Origin of breast cancer metastasis

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Aim

Abdominal organs are frequent targets of cancer cell invasion, but less known for their metastatic potential to other organs, particularly to breast. Our aim was to follow the common steps of abdominal tumor cell progression in rats upon i.p. administration, subrenal or subhepatic implantation of solid tumor and leukemia cells and mimicking their migration with inorganic colloidal particles.
The work was started at the University of Szeged and continued at the University of Debrecen.
Tumor diagnostics – Szeged University

\[ ^{113m} \text{Indium chloride, colloid, and macroaggregate prepared in } 1970 \]

Conclusion: after i.v. administration

**In-chloride:** acidic solution, circulated with blood could be used in small quantities (0.1-0.2 ml) (but not recommended)

**In-colloid:** accumulated in the liver (recommended and used)

**In-Fe-macroaggregate:** caused microembolization in the lungs of rats (the particle size was unreliable and large particles could cause death, thus it could not be used in human diagnostics (counterindicated))
$^{113m}\text{Indium}$  macroaggregate formation

- Without mixing (not to be used)
- Constant mixing (not to be used)
- Magnetic stirring (not to be used)

Distribution (%) vs. Particle size ($\mu$m)

......... life threatening particle size
Organ distribution of i.v. administered $^{113m}$In-colloid particles in rats

- Liver: 85-90 % (Kupffer cells)
- Spleen: 3-5 %
- Bone marrow: 5-10 %

Primary lymphoid organs:
- bone marrow, and the thymus

Secondary lymphoid organs:
- lymph nodes, tonsils, spleen, and
- "MALT" (mucosa-associated lymphoid tissue, Kupffer cells of the liver, microglia of the central nervous system)

Reticuloendothelial system (RES) = Mononuclear phagocytic system and lymphoreticular system
Heart scintigraphy with $^{113m}\text{InCl}_3$ solution

Banfalvi, G. Production of $^{1}\text{In}$-preparations suitable for diagnosis
PhD dissertation, 1971, University of Szeged, 1st Department of Medicine
Bánfalvi, G. Banfalvi, G. Production of $^{113m}$In-colloid suitable for liver scintigraphy PhD dissertation, 1971 Szeged, Department of Internal Medicine
Hepatomegaly
Human liver scintigraphy using $^{113}$In-colloid. In the upper right corner of the liver there was no colloid accumulation indicative of an abnormal process. Medical University, Szeged. 1st Department of Internal Medicine ($^{113}$In-colloid prepared by G. Banfalvi in 1970).
Colloid (nano) particles in the organism:

Similar to ultrafine particles, **nanoparticles** are sized between 1 and 100 nanometers.

**Colloid particles** are between 1 nm and 10 micrometers:
- viruses,
- bacteria,
- yeast cells,
- **cancer cells** (cancer cells often are different in their sizes and shapes)
- cell remnants,
- **carbon particles** (e.g. India ink)
- thin fibrous crystals of asbestos

Foreign particles are attacked by white blood cells:
- granulocytes,
- monocytes,
- lymhocytes

Debrecen cancer cells >
Tumor biology in Debrecen
Primer cells, cell culture, cell line

Clinical isolate (melanoma) → Part of tumor (2x2x2 mm) → Sliced tumor → Elastase digestion → Cell culture

Melanoma obtained from Department of Dermatology
Tumor cell line: Melanoma Debreceniensis (Me/De) (2008)

Establishment of He/De, Ne/De, My1/De, My2/De, Me/De cell lines
In model experiments we have used healthy mice and mice suffering in Severe Combined Immu-
deficiency (SCID) characterized by the inability to sustain an appropriate immune response against the tumor cell lines implanted. Tumor cell lines obtained from human tumors (liver, spleen, lung, kidney): implanted under the kidney capsule. Due to the absence of T and B lymphocytes tumor cells grow in SCID mice. Nanoparticles carrying the specific antitumor agent will be targeted to the sites of tumor growth. The comparison of syngenic model with the xenograft model in itself is expected to reveal the advantage of the presence of lymphocytes in supporting chemotherapy.
Local tumor formation upon administration of tumor cells by:

- subcutaneous (s.c.) injection

Local tumor growth:

intravenous (i.v.) administration

Subrenal rat implantation model

He/De, Ne/De, Me/De, My1/De, My2/De
hepatocarcinoma, nephroblastoma, melanoma, myeloid leukemia, myeloid leukemia

Tissue distribution of radioactivity after i.v. administration of $^{18}$FDG in Ne/De or He/De tumor-bearing rats

Metabolism:
- high in primary tumors and
- in PTNs
- low in other tissues

Metastatic potential of Ne/De and He/De cells, plasma, muscle, tumor, parathymic lymph nodes, thymus, liver and kidney expressed as DAR (differential absorption ratio).

Tissue distribution of $^{18}$FDG in the inner and outer part of the tumor

Outward tumor growth and inward necrosis of HeDe and NeDe tumors expressed as DAR (differential absorption ratio).

Metabolism:
- high in external part of primary tumors
- low at inner part of tumors

Subrenal implantation of tumor cells

Figure 3 Tumor growth after subrenal implantation of He/De cells. A. During the experimental surgery technique known as Subrenal Capsule Assay (SRCA): a) the left kidney of the rat was exposed, b) Gelasponge® disc containing $10^6$ He/De cells was placed under the capsule of kidney, c) kidney containing the implanted tumor cells was placed back in the retroperitoneum and the operative field was subjected to post-operative treatment, d) primary tumor formation 7 days after He/De cell implantation. B. Sagittal images of $^{18}$FDG-PET before and after implantation of He/De cells under the capsule of left kidney of rats. a) miniPET image before He/De cell implantation. PET images 3 days (b), 7 days (c), 14 days (d) and 18 days (e) after implantation. White arrows indicate the heart, black arrows the primary tumor in kidney, red arrows metastases in parathymic lymph nodes (PTNs).

Lagging angiogenesis during tumor formation (after subrenal implantation of HeDe cells)

Tumor growth outward with disruption of capillaries, blood and tumor cells pour out through the disruptions.

Subrenal implantation of nephroblastoma (NeDe) cells

Enlargement of parathymic lymph node (PTN)

Fig. 7. Enlargement of parathymic lymph nodes six days after tumor cell implantation. a. control PTN, b. tumor-bearing PTN. Bar: 0.5 cm.

Fig. 4. Tumor infiltration in parathymic lymph nodes at day 12 after tumor cell implantation.

Lipid formation in NeDe primary tumor

Fig. 6. Lipid droplets in primary tumor six days after tumor cell implantation. Staining: Sudan black B. Bar: 50 μm.

Fig. 10. Lipogenic pathway in NeDe tumor. In the mitochondria of tumor cells the terminal oxidation and the oxidative phosphorylation are not working under hypoxic conditions. In the absence of the normal aerobic metabolism the overproduction of acetylcoenzyme A induces fatty acid and lipid synthesis.

Metastasis in parathymic lymph nodes caused by nephroblastoma (NeDe) cell implantation

a) Elevated peripheral pressure in abdominal kidney tumor
b) produces an increased amount of fluid accumulating in the peritoneal cavity.
   (Uroperitoneum subsequent to kidney, ureter, bladder, or urethra rupture also cause an increased amount of effusion in the abdomen. Pleural neoplastic effusions are known to occur frequently (Ultmann JE: CA: Cancer J Clin 1962,12:42–50).

c and d) Rat mesenchymal renal tumor cells (Ne/De) transplanted under the kidney capsule of F344 rats pour through the disruptions of blood vessels into the retroperitoneum and cross the diaphragm and appear in the PTNs

Mimicking metastatic spread by India ink implantation in rats

Spread of metastasis
from organs to other organs primarily to the liver (well known)

from abdominal (peritoneal, retroperitoneal) tumors to parathymic lymph nodes (not well understood)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Primary tumor</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>central nervous system</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td>bone, lung, liver, brain</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>liver, bone, brain, adrenal gland</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>not specified</td>
</tr>
<tr>
<td>Colon</td>
<td></td>
<td>liver, lung, peritoneum</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>liver, lung, bone, brain</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td>lung, other bones</td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td>liver, lung, peritoneum</td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
<td>bone, lymph nodes, liver, lung</td>
</tr>
</tbody>
</table>

Metastasis: abdominal primary tumor → thoracic (PTN)

mammary lymph nodes
Metastatic spread of primary tumor to thoracal and parathymic lymph nodes in rat

Similar human metastatic spread of abdominal tumors to inner mammary lymph nodes

i) Disruptions in the abdominal primary tumor (arrows in a)

ii) Release of tumor cells through gastrointestinal bleeding of the primary tumor and their appearance in the abdominal space (a)

iii) Migration of tumor cells by pass the hiatuses of the diaphragm (b)

iv) Tumor cells exhaust the defense capacity of the parathymic organ

v) Unchecked tumor cells migrate from the inferior to the superior thoracal lymph nodes and drain to the vascular system (b).

Metastatic spread of abdominal tumors to mammary lymph nodes

What did others find?

- High radiotracer glucose analogue uptake in PTNs is in correlation with the inflammatory activity in the liver (1).
- After intraperitoneal administration of oncolytic measles virus-infected cells, the diaphragmatic stomata, thoracic lymphatic vessels, and parathymic lymph nodes contained numerous measles-infected cells (2).
- 9–45% axillary lymph node tumors drain to the internal mammary lymph nodes (IMLN) (3-6).
- In almost all patients the internal mammary lymph node tumor is a forerunner of a metastatic disease (7).
- Handley and Thackray concluded that the internal mammary glands may often be invaded before carcinoma has reached the axilla. Their clinical evidence indicates a relatively high incidence (~ 25%) of secondary spread to the breast (8).
- There is a direct anatomical connection between abdominal and mammary lymph nodes (9).

Summary

The migration of abdominal tumor cells can be summarized as:

i) Implanted tumor cells cause primary tumors and peripheral disruptions of primary tumors,

ii) Tumor cells are shedded into the abdominal cavity,

iii) Released tumor cells cross the stomata of the diaphragm,

iv) Tumor cells accumulate in thoracal, primarily in parathymic (rodent), inner mammary (human) lymph nodes,

v) After exhausting the defense capacity of parathymic lymph nodes, metastatic migration continues in the superior lymph node chain before the thoracic duct returns chyle to the vascular system,

vi) Colloidal carbon particles mimic faithfully the migration of tumor cells.
Conclusions

• The study of abdominal tumor spread to mammary lymph nodes has been neglected earlier due to the belief that mammary tumors are not metastases.

• The metastatic spread of tumor cells released from abdominal primary tumors to PTNs was found by using different cell lines including solid rat liver (He/De), kidney (Ne/De) and leukemia (My1/De, My2/De) cell lines.

• Why human parathymic lymph nodes have not been investigated earlier? Answer: they were investigated but were known as inner mammary lymph nodes (not to be confused with intra-mammary lymph nodes).

• The significance of potential spread of metastasis from the most often occurring abdominal primary tumors to mammary lymph nodes is emphasized. The spread of abdominal tumors cells to mammary lymph nodes provides a new explanation to the origin of breast metastasis and alternative for prevention.
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Thank you for your attention!