High-frequency Ultrasound Detection of Tumor Vascular Hypoxia as a Targeting Modality for Focused Ultrasound Ablation to Complement Chemoradiation

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Background
Fundamental Radiation Biology: Hypoxia


Palcic et. al, Radiation Research 1984
Background
Fundamental Radiation Biology: Hypoxia

Horsman et. al, Nature Reviews 2012
Meta-analysis showing a forest plot of the relationship between hypoxia imaging and the outcome to radiation therapy

Initial approach to reduce tumor hypoxia using ultrasound ablation to complement radiotherapy: Three basic steps of PET/MRI-guided FUS hypoxic-tissue ablation.
PET images of 4T1 and SCK mammary carcinomas, respectively. Attempt to target ‘most hypoxic’ regions with FUS.
Registration of MRI T2-weighted and $^{18}$F-miso PET images of three transverse slices of a 4T1 tumor. The hypoxic areas characterized by contours of tumor/muscle (T/M) ratio >1.2. The water bolus used is shown in the MRI T2-weighted images but not in the PET images.
MRgFUS ablation of the hypoxic region in rear-limb tumor implant:

**Advantages:** reduced ablation times for large tumors, improved radiation outcomes

**Disadvantages:** Difficult co-registrations, tedious set-up and targeting—are we really getting what we want?

**IS THERE ANOTHER WAY TO FIND AND TARGET ‘IMPORTANT’ HYPOXIA?**
Evidence of Hypoxemic Tumor Vessels: hypoxia is not a ‘yes’ or ‘no’ phenomenon.

- Radiation Resistance
- Chemotherapy Resistance
- Imunosuppressive Microenvironment
- Niche for Cancer Stem Cells
Background
Evidence of Hypoxemic Hypoxia

Perivascular oxygen tensions

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<table>
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<tr>
<td>Normal tissue vessels</td>
<td>72±13 mmHg</td>
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<tr>
<td>Tumor peripheral vessels</td>
<td>26±5 mmHg</td>
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<tr>
<td>Tumor central vessels</td>
<td>12±3 mmHg</td>
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Background:
Imaging Vascular Hypoxia

Provided by
Andrew Fontanella, Dewhirst Group
Hypoxia protects endothelial cells from radiation induced cell death
Vasculature key component of high dose radiation response....
Can we specifically detect and eliminate hypoxemic vessels?

Tumor Response to Radiotherapy Regulated by Endothelial Cell Apoptosis

Monica Garcia-Barros, François Paris, Carlos Cordon-Cardo,
David Lyden, Shahin Rafii, Adriana Haimovitz-Friedman,
Zvi Fuks, Richard Kolesnick
Evidence of indirect cell death caused by radiation induced vascular damage

Song CW, et al. unpublished
Detect hypoxia via pimonidazole adducts on the surface of hypoxic endothelium using contrast enhanced molecular ultrasound.
Detection of Hypoxemic Vessels in 4T1 murine breast tumor model

negative control – no pimonidazole

pimonidazole
MB<sub>pimonidazole</sub> in 4T1 mammary carcinoma

contrast mean power (linear a.u.)

Negative Control  MB<sub>pimonidazole</sub>  Muscle
4T1 Mammary Carcinoma
Evidence of Hypoxemic Vessels

CD31 – vessels
Pimonidazole - hypoxia
LnCap xenograft human prostate cancer model
Chronic Hypoxia seems to dominate

CD31 – vessels
Pimonidazole - hypoxia
MBpimonidazole in human prostate cancer

![Graph showing d.T.E. (a.u.) for different cell lines: Negative Control, LNCaP, Negative Control, PC3.](image)
Hyperthermia as a tool to understand changes in vascular hypoxia following therapy
Mild hyperthermia and 95% oxygen breathing combine to reduce vascular hypoxia: Link to improved radiation response observed in numerous studies.
A concept of selective drug/nanomedicine therapeutic targeting of vascular hypoxia
Inject pimonidazole i.p., allow adduct formation in hypoxic vessels
Infuse pimonidazole-targeted drug delivery vehicles to selectively target hypoxic vasculature.
Contrast-enhanced US analysis of vessel hypoxia in transgenic breast cancer model MMTV-Wnt-1
Colocalization analysis of hypoxic vessel presence: transgenic breast cancer also displays traits
Further importance of destroying/targeting the hypoxic, peri-vascular niche
Hypoxia linked to cancer stem cells
White: CD31+ endothelial cells, Red: ALDH stem cell marker, Green: PIMO, hypoxia in transgenic murine breast tumor line MMTV-Wnt-1
Conclusions

- Development of a novel method for detecting hypoxemic vessels with contrast enhanced US may lead to improved methods for specifically targeting/removing hypoxic tissue

- Resistance of tumor vasculature/stroma a major factor in overall tumor control with chemoradiation

- Potential new target for drug/nanomedicine delivery to areas associated with therapeutic resistance

- An ultrasound-based imaging and treatment approach against cancer initiating cells?
Acknowledgements

- Eduardo Moros, PhD, Moffitt Cancer Institute
- Joseph Levy
- Azemat Jamshidi-Parsian

Funding
Focused Ultrasound Surgery Foundation
NIH CA-44114
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