Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis

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Introduction

- Angiogenesis is pivotal for the progression of tumors.
- Endothelial cells (ECs) are an attractive target for therapy:
  - ECs are a homogeneous population; tumor type independent therapy.
  - ECs are the first to encounter the blood; easy drug delivery.
  - Endothelial cells are genetically stable; no drug resistance.
  - Multiple tumor cells depend on a single EC; avalanche of effect.
- Great efforts have been put to developing drugs targeting angiogenesis.
- Several drugs have been approved for use in the clinical setting.

Introduction

- Angiogenesis inhibitors targeting the VEGF signaling pathway have shown clinical benefit, survival of patients is increased with several months.
- Stable disease is not reached and the effects are only transient.
- Relapse to progressive disease suggests induction of resistance.
- The current study shows that tumors develop adaptive and invasive strategies to circumvent the inhibition of the VEGF signaling pathway.
Figure 1: Increased invasive phenotype after anti-VEGFR2 therapy

A

Control (end-stage) | Anti-VEGFR2 1 week | Anti-VEGFR2 4 weeks

H&E

Anti-T antigen

Anti-CD31

B

IT | IC1 | IC2

% viable area per animal

C

% total tumor per animal

12 wks | 13 wks | 14 wks | 15 wks | 16 wks
Considerations and questions

• No tumor growth curves or survival curves are presented.

• Vasculature/angiogenesis is not characterized.
  - Quantification of micro-vessel density
  - Vessel normalization
  - Vessel functionality, e.g. perfusion
  - EC proliferation

• Tumor cell invasion is scored qualitatively, not quantitatively.

• H&E staining is more difficult to interpret than T-Ag staining.

• Stainings for T-Ag and vasculature on different regions.

• Note: Control mice are dead at T=16 weeks.

Figure 2: Increased tumor invasion after tumor specific Vegf-A gene deletion
Considerations and questions

- Again, tumor growth and survival are not shown.
- Again, no characterization of vascular parameters, except for MVA.
- MVA is only compared within a genotype and not between genotypes.
- Why switch from anti T-Ag staining to anti-insulin staining?
- No confirmation of effective VEGF-A knockdown.
Figure 3: Increased incidence of lymph node and liver metastasis in anti-VEGFR2 treated animals

A

Lymph Node

Liver

H&E

Anti-T antigen IHC

100 μm

10 μm

B

C

Table: Incidence of metastasis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver Metastasis</th>
<th>LN Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VEGFR2</td>
<td>Yes: 7 (44%)</td>
<td>Yes: 11</td>
</tr>
<tr>
<td></td>
<td>No: 9 (60%)</td>
<td>No: 2</td>
</tr>
<tr>
<td>Control</td>
<td>Yes: 10</td>
<td>Yes: 15</td>
</tr>
<tr>
<td></td>
<td>No: 16</td>
<td>No: 15</td>
</tr>
</tbody>
</table>

* p < 0.05
** p < 0.01
Considerations and questions

- Again, tumor growth and survival are not shown.

- Other treatment scheme than in figure 1. In fig. 3, treatment started at 10 weeks and lasted for 10 days, whereas in fig. 1 treatment lasted for 1 or 4 weeks.

- No images of the control group are shown.

- It is counterintuitive that more liver metastasis are detected than lymph node metastasis.

- Mice were monitored until week 16 while the results from fig. 1 claim that all control mice die before they reach the age of 16 weeks.

**Figure 4: Increased life span and tumor reduction in sunitinib-treated RIP1-Tag2 animals**

![Graph showing increased life span and tumor reduction in sunitinib-treated RIP1-Tag2 animals.](image)
Considerations and questions

- Dull figure to demonstrate that sunitinib works.
- Treatment was continued for 5 weeks (B), which is different from the previous figures that emphasize that anti-VEGF treatment for only 1 week already induces clear changes.
- The survival curve refers to mice treated as from week 12 while the tumor burden refers to mice treated from week 10.
- The mice in (A) are treated continuously (> 7 weeks based on average survival difference) while mice in (B) received only 5 weeks treatment.
- What does this figure contribute to the paper?

Figure 5: Increased tumor invasion evoked by treatment with a multitargeted angiogenic kinase inhibitor
Considerations and questions

- Why not combined with figure 4?
- Only the perfused vessels are stained. It is not clear whether the treated tumors have less vessels or only less functional vessels.
- Again, treatment for 5 weeks for no apparent reason.
- Untreated controls in (B) and (C) are different from the data presented in Figures 1B and 3B.
- In (E), the number of metastasis detected in sunitinib treated mice is lower than in the control condition in figure 3C.
- Figure (C) lacks controls of untreated animals.
Figure 6: Effects of VEGFR-selective kinase inhibitors on an orthotopic mouse model of glioblastoma multiforme

A

WT GBM

Control | SU10944 | Sunitinib | VEGF-KO GBM

B

% Survival vs. Time (Days)

C

P values for comparison:

** indicates p < 0.01
Considerations and questions

- T-Ag staining not really clear.
- Tumor mass appears less in treated mice (less T-Ag detection) but increased in knock-out model.
- No characterization of vascular parameters
  Effect on vascular perfusion can be debated, why was perfusion used here and not staining?
- From 2 dimensional data it is not justified to conclude that 'GBM migrate alongside the blood vessels (perivascular invasion)'.
- Why is VEGF KO omitted from the survival curve?
- ‘Perivascular invasion’ scoring is arbitrary.
- the locations indicated by arrowheads do not really elucidate or clarify.
- GBM are known for their invasive phenotype and use of vessel co-option

Figure 7: Antiangiogenic treatment also provokes hypoxia in tumors and liver micrometastases
Considerations and questions

- Control treated animals (Aa and Ag) also have low vessel numbers but are apparently not hypoxic.
- The untreated WT tumor (Ad) is highly vascular as compared to Aa and Ag which are also WT and untreated.
- Why are different blood vessel stainings used (CD31, MECA 32)?

Conclusion of the paper

- VEGF targeted anti-angiogenesis therapy elicits an adaptive-evasive response involving an increased invasive phenotype and increased distant metastasis.
- The more effective the VEGF inhibition, the more pronounced the adaptive response.

Figure 8: Adaptive-evasive responses by tumors to anti-angiogenic therapies
Overall comments on this paper

- Inconsistency in experimental setup.
- Inconsistency between figures and experiments.
- Lack of proper controls.
- Many descriptive figures without any quantifications.
- No mechanistic insights.

Discussion

Proposed mechanisms that contribute to increased invasiveness.

- Activation of a pre-existing invasion program.
- Activation or elevated expression of matrix proteases.
- Epithelial-mesenchymal transition (EMT).
- Hypoxia-induced pro-invasive phenotype.
Discussion

The future of anti-angiogenesis therapy.

• Focus on the endothelium rather than on tumor-derived growth factors.
• Induction of vessel normalization.
• Combination therapy.
• Treatment scheduling.

Discussion

Suggestions for further reading.

• Review by Bergers and Hanahan.
  Modes of resistance to anti-angiogenic therapy. Bergers G, Hanahan D.
  Nat Rev Cancer. 2008;8(8).

• Comment by Carmeliet.
  Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited.