Modulating the tumour microenvironment: therapeutic strategies for enhancing tumor radioresponse

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Overview

• The tumour microenvironment
• Tumour vasculature
• IR effects on the tumour vasculature
• Approaches to biologically modify the tumour vasculature and improve radiation response
What is the tumour microenvironment?

The Evolution of Cancer View

The Reductionist View — — — Cancer as Complex Tissue

- Cancer cells
- Immature vessels
- Immune cells
- Fibroblasts
- Established tumor vessels
- Perivascular cells
- Endothelial cells

Hallahan and Weinberg. Cell 2000
Components of the tumour microenvironment

Consists of tumor cells and surrounding ‘normal’ cells and structures:

- **Vasculature**
- Extracellular matrix, secreted factors
- Immune cells
- Fibroblasts

Communication occurs through:
- secreted factors
- direct cell-cell contact
Communication within the tumour microenvironment – cell interactions and secreted factors

Fukumura et al. Microcirculation 2010
Why consider the tumour microenvironment?

• Collectively, the signals from secreted factors and direct cell-cell interactions influence tumor proliferation, survival and metastasis

• The microenvironment influences treatment response

• Our treatments influence the tumour microenvironment
Vasculature

- Vasculature is a major functional component in microenvironment
- Required for continued tumour growth and spread
Vasculature

- Tumour vasculature is abnormal:
  - Leaky, torturous, blind ends, shunts
  - Resulting in poor tumour perfusion and hypoxia
Tumour Vasculature

normal

tumour

Narang and Varia, Adv Drug Deliv Rev 2011
Ionizing radiation influences the vasculature
IR and vasculature response

As IR dose ↑, vascular density ↓

**FIG. 6.** Radiation dose–response relationship. The total growth area of the angiogenesis discs was measured using a digital video image analysis technique (see text for details). X irradiation was given on Day 11, and the discs were extracted on Day 20 after implantation. Each point represents the mean ± SE of four to six animals. The extent of total growth area achieved in unirradiated discs on Day 11 was 4.4 mm².

IR and vasculature response

As IR dose ↑, vascular density ↓

IR and vasculature response

As IR dose ↑, vascular perfusion can ↓

Tumor perfusion ↓

The dose of IR may determine vasculature response

- Appears to be a dose-dependent effect on vasculature function
- At **lower doses** (e.g., < 8 Gy) – no significant effect
- At **higher doses** (e.g., stereotactic RT), vascular ablation is believed to occur which may indirectly result in death of tumor cells
Ionizing radiation influences the vasculature – what are the biological mechanisms?
Potential mechanisms for IR effects on vasculature

• Induction of endothelial-cell apoptosis
• Alteration of angiogenic factors and cells in the microenvironment
  – VEGF
  – Circulating CD11b+ cells
A role for ceramide-mediated endothelial apoptosis?
Vasculature – important determinant of tumor radioresponse at high dose IR?

Tumors have marked resistance to single high dose radiation (15 Gy) when endothelial apoptosis is inhibited *This remains an area of controversy*
Ceramide-mediated endothelial apoptosis – dose dependent induction?

Fuks and Kolesnik, Cancer Cell 2005
Ceramide levels correlate with tumor response to SBRT in patients

Dubois et al., Radiother Oncol 2016
Summary

- Tumour vasculature is dysfunctional
  - Poor tumor perfusion and increased hypoxia
- IR can reduce vascular density and perfusion
- High dose IR may induce death of endothelial cells
  - May indirectly kill tumor cells by destroying vasculature
IR can alter angiogenic factors in the tumour microenvironment
IR influences angiogenic factors

- IR promotes tumour secretion of VEGF

10, or 20 Gy. Conditioned medium was collected every 24 h, and VEGF levels were normalized to cell number. A dose-dependent increase in VEGF secretion was observed for all doses of IR ($P < 0.05$). ■, 0 cGy; □, 500 cGy; ■, 1000 cGy; □, 2000 cGy. Data are presented as means: bars, SE. C. VEGF expression in human tumor cell

Gorski et al. Cancer Res 1999
IR induced tumour secretion of VEGF

This may promote angiogenesis and support continued tumour growth.
IR influences angiogenic factors

• IR induces SDF-1 secretion from tumors which promotes infiltration of circulating immune cells (CD11b+ myelomonocytes) from bone marrow – mediates vasculogenesis
Restoration of tumor vasculature post-irradiation by BM-derived cells

Large dose of IR ablates vasculature

Tumor secretion of SDF-1 recruits CD11b+ cells to re-establish vasculature

Tseng et al., BJC 2011.
Circulating BM-derived CD11b+ myelomonocytes

- Following tumor irradiation, CD11b+ myelomonocytes are recruited (via SDF-1) and promote tumor revascularization
- Blocking this process delays tumor regrowth post-irradiation

Ahn et al., PNAS 2010 (Brown lab)  
Kozin et al., Cancer Res 2010 (Jain lab)
Summary

• IR can induce tumour secretion of VEGF
  – Potentiate tumor angiogenesis and tumor growth
• High dose IR may ablate tumor vasculature
• Re-establishment of vasculature involves SDF-1-mediated recruitment of CD11b+ cells
• Blocking SDF-1 or CD11b+ (to prevent re-establishment of vasculature) can improve tumor radiation response
How can we biologically target the vasculature to favorably influence tumor radiation response?
Biological vascular modifiers

**Improve** vascular function:
- VEGF blockade
- Ang-1 agonist
- Vasodilators

**Decrease** or **disrupt** vascular function:
- Vascular disrupting agents (VDAs)
- Notch blockade

How does modification of the vasculature influence tumor radioresponse?
Vascular modifiers

Improve vascular function:
Deliver more oxygen to tumour to **before** radiation

- **VEGF**
  - Ang-1
  - Vasodilators
VEGF and tumor angiogenesis

- **VEGF is a major pro-angiogenic factor**
  - Secreted by tumors and stromal cells
  - Binds to VEGF receptors on endothelial cells
  - Increases endothelial cell proliferation, survival and migration
  - Causes vasodilation and increased vessel permeability
  - May have protective effects on tumor cells (independent of vasculature)
VEGF and tumor angiogenesis

Hicklin and Ellis, JCO 2005
Inhibiting the VEGF pathway

- **Antibodies** against:
  - VEGF (bevacizumab, VEGF-trap),
  - VEGF receptor (DC101)
- **Tyrosine-kinase inhibitors**

Improve tumor vascular function by inhibiting VEGF pathway
Early treatment with VEGF blockade may improve vascular function
Normalization of tumor vasculature

Goel et al., Physio Rev 2011
Vascular normalization window

No treatment

Early phase

DC101

Late phase

Low pericyte coverage
Thick BM

Legend Key
- pericytes
- blood vessels
- BM
- Ang-1

α-Tie2 Ab
Tie2 peptide
siAng-1
MMP-Inhibitor

↑ Pericyte coverage
↓ BM thickness
↑ Ang-1 expression
↓ Vessel diameter
↓ Hypoxia
↑ Radiation response

↓ Pericyte coverage
↓ BM thickness
↓ Vessel diameter and density

Lin and Sessa. Cancer Cell 2004
Vessel normalization and tumor radioresponse

- VEGFR2 blockade may temporarily ‘normalize’ tumor vasculature (normalization window)
- During this period, tumor hypoxia is reduced and tumor radioresponse is improved

Winkler et al. *Cancer Cell* 2004
Improved perfusion in GBM pts following anti-VEGF TKI correlates with OS

- Cediranib (TKI) can ‘normalize’ and improve perfusion in subset of GBM pts, and this improves OS

Sorensen et al. Cancer Res 2012
VEGF-blockade and IR trials

• Phase 1 and 2 trials show promising results

Preoperative Radiation Therapy With Concurrent Capecitabine, Bevacizumab, and Erlotinib for Rectal Cancer: A Phase 1 Trial

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RTOG 0417: Efficacy of Bevacizumab in Combination With Definitive Radiation Therapy and Cisplatin Chemotherapy in Untreated Patients With Locally Advanced Cervical Carcinoma

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VEGF-blockade and IR trials

• Phase 3 trial:
  – **AVAglio**: GBM pts RT + TMZ +/- bevacizumab; addition of bevacizumab increased PFS (10.6 mo vs 6.2 mo placebo), increased 1 yr OS (72% versus 66%, p=.049) although OS not significant at 2 year (33.9% versus 30.1%, p=.010).
  – More patients were able to discontinue steroids with bevacizumab (66% versus 47%)

Gilbert et al. *NEJM* 2014
Limitations:

• Definition of the ‘window’ during which to initiate VEGF blockade during IR
  – If started too late, could antagonize radiation (‘overpruning’ of blood vessels → hypoxia)

• Some tumors/vasculature possess intrinsic resistance to VEGF due to bypass pathways
  – Require biomarkers to predict which tumors will respond
  – Additional targeting of bypass pathways
Summary

• Blockade of VEGF pathway can normalize tumor vasculature
  – Less torturous, more stable, less leaky, reduced hypoxia

• This is a transient period (‘window’)
  – IR may be antagonized beyond this period, due to excessive vessel pruning, less stable vessels and increased hypoxia!
Vascular modifiers

Improve vascular function:
Deliver more oxygen to tumor to before radiation

• VEGF

• Ang-1

• Vasodilators
**Angiopoietin-1**

- Ang-1 opposes effects of VEGF
  - Produced by pericytes (vascular support cells)
  - Binds to Tie2 on endothelial cells
  - Improves vascular stability
  - Reduces vessel branching and leakiness
  - Tie2 expressed on immune cells (may also have anti-inflammatory effects)

*Xu et al. Contra 2005*
Angiopoietin-1 stabilizes tumour vasculature

Stabilized, mature vessels (High Ang1)

Destabilized, immature vessels (Low Ang1)
Angiopoietin-1 stabilizes tumour vasculature and improves perfusion

Angiopoietin-1 corresponds with decreased tumour hypoxia

Winkler et al. *Cancer Cell* 2004
Angiopoietin-1

• Administration of Ang-1 is predicted to improve tumor radioresponse by vessel normalization

• Vascular normalization, reduced hypoxia: impair tumor regrowth after irradiation?
Vascular modifiers

Improve vascular function:
Deliver more oxygen to tumor to **before** radiation

- VEGF
- Ang-1

- **Vasodilators**
Vasodilation – improvement in tumor perfusion
Vasodilators

• Vasodilators (nicotinamide, hydralazine, PARP inhibitors) can increase tumor perfusion and decrease acute hypoxia

• Radiation is known to impair nitric oxide production and vasodilation (may partly contribute towards reduced tumour perfusion)
Nicotinamide improves tumour radioresponse

Nicotinamide reduces tumour hypoxia and decreases tumour cell survival following irradiation
ARCON – improving tumour oxygenation and radioresponse in patients

- **ARCON** (accelerated RT with carbogen (98% O₂) and **nicotinamide**) clinical trials show promise in improving radioresponse for laryngeal and bladder cancer

98% O₂ → **improved tumour oxygenation**

nicotinamide → vasodilation
ARCON in laryngeal cancer

- Ph3 trial: cT2-T4 laryngeal ca randomized to accelerated RT (AR) (N=174) or ARCON (N=171)
- 5 yr local control: 78% for AR, 79% for ARCON (p=0.8)
- 5 yr regional control: 86% for AR vs 93% for ARCON (p=0.04)
- Improved regional control for ARCON was significantly higher in hypoxic tumours (60% for AR vs 100% for ARCON (p=0.01)
- Need proper patient selection

Janssens et al., JCO 2012
ARCON in laryngeal cancer

- Pts with low pretreatment hemoglobin levels had improved locoregional control and disease free survival with ARCON

Janssens et al., CCR 2014
ARCON in bladder cancer

Purpose
Phase II clinical studies suggest that hypoxic modification with carbogen and nicotinamide (CON) may increase the efficacy of radiotherapy (RT).

Patients and Methods
Three hundred thirty-three patients with locally advanced bladder carcinoma were randomly assigned to RT alone versus RT with CON. A schedule of either 55 Gy in 20 fractions in 4 weeks or 64 Gy in 32 fractions in 6.5 weeks was used. The primary end point was cystoscopic control at 6 months ($C_{6}$) and secondary end points were overall survival (OS), local relapse-free survival (RFS), urinary and rectal morbidity.

Results
$C_{6}$ was 81% for RT + CON and 76% for RT alone ($P = .3$); however, just more than half of patients underwent cystoscopy at that time. Three-year estimates of OS were 59% and 46% ($P = .04$) and 3-year estimates of RFS were 54% and 43% ($P = .06$) for RT + CON versus RT alone. Risk of death was 14% lower with RT + CON ($P = .04$). In multivariate comparison, RT + CON significantly reduced the risk of relapse ($P = .05$) and death ($P = .03$). There was no evidence that differences in late urinary or GI morbidity between treatment groups or between fractionation schedules were significant.

Conclusion
RT + CON produced a small nonsignificant improvement in $C_{6}$. Differences in OS, risk of death, and local relapse were significantly in favor of RT + CON. Late morbidity was similar in both trial arms. Results indicate a benefit of adding CON to radical RT.

*J Clin Oncol 28:4912-4918. © 2010 by American Society of Clinical Oncology*

Hoskin et al. *JCO* 2010

- Carbogen and Nicotinamide improved OS and reduced relapse in bladder cancer
Summary

• Vasodilators improve tumour perfusion, oxygenation and radiation response
• Clinical trials of nicotinamide and carbogen with radiotherapy show improved locoregional control and disease-free survival (H&N ca), and overall survival (bladder ca)
Vascular modifiers

Decrease or disrupt vascular function:
Starve tumours of oxygen and nutrients after radiation

• Vascular disrupting agents (VDAs)
• Notch pathway
Vascular disrupting agents (VDAs)

- VDAs cause rapid shutdown of existing tumour vasculature (endothelial apoptosis), causing extensive tumour kill

Gridelli et al. *The Oncologist* 2010
Vascular disrupting agents

- VDAs have little effect as sole therapy
- VDAs potentiate tumour radioresponse when given after IR

Central necrosis caused by VDA (ZD6126) treatment (right panel)

Davis et al. *Cancer Res* 2002
Vascular disrupting agents (VDAs)

• VDA (ZD6126) and fractionated RT

Timing of administration very important
Vascular disrupting agents (VDAs)

Clemenson et al. *Crit Rev Onc/Hem* 2013
Vascular modifiers

Decrease or disrupt vascular function:

Starve tumours of oxygen and nutrients after radiation

• Vascular disrupting agents (VDAs)
• Notch pathway
Notch pathway

- Notch ligands and receptors are expressed on vasculature, tumor cells
- Mediated by direct cell-cell interactions (e.g., tumor cell with endothelial cell)
- Regulates tumor angiogenesis and other tumorigenic processes
DLL4-Notch blockade decreases tumour perfusion
Notch blockade and irradiation lead to supra-additive tumour growth delay
Summary

• Vascular disrupting agents cause acute collapse of tumour vasculature
• Anoxia induces tumour necrosis
• Notch inhibition impairs tumour angiogenesis and induces tumour necrosis after radiation
• Appropriate administration of these agents will be important – (after radiotherapy)
Summary

- IR can affect vasculature
  - At high doses - ceramide-mediated endothelial apoptosis
- IR induces tumour secretion of VEGF
  - May promote tumour angiogenesis
- IR can induce SDF-1 and recruit CD11b+ cells for revascularization
Summary

• Biological modulation of vasculature
  • Improve blood flow and increase tumour oxygenation prior to or early in radiation treatment:
    – VEGF blockade
    – Ang1
    – Vasodilators
  • Disrupt blood flow and starve tumour after radiation is delivered:
    – Vascular disrupting agents,
    – Notch inhibitor
    – Anti-SDF1 or CD11b antibody
Summary

• Timing of administration for vascular modifying agents with IR is important
• Biomarkers for appropriate patient selection are needed
Summary

• The tumour microenvironment profoundly influences response to treatment
• Modifying the microenvironment can enhance tumour radiation response