Irradiation triggers molecular and transcriptional shifts in tumor endothelial cells, supporting their activation and enhancing immune response

Iva Santek[1,2], Tim Bozic[1], Gregor Sersa[1,3], Bostjan Markelc[1]

[1]Department of Experimental Oncology, Institute of Oncology Ljubljana, Zaloska cesta 2, SI-1000 Ljubljana, Slovenia
[2]Faculty of Medicine, University of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia
[3]Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, SI-1000 Ljubljana, Slovenia

*isantek@onko-i.si

Background

Abnormal tumor vasculature is marked by inadequate blood flow and oxygen delivery, causing the formation of hypoxic areas, resistant to radiotherapy (RT). Irradiation (IR) affects not only cancer cells but also the tumor microenvironment, including tumor blood vessels. Intriguingly, besides triggering apoptosis of tumor endothelial cells (TECs), IR can also lead to vascular normalization/remodeling or TEC activation, potentially alleviating hypoxia and facilitating immune cell infiltration. However, the role of IR-induced alterations of tumor vasculature and TECs on the tumor response to RT remains poorly understood.

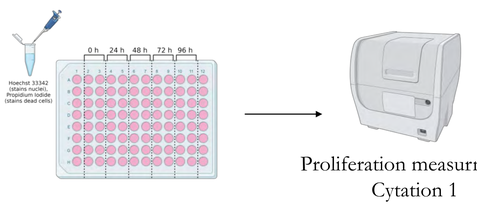
Aim

To clarify how vasculature responds to IR, focusing on its remodeling and TEC activation.

Methods

1) In vitro IR of ECs

0, 2, 4, 6, 8 and 10 Gy



Proliferation measurments Cytation 1

2) Transcriptome analysis of irradiated ECs

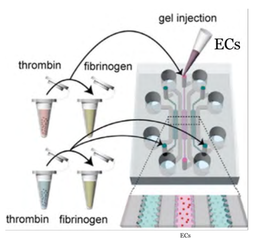
0, 2 and 5 Gy

RNA isolation 24 and 72 h after IR

RNA sequencing

RStudio analysis

3) Immunofluorescence of established vasculature-on-a-chip model



VCAM-1
VWF
CD31

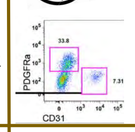
70 kDa Dextran

4) TEC transcriptomic analysis

C57BL/6 (MC38 colon carcinoma)

0 and 15 Gy

tumor isolation 48 h after 15 Gy IR

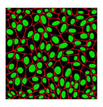


bulk RNA sequencing of TECs
dr Markelc, GSE168481

5) Immunofluorescence of fresh-frozen tumor sections

Balb/c (CT26 colon carcinoma)

0 or 5x5 Gy

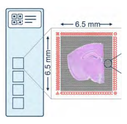


immunofluorescence of fresh-frozen control and IR samples

6) Spatial transcriptomic analysis of irradiated murine tumors

Balb/c (CT26 colon carcinoma)

FFPE tumor sections



Control (no IR) vs 48 h and 7 days after 5x5 Gy IR

Conclusions

Irradiation:

1) reduces EC proliferation and survival
2) alters TEC gene expression and usage of pathways, supporting TEC activation and augmented anti-tumor immune response

Results

1) In vitro IR of ECs

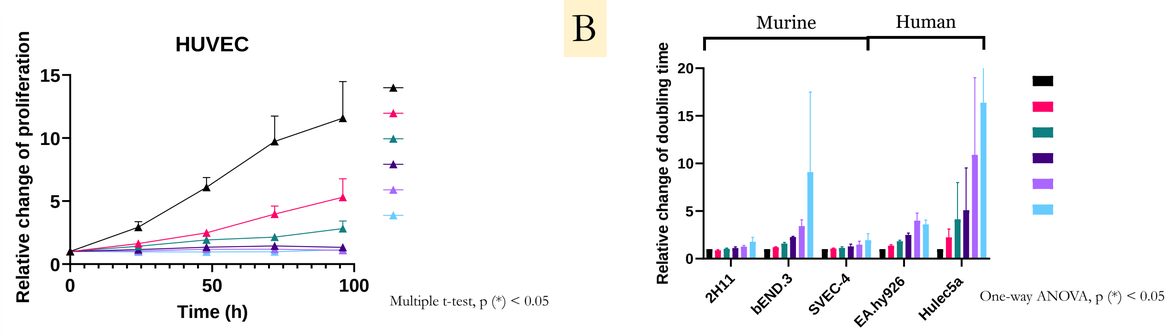


Fig 1: ***In vitro*** **IR of murine and human ECs with IR doses from 0 to 10 Gy: (A)** Relative change of cell proliferation of human endothelial cell line HUVEC. **(B)** Relative change of doubling time after IR.

2) Transcriptome analysis of irradiated ECs in vitro

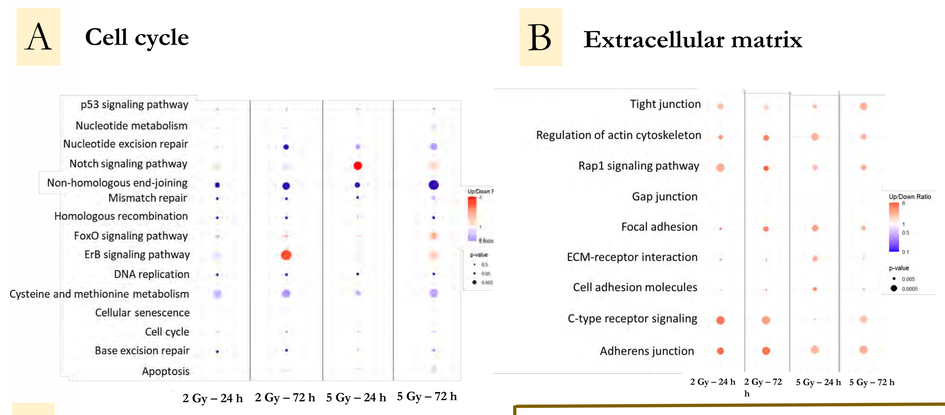


Fig 2: ***RNA sequencing analysis of HUVEC after IR.*** RNA was collected 24 or 72 h after IR with 2 or 5 Gy. Transcriptome of irradiated samples was compared to non-irradiated control samples. KEGG enriched pathways related to **(A)** Cell cycle, **(B)** Extracellular matrix, **(C)** Immune response. n = 5 per group.

3) Immunofluorescence of established vasculature-on-a-chip

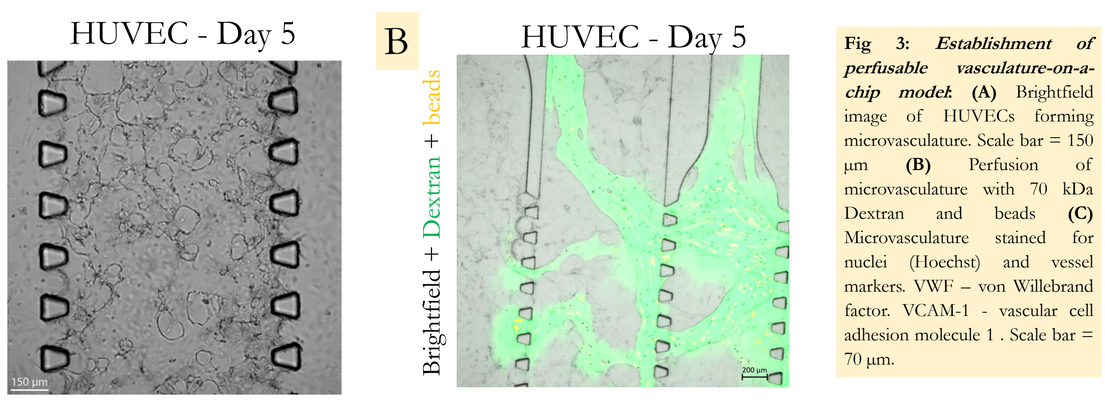


Fig 3: ***Establishment of perfusable vasculature-on-a-chip model*****: (A)** Brightfield image of HUVECs forming microvasculature. Scale bar = 150 µm **(B)** Perfusion of microvasculature with 70 kDa Dextran and beads **(C)** Microvasculature stained for nuclei (Hoechst) and vessel markers. VWF – von Willebrand factor. VCAM-1 - vascular cell adhesion molecule 1 . Scale bar = 70 µm.

4) TEC transcriptomic analysis

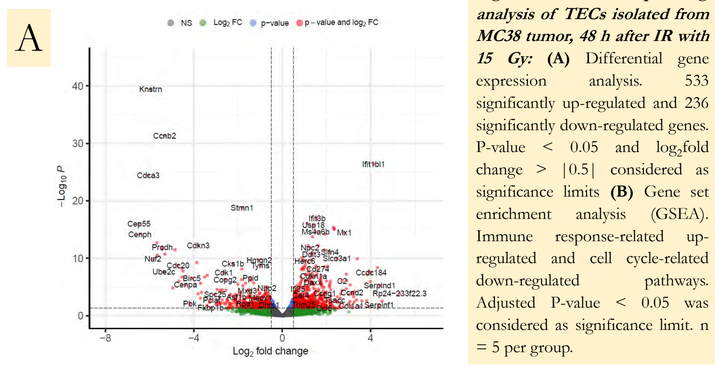


Fig 4: Bulk ***RNA sequencing analysis of TECs isolated from MC38 tumor, 48 h after with 15 Gy:*** **(A)** Differential gene expression analysis. 533 significantly up-regulated and 236 significantly down-regulated genes. P-value < 0.05 and $\log_2$fold change > |0.5| considered as significance limits **(B)** Gene set enrichment analysis (GSEA). Immune response-related up-regulated and cell cycle-related down-regulated pathways. Adjusted P-value < 0.05 was considered as significance limit. n = 5 per group.

5) Immunofluorescence of fresh-frozen irradiated tumors

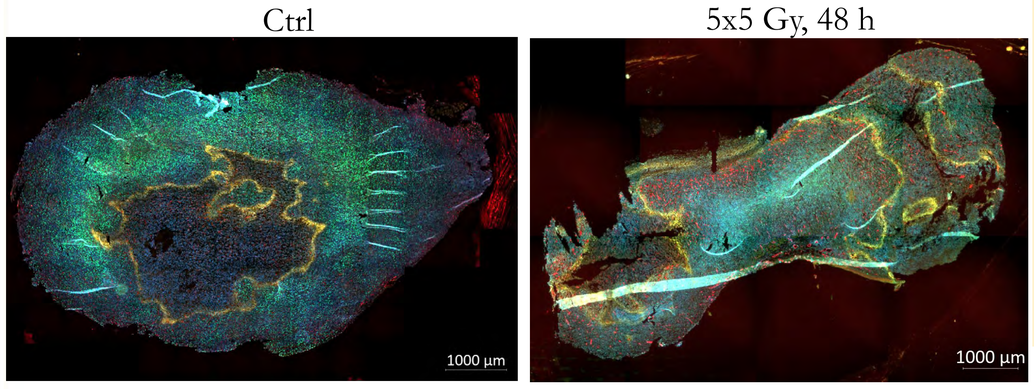


Fig 5: Tumor sections stained for immune-response and TECs activation markers. Frozen tumor sections from control CT26 tumors and CT26 tumors 48 h after 5x5 Gy IR. CD31 - platelet endothelial cell adhesion molecule 1 (PECAM-1), EdU - a thymidine analogue used as proliferation marker, EF5 – a nitroimidazole derivative used as marker for hypoxia Scale bar = 1000 µm.

6) Spatial transcriptomic analysis of irradiated murine tumors

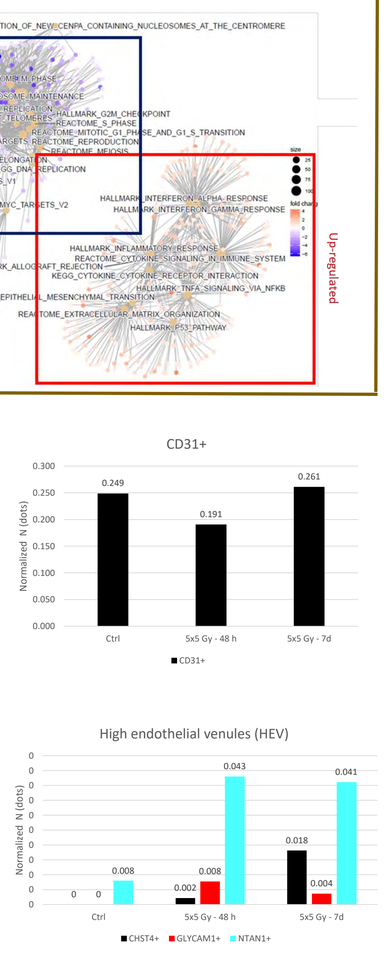


Fig 6: Spatial transcriptomic analysis of CT26 tumors 48 h or 7 d after 5 x 5 Gy IR. (A) Spatial expression of EC marker CD31 and HEV markers CHST4, GLYCAM1, and NTAN1. CD31- platelet endothelial cell adhesion molecule 1 (PECAM-1), CHST4 - carbohydrate sulfotransferase 4, GLYCAM1 - glycosylation-dependent cell adhesion molecule-1, NTAN1 - N-terminal asparagine amidase, CD8 – cluster of differentiation 8.