Mutual Growth Inhibition Between Metastases

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Introduction

- Despite progress on many fronts in cancer research, metastasis remains the most intractable problem and accounts for most cancer deaths
- It is critically important to this research that we understand the interactions of metastatic cells with their host environment
- Fuchs (1882) and Paget (1889) proposed the hypothesis that characteristics of malignant cells as well as destination tissues determine whether metastases become successfully established in secondary sites

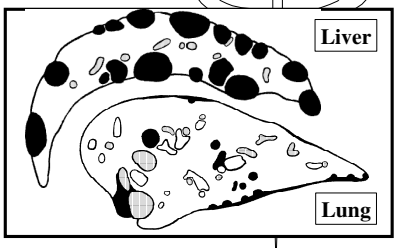
Paget's Seed & Soil Hypothesis

"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 … Then as regards metastasis. Here, too, we shall find evidences of predisposition; we shall see that one remote organ is more prone to be the seat of secondary growth than another … [the] frequency of secondary disease of the liver is of course a familiar fact; but it acquires fresh interest when we contrast it with the immunity enjoyed by other organs. The spleen has, so to speak, the same chances as the liver; its artery is even larger than the hepatic artery; it cannot avoid embolism. Yet the liver was the seat of cancer in 276 cases; the spleen in 18 only. Such a disproportion cannot be due to chance."

Paget, S., *The distribution of secondary growths in cancer of the breast.* Lancet, 1889. **1**: p. 571-573.

Disproportionate Distribution

- **Disproportionate tumor cell distribution to target organs**
- **Also preferential location within organs**



B16F1/F10 metastases

Dingemans, K.P., R. van Spronsen, and E. Thunnissen, *B16 melanoma metastases in mouse liver and lung: I. Localization.* Invasion Metastasis, 1985. **5**: p. 50-60.

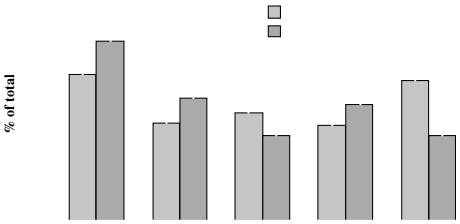
Location Preference in Lung

- **2.5 x 10^5 B16F10 cells, labeled with fluorescent nanospheres, injected to target mouse lung**
- **Location analyzed at initial arrival in lung and every day as metastases grew**

Results:

1. **No significant initial preference: cells went wherever space was available in the lung**
2. **No significant preference in location as cells began dividing, up to day 4**
3. **Highly significant preference by day 10**
4. **Declining significance as space filled by day 14**
5. **Preferential growth but not apoptosis**

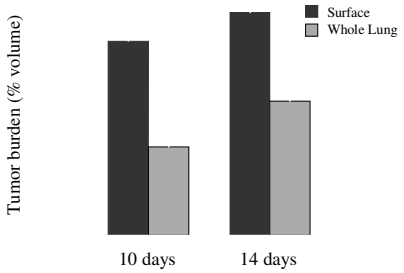
Initial distribution in lung



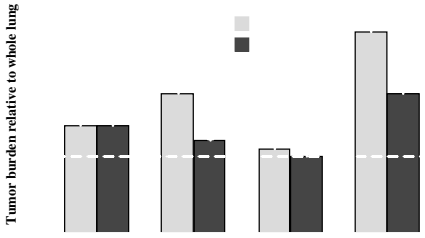
Location relative to lung structures > 100 microns

Cameron, M.D., et al., *Temporal Progression of Metastasis in Lung: Cell Survival, Dormancy, and Location Dependence of Metastatic Inefficiency.* Cancer Research, 2000. **60**: p. 2541-2546.

Tumor burden at lung surface and in whole lung, days 10 and 14



Distribution of tumor burden, days 10 and 14



Location Preference in Lung

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Next Questions:

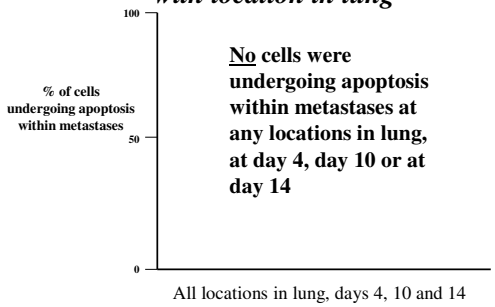
1. **Preferential cell death (apoptosis)?**
2. **Preferential growth?**

Tests for Apoptosis and Proliferation

Adjacent serial sections of lungs were prepared with the following:

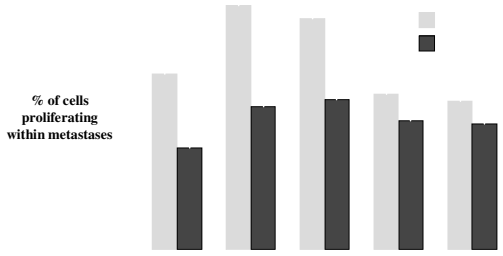
- **S100 used to identify melanoma cells**
- **Ki-67 used to assess proliferation**
- **TUNEL assay to assess apoptosis**

Differences in apoptosis with location in lung



<u>No</u> cells were undergoing apoptosis within metastases at any locations in lung, at day 4, day 10 or at day 14

Differences in proliferation with location in lung

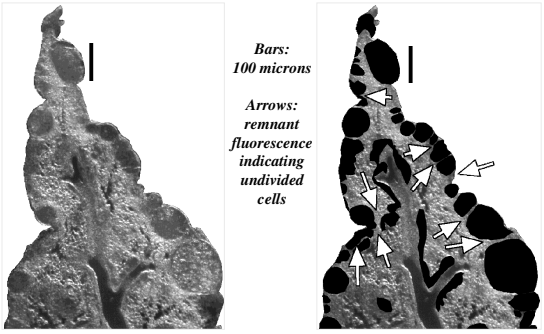


Location Preference

Preferential distribution is real but transitory:

- **Not caused by**
  - **Initial distribution on entry to the organ**
  - **Preferential cell death or loss**
- **Distribution is due to preferential growth rates, especially at surface**
- **Location preference is transitory**
  - **Increases after start of cell division (day 4)**
  - **Decreases as space is filled by tumour**

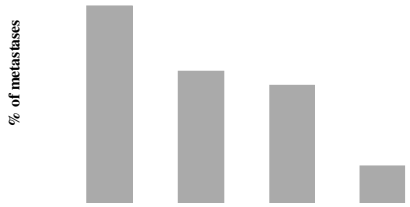
Part of lung thick section at 14 days



Bars: 100 microns

Arrows: remnant fluorescence indicating undivided cells

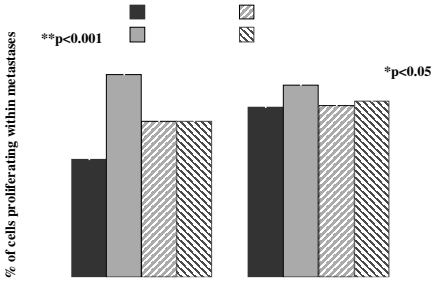
Metastases with cells retaining nanosphere fluorescence



More preferential growth?

- **Distribution of metastases was due to preferential growth rates, especially at surface**
- **Remnant fluorescence at margins between lesions indicated fewer cell divisions in those areas**
- **Was preferential growth occurring even within metastases?**

Mutual inhibition between metastases



Mutual inhibition

Inhibition of metastases by presence of a primary: anecdotal surgical evidence and published papers

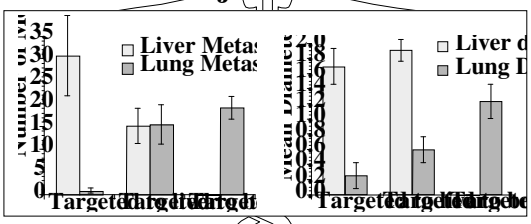
1. Guba, M., et al., *A Primary Tumor Promotes Dormancy of Solitary Tumor Cells before Inhibiting Angiogenesis.* Cancer Res, 2001. **61**(14): p. 5575-5579.
2. O'Reilly, M.S., et al., *Endostatin: an endogenous inhibitor of angiogenesis and tumor growth.* Cell, 1997. **88**: p. 277-285.
3. Camphausen, K., et al., *Radiation Therapy to a Primary Tumor Accelerates Metastatic Growth in Mice.* Cancer Res, 2001. **61**(5): p. 2207-2211.

Mutual inhibition: dual injection experiment

- Prompted by Folkman's laborious isolation of a factor in urine of mice with primary tumors that prevented growth of metastases
- Double injection experiment to target both liver and lungs of mice with B16F1 melanoma (preferential for liver)
- 3 groups: Lungs/sham, liver/sham, dual (and double sham controls); blood samples also collected for analysis

Folkman, J., *Tumor angiogenesis.* Adv Cancer Res, 1985. **43**: p. 175-203.

Dual injection results

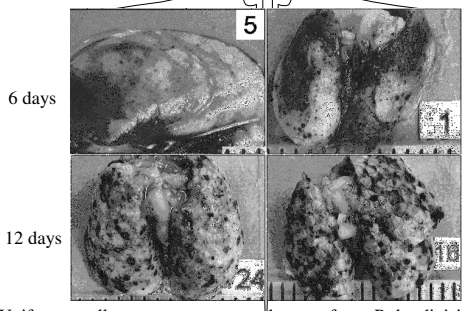


- Ambiguous results:
  - dual 50% fewer liver metastases but this was not significant
  - dual > 2-fold reduction in lung metastases diameter ($p = 0.0384$)
- Would results be reversed if B16F10 cells (preferential to lung) were used instead of B16F1 cells?

Mutual inhibition: within same organ

- Ann Chambers group found wide variation in number, size, and distribution of melanoma metastases in mice injected with $\leq 10^5$ cells
- Using higher density of (1) 2.5×10^6 and (2) 2.5×10^5 B16F10 cells injected to target lung produced consistent and different results
- Most metastases did not coalesce up to 14 days after injection, most grew as mounds at surface of lungs, remarkably uniform in size, shape, and distribution
- Results: Size, distribution and morphology depend on density of metastases

B16F10 Lung Metastases



Uniform small metastases at mouse lung surface. Ruler divisions 1 mm

Conclusions

Seed and Soil: Paget was right

- **Metastatic growth is a an example of ecology at the cell level**
- **Inhibition of metastases studied in several ways:**
  - **Inhibition by a primary; metastatic growth accelerates if primary removed surgically or by radiation treatment**
  - **Inhibition by distant metastases in other organs: inconclusive evidence that growth is less rapid**
  - **Inhibition by metastases in same organ: transitory effect depending on density of metastases**
- **For metastases > 1 mm, antiangiogenesis factors**
- **For small crowded metastases:**

More questions

Future Work: Causes of Mutual Inhibition in Closely Spaced Metastases

- **Depletion of resources between adjacent lesions (signal molecules, nutrients, oxygen, waste removal)?**
- **Inter-cell signaling: unknown inhibitory factors secreted into inter-lesion space?**

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Resource Competition Between Metastases

- **Rate of depletion of nutrients in growth medium and rate of buildup of waste products can be measured in culture for different densities of cells**
- **Probes can assay factors in extra cellular matrix and cell respiratory rate *in situ* within and between lesions**

Cell Communication

4 primary types of cell-cell communication, which vary in speed and selectivity of signal:

1. **Contact dependent signaling**
2. **Paracrine signaling: signals released into extra-cellular matrix (ECM) act on self and neighbours**
3. **Synaptic signaling (mainly in neurons)**
4. **Endocrine signaling (hormones secreted and carried in blood) - acts over long distances**

The first 2 can be considered relevant in closely spaced metastases in the same organ

Contact signaling through cell-cell junctional complexes

- **Most cancer cells lack intercellular communication through junctional complexes**
- **Cell coupling ratio: ratio of transmembrane potential ΔV of adjacent cell to ΔV of cell with ionic current**
- **In normal epithelial cells: membrane resistance at cell surface of an isolated cell is factor of 10^4 > that of a coupled cell inter-cell membrane; coupling ratio is 0.5 to 0.9; permits rapid diffusion of dyes such as fluorescein, large molecules (e.g. BSA, MW 67000) via junctions**
- **In cancer cells, inter-cell membrane resistance ~ surface membrane resistance; coupling ratio <0.02**
- **Cells within the same lesion might use this communication, but are not connected to cells of nearby lesions**

Paracrine signaling through extra-cellular matrix (ECM)

- **Signal strength is proportional to density of signaling cells of same type**
- **In cancer cells, autocrine signaling often overcomes normal controls on proliferation**
- **Many enzyme-linked cell surface receptors are oncogenes implicated in this type of signaling: e.g. 30% of all human tumors have *ras* mutations**
- **Neighbouring cells compete for extracellular signal proteins: mitogens, growth factors, survival signals as well as nutrients and oxygen**
- **Normal cells stop proliferating when they contact neighbors on all sides: *density-dependent inhibition of cell division* reflects the ability of a cell to deplete local medium of extracellular mitogens and other factors, thus depriving its neighbours**
- **Cancer cells often do not require extracellular mitogenic signals, and can proliferate without them**