Ninth European International Kidney Cancer Symposium
25-26 April 2014
The Convention Centre Dublin
with the Gibson Hotel
Dublin, Ireland
KidneyCancer.com
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Epithelial to Mesenchymal Transition (EMT), Metastasis and the Metastatic Microenvironment

Isaac P. Witz
Tel Aviv University
Tel Aviv, Israel

Dublin, April 2014
The Metastatic Cascade

1) Tumour cell growth
2) Angiogenesis
3) Invasion
4) Intravasation
5) Survival in circulation
6) Arrest in new organ
7) Extravasation

Single cell
Death
"Dormant"
Clinically undetectable metastasis

Micrometastasis
Proliferation
"Dormant"
Growing metastasis

Disseminated tumor cells

Cell death

Clinically detectable metastasis
SITE-SPECIFIC METASTASIS

“When a plant goes to seed its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil.”
Stephan Paget, The Lancet, 1889

seed = tumor;
soil = metastatic microenvironment
From the Primary Tumor to the Metastatic Site

Primary tumor

Chemokine-Chemokine Receptor Axes

Cancer cells

Epithelial to Mesenchimal Transition

Cancer cells

The metastatic site

Ligand

Lungs

Liver

Brain
Epithelial-Mesenchymal Transition (EMT)

- A process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties
- Cells undergoing EMT are characterized by phenotypic changes such as loss of the epithelial marker E-cadherin and a gain of the mesenchymal marker vimentin
- Many of the EMT inducers originate in the tumor microenvironment
- Initiation of metastasis requires invasion, which is enabled by EMT
Epithelial-Mesenchymal Transition (EMT)
EMT leads to metastasis

1. EMT signals at tumor margin
2. Partial EMT state facilitates motility and invasion into stroma
3. Mesenchymal phenotype facilitates intravasation and anoikis resistance during dissemination and extravasation
4. Migrating cancer stem cell
5. Survival and dormancy at distant site
6. Exit from dormancy and early colonization
7. Colonization: proliferation and formation of macrometastasis

Epithelial cell → Quasi-epithelial cell → Quasi-mesenchymal cell → Mesenchymal cell
Partial EMT

WL Tam & RA Weinberg, Nat Med. 2013
EMT occurs in renal cell carcinoma

- Snail, a major regulator of EMT, is predominantly expressed in high-grade RCC, and high Snail expression is a bad prognostic factor (Mikami et al. Med Mol Morphol. 2013).
- miR-145 functions as tumor suppressor in RCC. It regulates numerous genes involved in EMT (Lu et al. J Cancer Res Clin Oncol. 2014).
- Vimentin may function as an oncogene and is regulated by tumor suppressive miR-138 (Yamasaki et al. Int J Oncol. 2012).
- A high ZEB2 expression in RCC may facilitate the acquisition of an aggressive phenotype. ZEB2 overexpression is an independent biomarker for the poor prognosis of patients with RCC (Fang et al. PLoS One. 2013).
- Mutations of the von Hippel-Lindau (VHL) gene are major risk factors for the development of familial and sporadic RCC. VHL mutations up-regulate and stabilize HIF, which in turn activates many downstream molecules, including EPO. EPO may also stimulate epithelial-mesenchymal transition (EMT) in RCC (Morais et al. BMC Cancer. 2013).
- Hypoxia induces EMT in RCC cells through down regulation of miR-30c, which leads to subsequent increase of Slug expression and repression of E-cadherin. A potential application of miR-30c in RCC treatment? (Huang et al. Cancer Sci 2013).
- TNF-α induced EMT and promoted invasion and tumorigenicity of RCC by repressing E-cadherin, up-regulating vimentin, activating MMP9 (Ho et al. Mol Cancer Res. 2012).
The Metastatic Cascade

It is the *metastatic* microenvironment that regulates progression.

Sharon F. McGee et al (2010), RCSI
THE TUMOR MICROENVIRONMENT (TME)

- Resident cells (e.g. endothelial cells, fibroblasts)
- Infiltrating cells (e.g. lymphocytes, macrophages, fibroblasts)
- ECM (e.g. collagen, fibronectin)
- Released molecules (e.g. cytokines, chemokines, antibodies, proteases, angiogenic factors)
- Hypoxia
- Drugs

These factors may interact with tumor cells & with non-tumor cells in the TME
These interactions regulate gene expression in non-tumor cells or cancer cells

Outcome: progression; dormancy or death
The metastatic microenvironment

primary melanoma
The metastatic microenvironment

• What directs tumor cells to different organ sites?
• Different organ sites - different microenvironments - microenvironmental signals differ from organ to organ
• Do tumor cells in one metastatic site differ from tumor cells in other sites? site specific metastatic signature?
• What keeps micrometastasis in a state of dormancy?
• What awakens dormant micrometastases? - organ specific survival and growth factors?
Human to Mouse Xenograft Models

Non-metastatic & metastatic variants originate from the same human tumor i.e. they have an IDENTICAL GENETIC BACKGROUND.

Genetic, proteomic and/or transcriptomic differences between such variants may therefore be attributed to their differential metastatic capacity.
Neuroblastoma: The most common solid malignant tumor in children

Interactions of disseminated neuroblastoma cells with the lung microenvironment
Adrenal inoculation of human NB cell lines: SH-SY5Y MHH-NB11

Nevo et al. Neoplasia, 2008,
Edry-Botzer et al. Am J. Pathol, 2011,
Lung Metastasis in Mice Xenografted Orthotopically with Cells from Local NB Tumors or from Lung Mets

Lungs of mice with macro metastasis

(cDNA)

Real Time PCR

Lungs of mice with no overt metastasis

(cDNA)

- Human NB metastases in mouse lung

(β2 microglobulin)

Normalizing gene (RS9)

Lungs of mice with Maco metastasis

Lung of mice with Micro metastasis

Lung of mice with Macro metastasis

Lung of mice with Micro metastasis

Normalized fluorescence

Normalized fluorescence

Cycle

Cycle

β2M/RS9 mRNA copies

0 1000 2000 3000 4000 5000 6000

Micro

Macro

Lungs of mice xenografted orthotopically with metastases contain more human NB cells than lungs of mice xenografted with cells from local tumors

Edry-Botzer et al. Am J. Pathol. 2011
Micrometastasis

• Dormant tumor cells; Disseminated tumor cells micrometastases

• Are micrometastases the progenitor cells for metastases? What keeps them dormant?

• Do dormant micrometastases “wake up”? If yes what wakes them up? Are genomic/epigenomic alterations in the dormant tumor cells involved? Is it the metastatic microenvironment? Both?

• Can the “awakening” be inhibited?
NB lung micro-metastatic cells:
- form tumors in the orthotopic site (adrenal)
- proliferate in-vitro hence
they are dormant in the lung microenvironment
What keeps micro-metastasis dormant in the metastatic site?

“Why do we not all die of cancer at an early age?”


Microenvironmental control
Non-immunological surveillance
Hypothesis:
Organ (specific?) factors restrain the proliferation of metastatic tumor cells
Are Lung-Derived Soluble Factors Involved in Keeping NB Micrometastases Dormant?
**Lung-Derived Factors Reduce NB Viability**

**Cell viability**

![Graph showing cell viability](image)

- MicroNB: ***** P<0.005
- MacroNB: ** ** P<0.01

**ERK phosphorylation**

![Graph showing ERK phosphorylation](image)

- MicroNB: ** *** P<0.005
- MacroNB: ** ** P<0.01

* - P<0.05, ** - P<0.01, *** - P<0.005
Lung-Derived Factors Cause Apoptosis and G0-G1 Arrest in NB Cells

Cell cycle arrest

Apoptosis

* - P<0.01, ** - P<0.005
Identifying the Inhibitory Factor in Lung-Derived Factors

- Lung-derived factors
  - HPLC separation
    - Purification of the inhibitory factor from the bio-active HPLC fraction
      - Identification of the inhibitory factor
Significance

Endogenous metastasis-restraining bioactive factors could:

• Regulate tumor progression
• May overcome drug resistance
• Serve as an antitumor agent
Emphasis:
Primary tumor

The metastatic microenvironment

Micrometastasis

Macrometastasis

Cancer cell

Migrating cancer cell

Endothelial cell

Chemokine receptor

Chemokine

From: Mamman & Witz in: The Tumor Microenvironment, Springer, Shurin, Umansky & Malyguine editors, 2013
Acknowledgements

Witz Group
Roman Bengaiev
Shlomit Ben-Menachem
Dr. Liat Edri-Botzer*
Dr. Sivan Izraely
Anat Klein
Shelly Mamman
Tsipi Meshel
Osnat Naftali
Dr. Ido Nevo*
Maya Rappaport
Dr. Orit Sagi-Assif
Inna Zoubrilov

Tel Aviv University
Dr. Neta Erez, Dr. Marcello Ehrlich, Dr. Metsada Pasmanik-Chor,
The Chaim Sheba Medical Center, Tel Hashomer
Dr. Galia Tsarfaty
John Wayne Cancer Institute, Santa Monica, CA.
Dr. Dave S.B. Hoon
Institute of Human Virology, University of Maryland, Medical School, Baltimore, MD
Dr. Wuyuan Lu
Department of Cell Biology, Institut Cochin, Paris
Dr. Clara Nahmias, Dr. Pierre-Olivier Couraud
DKFZ, Heidelberg
Dr. Manfred Schwab, Dr. Larissa Savelyeva,
Dr. Tobias Bäuerle, Dr. Frank Westermann
Institute of Cancer Research, CCC, Medical U.
Vienna
Dr. Walter Berger, Dr. Christine Pirker, Dr. Michael Grusch
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