Center for Translational Research in Oncology
Instituto do câncer do estado de São Paulo
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VISION: ICESP will interface the Secretary of Health and the Academic Network for Cancer Research, allowing for the completion of translational projects of interest to Public Health in the area of Oncology.
The tumor microenvironment as a target for experimental combination therapy in tumors

Universidade de São Paulo, ICESP/FM

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Support: FAPESP, CNPq, NIGMS/NIH Consortium for Functional Glycomics and UICC-YY grant
Galectins, a family of $\beta$-galactoside binding lectins, have a conserved carbohydrate recognition domain (CRD).

**Proto**

Galectins 1, 2, 5, 7, 10, 11, 13, 14, 15

**Chimera**

Galectin-3

**Tandem Repeat**

Galectins 4, 6, 8, 9 and 12
Galectins have a highly conserved secondary structure with internally oriented hydrophobic residues in β strands in the β-sandwich of the galectin fold. Prototypical galectins are found as dimers in the extracellular space, galectin-3 is found as a pentamer/aggregates in the extracellular space.
Galectin-3 amplifies the intensity of macrophage-dependent immune responses and interferes with B cell differentiation into plasma cells (Notch signalling).

Monocyte/Macrophage

Infection-related granulomatous inflammation

Th1
Toxoplasma gondii
Rhodococcus equi

Th2
Schistosoma mansoni

P. brasiliensis

Tumor-associated inflammation

M1

M2

B cell/Plasma cell


Oliveira et al. 2007, J. Leuk. Biol. 82:300-310
Oliveira et al. 2009, Glycobiology 19:1248-1258
Galectin-3 expression is dynamic, responding to microenvironmental stimuli such as hypoxia and nutrient deprivation found in glioma pseudopalisades.
Galectin-3 expression is induced and the protein accumulates in cells exposed to hypoxia and **serum deprivation**.
Upregulation of gal-3 in hypoxia and serum deprivation depends on HIF-1α and NF-κB

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0111592
Gal-3 knockdown sensitizes cells to cell death in oxygen and nutrient deprivation in the NG97ht cell line.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0111592
U87MG glioma cells transduced with gal-3 shRNA demonstrate decreased tumor size and growth.

http://127.0.0.1:8081/plosone/article?id=info:doi/10.1371/journal.pone.0111592
Galectin-3 is present in lamellipodia of migrating cells

Melo et al. (2011) Plos one e29313
Gal-3 null (Σ12) cells are more adherent and less migratory on laminin-1 surfaces than gal-3 +/+ cells (S12)

Cell Adhesion

Cell Migration

Melo et al. (2011) Plos one e29313
Gal-3 expression in gal-3 null cells rendered them less adherent and more migratory on laminin-1 surfaces.

Cell Adhesion

- P<0.01

Cell Migration

- p<0.05
- p<0.01

Melo et al. (2011) Plos one e29313
Extracellular galectin-3 destabilizes focal adhesion plaques

Melo et al. (2011) Plos one e29313
Cell migration triggered by galectin-3 is associated with AKT phosphorylation; it is dependent upon PI-3K activation.
Increasing complexity: the role of galectin-3 in the build up of a vascularized tumor.

Tm1 melanoma cells were selected from a “normal” melanocyte cell line and do not express galectin-3.
Tm1 tumor growth is impaired in galectin-3 deficient mice (KOTm1 or KON3).

Galectin-3, either from tumor or stromal origin, stimulates melanoma growth.

Machado et al. (2014) Cancer Med. 3:201-14
Absence from galectin-3 from both tumor cells and stromal cells (KON3) favor necrosis, associated with decreased/unsustained angiogenesis.
VEGF expression is decreased within tumors devoid of galectin-3. TGF-β accumulates in VEGF poor tumors (a compensatory mechanism?)

Machado et al. (2014) Cancer Med. 3:201-14
Macrophage homing to tumors is altered in the absence of galectin-3. Galectin-3 deficient macrophages produce less VEGF than wild type macrophages (attenuated M2 phenotype of galectin-3 deficient macrophages)
Davanat, a pectin that acts as a galectin inhibitor, may be used to control tumor growth.
Summary-1

Galectin-3 and “the run or die” hypothesis

✔ Galectin-3 expression is increased in stressed tissue microenvironments associated with tumor cell migration and tissue remodelling, inducing angiogenesis.

✔ Endogenous galectin-3 accumulated in tumor cells exposed to hypoxia and nutrient deprivation. Under these conditions, endogenous galectin-3 favoured survival (*not a lectin function*).

✔ Extracellular galectin-3 is targetable by pectins, which may be used to disrupt tumor cell migration and tumor-associated angiogenesis.
Imaging the tumor microenvironment in experimental melanoma models

Angiogenesis and vascular function control as targets for combination therapy in experimental tumors

Vasoactive peptides and their angiogenic/vascular permeability functions

• Angiotensin II antagonists and bradykinin antagonism
• Imag(in)ing tumor vasculature function and interfering with tumor perfusion
Angiotensin II, Bradykinin and des-Arg-Bradykinin control the vascular tonus and other endothelial cell functions. Angiotensin converting enzyme (ACE) connects both systems.

Decrease vascular tonus

Increase vascular tonus

Losartan, candesartan

AT1R

AT2R

ACE

Angiotensin II

Bradykinin

Angiotensin I

Kininogen

B2R

B1R
PRESENCE OF AT1 RECEPTORS AND ANGIOTENSIN II IN HUMAN MELANOMA TISSUES

AT1 Receptor

Angiotensin II

Maximal tolerated dose of Losartan

Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanotherapeutics in tumors

Benjamin Diop-Frimpong\textsuperscript{a,b,c}, Vikash P. Chauhan\textsuperscript{a,c}, Stephen Krane\textsuperscript{d}, Yves Boucher\textsuperscript{a,1,2}, and Rakesh K. Jain\textsuperscript{a,1,2}

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Contributed by Rakesh K. Jain, December 21, 2010 (sent for review October 20, 2010)
Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels.

Angiotensin II, Bradykinin and des-Arg-Bradykinin control the vascular tonus and other endothelial cell functions. Angiotensin converting enzyme (ACE) connects both systems.

Angiotensinogen $\xrightarrow{\text{Renin}}$ Angiotensin I $\xrightarrow{\text{ACE}}$ Angiotensin II

Kininogen $\xrightarrow{\text{Kallikreins}}$ Bradykinin $\xrightarrow{\text{Carboxypeptidases}}$ des-Arg<sup>9</sup> Bradykinin

B2R $\xrightarrow{\text{Bradykinin}}$ B1R $\xrightarrow{\text{des-Arg}<sup>9</sup> - Bradykinin}}$

R-715, R-954

Decrease vascular tonus Increases vascular tonus
Increase vascular permeability Induces angiogenesis
The BKR1 antagonists R715 and R954 increased doxorubicin uptake within tumors.

CD31/Doxorubicin

...adverse effects of R954/R715 include a transient increase in blood pressure.
Doppler studies

B16-F10 melanoma

Chammas et al., in preparation
Both angiotensin II and its receptor (AT1) are present within the tumor microenvironment of human melanomas and murine melanomas.

The antihypertensive agent Losartan has a dual function, controlling not only the vascular tonus, but also controlling angiogenesis.

*Off label indications of old drugs (Losartan, e.g.) may help managing cancer patients.*

*Opportunity for an academic clinical trial*

Bradykinin receptor 1 antagonists may lead to secondary local and transient hypertension, favouring drug delivery to experimental tumors. Transient increase in tumor perfusion can also be induced through usage of hypertonic saline solutions.

Multimodality imaging allowed for devising a strategy of combination therapy to improve drug delivery.
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www.icesp.org.br

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