Tumor hypoxia: Causes, characterization and clinical implications

Univ.-Prof. Dr. med. P. Vaupel, M.A.

Dept. of Radiotherapy & Radiation Oncology,
Klinikum rechts der Isar
Technical University, Munich, Germany

1st Symposium of the SFB 824, Alpbach 2010
Methods currently available or undergoing development for detection of tumor hypoxia in the experimental and clinical setting (selection)

1. Minimally invasive microsensor techniques for direct tissue $pO_2$ measurements
   - polarographic $O_2$ sensors
   - luminescence-based optical sensors

2. Noninvasive detection of sensitizer adducts
   - e.g., FMISO-PET, FAZA-PET, Cu-ATSM-PET

3. Nuclear magnetic resonance spectroscopy and imaging techniques
   - blood oxygen level dependent (BOLD) MRI
   - dynamic contrast-enhanced (DCE) MRI
Recess–type pO$_2$ microelectrode (experimental, 15 µm tip diameter)

Eppendorf microsensor (clinical setting)
Methods currently available or undergoing development for detection of tumor hypoxia in the experimental and clinical setting (cont'd)

4. Techniques for intravascular $O_2$ detection
   - cryospectrophotometry ($HbO_2$ saturations)
   - near-infrared spectroscopy ($HbO_2$ saturations)
   - phosphorescence imaging

5. Electron paramagnetic resonance (EPR) oximetry

6. Immunohistochemistry (IHC) with exogenous hypoxic markers
   - e.g., MISO, PIMO, EF5

7. IHC with “endogenous hypoxia markers” (biomarkers are not hypoxia-specific !)
   - HIF-1$\alpha$
   - HIF-dependent downstream proteins
     (e.g., CA-IX, GLUT-1, VEGF)
Oxygenation of Normal Breast vs. Breast Cancer

Normal breast
n = 16
1,000 measurements
Median pO₂ = 65 mmHg

Breast cancer
Primary tumors Ib-IV
n = 15
851 measurements
Median pO₂ = 10 mmHg

Oxygenation of Normal Cervix vs. Cervical Cancer

Normal cervix
Nullipara
n = 7
432 measurements
Median pO₂ = 42 mmHg

Squamous cell carcinomas of the uterine cervix
Primary tumors Ib-IV
n = 116
10,043 measurements
Median pO₂ = 10 mmHg
Oxygen Consumption vs Oxygen Supply: Balance in Normal Tissues and Imbalance in Solid Tumors

- **Normal tissue**:
  - Normoxia
  - Hypoxia
  - Hyperoxia
  - Oxygen consumption
  - Oxygen supply (perfusion, diffusion, cHb)

- **Tumor**:
  - Normoxia
  - Hypoxia
  - Hyperoxia
  - Oxygen consumption
  - Oxygen supply (perfusion, diffusion, cHb)

Factors compromising $O_2$ supply

- tumor hypoxia
- inadequate perfusion
Differences in vasculature between normal tissues and tumors

Colon vs. Colon carcinoma
Subcutis vs. Melanoma
Skeletal muscle vs. Sarcoma

Konerding, Vaupel (Mainz)
Variability of Blood Flow in Solid Tumors

BLOOD FLOW (ml·g⁻¹·min⁻¹)

- Carotid Body
- Adrenals, Thyroid
- Kidneys, Heart (strenuous exercise)
- Spleen
- Liver
- Heart (at rest), Breast (lactating)
- Pancreas, Skin (heat load)
- Brain, Skeletal Muscle (hard work)
- Intestine, Prostate, Uterus (pregnant)
- Red Marrow, Lymphoid Tissues
- Testes, Ovaries
- Spinal Cord
- Bronchial Blood Flow
- Skin
- Breast (post menopausal)
- Skeletal Muscle (resting)
- Adipose Tissue
Factors compromising $O_2$ supply

- diffusion distances $\uparrow$
- inadequate perfusion

- tumor hypoxia
Intervascular $O_2$ and glucose profiles
Factors compromising $O_2$ supply

diffusion distances $\uparrow$

inadequate perfusion

adverse diffusion geometry

tumor hypoxia
Factors compromising $O_2$ supply

- Up to 25% increase in diffusion distances
- Inadequate perfusion
- Adverse diffusion geometry
- HbCO formation

*Up to 25% in heavy smokers*
Factors compromising $O_2$ supply

- Blood supply from venous side
- Hypoxemia
- HbCO formation
- Adverse diffusion geometry
- Inadequate perfusion
Factors compromising O₂ supply

Therapy-induced

- anemia
- diffusion distances ↑
- adverse diffusion geometry
- HbCO formation

Tumour-associated

- inadequate perfusion
- hypoxemia

Tumor hypoxia
Characteristics of oxygenation status in cancers of the uterine cervix - I
Höckel & Vaupel (since 1991)

Results from pretreatment pO₂ measurements in conscious patients:

- Oxygenation in tumors is heterogeneous and compromised as compared to normal tissues
- On average, the median pO₂ values in primary cancers of the uterine cervix are lower than those in the normal cervix
- Many cervical cancers contain hypoxic tissue areas (≈ 60% in SCC, ≈ 66% in AC)
- Tumor oxygenation is not regulated according to the metabolic demand as is the case in normal tissues
- Major causative factors for the development of hypoxia are limitations in perfusion and diffusion as well as tumor-associated anemia
- In cervix cancers of moderately/severely anemic patients severely hypoxic areas are more frequently found than in non-anemic patients
- Anemia (in approx. 30% of patients at diagnosis) contributes to the development of hypoxia, especially in low-flow tumor areas
Hypoxic Tumor Fraction as a Function of Hb Concentration and Blood Flow Rate (TBF)

Hypoxic tissue fraction ($pO_2 < 1$ mmHg) (%)

- TBF = 0.2 ml/g/min
- TBF = 0.3 ml/g/min

Hb concentration (g/dl)

Vaupel (2001)
Characteristics of oxygenation status in cancers of the uterine cervix - II

• There is no characteristic topological distribution of $O_2$ tensions within cervix cancers (periphery vs. center)

• Tumor-to-tumor variability in oxygenation is greater than intra-tumor variability

• Tumor oxygenation is independent of various patient demographics (e.g., age, menopausal status, parity)

• Tumor oxygenation and the extent of hypoxia are independent of clinical size, FIGO stage, histological type (SCC vs. AC), grade and lymph node status

• Higher pathological tumor stages (pT stages) correlate with significant poorer tumor oxygenation

• Local recurrences of cervix cancers have a higher hypoxic fraction than the primary tumors

• Hypoxic tumors with low apoptotic index are highly aggressive

• Microregional expression of HIF-1$\alpha$, GLUT-1 and CA IX do not correlate with oxygenation status

• Hypoxia is the prototypical (but not the only) driving force for the HIF pathway
• Fluctuating spatial and temporal oxygen gradients (4D-heterogeneity) may be key factors driving adaptive processes and malignant progression

• In contrast to malignant cervical cancers and leiomyosarcomas of the uterus, benign leiomyomas are severely and uniformly hypoxic
  Median $pO_2 = 1$ mmHg without steep $O_2$ gradients, “static” hypoxia

Pretherapeutic oxygenation status of locally advanced human tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>n</th>
<th>Median pO₂ (mmHg)</th>
<th>HF 2.5 (%)</th>
<th>HF 5 (%)</th>
<th>HF 10 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix cancers</td>
<td>730</td>
<td>9</td>
<td>28</td>
<td>44</td>
<td>59</td>
</tr>
<tr>
<td>Head &amp; neck cancers</td>
<td>592</td>
<td>10</td>
<td>21</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Soft tissue sarcomas</td>
<td>283</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>59</td>
</tr>
<tr>
<td>Breast cancers</td>
<td>212</td>
<td>10</td>
<td>30</td>
<td>47</td>
<td>50</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>190</td>
<td>10</td>
<td>45</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Gliomas</td>
<td>104</td>
<td>13</td>
<td>26</td>
<td>30</td>
<td>49</td>
</tr>
</tbody>
</table>

HF 2.5 = fraction of pO₂ values ≤ 2.5 mmHg;
HF 5 = fraction of pO₂ values ≤ 5 mmHg
HF 10 = fraction of pO₂ ≤ 10 mmHg
## Oxygenation status of normal tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>median $pO_2$ (mmHg)</th>
<th>HF 2.5 (%)</th>
<th>HF 5 (%)</th>
<th>HF 10 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>42</td>
<td>8</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Subcutis</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>25-30</td>
<td>0</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Breast</td>
<td>52</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Brain</td>
<td>24-27</td>
<td>0</td>
<td>3-8</td>
<td>13</td>
</tr>
</tbody>
</table>

Hypoxia is an independent, adverse prognostic factor for tumour progression (cervical cancer)

Prognostic significance of tumor hypoxia (selection of data, multivariate analysis)

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Hypoxia detection via</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancers of the uterine cervix</td>
<td>direct $pO_2$ measurements</td>
<td>DFS, OS</td>
</tr>
<tr>
<td></td>
<td>HIF-1α (IHC)</td>
<td>DFS, OS</td>
</tr>
<tr>
<td></td>
<td>GLUT-1 (IHC)</td>
<td>MFS</td>
</tr>
<tr>
<td></td>
<td>CA IX (IHC)</td>
<td>DFS, OS, MFS</td>
</tr>
<tr>
<td></td>
<td>Cu-ATSM-PET</td>
<td>OS, PFS</td>
</tr>
<tr>
<td></td>
<td>DCE-MRI</td>
<td>DFS</td>
</tr>
<tr>
<td>Head &amp; neck cancers</td>
<td>direct $pO_2$ measurements</td>
<td>LC, OS</td>
</tr>
<tr>
<td></td>
<td>HIF-1α (IHC)</td>
<td>OS</td>
</tr>
<tr>
<td></td>
<td>GLUT-1 (IHC)</td>
<td>DFS, OS</td>
</tr>
<tr>
<td></td>
<td>CA IX (IHC)</td>
<td>OS</td>
</tr>
<tr>
<td></td>
<td>F-MISO-PET</td>
<td>LC</td>
</tr>
<tr>
<td></td>
<td>PIMO-binding</td>
<td>LC</td>
</tr>
</tbody>
</table>

DCE = dynamic contrast enhanced, DFS = disease-free survival, IHC = immunohistochemistry, LC = local control, MFS = metastasis-free survival, OS = overall survival, PFS = progression-free survival
Effects of hypoxia

- Restrained proliferation
- Differentiation
- Apoptosis
- Necrosis

Normal cells and the majority of cancer cells

"positive" effects
Effects of hypoxia

Hypoxia

Anti-proliferative effects
- Restricted proliferation
- Differentiation
- Apoptosis
- Necrosis

Pro-proliferative effects
- Adaptive processes
- Aggressive phenotype
- Tumour progression
- Acquired treatment resistance

Normal cells and the majority of cancer cells
"positive" effects

Only a minority of cancer cells but decisive for prognosis
"negative" effects
Hypoxia-mediated proteome changes

The role of HIF-1α

Hypoxia <1% O₂ → HIF-1-dependent

Hypoxia-related gene expression, proteome changes

- Cellular adaptation: metabolism, cell survival & proliferation, apoptosis protection
- Local adaptation: angiogenesis, invasion
- Systemic adaptation: O₂ transport capacity (EPO)

Aggressive phenotype, tumour progression

Poor prognosis

HIF-1 = Hypoxia Inducible Factor-1
Hypoxia-mediated proteome changes

The role of HIF-1α

Hypoxia <1% O₂

HIF-1-dependent

Hypoxia-related gene expression, proteome changes

Cellular adaptation: metabolism, cell survival & proliferation, apoptosis protection

Local adaptation: angiogenesis, invasion

Systemic adaptation: O₂ transport capacity (EPO)

Aggressive phenotype, tumour progression

Poor prognosis

HIF-1 = Hypoxia Inducible Factor-1
Hypoxia-mediated proteome changes (cont'd)

Hypoxia <1% O₂

Other transcription factors (e.g. NF-κB, AP-1)

Hypoxia-related gene expression, proteome changes

Cellular adaptation: resistance to apoptosis

Local adaptation: angiogenesis, invasion

Systemic adaptation: suppression of anti-cancer immune response

Aggressive phenotype, tumour progression

Poor prognosis

NF-κB = Nuclear Factor-κB
AP-1 = Activating Protein-1

Redundancy in biological mechanisms in malignant tumours!
Hypoxia-Mediated Genome Changes

Hypoxia

< 0.7 mmHg
(< 0.1% O₂)

+/- Reoxygenation
+/- spatio-temporal heterogeneities

Selection pressure

Clonal selection

Point mutations,
chromosomal aberrations,
gene amplification,
polyploidy

Genetic instability

New genetic variants

Aggressive/resistant phenotype, tumour progression

Poor prognosis
Hypoxia as a driving force in malignant progression: 4 possible levels of intervention

- at the **transcriptome level** leading to hypoxia-induced changes in gene expression coordinated by a special set of transcription factors, such as HIFs, NF-κB, AP-1, indicating redundancy in biological mechanisms in malignant tumors below 1% $O_2$
- at the **proteome and metabolome level** via adaptive gene expression, post-transcriptional and post-translational modifications below 1% $O_2$
- at the **genome/epigenome level** by increasing genomic and epigenomic instability (below 0.1% $O_2$)
- at the **cell population level** by clonal selection and clonal expansion according to phenotype fitness
Tumor Hypoxia

Chicken or Egg?
The vicious circle of tumour hypoxia

Tumour hypoxia (hostile microenvironment)

Changes in gene expression
Proteome changes
Genome changes
Clonal selection

Malignant progression
\[ \uparrow \text{Aggressiveness} \]

Tumour propagation

Hoeckel & Vaupel, J Natl Cancer Inst 2001;93:266–76
Tumor Hypoxia and Therapeutic Resistance - I (direct mechanisms)

• reduced generation of free radicals (some chemotherapy, photodynamic therapy)
• reduced “fixation” of DNA damage (X- and γ-rays)
Tumor Hypoxia and Therapeutic Resistance – II (indirect mechanisms)

- cell cycle arrest
- decreased cell proliferation rate
- modulation of gene expression
- changes in proteome ($pO_2 < 7 \text{ mmHg}$)
- genetic instability, clonal selection ($pO_2 < 1 \text{ mmHg}$)

applies to:
- chemotherapy
- RT
- hormone therapy
Key information

Tumor Hypoxia

< 2% O₂

< 1% O₂
(< 7 mmHg)

Changes in Gene Expression
Proteome Changes

Resistance to Therapy

Tumor Propagation
Poor Prognosis

< 0.1% O₂
(< 0.7 mmHg)

Genome Changes
Genomic Instability
Genomic Heterogeneity
Clonal Selection

Malignant Progression

Malignant Progression
Cryosections from xenografted tumors were (immuno-) fluorescently stained with:

- Pimonidazole (hypoxia)
- Hoechst 33342 (perfusion)
- CD31 (vessels)

Four patterns in non-necrotic tissue could be visualized:
### Hypoxia subtypes based on causative mechanisms (I)

<table>
<thead>
<tr>
<th>Type of hypoxia</th>
<th>Subtype of hypoxia</th>
<th>Causative mechanism(s)</th>
<th>Examples (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hypoxia*</td>
<td>ischemic hypoxia due to transient flow stop</td>
<td>temporary shut-down of flow</td>
<td>temporary obstruction of vessel lumen by tumor cells, blood cells and/or fibrin aggregates; arterial vasomotion, vascular remodeling</td>
</tr>
<tr>
<td></td>
<td>hypoxemic hypoxia</td>
<td>severe reduction of oxygen content in tumor microvessels</td>
<td>transient plasma flow in tumor microvessels, temporal fluctuations in red blood cell flux; flow reversal, sluggish blood flow</td>
</tr>
</tbody>
</table>

* Terms alternatively used: cyclic, intermittent, fluctuating, transient or repetitive hypoxia (< 2 h)
## Hypoxia subtypes based on causative mechanisms (II)

<table>
<thead>
<tr>
<th>Type of hypoxia</th>
<th>Subtype of hypoxia</th>
<th>Causative mechanism(s)</th>
<th>Examples (selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic hypoxia</strong></td>
<td>diffusion-limited hypoxia</td>
<td>surpassing the maximum oxygen diffusion distance in the perivascular space</td>
<td>expansion of the intervascular space (low vascular density)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>adverse diffusion (vascular) geometry</td>
<td>concurrent vs. countercurrent tumor microvessels; centrifugal vs. centrifugal diffusion field*** (approx. 2.7 times greater hypoxic area in the latter at otherwise identical boundary)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>exaggerated longitudinal intravascular oxygen gradients</td>
<td>elongated, tortuous (convoluted) microvessels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>diverging oxygen-rich (arterial) blood to the venous side of the tumor vascular bed</td>
<td>shunt perfusion through arterio-venous anastomoses</td>
</tr>
<tr>
<td></td>
<td>hypoxemic hypoxia</td>
<td>reduced oxygen content in the blood supplying the tumor</td>
<td>tumor-associated and/or therapy-induced anemia (anemic hypoxia)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tumors/tumor areas supplied from the venous side (i.e., supply with partly deoxygenated blood)</td>
<td>primary or metastatic liver tumors that are (at least partially) supplied by branches of the portal vein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“functional” anemia by hemoglobin (Hb) blockage</td>
<td>carboxyhemoglobin (HbCO) formation in heavy smokers (reduced carrying capacity for oxygen in blood)</td>
</tr>
<tr>
<td></td>
<td>hypoxia due to compromised perfusion of leaky microvessels</td>
<td>abolished perfusion pressure differences between up- and downstream tumor microvessels</td>
<td>transmural coupling between (chronically high) interstitial fluid pressure and microvascular pressure due to the high permeability of microvessels</td>
</tr>
</tbody>
</table>

---

** Term alternatively used: sustained hypoxia (> 2h)
*** Centrifugal diffusion: tumor cord supplied by a tumor microvessel running longitudinally within the cord center or along the cord’s circumference
Hypoxia due to compromised perfusion of leaky microvessels
Pathophysiological consequences

Refining these subtypes of hypoxia has immense impact on our understanding of tumor biology, pathophysiological consequences, radiation efficacy, drug delivery and waste (e.g., lactic acid, adenosine) removal, etc:

<table>
<thead>
<tr>
<th>Type of hypoxia</th>
<th>Subtype of hypoxia</th>
<th>Perfusion</th>
<th>Oxygen supply</th>
<th>Nutrient supply (e.g., glucose)</th>
<th>Waste removal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute hypoxia</strong></td>
<td>ischemic hypoxia</td>
<td>flow stop</td>
<td>abolished</td>
<td>abolished</td>
<td>abolished</td>
</tr>
<tr>
<td></td>
<td>hypoxemic hypoxia</td>
<td>maintained</td>
<td>strongly reduced</td>
<td>maintained</td>
<td>maintained</td>
</tr>
<tr>
<td><strong>Chronic hypoxia</strong></td>
<td>diffusion-limited hypoxia</td>
<td>maintained</td>
<td>reduced to abolished (distance dependent)</td>
<td>reduced to abolished (distance dependent)</td>
<td>reduced to abolished (distance dependent)</td>
</tr>
<tr>
<td></td>
<td>hypoxemic (anemic) hypoxia</td>
<td>maintained</td>
<td>reduced</td>
<td>maintained</td>
<td>maintained</td>
</tr>
<tr>
<td></td>
<td>IHT*-induced</td>
<td>reduced</td>
<td>reduced</td>
<td>reduced</td>
<td>reduced</td>
</tr>
</tbody>
</table>

* Interstitial hypertension in tumor center
Key messages (I)

1. Hypoxia is a characteristic feature of locally advanced solid tumors
2. Pathogenesis of tumor hypoxia is multifactorial (e.g., poor perfusion, anemia)
3. Hypoxia has an ominous impact on tumor progression
Key messages (II)

4. Hypoxia ($pO_2 < 10 \text{ mmHg}$) may cause changes in gene expression and in the proteome
5. Hypoxia ($pO_2 < 1 \text{ mmHg}$) may cause changes in the genome
6. Hypoxia may trigger invasive and metastatic behavior in cancer cells
Key messages (III)

7. Hypoxia may diminish the apoptotic potential of transformed cells
8. Hypoxia may, in turn, create an aggressive phenotype
9. Hypoxia can compromise the therapeutic outcome via direct and/or indirect mechanisms
Thank you for your kind attention!

For questions not addressed in the following discussion please contact:

vaupel@uni-mainz.de
or
peter.vaupel@lrz.tum.de