THE MAJOR CAUSE OF DEATH FROM CANCER IS DUE TO METASTASES THAT ARE RESISTANT TO CONVENTIONAL THERAPY
THE PATHOGENESIS OF METASTASIS

TRANSFORMATION

ANGIOGENESIS

MOTILITY & INVASION

Capillaries, Venules, lymphatic vessels

EMBOLISM & CIRCULATION

TRANSPORT

MULTICELL AGGREGATES
(lymphocytes, platelets)

RESPONSE TO MICROENVIRONMENT

TUMOR CELL PROLIFERATION & ANGIOGENESIS

EXTRAVASATION INTO ORGAN PARENCHYMA

ADHERENCE

ARREST IN CAPILLARY BEDS

TRANSPORT

METASTASIS OF METASTASES

METASTASES
METASTATIC INEFFICIENCY

LESS THAN 0.01% OF CELLS THAT ENTER THE CIRCULATION SURVIVE TO ATTACH IN CAPILLARY BEDS OF DISTANT ORGANS
DOES THE DEVELOPMENT OF METASTASES REPRESENT

CHANCE SURVIVAL OF TUMOR CELLS

OR

SELECTIVE GROWTH OF SPECIALIZED TUMOR CELLS?
in vivo Enrichment for Metastatic Cells

One Step Selection
METASTATIC TUMOR CELL VARIANTS:

1. ARISE DURING METASTASIS BY THE PROCESS OF ADAPTATION.

2. PREEXIST IN THE PARENTAL NEOPLASM.
CANCER METASTASIS

THE PROCESS IS HIGHLY SELECTIVE FOR PREEXISTING METASTATIC CELLS

METASTASES ARE CLONAL IN ORIGIN

TUMOR CELLS IN GENERAL AND METASTATIC CELLS IN PARTICULAR ARE GENETICALLY UNSTABLE

CANCERS ARE BIOLOGICALLY HETEROGENEOUS
METASTASIS CAN NOT BE A RANDOM PROCESS BECAUSE PATTERNS OF METASTASIS ARE PREDICTABLE.
‘SEED AND SOIL’ HYPOTHESIS

- 735 Breast Cancer Patient Records Analyzed
- Discrepancy Between Blood Supply and Frequency of Metastases in Various Organs

Stephen Paget, M.D.
Lancet 1: 571-573, 1889
SEED AND SOIL HYPOTHESIS

- PATTERNS OF METASTASIS ARE PREDICATABLE.
- CERTAIN TUMOR CELLS (SEED) HAVE AN AFFINITY FOR CERTAIN ORGANS (SOIL).
- METASTASIS OCCURS ONLY WHEN THE SEEDS AND THE SOIL ARE COMPATIBLE.

DR. STEPHEN PAGET, 1889
Organs from 1-day-old mouse

1-mm³ fragments

Fragments implanted into quadriceps femoris

3 weeks later

Lung-specific melanoma cells

4 weeks later

Hemodynamic

or

Hemodynamic seed and soil
Observations from a ploughman

"What is it that decides what organs shall suffer a case of disseminated cancer?" This question intrigued Stephen Paget, assistant surgeon to the West London Hospital and the Metropolis, hospital, whose self-penetrating paper of 1889 records his careful analysis of case histories that led to the visionary soil and seed hypothesis of metastasis.

"When a plant goes to seed, its seeds are carried in all directions," he wrote. "But they can only live and grow if they fall on congenial soil." This idea was at odds with one prevalent theory of the time, which stated that cancer cells, having been ignited through the body in the blood or lymph, could lodge in a tissue and persuade the surrounding cells to grow similarly. However, Paget followed the school of thought that all cancer cells could continually develop wherever they settled, but grew only in certain organs that were somehow predisposed to a secondary cancer.

Paget reasoned that if the organs where secondary tumours arose were "passive" in the process, then these cancers would be distributed randomly. By analyzing 716 case histories of fatal breast cancer, he found that metastases formed in the liver far more often than in any other organ — even those such as the spleen that could be considered to have the same exposure to the cancer cells because of similar blood flows.

This was enough to persuade Paget that sites of secondary growths are not a matter of chance, and that some organs provide a more fertile environment than others for the growth of certain metastases. "The best work in the pathology of cancer is now done by those who ... are studying the nature of the soil," he noted. "They are like scientific botanists, and he who turns over the records of cases of cancer is only a ploughman, but his observation of the properties of the soil may also be useful."

This proved to be the case and, although it languished in the shadows for many years, the seed and soil hypothesis was revived fully in 1980 by Ian Hart and Russell Fidler. By this time, clinical observations had established that certain organs were, indeed, more susceptible to metastasis, even after specific properties of the tumour cells and other host factors had been accounted for. So, Hart and Fidler examined whether the locations of metastases exist merely because tumour cells tend to come to rest in particular organs — for instance, because the blood capillaries are more narrow — or because the distributed cells can only grow at particular sites, in accordance with the Paget hypothesis. Using mice, they grafted kidney, ovary and lung tissue under the skin or into the muscle, and showed that the transplanted tissues established their own blood supply. They then injected the mice with metastases. Metastases developed in the grafted lung and ovary tissues but not in the skin tissues, thereby showing a distinct preference.

Notably, radioactive labelling of the injected cells showed that they were equally likely to be trapped in the kidney tissue as in either of the other transplants. So, just landing in a tissue is not sufficient for cancer cells to develop a secondary tumour, rather, some property of the tissue itself must sustain the new growth. The idea that cancer cells require some "environment" from their environment to develop still motivates research today, with the focus now being on unravelling the molecular mechanisms that bring seed and soil together to promote metastases.
CANCER METASTASIS

PRIMARY NEOPLASMS ARE HETEROGENEOUS.

THE PROCESS IS HIGHLY SELECTIVE FOR PREEXISTING METASTATIC CELLS.

THE PROCESS DEPENDS ON THE INTERACTION OF TUMOR CELLS WITH HOST FACTORS.
Brain metastasis

20-40% of all cancer patients develop CNS metastasis

In adults the primary tumors are:

- Lung (50-60%),
- Breast (15-20%),
- Melanoma (8-10%)
- GI tract (5-7%)

Brain metastasis

For untreated patients the median survival is 1-2 months.

Conventional treatment (radio-chemotherapy) can extends the median survival to 4-6 months.

Kehrli P et al Neurochirurgie 1999;
BRAIN METASTASES ARE RESISTANT TO CHEMOTHERAPY

BLOOD – BRAIN – BARRIER
MODELS FOR HUMAN CANCER METASTASIS MUST EMPLOY:

- RELEVANT TUMOR CELLS \textbf{(SEED)}
- RELEVANT ORGAN MICRENVIROMENT \textbf{(SOIL)}

Orthotopic implantation
Imaging of melanoma brain metastasis

MRI T1 Weighed Image
Without contrast agent (gadolinium)

MRI T1 Weighed Image
With contrast agent (gadolinium)

UHrad.com (University Hospitals of Cleveland)
EXPRESSION OF VPF-VEGF
## Brain metastasis and vascularization

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Brain mets. Incidence</th>
<th>Survival (days)</th>
<th>MVD</th>
<th>Large vessels (%)</th>
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### H&E

- KM12SM
- PC14PE6
- PC14Br
- H226
- SN12PM6
- Tumor free

### CD31

- KM12SM
- PC14PE6
- PC14Br
- H226
- SN12PM6
- Tumor free
Expression of angiogenic cytokines

mRNA expression *in vitro*

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VEGF expression *in vivo* (brain)

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<td><strong>Tumor free</strong></td>
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Transfection of sense or antisense VEGF gene
Antisense-VEGF165 gene transfection
TRANSFECTION OF THE VEGF121 OR 165 GENES INTO H226 CELLS DID NOT RESULT IN PRODUCTION OF BRAIN METASTASIS
EXPRESSION OF VPF-VEGF IS ESSENTIAL BUT NOT SUFFICIENT FOR PRODUCTION OF BRAIN METASTASIS
BRAIN METASTASES ARE RESISTANT TO CHEMOTHERAPY

THE BRAIN MICROENVIRONMENT: ASTROCYTES
Astrocytes in the Brain microenvironment

Functions of astrocytes:
- Transport nutrients from blood to neurons
- Protect neurons
- Participate in neuronal signal transmission
- Maintain homeostasis: \([k^+]\), \([Na^+]\), \([PH]\), \(H_2O\)

Activated Astrocytes Protect Neurons

ASTROCYTES IN PHYSIOLOGY
SUPPLY GLUCOSE AND OXYGEN TO NEURONS
SURVIVAL OF NEURONS
AND ENDOTHELIAL CELLS

ASTROCYTES IN PATHOLOGY
?
Lung cancer brain metastasis (clinical specimen)

Astrocyte staining with GFAP (glial fibrillary acidic protein)
Lung Cancer

Astrocytes/tumor region    Tumor free
ACTIVATED ASTROCYTES IN BRAIN METASTASES

Breast Cancer

Lung Cancer

Melanoma
3LL-LM in brain (paraffin 10X) (11/16/2006) GFAP / PCNA
ACTIVATED ASTROCYTES EXPRESS GFAP

In response to:

Hypoxia
Inflammation
VEGF
IL-6
IL-8
Isolation of Astrocytes from \( H-2K^b-tsA58 \) Mice

**Background:** CBA/Ca x C57BL/10

1. Harvest Brain
2. Rotate shaking 250 RPM Overnight
3. Evaluate GFAP Expression

**Growing Conditions**
- Permissive: 33°
- Non-Permissive: 37°

*Langley et al.,*
Expression of SV40 Large T Antigen and Cell Proliferation of Immortal Mouse Astrocytes

A.

0  24hr  48hr  72hr

SV40 large T antigen

β-actin

B.

Absorbance (570 nm)

Time (hours)

33°C

37°C
Immortalized Astrocytes from $H-2K^b$-tsA58 Mice

Control

GFAP

Glutamate Receptor 1

NMDA Receptor
CO-CULTURE EXPERIMENTS
Mouse Astrocytes Enhance Resistance of PC14Br4 Cells to Taxol (72 hr)

Apoptosis (% sub G0/G1 cells)

- PC14Br4/GFP
- Plus Astrocyte

Taxol (ng/ml)

0 5 10
Vinblastine (µg/ml)

- MDA 231 Br3
- + Astrocytes

Percent Cytotoxicity

Vinblastine (µg/ml):
- 0.1
- 1
- 10

Error bars indicate variation in the data.
MDA231 /astrocytes  Taxol protection assay (TUNEL)

Astrocytes  |  MDA231  |  Astrocytes + MDA231

TUNEL
5±2

DAPI

MERGE
MDA231 / 3T3  Taxol protection assay (TUNEL)
IS THE PROTECTION FROM CHEMOTHERAPEUTIC DRUGS MEDIATED BY SECRETED FACTORS OR IS IT CONTACT DEPENDENT?
Tumor cells

Astrocytes

0.4uM
Trans-well

Tumor cells

Astrocytes
IS THE PROTECTION DEPENDENT ON GAP - JUNCTION CHANNELS (GJC) ?
IS THE PROTECTION OF TUMOR CELLS FROM CHEMOTHERAPEUTIC DRUGS ASSOCIATED WITH ALTERED EXPRESSION OF SURVIVAL GENES?
EXPERIMENTAL DESIGN

- Human cancer cell (MDA-MB-231Br3 or PC14Br4)
- Mouse cell (Astrocytes or NIH3T3)

Cancer cell only (sensitive to apoptosis)
Co-culture with astrocytes (resistant to apoptosis)
Co-culture with fibroblasts (sensitive to apoptosis)
Astrocytes or Fibroblast only

RNA Extraction
Labeling
Hybridization

Human Microarray
Mouse Microarrays

Resistance signature from carcinoma cells
Co-opted signature from astrocytes
MDA-MB-231 (1069)
PC14Br (594)

Human cancer cells
Co-cultured mouse cells

Up-regulated genes when co-cultured with astrocytes
GSTA1, HSPCAL, BCL2L1, BCLXL, TNFSF7, TWIST1

Down-regulated genes when co-cultured with astrocytes
DLK1, NXN, CTNNB1, CREM
ASTROCYTE PROTECTION OF TUMOR CELLS FROM CHEMOTHERAPEUTIC DRUGS IS ASSOCIATED WITH INCREASED EXPRESSION OF SURVIVAL GENES
ASTROCYTE MEDIATED PROTECTION OF PC14 LUNG CANCER CELLS FROM CYTOTOXIC DRUGS

Permanent vs Transient

Tumor cells/Astrocytes

72 hours Taxol

Tumor cells/Astrocytes

72 hours

Tumor cells/3T3

Astrocytes (sorted out)

Tumor cells/3T3

3T3 (sorted out)

Tumor cells

Astrocytes

3T3

Tumor cells

Astrocytes

3T3

72 hours Taxol

(40±4%) (73±3%)

(34±3%) (71±2%)

(P<0.01) (P<0.01)
GSTA5, glutathione S transferase 5
HSPCAL, heat shock 90kDa protein 1-like
BCL2L1 (anti-apoptosis gene)
BCXL (anti-apoptosis gene)
TNFSF7 (CD70, CD27L)
TWIST1

**Activation of NFκB**
ASTROCYTES

SUPPLY GLUCOSE AND OXYGEN TO NEURONS

ASSURE SURVIVAL OF NEURONS AND ENDOTHELIAL CELLS

AND

TUMOR CELLS
THERAPY OF CANCER METASTASIS

OBSTACLES

BIOLOGICAL HETEROGENEITY

RAPID EMERGENCE OF RESISTANT VARIANT CELLS

PROTECTION BY THE MICROENVIRONMENT
THERAPY OF METASTASIS MUST BE DIRECTED AGAINST THE

METASTATIC CELLS AND THE ORGAN MICROENVIRONMENT
THANK YOU