was observed after preoperative RT. Specific assessment of lymphocytes in the scar area for patients in pCR and additional analyses of lymphocytes subtypes together with PD1 and FOXP3 Are planned and could be an innovative way to use lymphocytes dynamics after RT to guide the use of RT and immunotherapy combination in the future.

OC-0154 Radiation abrogates fibroblast-mediated immunosuppressive effects on dendritic cells

T. Hellevik[1], R. Berzaghi[2], M.A. Akhtar[2], S. Tornaas[2], I. Martinez-Zubiaurre[2]
[1]Univeristy Hospital of Northern Norway, Department of Radiation Oncology, Tromsø, Norway; [2]University of Tromsø, Clinical Medicine, Tromsø, Norway

Purpose or Objective

Recent reviews have highlighted the impact of cancer-associated fibroblasts (CAFs) in tumor development, dissemination and treatment resistance. Reactive CAFs - in contrast to normal fibroblasts - secrete a myriad of immunomodulatory soluble factors at high levels, which directly influence tumor immunity and inflammation, and promote an immunosuppressive tumor microenvironment. However, little is known about the mechanisms by which CAFs modulate immune responses after radiotherapy (RT). Previous studies by our group have uncovered the phenotypical and functional changes provoked by (high-dose) RT on human lung CAFs, characterized by induction of premature cellular senescence, as well as reduced proliferation, migration, and invasion rates. Changes have also been observed on the secretory profile by CAFs. The conditioned medium (CM) from irradiated-CAFs reduces the migratory capacity of endothelial cells, inhibits angiogenesis, and exerts a powerful immunosuppressive effect over activated T-cells. However, neither low-dose nor high-dose RT is affecting baseline CAF-mediated immunosuppressive effects. The present study focuses on effects of ionizing radiation, applied in different regimens, on CAF-mediated immunoregulation of macrophage polarization and dendritic cell phenotype and functions.

Material and Methods

CAFs were isolated by the outgrowth method from freshly resected NSCLC tumor specimens and characterized by the presence of lineage-specific markers. Highly pure CAF cultures (at passage 3) were irradiated with high-energy photons delivered as a single-high dose of 18 Gy or three consecutive daily doses of 6 Gy (3x6 Gy). CAF-CM were collected from (sham) irradiated cultures to examine CAF-induced paracrine effects on macrophage phenotype and dendritic cell differentiation, activation and function.

Results

We show that CAF-CM from non-irradiated cultures induces A) M2-polarization of monocyte derived-macrophages, promoting elevated surface expression of markers CD206 and CD163; and B) a tolerogenic phenotype of monocyte-derived DCs with decreased expression of differentiation surface markers (CD14, CD1a, CD209), decreased expression of maturation surface markers (CD80, CD86, CD40 and HLA-DR) and decreased functional capacities (migration, antigen-uptake, and T-CD4 cell priming). Interestingly, CM from CAFs irradiated with a single-high dose, or more prominently, with a fractionated medium-high dose (3x6 Gy) *reverts* the CAF-mediated tolerogenic induction on DCs. No significant differences in M2- or M1-macrophage polarization were observed between irradiated and non-irradiated CAFs.

Conclusion

Our results unveil advantageous effects elicited by defined radiotherapy regimens by reversing the baseline CAF-mediated immunosuppressive actions on DC phenotypes and functions.

OC-0155 Lxr Signaling Regulates Macrophage Survival and Phenotype Polarization Response To Ionizing Radiation

P.C. Lara Jimenez[1], C. Tabraue[2], M. Mirecki[3], J.V. De La Rosa[4], F. López-Blanco[5], L. Bosca[6], L. Fernandez[5], A. Castrillo[7]
[1]Hospital Universitario San Roque/Universidad Fernando Pessoa Canarias, Oncology, Las Palmas de Gran Canaria-Ca, Spain; [2]Unidad Consejo Superior de Investigaciones Cientificas, Grupo de investigación en Medio Ambiente y Salud, Las Palmas, Spain ; [3]Universidad de Las Palmas de Gran Canaria, Ciencias Clínicas, Las Palmas de Gran Canaria, Spain; [4]Unidad del Consejo Superior de Investigaciones Científicas, Grupo de Estudio en Salud y Medio Ambiente, Las Palmas de Gran Canaria, Spain; [5]Universidad de Las Palmas de Gran Canaria, Ciencias Clinicas, Las Palmas de Gran Canaria, Spain ; [6]Unidad de Consejo Superior de Investigaciones Científicas, Grupo de Investigación en Medio ambiente y salud, Las Palmas de Gran Canaria, Spain; [7]Unidad del Consejo Superior de Investigaciones Científicas, Grupo de Investigación en Medioambiente y Salud, Las Palmas de Gran Canaria, Spain

Purpose or Objective

Macrophages are a major cellular component of murine and human tumors, where they are commonly termed tumor-associated macrophages (TAMs). Recent studies indicate that ionizing radiation not only polarizes macrophages towards a M1-like phenotype leading an effective antitumor response, but also decreases macrophage survival through a pyroptosis mechanism.

The purpose of this study was to evaluate the role of LXR in the response of macrophages to irradiation, analyzing both cell viability and functional activity of the macrophage, as a possiblestrategy focused to deplete TAMs or re-program them towards a M1-like phenotype, in the context of cancer treatment with radiotherapy.

Material and Methods

Primary and immortalized murine bone marrow macrophages (BMDMs) from wild type or LXR double knock-out mice were exposed to X-ray irradiation. Subsequently, analysis of LXR signaling on cell proliferation and cytotoxicity induced by ionizing radiation was determined by time-lapse photomicroscopy. Genotoxic cell damage was evaluated by western blot of - H2AX and p53. Pyroptosis was analyzed through cell viability assay, lactate dehydrogenase (LDH) release assay and western-blot of caspase-1 active protein. We analyzed the expression of classic polarization markers, such as inducible nitric oxide synthase (iNOS) and interleukin-6 (IL-6) by real time RT-QPCR of messenger RNA levels of these M1 markers.

Results

Genetic and pharmacological inactivation of LXR induced radio sensitivity of macrophages.(figure 2) LXR deficiency decreased cell proliferation and enhanced cytotoxicity induced by ionizing radiation, in both immortalized and primary BMDMs. Protein levels of γ- H2AX and p53, both involved in response to cell damage, were exacerbated in LXR-deficient macrophages exposed to irradiation. Cell membrane damage augmented and cell viability decreased in LXR deficient macrophages compared to LXR-WT macrophages in response to irradiation. In addition, LXR deficiency enhanced both caspase-1 activation and LDH release in BMDM(figure 4) exposed inflammasome activators. Expression of pro-inflammatory markers IL-6 and iNOS was markedly higher in irradiated LXR-deficient macrophages than in LXR-WT macrophages(figure 4). This results show the role of LXR suppression favoring M1-polarization and therefore tumor control.

Fig.2

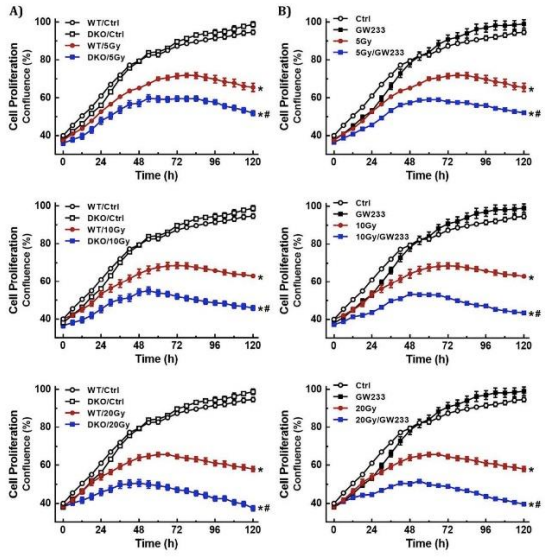
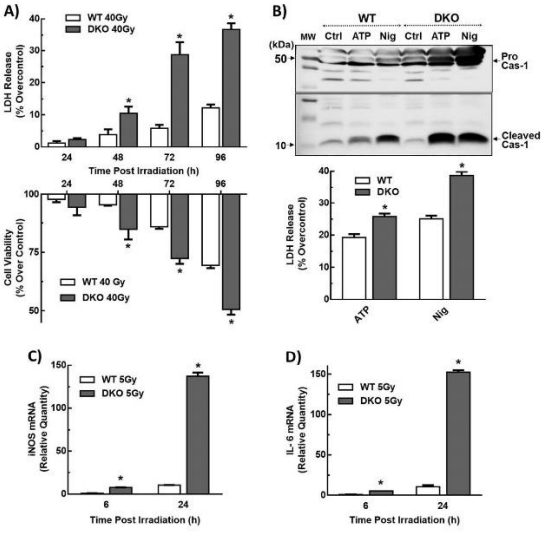


Fig.4



Conclusion

The present work identifies LXR transcription factors as potential therapeutic targets to enhance the suppressive effects of radiotherapy on tumor growth, through induction of macrophage cell death and polarization towards a pro-inflammatory phenotype

OC-0156 High-intensity focused ultrasound and radiotherapy: a promising combination?

G. Adema[1], R. Van den Bijgaart[1], T. Raaijmakers[2], M. Den Brok[1], M. Hoogenboom[3], J. Fütterer[4], J. Bussink[1]

[1]UMC St Radboud Nijmegen, Radiation Oncology, Nijmegen, The Netherlands; [2]UMC St Radboud Nijmegen, Radiation Oncology and Anesthesiology- Pain Management and Palliative Care, Nijmegen, The Netherlands ; [3]UMC St Radboud Nijmegen, Radiology and Nuclear Medicine, Nijmegen, The Netherlands ; [4]UMC St Radboud Nijmegen and University of Twente, Radiology and Nuclear Medicine and MIRA Institute for Biomedical Technology and Technical Medicine, Nijmegen, The Netherlands

Purpose or Objective

In interventional oncology, a large variety of *in situ* tumor destruction techniques like cryo or thermal ablation, radiotherapy (RT) or high-intensity focused ultrasound (HIFU) are successfully applied. Although diverse in technology and the way of inducing cell death, ablative techniques share one key feature: they create *in situ* availability of ablated tumor material. During the efforts of the body to clear this dead material there is a time frame in which the immune system is actively controlling immune responses directed towards antigens from this antigen 'depot' [1]. The ability to stimulate the immune system upon scavenging antigens from dead tumor cells has led to the concept that *in situ* tumor destruction can be used to achieve systemic '*in vivo* vaccination' against tumors, ultimately leading to the elusive 'abscopal effect' [2,3]. The aim of this study is to understand which (combination of) techniques results in the most effective release of tumor antigens, creates the most immunostimulatory environment from a molecular perspective, or combines most effectively with optimally timed immune stimulating therapies.

Material and Methods

We have established multiple different ablation technologies in murine tumor models, including a MR-guided HIFU setup that can be used for thermal and mechanical destruction of murine tumors, including a melanoma (B16F10), a thymoma (EL4) and neuroblastoma (9464D) model [4]. Using these models we have explored the impact of multiple ablation treatments with immunotherapy, including the administration of different immune adjuvants. As read outs we have performed immune activation parameters (DC maturation, cytokine production), analysis of immune responses (T cell activation, proliferation and cytotoxicity) as well as tumor growth experiments.

Results

Our data demonstrate that a tumor can serve as its own antigenic vaccine after different types of ablation, provided that additional immune activating signals are concomitantly given [5-7]. We have further established a HIFU based protocol to induce mechanical rather than thermal ablation of tumors. This mechanical HIFU based ablation of tissues induces complete subcellular fragmentation rather than tissue coagulative necrosis. RT is the main treatment modality for cancer, and around 50% of all cancer patients receive RT. Currently we are investigating the combination of HIFU and RT ablation with immunotherapy.

Conclusion

Both HIFU and RT are non-invasive ablation techniques and can have immune modulatory effects [8,9]. Despite the obviously changing immunological parameters, their ablation-induced immunomodulation alone appears insufficient to generate consistent protective antitumor immunity. Combining HIFU with RT and immunotherapy may be more effective than either therapy alone, and is expected to be key to achieve systemic, long-lasting, antitumor immunity.

OC-0157 Radiation and immunotherapy to fight cancer: a 'pushing the gas and releasing the brakes' approach.

V. Olivo Pimentel[1], D. Marcus[1], A. Van der Wiel[1], R. Biemans[1], N.G. Lieuwes[1], D. Neri[2], J. Theys[1], A. Yaromina[1], L.J. Dubois[1], P. Lambin[1]

[1]Department of Precision Medicine- The M-Lab- GROW - School for Oncology and Developmental Biology, Maastricht Comprehensive Cancer Centre- Maastricht