New Indigo workshop on “Antiparasitic and Antitumour drugs”
IBMC, 9th September, 2011

Targeting angiogenesis in cancer

Raquel Soares
Department of Biochemistry
Angiogenesis is required for tumors to grow beyond 1-2 mm$^3$
Angiogenesis results in Metastization
Angiogenesis blockage can be a good strategy to prevent tumor growth

Tumor angiogenesis: therapeutic implications

Angiogenesis is a complex process
Pro- and anti-angiogenic factors

**Inhibitors:**
- Thrombospondin-1
- The statins: Angiostatin, Endostatin, Canstatin, Tumstatin

**Activators:**
- VEGFs
- FGFs
- PDGFB
- EGF
- LPA

Nature Reviews | Cancer
1) Estrogens as potential targets for angiogenesis inhibition
Estradiol induces Notch1 expression and activity, enhancing angiogenesis in breast cancer *in vitro* and *in vivo*.

Notch signaling pathway

Notch:

- Highly conserved transmembrane receptor family (Notch1-4)
- Regulates cell migration in embryogenesis
- Is involved in angiogenesis
NOTCH signaling inhibits tip cell response in stalk cells

Tip cell numbers further increase through slightly elevated growth
Conclusions:

Estradiol induces expression of Notch1 in EC

enables the assembly of normalized functioning vessels

Mural cells’ recruitment is a PDGF gradient-dependent event

IMATINIB

- Also known as Gleevec®, Glivec® or STI571

- Potent inhibitor of tyrosine kinase receptors such as PDGFR-α and -β, c-Kit, Bcr-Abl protein

- FDA approved for chronic myeloid leukaemia (CML) gastrointestinal stromal tumours (GIST)
Imatinib inhibits PDGF signaling in Smooth Muscle Cells

2) Is Imatinib an angiogenic mediator?
**Elucidating Progesterone Effects in Breast Cancer: Cross Talk With PDGF Signaling Pathway in Smooth Muscle Cell**

Raquel Soares,1* Susana Gaerreiro,1 and Mónica Botelho2

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2IPATIMUP, Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Progesterone/control ratio</th>
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<tr>
<td>DNA repair</td>
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<tr>
<td>BARD</td>
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<td>Aurora-associated protein</td>
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**Anti-angiogenic effects of imatinib target smooth muscle cells but not endothelial cells**

Ana Rocha · Isabel Azevedo · Raquel Soares

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**Thin Cigarette**

**Bold Cigarette**
MELANOMA: THE MODEL

Miller and Mihm, 2006

C-Kit

PDGF
Investigate the anti-angiogenic effect of imatinib on vascular endothelium and mural cells, taking melanoma as a model
RESULTS: *in vivo*

IMATINIB EFFECTS’ ON MELANOMA ANGIOGENESIS

C57Bl/6 mice inoculated with B16 mouse melanoma cell line

**TUMOR GROWTH**

![Graph showing tumor growth over time with control and Imatinib treatments.](image)
RESULTS: *in vivo*

**IMATINIB EFFECTS’ ON MELANOMA ANGIOGENESIS**

Imatinib therapy decreased proliferation and increased apoptosis on tumour cells.
**RESULTS: in vivo**

**IMATINIB EFFECTS’ ON MELANOMA ANGIOGENESIS**

Imatinib affects vasculature

*Imatinib results in absence of support cells in mouse melanoma*

**Aortic ring assay**

Control  
PDGF  
PDGF + Imatinib

**SMA staining**

Control  
Imatinib

Imatinib inactivates PDGFR signaling (decreased P-ERK expression) in mouse melanoma

This work showed that imatinib:

- Abrogated B16 melanoma cells proliferation
- Increased B16 melanoma cells apoptosis
- Reduced vessels’ number and decreased the percentage of mural cells-stabilized vessels

Imatinib has a double effect *in vivo*
Distinct cell types contribute to angiogenesis
Angiogenesis and inflammation work together
Polyphenols

Present in diet (fruits, vegetables, beverages)

Anti-oxidant, Anti-inflammatory properties
3) Diet-derived polyphenols as potential anti-angiogenic agents
Polyphenols

Xanthohumol (XN)

- Prenylated chalcone
- Used in beer production (*Humulus lupulus*, L.)
- Metabolized to isoxanthohumol (IXN) and 8-prenylnaringenin (8PN)

Effects in angiogenesis?
XN, IXN and 8PN affect capillary-like structures formation
Beer-derived polyphenols effects in angiogenesis in C57Bl/6 mice

Effect of polyphenols in rat skin wound healing

Effect of polyphenols in rat skin wound healing

Effect of polyphenols in rat skin wound healing
Microvessel density

XN, IXN and 8PN interfere with gene expression profile in endothelial and smooth muscle cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>HUVEC</th>
<th>HASMC</th>
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<tr>
<td></td>
<td>XN</td>
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Polyphenols effects on TNF expression

ELISA assay

NF-κB promotes inflammation and angiogenesis in endothelial cells.
XN prevents angiogenesis *in vitro* (HUVEC) and inhibits NFκB activity

XN inhibits inflammation and angiogenesis in *in vivo* breast carcinoma

XN impairs inflammation and angiogenesis in *in vivo* breast cancer through NF-κB

Do platelets contribute to the angiogenic phenotype?

OPINION

Contribution of platelets to tumour metastasis

Laurie J. Gay and Brunhilde Felding-Habermann

Abstract | Extensive experimental evidence shows that platelets support tumour metastasis. The activation of platelets and the coagulation system have a crucial role in the progression of cancer. Within the circulatory system, platelets guard tumour cells from immune elimination and promote their arrest at the endothelium, supporting the establishment of secondary lesions. These contributions of platelets to tumour cell survival and spread suggest platelets as a new avenue for therapy.
Could platelet-accumulating polyphenols prevent tumour metastasis?

Rita Negrão, Delfim Duarte, Raquel Costa and Raquel Soares

We read with great interest the Review by Gay and Felding-Habermann (Contribution of platelets to tumour metastasis. Nature Rev. Cancer 11, 123–124 (2011))\(^1\), which discussed the observation that cancer patients usually present signs of throm-

We have been studying the effects on angiogenesis and inflammation of a group of naturally derived compounds, polyphenols, which have established anti-oxidant, anti-inflammatory, anti-angiogenic and antitumour properties. The topical administration of
Conclusions

Beer-derived polyphenols (XN and IXN) impair oxidative stress, inflammation and angiogenesis, three processes associated with cancer progression.

Therefore, they might be helpful cancer therapeutic/preventive agents.
Take-home message

- Angiogenesis is a crucial event in cancer
- Estrogens lead to normalized vessels
- Some cancer therapy agents also affect vascular wall cells
- Angiogenesis strongly associates with inflammation and oxidative stress in cancer (and other diseases)
- Anti-inflammatory and anti-oxidant agents (e.g. polyphenols) might be useful in controlling tumor angiogenesis
Angiogenesis blockage can be a good strategy to prevent tumor growth
Angiogenesis inhibition results in hypoxia

Hypoxia induces Angiogenesis
Normalizing vs inhibiting vascularization
Acknowledgements

Ana Pirraco
Ana Rita Silva
Ana Sofia Rocha
Ângela Castela
Carla Costa
Delfim Duarte
Pedro Coelho
Raquel Costa
Rita Negrão
Susana Guerreiro

Funding:
• ERAB – European Research Advisory Board
• FCT
• Fundação Prof Ernesto Morais
• iBeSa
• Novartis Oncology
• Portugal-Spain international Nanotechnology Laboratory