BIOLOGICAL MECHANISMS AND SIGNIFICANCE OF NODAL METASTASIS

Selected aspects

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W. S. Halsted: Lymph node metastasis is the first step in distant metastasis.

B. Fisher: Metastatic cancer is a systemic disease from the onset.
### INVASION & METASTASIS: cause of cancer-specific death

<table>
<thead>
<tr>
<th>TUMOR</th>
<th>SPREAD</th>
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<tbody>
<tr>
<td></td>
<td>Local</td>
</tr>
<tr>
<td>Brain</td>
<td>100%</td>
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<tr>
<td>Head- Neck</td>
<td>75%</td>
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<tr>
<td>Colorectal</td>
<td>25%</td>
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<tr>
<td>Prostate</td>
<td>5%</td>
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<tr>
<td>Breast</td>
<td>5%</td>
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<tr>
<td>Kidney</td>
<td>5%</td>
</tr>
<tr>
<td>Melanoma</td>
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LYMPH NODE METASTASIS: DIAGNOSIS, THERAPY, PROGNOSIS

LYMPH NODE METASTASIS (LNM):
METASTATIC CANCER: COMMUNICATING ECOSYSTEMS
THE ELEMENTS
Metastatic Cancer Ecosystems

Cancer Cells (SEED) + Host Cells (SOIL)
Mammary Cancer: Post Radiotherapy Cytotoxic T Lymphocytes

Formenti & Demaria, Lancet Oncol 10: 718, 2009
Colorectal Cancer
Cancer-associated fibroblasts

Provided by P. Demetter
HOST CELLS CONTRIBUTE TO TUMOR MALIGNANCY

14-day collagen invasion

HCT-8/E11  

HCT-8/E11R1

control  

+ myofibroblasts

HOST CELLS CONTRIBUTE TO TUMOR MALIGNANCY

Van Hoorde et al., Int J Cancer 88: 751, 2000
THE TUMOR ECOSYSTEM: MULTIPLE CELL TYPES AND SUBTYPES

ANGIOGENESIS

OSTEOLYSIS

IMMUNOMODULATION

INFLAMMATION

THE TUMOR ECOSYSTEM: MULTIPLE CELL TYPES AND SUBTYPES

NEUROGENESIS

ANGIOGENESIS

OSTEOPLASIA

OSTEOLYSIS

IMMUNOMODULATION

INFLAMMATION

THE TUMOR ECOSYSTEM: MULTIPLE CELL TYPES AND SUBTYPES

NEUROGENESIS

ANGIOGENESIS

OSTEOPLASIA

OSTEOLYSIS

IMMUNOMODULATION

INFLAMMATION
VEGFA/VEGFR2: Angiogenesis and Immunomodulation

M2

LEC

CD8+TC

CC

VEGFA

CD8

VEGFR2

MERGE

VEGF/VEGFR: Molecular Complexity

Modulated at various levels (DNA, RNA, miRNA, protein etc.)

Members of molecular families

Multidomain proteins

Direct interaction with various other molecules

Participation at complex signaling

Mediators of multifunctional responses

Secker and Harvey, Dev. Dyn. Nov. 14, 2014
THE LYMPH NODE AND ITS VASCULAR CONNECTIONS

THORACIC DUCT

LYMPH NODES

EFFERENT LV

NORMAL TISSUE

PRIMARY TUMOR

LYMPH NODES

AFFERENT LV

ARTERIAL CIRCULATION

VENOUS CIRCULATION
THE LYMPH VESSELS: LYMPHENDOTHELIAL CELLS (LECs)

LECs MARKERS:

LYVE1
PODOPLANIN (D2-40 Mab)

The microenvironment of each organ (the “soil”) influences the survival and growth of tumor cells (the “seed”).

THE PREMETASTATIC NICHE

The tumor prepares the soil before arrival of the cancer cells

Paget S., Lancet 1: 571 (1889)
The Pre-metastatic Niche

“Der prekanzerose Sinuskatarrh.

Hyperplasie der Lymphatischen Gewebes …. 

Ein notwendige Vorläufer, der den Boden für das Angehender sekundären Geschwulst prepariere”.

Hans E. Walther, Krebsmetastasen
Benno Schwabe & Co Verlag Basel, 1948
PREMETASTATIC NICHE IN LYMPH NODES
EXPERIMENTAL

K14/GFP
K14/VEGFA

X

K14/GFP/VEGFA

Multistep skin carcinogenesis

PREMETASTATIC NICHE IN LYMPH NODES: LYMPHANGIOGENESIS AND LYMPHATIC ENLARGEMENT

Lymphangiogenesis: LECs ARE KEY TARGET CELLS

Li & Li, Cancer Letters 357:438, 2014; Stacker et al; Nat Rev Cancer 14: 159, 2014
HYPOXIA and VEGF and LYMPHANGIOGENESIS

proline hydroxylase

+ O₂

normoxia

HIF-1α

hypoxia

recognition and binding by pVHL and two other proteins

poly-ubiquitylation

degradation in proteasome

OH OH

OH OH

HIF-1α

HIF-1β

transcription

VEGF gene

VEGF

transcription
LYMPHANGIOGENESIS: VEGFC-VEGFR2 ..........AND OTHERS.
Protease-activated receptor-2 (PAR-2) An Inhibitor of Lymphangiogenesis
LYMPH NODES AND TUMOR IMMUNITY

TUMOR

VESSEL

DENDRITIC CELL

TAA

HELPER CELL

LYMPH NODE

CYTOTOXIC T CELL

T AA

Co

TCR
VISUALIZING IMMUNE CELL INTERACTIONS INSIDE LYMPH NODES

Immune Synapses

From Stoll S et al., 2002 Science 296:1873
THE IMMUNE BALANCE IN UNTREATED MALIGNANT TUMORS

STAT3<sup>P</sup> vs. STAT1<sup>P</sup>

Proliferation, Motility, Immune escape, Invasion, Angiogenesis, Metastasis vs. Immune surveillance, Cytotoxicity, Apoptosis

IMMUNOSUPPRESSIVE vs. IMMUNOENHANCING

Oliveira M. & Velho S., in: Kevin M.

Haigis Editor Springer, 2013
LECs CONTRIBUTE TO TUMOR IMMUNE SUPPRESSION

Podgrabinska and Skobe, Microvascular Research 95: 46, 2014
Material & Methods

TRANSFECTANT CELLS

SIGNALING Pathways

BIOLOGICAL TESTING

IN VITRO : Cellular activities

IN VIVO: Lymphangiogenesis; LNM

TRANSGENIC ANIMAL MODELS

SYNGENEIC

IMMUNE-SUPPRESSED (Nu/Nu etc.)

HUMAN TUMORS

PATHOLOGY

REGIONAL FAILURE/CONTROL
Is Lymphangiogenesis causally related to LNM?

Podgrabinska and Skobe, MicrovascularResearch 95: 46, 2014
Data from Karaman & Detmar, J Clin Invest 124: 922, 2014
with less order and coordination than observed in developmental EMT. Numerous inducers of EMT in cancer cell lines have been identified including Transforming Growth Factor-β (TGF-β), Wnt, Snail/Slug, Twist, and Six1, and these oncogenic EMT inducers are also critical during developmental EMT. These regulators will be discussed more below.

In the context of epithelial cancer, EMT provides a mechanism for tumor cells to leave the primary tumor and invade into the local tissue and blood vessels, setting the stage for metastatic spread (Fig. 2). Therefore, EMT is hypothesized to contribute to tumor progression, and indeed clinical evidence suggests that regulators of EMT in cancer cells correlate with poor patient outcomes and tumor aggressiveness. However, unlike developmental EMT, which typically proceeds stepwise under the tight control of morphogenic signals and generally correlates with a cell fate change, oncogenic EMT occurs in the context of unpredictable genetic changes present in the tumor cells, as well as an abnormal local tumor environment. Therefore, EMT in cancer is much more difficult to predict and observe in vivo, since only a subset of tumor cells may undergo EMT at any one time. In colon cancer, cells exhibiting properties of EMT have been specifically localized to the periphery of the tumor where they are primed to undergo EMT likely by exposure to cytokines and an extracellular milieu that promotes EMT. Much of the recent work on inducers of EMT has utilized tissue culture models to assess the epithelial and mesenchymal phenotype of cancer cell lines. However, numerous EMT inducers characterized in cell culture systems can initiate metastatic spread in animal models, and in fact correlate with poor prognosis in human cancer. Therefore, misexpression of EMT regulators in cancer appears to have clinical significance. Importantly, the reactivation of developmental programs in cancer offers the potential to identify new drug targets that inhibit the metastatic process and may also be specific to the cancer, leaving the fully differentiated adult epithelium unaffected.

Examples of Developmental EMT
During development the first instance of EMT occurs during gastrulation, giving rise to the primary mesenchyme and the formation of the three primitive germ layers (Fig. 3a). Signaling molecules emanating from the Spemann-Mangold organizer, a region of the embryo critical for axis formation and neuralization, act on cells at the primitive streak to induce EMT and to specify cell fate. During gastrulation, the first observable evidence of EMT is the breakdown of the basement membrane underlying the epiblast. Subsequently, the action of Fibroblast Growth Factor (FGF) on the cells in the primitive streak results in an upregulation of the zinc finger transcription factor Snail, repressing E-cadherin and destabilizing cell-cell junctions. Additional signals...
Colorectal Cancer

Primary Tumor

? EMT?

EMT

MET

Lymph Node Metastasis

Courtesy of P. Demetter
DO METASTASES METASTAZISE?

PT

N

M1

M2

NO ?

YES ?
**THE CANCER CELL POPULATION IS HETEROGENEOUS**

**Advanced Stage Primary Tumor**

**DISSECTION & SEPARATION**

- **MIC**
  - Leads to **LUNG METASTASIS**

- **MIC**
  - Leads to **BONE METASTASIS**

- **CSC**
  - Tumorigenic
  - Leads to **NO METASTASIS**

- **Non-CSC**
  - Non-Tumorigenic
  - Leads to **NO METASTASIS**

Genetic Analysis of Human Cancers

Yashida et al. 2010, Nature 467: 1114
CONCLUSION

LYMPH NODES,
NORMAL, PRE-METASTATIC AND METASTATIC
ARE ELEMENTS OF THE COMMUNICATING ECOSYSTEM OF METASTATIC CANCER.

NONE OF THE COMPONENTS OF THESE LYMPH NODES,
CELLULAR AND MOLECULAR
SHALL BE OVERLOOKED WHEN ANALYZING
THE DIAGNOSTIC, THERAPEUTIC AND PROGNOSTIC VALUE OF
LYMPH NODE METASTASIS

Ghent, 14 February 2015