson West of Scotland Cancer Cente, Wolfson Wohl Translational Cancer Research Centre, Glasgow, United Kingdom*

Glioblastoma (GBM) is the most common primary brain tumour with dismal prognosis. Tumours exhibit inherent resistance to radiation and chemotherapy which has been attributed to a subpopulation of cancer cells termed 'GBM stem-like cells' (GSC) characterised by multipotentiality and potent tumorigenic capacity. The use of established cancer cell lines in simplified two-dimensional (2D) *in vitro* cultures might explain the observed discrepancy between pre-clinical and clinical responses to cytotoxic treatments. We developed a customised, 3D GSC culture system using a polystyrene scaffold (Alvetex®) that recapitulates key histological features of GBM including high cellularity and sparse extracellular matrix (ECM) and compared it to conventional 2D GSC cultures. 2D and 3D cultures of three different primary GSC lines exhibited similar radiation sensitivities as measured by clonogenic survival. Previous studies have demonstrated radiopotentiating efficacy of the epidermal growth factor receptor (EGFR) inhibitor erlotinib against GBM cell lines in 2D cultures; however it failed in GBM clinical trials. Thus we evaluated the radiation modifying effects of erlotinib on 2D and 3D GSC cultures. Erlotinib enhanced radiosensitivity of 2D GSC cultures but had no effect on radiation responses of 3D GSC or in neurosphere formation assays, where cells grow in 3D conditions devoid of a scaffold or extrinsic ECM. We next examined VEGF inhibition, since anti-VEGF therapy in combination with standard radio-chemotherapy increases progression-free survival of GBM patients. VEGF deprivation was associated with significant radiosensitisation of 3D GSC cultures but had no effect on 2D GSC. Erlotinib treatment of VEGF-deprived 3D cultures increased radiation resistance of 3D cells to the same extent as VEGF addition, indicating epistasis. EGFR has been shown to regulate repair of radiation-induced double-strand breaks by activating the non-homologous end-joining (NHEJ) repair protein DNA-PKcs. A correlation between radiosensitivity, increased gH2AX foci and phospho-DNA-PK nuclear foci after radiation treatment was observed. In contrast, increased numbers of foci of the homologous recombination (HR) repair protein Rad51 were observed in radioresistant populations. Our results show that in the 3D model, VEGF signalling is required for optimal NHEJ activation with fast kinetics. This effect allows access to HR repair proteins at the remaining unrepaired DSBs at later time points, facilitating their repair and conferring radiation protection. Detailed analysis of the signalling pathways involved in the radiation resistance conferred by VEGF and EGFR signalling in the 3D and 2D models respectively demonstrated a radioprotective role of the downstream signaling molecule Akt. Specific inhibition of Akt using the small molecule inhibitor MK-2206 increased radiation sensitivity to the same extent as VEGF deprivation in 3D cells or erlotinib treatment in 2D cells, and no additivity was observed when these agents were combined. Our results for erlotinib treatment and VEGF deprivation in the 3D model recapitulate data from clinical trials, and suggest novel therapeutic targets for GBM. The 3D-specific effects of this panel of molecularly targeted agents strongly support the clinical relevance of this 3D model and its potential value in preclinical studies.

SP-0586

Radiotherapy supports tumour-specific immunity

M. Van den Broek[1]

[1]*University of Zürich, Institute of Experimental Immunology, Zurich, Switzerland*

Tumour-specific immunity occurs in cancer patients but has insufficient potential to control or eliminate the tumour. Strengthening this response through immunotherapy may lead to a durable, systemic response that may also control (development of)metastases.

Radiotherapy - a standard treatment for cancer - acts through induction of DNA damage in cancer cells. Although this treatment was thought to e immuno suppressive for a long time, recent data show that radiotherapy can support tumour-specific immunity. In fact, there is accumulating evidence that immune stimulation is an integral part of this therapy.

Using preclinical cancer models we showed that the efficacy of radiotherapy crucially depends on $CD8^+$ T cells and dendritic cells. Radiotherapy induces activation of tumour-associated dendritic cells and accumulation of $CD8^+$ T cells with protective effect or function within the tumour (1).

These results prompted us to investigate whether similar changes occur in cancer patients and we compared the immune signature in paired biopsies that were obtained from sarcoma patients before and after radiotherapy. Most patients showed a significant upregulation of molecules and cell types associated with protective immunity and a concomitant downregulation of such characteristic for immune regulation/suppression. Importantly, those patients with the strongest changes towards protective immunity survived longer after radiotherapy (2, 3).

Because it is largely unknown how radiotherapy supports tumour-specific immunity, we performed a semi-unbiased transcript analysis to identify pathways that change significantly upon radiotherapy. We found that radiotherapy induces transient and local activation of the classical and alternative pathway of complement in murine and human tumours, which results in local production of the anaphylatoxins C3a and C5a. Complement activation and subsequent production of anaphylatoxins happens downstream of radiotherapy-induced necrosis. The local production of C3a andC5a is crucial to clinical efficacy of radiotherapy and concomitant stimulation of tumour-specific immunity (4).

Radiotherapy influences a plethora of pathways, which we are currently identifying, because we think that selectively promoting or inhibiting particular pathways in the context of radiotherapy may further promote tumour-specific immunity and increase the therapeutic efficacy.Because chronic inflammation is immunosuppressive whereas acute inflation supports immunity, we are comparing chronic radiotherapy (low-dose given in multiple fractions during weeks) with radiotherapy that includes radiation holidays (limited fractions of high-dose given with substantial breaks) with respect to efficacy and immune stimulation.

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Symposium: WBRT for brain metastases- the end of an era?

SP-0587

Whole brain radiotherapy for brain metastases - the end of an era?

P. Mulvenna[1]

[1]*Freeman Hospital, Northern Centre for Cancer Care, Newcastle-upon-Tyne, United Kingdom*

Summary: Whole Brain Radiotherapy (WBRT) may be administered with either prophylactic or palliative intent. I will discuss both these approaches and how they fit into our management of metastatic brain disease in the 21st century.

Background: The use of Whole Brain Radiotherapy (WBRT) emerged as standard management for patients with brain metastases during the latter half of the 20th century (1,2,3).