Tumor Hypoxia at the Molecular, Cellular, and Patient Level

Ted Graves (eg Graves@stanford.edu)  May 19, 2014
• Tumor oxygenation
• The hypoxic tumor phenotype
• Molecular and cellular effects of hypoxia
• Integrative approaches to studying tumor hypoxia
  • Molecular imaging
  • Computational models
Tumor Hypoxia

Tumor oxygenation • Hypoxic phenotype • Molecular effects • Imaging • Modeling • Summary

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Tumor

100-180 µm

Necrosis

Stroma

Hypoxic

Tumor

100-180 µm

Necrosis

Stroma
Tumor Vasculature

Colon Subcutis Skeletal Muscle

Colon Carcinoma Melanoma Sarcoma
Vasculature and Hypoxia

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Normal

Tumor

Temporary Occlusion

Hypoxia

AV Shunt

Blind Ends

Break in Vessel Walls
Chronic Hypoxia

Hypoxia (EF5)

Blood vessels (CD31)

Glioma
Acute Hypoxia

Tumor oxygenation • Hypoxic phenotype • Molecular effects • Imaging • Modeling • Summary

Hoechst 33342 → 20 minutes → DiOC7 → Sacrifice
## Tumor Hypoxia

### Tumor oxygenation

- Hypoxic phenotype
- Molecular effects
- Imaging
- Modeling
- Summary

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### Table 1 | Oxygenation of Tumours and the Surrounding Normal Tissue

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>Median Tumour pO$_2$ * (Number of Patients)</th>
<th>Median Normal pO$_2$ * (Number of Patients)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioblastoma</td>
<td>4.9 (10)</td>
<td>ND</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>5.6 (14)</td>
<td>ND</td>
<td>129</td>
</tr>
<tr>
<td>Head and neck carcinoma</td>
<td>12.2 (30)</td>
<td>40.0 (14)</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>14.7 (23)</td>
<td>43.8 (30)</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>14.6 (65)</td>
<td>51.2 (65)</td>
<td>132</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>7.5 (17)</td>
<td>38.5 (17)</td>
<td>Q. Le (personal communication)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>10.0 (15)</td>
<td>ND</td>
<td>133</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>2.7 (7)</td>
<td>51.6 (7)</td>
<td>134</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>5.0 (8)</td>
<td>51 (8)</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>5.0 (74)</td>
<td>ND</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>3 (86)</td>
<td>ND</td>
<td>137</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>2.4 (59)</td>
<td>30.0 (59)</td>
<td>138</td>
</tr>
<tr>
<td>Soft-tissue sarcoma</td>
<td>6.2 (34)</td>
<td>ND</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>18 (22)</td>
<td>ND</td>
<td>140</td>
</tr>
</tbody>
</table>

*pO$_2$ measured in mmHg. Measurements were made using a commercially available oxygen electrode (the ‘Eppendorf’ electrode). The values shown are the median of the median values for each patient. ND, not determined; pO$_2$, oxygen partial pressure.

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Hypoxic cancers exhibit:

- Resistance to radiotherapy
  - Oxygen dependence of DNA damage
- Resistance to chemotherapy
  - Poor drug delivery
  - Decreased cell proliferation
- Aggressive phenotype
- Genomic instability
- Reduced apoptosis
- Increased metastasis

L.H. Gray et al., Brit. J. Cancer, 1953
Hypoxia and Proliferation

Labeled with pimonidazole

Labeled with PCNA

J.A. Raleigh et al., Acta Oncol., 1995
**Cells exposed To Hypoxia**

**Lung metastasis**

**Table I. Metastatic potential of oxic vs hypoxic tumor cells**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Treatment</th>
<th>Metastatic Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>KHT Fibrosarcoma</td>
<td>oxic</td>
<td>1.2 X 10^-3</td>
</tr>
<tr>
<td>KHT Fibrosarcoma</td>
<td>hypoxic</td>
<td>1.7 X 10^-2</td>
</tr>
<tr>
<td>B16 Melanoma</td>
<td>oxic</td>
<td>5.5 X 10^-4</td>
</tr>
<tr>
<td></td>
<td>hypoxic</td>
<td>5.0 X 10^-3</td>
</tr>
<tr>
<td>SSCVII</td>
<td>oxic</td>
<td>5.6 X 10^-4</td>
</tr>
<tr>
<td></td>
<td>hypoxic</td>
<td>3.1 X 10^-3</td>
</tr>
</tbody>
</table>
Hypoxia and Outcome

Tumor oxygenation • Hypoxic phenotype • Molecular effects • Imaging • Modeling • Summary

Head and neck cancer

Prostate cancer

D.M. Brizel et al., Radiother. Oncol., 1999

B. Movsas et al., Urology, 2002
Hypoxia and Protein Synthesis

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Hypoxia coordinates the simultaneous induction and repression of specific sets of genes.
• Hypoxia regulates about 1.5% of the genes in the genome

• The transcription factors HIF-1, Egr, Jun, NF-κB, and p53 are all hypoxia-responsive.
HIF-1 is an oxygen-sensitive transcription factor that is upregulated in a number of tumors.
Regulation of HIF-1

Prolyl Hydroxylation of HIF1α

\[
\text{PHD2} + \text{Fe}^{2+} + 2\text{OG} + \text{O}_2 + \text{Asc} \leftrightarrow \text{PHD2} \cdot \text{Fe}^{2+} \cdot 2\text{OG} \cdot \text{O}_2 \cdot \text{Asc} \rightarrow \text{PHD2} + \text{CO}_2 + \text{SC}
\]

VHL-Initiated Degradation

\[
\text{HIF1}_\alpha_{\text{hydroxylated}} + \text{VHL} \cdot \text{Elongin} \cdot \text{Elongin} \leftrightarrow \text{HIF1}_\alpha_{\text{hydroxylated}} \cdot \text{VHL} \cdot \text{Elongin} \cdot \text{Elongin} \rightarrow \text{HIF1}_\alpha_{\text{Degradation~Products}}
\]

Iron Oxidation & Reduction

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^+ + \text{OH}^-
\]

\[
4\text{Fe}^{3+} + 2\text{Asc} + \text{O}_2 \rightarrow 4\text{Fe}^{2+} + 2\text{dehydroAsc} + 2\text{H}_2\text{O}
\]
HIF-1 and Outcome

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Nasopharyngeal carcinoma

Lung cancer

Breast cancer

S.J. Kim et al., Lung Cancer, 2005


J. Dales et al., Int. J. Cancer, 2005
Molecular imaging is an inherently integrative method with a variety of applications to systems biology:

- Imaging is non-invasive and conducive to longitudinal measurement
- A variety of imaging approaches exist to interrogate a range of molecular, cellular, and physiologic processes
- Probe-based imaging relies on the dynamic distribution and uptake of a contrast molecule and thus reports on multiple physiologic parameters
FDG PET signal is dependent on:

- Vascular delivery
- Cell number
- GLUT expression
- Hexokinase expression

**Reaction Diagram**

- Hexokinase
- Phosphohexose isomerase
- Glucose-6-phosphatase Slow!!!

**Chemical Formulas**

- Glucose-6-P
- Phosphoenolpyruvate
- Pyruvate

**FDG PET**

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Hypoxia and FDG


Human head/neck cancer cell lines

*In vitro* uptake assay

$r=0.24$ for all tumors ($n=49$)

$r=0.41$ ($p=0.04$) for head/neck ($n=26$)
Fig. 2. Hematoxylin-eosin images (a, d, g), composite images (b, e, h) showing Hoechst 33342 (blood flow) in blue, pimonidazole stain (hypoxia) in green, and bromodeoxyuridine (cell proliferation) in red, and $^{18}$F-FDG autoradiograms (c, f, i) for each of the three tumors.
2-nitroimidazole uptake is dependent on:

- Vascular delivery
- Cell number
- Reductase expression
- Oxygen
Multimodal Imaging

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Graves et al., Clin Cancer Res, 2010
Hypoxia PET and Prognosis

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$^{18}$F-Fluoromisonidazole (FMISO)

### Variation in Mean EF5 T/M in Different Cell Line Derived Subcutaneous Tumors

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>T/M Value</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29</td>
<td>1.2</td>
<td>(n=9)</td>
</tr>
<tr>
<td>A549</td>
<td>1.5</td>
<td>(n=9)</td>
</tr>
<tr>
<td>RKO</td>
<td>2.0</td>
<td>(n=7)</td>
</tr>
<tr>
<td>22B</td>
<td>2.5</td>
<td>(n=10)</td>
</tr>
<tr>
<td>FaDu</td>
<td>3.0</td>
<td>(n=10)</td>
</tr>
<tr>
<td>SAS</td>
<td>2.8</td>
<td>(n=6)</td>
</tr>
<tr>
<td>SAS</td>
<td>3.2</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

**FAZA**

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Hypoxia Imaging for Prognosis?

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R.S. Ali et al., in preparation
Effect of Total Dose on Post-Tx Response
EF5 and RT Response

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1 x 10Gy

1 x 40Gy

Normalised Post-RT Volume vs. Days Post Treatment

- T/M < 2.5
- T/M > 2.5

p < 0.05
EF5 and RT Response

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2 x 5Gy

Days Post Treatment

Normalised Post-RT Volume

Days Post Treatment

Normalised Post-RT Volume

2 x 10Gy

T/M < 2.5

T/M > 2.5

Days Post Treatment

Normalised Post-RT Volume

Days Post Treatment

Normalised Post-RT Volume

4 x 5Gy

T/M < 2.5

T/M > 2.5

Normalised Post-RT Volume

0 10 20 30 40 50 60

Days Post Treatment

2 x 10Gy

Normalised Post-RT Volume

0 10 20 30 40 50 60

Days Post Treatment

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Intertumoral Heterogeneity

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T/M = 3.2

T/M = 3.3

Tumor Volume (mm³)

Days Post Radiation
Intertumoral Heterogeneity

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T/M = 2.2

T/M = 2.4
Models of Hypoxia

Molecular and cellular-level models

* Molecular interaction maps

* Chemical-kinetic models
  Nguyen et al., J. Cell Sci., 2013

* Genetic models
  Yu et al., PLoS Comp. Biol., 2007

Tissue-level models

* Radiobiological simulations
  Stamatakos et al., Proc. IEEE, 2002
HIF-1 Modeling

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Distinct reactive oxygen species conditions can explain the difference in HIF-1 signaling and cellular response in cancer and ischemia.

Radiobiological Modeling

G. Stamatakos et al., Proc. IEEE, 2002
Image-Based Modeling

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CT

18FDG-PET

MRI

In vitro/molecular

Computational

In vivo/translational

Feed info

Prediction

Feed info

Prediction
Image-Based Modeling

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Image-Based Modeling

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Conclusions

• Lack of oxygen in cancer is a long-recognized and studied aspect of the tumor microenvironment

• Hypoxia drives a variety of phenotypic changes in cancer through both direct and indirect mechanisms

• Transcription factors, most notably HIFs, act as cellular oxygen sensors to mediate a molecular response to the absence of oxygen

• Imaging methods can shed light on hypoxia at the cellular to the patient level, but interpretation and prediction based on these images remains challenging

• Quantitative modeling techniques have been applied to understand hypoxia at the molecular, cellular, and patient levels

**Points to ponder while reading:**

1. What are the rules on which the model is predicated, and how generalizable is the model as a whole?
2. How was the model validated?
3. What actionable scientific or preclinical hypotheses or information was obtained through the modeling process?