ENHANCING RADIOSENSITIVITY BY MODULATING ANGIOCRINE SIGNALING WITHIN THE TUMUOR MICROENVIRONMENT

Rebecca Drake, CoL Centre Symposium, 02/03/21

BACKGROUND

Radiotherapy (RT) has been a mainstay of cancer treatment since the early 1900s with 50% of all cancer patients now receiving RT as part of their treatment and contributing to 40% of cured cancers[1].

63% of breast cancer patients receive radiotherapy as part of their primary treatment [2] usually a 3-week course of whole breast external beam RT (40gy in 15 fractions) following surgery or as an adjuvant to chemotherapy or immunotherapy[3]. (additional boost to tumour bed in some)

Breast cancer remains the 2nd leading cause of cancer deaths in women. As cancer diagnosis rises, an estimated 16% increase in RT courses are expected by 2025 across Europe[4].

Development of resistance is the main limitation of RT (occurring as loco-regional relapse or distant metastasis), as well as normal tissue toxicity[5].

RT efficacy could be improved with a greater understanding of the basic **biological responses to radiation**, the **mechanisms of radioresistance** and research into **combination treatment**

Endothelial-cell FAK deletion sensitises cancer cells to radiotherapy *in vivo*

Focal Adhesion Kinase **(FAK)** is a non-receptor tyrosine kinase, which in endothelial cells (ECs), has been shown to regulate tumour cell responses to radiation, without affecting blood vessel function.

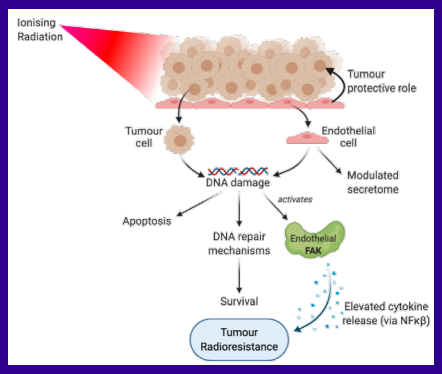


Figure 1 – Diagram presenting findings from publication: *Tavora, B; et al. Endothelial-cell FAK targeting sensitizes tumours to DNA-damaging therapy. Nature. 2014 Oct 2;514(7520):112-6.*

Endothelial cell-FAK deletion in mice (EC-FAK^{KO}) enhanced the radiosensitivity of **subcut tumours** and inhibited their growth compared to controls (EC-FAK^{WT})[6].

The effect of EC-FAK loss on tumour growth was only present upon radiation treatment but was associated with enhanced NF-κβ signaling and elevated cytokine release[6].

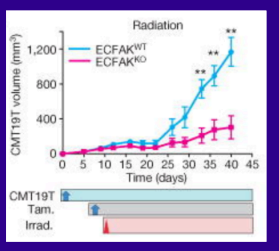
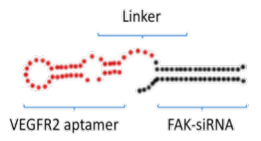


Figure 2 – Graph extracted from *Tavora et al. 2014*

RESEARCH AIMS

EXPERIMENTAL STRATEGIES

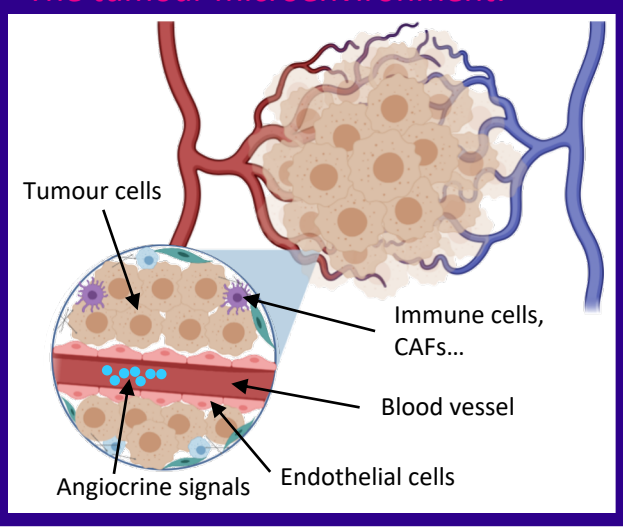
1. To investigate the effects of radiation on endothelial cells and characterise TME-derived angiocrine signatures associated with radioresistance/radiosensitivity	• Characterise **blood vessel phenotypes** and **endothelial DNA damage** with radiation in *Wt* orthotopic mouse models • Look at the histopathological responses to radiation involved in cancer progression • Analyse effects of irradiated endothelial/tumour cell conditioned medium transfer on each others response to radiation (also in 3D co-cultures) • Identify *in vivo* effects of radiation on endothelial proteomic signatures and endothelial gene expression
2. To unravel the molecular pathways involved in endothelial cell responses to radiation-induced DNA damage and FAK-regulated DNA repair	• Investigate the effect of **EC-FAK KO** on radiation response (orthotopic breast cancer mouse model) • Targeted analysis of endothelial secretome, directed by Aim 1 findings (MS & phosphoproteomics of radiated/non-irradiated and radioresistant/radiosensitive) • In vitro characterisation of endothelial cell responses to radiation-induced DNA damage and FAK-regulated DNA repair (using FAK inhibitors)
3. To develop novel endothelial-specific radiosensitising aptamers to improve radiotherapy efficacy	• Test FAK aptamers in vitro in endothelial/tumour cell co-cultures (also 3D spheroids), evaluate radiation responses • *In vivo* testing of endothelial-specific FAK aptamers (conjugated vegf-r2-siFAK RNA) • Validate molecular targets downstream of FAK, explore radiosensitising potential



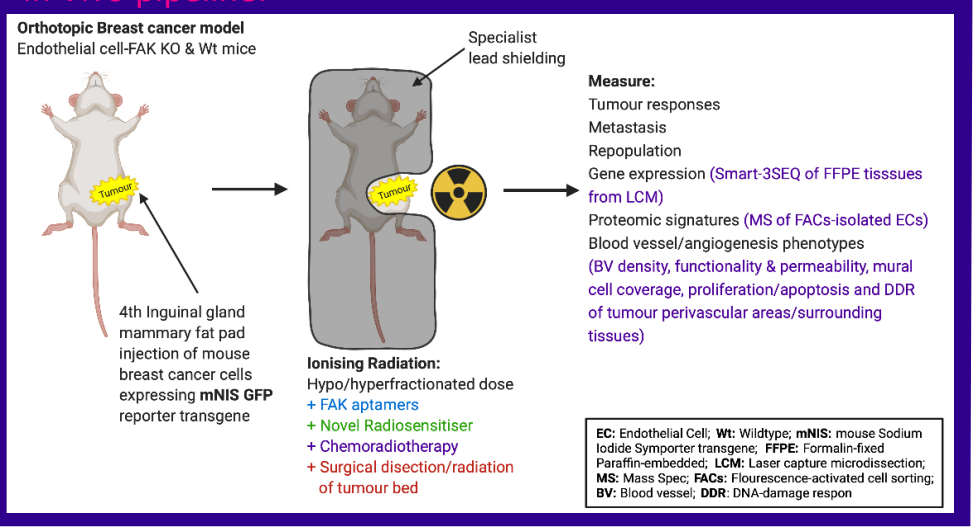
GRAPHIC SUMMARY

What are the effects of radiation on TME-derived endothelial cells and their angiocrine signals? → What are the molecular pathways regulating radiosensitivity? → How can we target these to enhance radiotherapy efficacy?

The tumour microenvironment:



In Vivo pipeline:



CLINICAL TRANSLATION

- Need to validate targets from mass-spec in a clinical context and incorporate human relevance to enable clinical translation of in vivo findings
- Have an established collaboration with Nottingham cohort (ER+ BC) which has already shown that high EC & tumour cell FAK & high BV density associated with poor outcomes to treatment (surgery, RT & 5yr tamoxifen)[7]
- Analyse histopathology/IMC of radiotherapy-treated tumour tissue samples (pre/post-treatment matched, radiosensitive/resistant)
- Characterisation of angiocrine signatures associated with radioresistance in a clinical setting
- Identify prognostic biomarkers to assist in clinical decision making and personalization of radiotherapy

References:

1. Public Health England (2017) Radiotherapy activity across England, National Cancer Registration and Analysis Service (NCRAS)
2. Office for National Statistics, Cancer survival by stage at diagnosis for England(link is external), 2019.
3. NHS ENGLAND (2016) Clinical Commissioning Policy: Radiotherapy after primary surgery for breast cancer
4. Borras JM, et al. (2016) How many new cancer patients in Europe will require radiotherapy by 2025? An ESTRO-HERO analysis. Radiother Oncol.
5. Wei W, Lewis MT. Identifying and targeting tumor-initiating cells in the treatment of breast cancer. Endocr Relat Cancer. 2015 Jun;22(3):R135-55
6. Tavora, B; et al. Endothelial-cell FAK targeting sensitizes tumours to DNA-damaging therapy. Nature. 2014 Oct 2;514(7520):112-6.
7. Roy-Luzarraga M, et al. (2020) Association of Low Tumor Endothelial Cell pY397-Focal Adhesion Kinase Expression With Survival in Patients With Neoadjuvant-Treated Locally Advanced Breast Cancer. JAMA Netw Open. 1;3(10)