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TITLE: Pathogenesis and Blood-Brain Barrier Heterogeneity of Breast Cancer Brain Metastasis

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The purpose of this study was to elucidate the pathogenesis of breast cancer brain metastasis and the role of the BBB in the process. Green fluorescent protein (GFP) expressing breast cancer cells were injected into the left ventricle of nude mice and the progression of brain metastases analyzed. Our data revealed that: i) 80% hematogenous metastatic cells homing in the brain extravasated and grew along blood vessels; ii) 20% metastatic cells attached to the microvessel wall did not extravasate immediately but proliferate within the vasculature, this leads to thrombosis-like complications such as infarction of brain parenchyma; iii) the cancerous thrombus can serve as a sustained releasing source of tumor cells to the downstream area through blood flow, making the spread of tumor cells extremely quick; iv) continuing intravascular tumor expansion led to disruption of blood vessels and BBB integrity; v) the overflowing metastatic cells proliferate and migrate along the host vasculature perivascularly to far distant sites and regain the protection of the BBB. vi) function of BBB is heterogeneous in different regions of tumor mass. These observations provide rationales for early diagnosis and treatment and improve our understanding of the role of BBB in chemotherapy of brain tumors.
<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover</td>
<td></td>
</tr>
<tr>
<td>SF 298</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Body</td>
<td>5-20</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>21</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>22</td>
</tr>
<tr>
<td>Conclusions</td>
<td>23</td>
</tr>
<tr>
<td>References</td>
<td>24-25</td>
</tr>
<tr>
<td>Appendices</td>
<td>26-31</td>
</tr>
</tbody>
</table>
Introduction

Brain metastases are the most frequently occurring intracranial tumors, outnumbering primary brain tumors by a ratio of 10:1 (Shaffrey et al., 2004) and presenting the major causes of systemic cancer morbidity and mortality (Nathoo et al., 2005). Their incidence is increasing because of improved cancer therapy for the systemic disease (Shaffrey et al., 2004; Tosoni et al., 2004). Among patients with breast cancer, at least 10-15% of them develop brain metastases, making breast cancer the second most common cause of brain metastases (Boogerd, 1996; Ewend et al., 2001; Fenner and Possinger, 2002). Patients with brain metastases have a generally poor outcome with a median survival after diagnosis of approximately 4 months (Bradley and Mehta, 2004). It is predicted that brain metastases will become more problematic with the improved systemic control of metastatic diseases, because it is believed that most systemically administered agents have poor central nervous system penetration.

However, our knowledge about how brain metastases develop has, for the most part, been extrapolated from observation in other tissues. This has led to the concept that brain metastasis develops after extravasation of metastatic tumor cells into the surrounding tissue. Dogma also dictates that the blood-brain barrier (BBB) prevents the delivery of therapeutic agents through the BBB (Ewend et al., 2001; Landonio et al., 2001). We have developed a unique breast cancer brain metastasis model that allows us to visualize the development of brain metastasis and monitor BBB function with unprecedented high resolution. High expression of green fluorescent protein (GFP) allows us to trace the very few tumor cells and find the early tumor metastasis, and intravenous injection of rhodamine conjugated albumin facilitates monitoring the function of BBB under fluorescence microscope. Serendipitous observations indicate that our current concepts about pathogenesis and BBB function of brain metastasis (Fenner and Possinger, 2002; Walsh, 1996) might be incorrect. Accordingly, it is very important to further understand the mechanisms of the brain metastasis of cancer in order to design effective and specific therapeutic interventions for this devastating complication in cancer. Advances in this understanding of the pathobiology of brain metastasis may lead to novel targeted treatment paradigms and a better prognosis for patients with brain metastatic disease.

Based on this background, we proposed a new concept on the pathogenesis of breast cancer brain metastasis. The results obtained with financial support from DOD support most of what we hypothesized and tremendously broadened and enriched our understanding of the mechanisms of brain metastasis and the role of chemotherapy in brain tumors.
Pathogenesis and blood-brain barrier heterogeneity of breast cancer brain metastasis

**Background:** Brain metastases are the most frequently occurring intracranial tumors, outnumbering primary brain tumors by a ratio of 10:1 (Shaffrey et al., 2004) and representing the major causes of systemic cancer morbidity and mortality (Nathoo et al., 2005). Their incidence is increasing because of improved cancer therapy for the systemic disease (Shaffrey et al., 2004; Tosoni et al., 2004). Among patients with breast cancer, at least 10-15% of them develop brain metastases, making breast cancer the second most common cause of brain metastases (Boogerd, 1996; Ewend et al., 2001; Fenner and Possinger, 2002). Most of these patients die within a few months of diagnosis since many systemic therapies are ineffective within the brain (Bradley and Mehta, 2004). Our knowledge about how brain metastases develop has, for the most part, been extrapolated from observation in other tissues. This has led to the concept that brain metastasis develops after extravasation of metastatic tumor cells into the surrounding tissue. Dogma also dictates that the blood-brain barrier (BBB) prevents the delivery of therapeutic agents through the BBB (Ewend et al., 2001; Landonio et al., 2001). We have developed a unique breast cancer brain metastasis model that allows us to visualize the development of brain metastasis and monitor BBB function with unprecedented high resolution. Serendipitous observations indicate that our current concepts about pathogenesis and BBB function of brain metastasis (Fenner and Possinger, 2002; Walsh, 1996) may be incorrect. The development of rational therapeutic strategies for the treatment of brain metastasis requires a complete understanding of the mechanism by which lesions develop.

**Rationale/Purpose:** We hypothesized that the anatomic structure of BBB prevents tumor cell extravasation. This results in local intravascular growth that embolizes and ultimately ruptures the vasculature. Once tumor ruptures the vessel wall, the characteristic perivascular growth spreads tumor cells far from ruptured site and then gains BBB protection. This model dictates that brain metastasis has a prolonged intravascular growth stage and the BBB is functionally heterogeneous in different tumor areas. Based on the unique pattern of brain metastasis proposed here, we assumed that the function of BBB in brain metastatic tumors should be temporally and spatially heterogeneous.

**Objectives:** The objectives of this proposal were to clarify the primary mechanism by which breast cancer develop brain metastasis with the emphasis on identifying the role of BBB in the process. The different models of breast cancer brain metastases developed in this laboratory allow us to visualize the whole procedure of brain metastasis development, interaction of tumor cells with BBB and the dynamic changes of the function of BBB with unprecedented clarity in direct in vivo natural tumor settings. By serial examination of the interactions between breast cancer brain metastasis and host vessels at different stages of brain metastasis growth, we would be able to provide a general framework of the mechanisms that underlie brain metastatic process. The proposal had the following specific aims:

1. Determine how breast cancer brain metastases initiate and grow with the particular emphasis on their interaction with the BBB.
2. Determine the changes in the function of the BBB in brain metastasis at different stages of tumor progression.

**Original experimental design:**
Analyses of the brain metastatic process and function of the BBB in brain metastasis at different stages of tumor progression.

Months 1–12: Nude mice will be injected with MDA-MB435GFP breast cancer cells into the left ventricle of the heart. At weekly intervals mice will be injected i.v. with rhodamine-albumin and sacrificed.

A) Superficial tumors will be localized by green fluorescent protein (GFP) using stereoscopic fluorescence microscopy. Tumor bearing regions will be further analyzed for supporting vasculature and intact/permeable BBB by red fluorescence (rhodamine-albumin) imaging. The areas will be surveyed, mapped and recorded.

B) Confocal microscopy – Thick slide frozen sections will be prepared and, where appropriate, fixed and stained with eosin-yellow CD31 antibodies to identify supporting endothelial cells. This will facilitate three-dimensional three color imaging of tumor (green fluorescence), blood vessel walls (yellow fluorescence) and patency of the BBB (red fluorescence).

Materials and Methods:

Mice. Female athymic nude mice were purchased from the Animal Production Area of the National Cancer Institute-Frederick Cancer Research Facility (Frederick, Md.). The animals were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States department of Agriculture, Department of Health and Human Services, and National Institutes of Health. All the mice were used when they were 8- to 12-weeks old.

Establishment of GFP-expressing MDA-MB 435 (MDA-MB 435GFP) and 4T1 cell lines. The mammary carcinoma MDA-MB-435 (human) and 4T1 (mouse) cell lines were transfected with a plasmid, pEGFP-N1, that constitutively expresses an enhanced version of the GFP (Clontech Laboratories, Inc., Palo Alto, CA). FuGENE 6 Transfection Reagent (Roche Molecular Biochemicals, Indianapolis, IN) was used to aid the transfections. A protocol recommended by the manufacturer was followed. G418 selection at 500 µg/ml started 2 days after transfection. Two to 3 weeks later, colonies that emerged in Petri dish cultures were examined for GFP expression under a fluorescence microscope. Those colonies with robust GFP expression were picked, pooled, and expanded for further experiments.

Intracardiac injection of tumor cells. Female nu/nu mice were anesthetized by i.p. injection of Nembutal (45 mg/kg of diabutal (50 mg/ml)/saline/ethanol/propylene glycol 10/63/7/18). The anterior chest wall were scrubbed with 70% alcohol. A 30 gauge needle on a tuberculin syringe were inserted slowly into the second intercostal space 3 mm to the left of the sternum and aimed centrally. The spontaneous and continuous entrance of pulsating red blood into the transparent needle hub indicated proper positioning of the needle into the left ventricle of the heart. Gentle aspiration of the syringe were necessary to determine if the needle is in the left ventricle of the heart if red blood failed to enter the needle hub. 10^6 cells in 0.1ml saline were injected into the left ventricle of the heart over a 20-40 second period.

Selection of MDA-MB 435GFP cells preferentially metastasizing to brain. MDA-MB 435GFP cells were injected into the left cardiac ventricles of nude mice, brain metastases were harvested when mice became moribund, tumor cells were cultured, amplified and then injected back into the left heart of the nude mice to generate brain metastases again. By selecting brain
Weixin Lu

metastatic lesions for four cycles we established a unique human breast cancer cell line expressing green fluorescent protein that preferentially metastasizes to the brain. At least 80% of nude mice died of brain metastasis 2-3 months after the cell line was injected into their left cardiac ventricle.

Labeling of vasculature in vivo. Systemic labeling of vascular network was done by i.v. injection of 1 mg rhodamine conjugated albumin (Molecular Probes, Eugene, OR).

Examination of brain metastases and function alteration of BBB. At every time point or when the mice show signs of morbidity, the animals were injected i.v. with Rhodamine-albumin, 0.5-1 hr later the mice euthanized by inhalation of carbon dioxide in a closed chamber. The brains were removed and examined under fluorescence stereoscope ((Leica Model LZ12), equipped with narrow bandpass excitation and emission filters mounted in a selectable filter wheel (Ludl Electronic Products, Hawthorne, New York). Real-time images were directly captured with an Evolution MP camera (Media Cybernetics Inc., Silver Spring, Maryland or by frame capture from videotaped images.) or a Zeiss LSM 510 laser scanning confocal microscope (LSM510, Carl Zeiss Inc. Thornwood, New York) with krypton-argon and helium-neon lasers. The brain metastases were also harvested, frozen sections cut and fixed immediately in cold acetone for 5 min. Fluorescence microscopy for Rhodamine-albumin permeability in brain tissues was performed with an epifluorescence microscope equipped with a mercury vapor lamp and appropriate narrow bandpass excitation and emission filters (Ludl Electronic Products, Hawthorne, New York).

Immunostaining for CD31. Since the rhodamine labeled albumin was washed away and green fluorescence of GFP tumor cells quenched during the process of staining, so we failed to complete three-dimensional three color imaging of tumor (green fluorescence). We performed our routine CD31 staining and the results demonstrated the points all the same as we proposed. Briefly, acetone-fixed frozen sections were sequentially incubated with rat anti-mouse CD31 (PharMingen, San Diego, California), peroxidase-conjugated goat anti-rat antibodies (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) and diaminobenzidine.

Results:

Sequential observation of pathogenesis of brain metastasis using MDA-MB-435GFP and 4T1GFP revealed the complexity of mechanisms by which breast cancer develop brain metastases and functional heterogeneity of BBB in brain metastases.

Coexistence of different mechanisms in breast cancer brain metastases.

1) Extravasation and extravascular growth. At weeks 1 and 2, among 28 superficial MDA-MB-435GFP metastatic nodules consisting of cells cluster or a single cell, 22 of 28 were extravascular (Fig. 1), indicating that extravasation was the dominant mechanism of brain metastases. Perivascular nodular foci most located around capillaries and arterioles. The single cell outside vessels at week 1 indicated that some metastatic cells did not go proliferation immediately after tumor cell inoculation. After extravation, metastatic cells migrate and grow towards the adjacent blood vessels of the neighbor vessel network, and then proliferate along the vasculature perivascularly to distant sites (Fig. 2). For brain metastases at advanced stage tumor cells did not grow randomly at the tumor periphery, but aligned themselves longitudinally along the blood vessels. The longitudinal alignment could be continuous or discontinuous, indicating the migration of tumor cells along the
vasculature. And perivascular tumor growth could encompass the vascular perimeter completely or just partially (Fig. 2c). When cells divided and migrated along an existing vessel, they could spread almost as far as the whole hemisphere of brain without notable tumor nodule.

2) **Intravascular proliferation, vessel rupture, and extravascular expansion.** 6 of 28 metastatic nodules were intravascular at weeks 1 and 2. The intravascular nodules could also be found in brain at late stages and the nodular foci were larger. They were confined in arterioles or intermediate-size arteries and extended as strings in the shape of vessel, CD 31 staining showed that tumor cells were surrounded by CD 31 positive endothelial cells (Figs. 3). Cell division must have been occurring within the brain vasculature since strings of cells were never present at times earlier than 3 days. In our system, both vasculature and tumor cells were clearly visible under fluorescence microscope. The results suggest that the intravascular stage of brain metastasis be much more prolonged than has been predicated from other organ metastasis models. In addition to the direct observation of intravascular growth of metastatic cells, there were two additional evidences supporting the intravascular origin of brain metastasis. The first was the occlusion of the blood vessels (Fig. 4). This feature was quite apparent by the atrophy of local tissue under dissecting microscope and the necrosis of brain tissue during the early brain metastasis in H/E slides. The necrotic areas usually occurred in the deep parenchyma of the brain, and the size usually depended on the size of the blood vessel occluded. The second was the releasing of tumor emboli into the downstream area of brain. This phenomenon was clearly demonstrated in 4T1 cells model in Fig. 5. The shaded tumor cells from the intravascular metastatic colony could become the constant source of releasing tumor emboli to distant regions, causing the metastases of metastasis. Although the intravascular pattern of brain metastasis was not the dominant mechanism, it could easily lead to extensive tumor dissemination through the vasculature and damage the host tissues located in the downstream area by blocking blood flow. In the late stages (when the mice were moribund or 30 days later after tumor cell inoculation), continuous intravascular proliferation of tumor cells led to disruption of the surrounding blood walls (fig 6). After disruption of blood vessels, a completely different event took place. The overflowing (escaped) metastatic cells migrate and grow towards the adjacent blood vessels of the neighbor artery network, and then proliferate along the vasculature perivascularly to distant sites.

**Heterogeneity of BBB function.** At weeks 1-2, Rhodamine-albumin was not leaky if the tumor nodules were inside the vessels (Fig. 7). If the tumor cells were outside the vessels, the vessels could be leaky (Fig. 8) or not leaky (fig 1). When at the late stages of metastasis, the tumor size became bigger; the vessels became leaky, especially in the tumor center. But in the periphery of metastasis, the vessels were heterogeneous to the penetration of rhodamine-albumin, indicative of the heterogeneity of BBB function in brain tumors (Fig. 9).

**Discussion:**

High-resolution observation of GFP-labeled tumor cells metastases in isolated, perfused lung made Al-Mehdi at al conclude that hematogenous metastasis originates from the proliferation of attached intravascular tumor cells rather than extravasated ones (Al-Mehdi et al., 2000). This contradicts the traditional view that circulating metastatic cells must extravasate before proliferating into colonies (Fidler, 1990; Koop et al., 1995). This issue is extremely important for brain metastasis in that intravascular metastasis formation would offer us the opportunity to cure brain metastases by developing new approaches to early detection and giving intravascular drugs
despite the BBB existence. In fact, intravascular growth has been described in B16 melanoma brain metastases (Alterman and Stackpole, 1989; Raz and Hart, 1980), but it was termed as an exception because it has not been observed in other tumor systems and B16 melanoma brain metastasis almost always occurred in leptomeninges and the B16 tumor cells within the leptomeninges artery were enmeshed in large fibrin-like clots that disable tumor cells undergo active extravasation. Current concept dictates that brain metastases begin from malignant cells extravasation (Kawaguchi et al., 1982; Walsh, 1996). The view that tumor cells proliferate after their penetration of BBB is very discouraging and devastating, this makes the diagnosis and treatment of early brain metastasis extremely difficult because of the specialty of BBB in hindering penetration of most systemically administered agents. Although the idea that tumor cells can affect BBB permeability (Kohn et al., 1989; Stewart et al., 1987; Zhang et al., 1992), the interpretation of this result in terms of the inefficacy of chemotherapy remains a fundamental problem.

The in vivo application of GFP-labeled tumor cells (Hoffman, 1998) allows us to observe pathogenesis of brain metastasis with unprecedented spatial resolutions. This greatly facilitates a broad investigation of development of brain metastasis. This is the first clear evidence that the same tumor cells can establish metastases by different mechanisms – extravasation and intravascular proliferation. The reasons why some brain metastases began intravascularly and some extravascularly, whether they were due to the difference of biological properties in tumor cells or the different vascular structure of various locations, remain unknown. The two deadly complications of intravascular growth of brain metastasis – the occlusion of blood vessel and the constant releasing of tumor cells into the downstream area- explain why brain metastasis is so calamitous and emphasize the necessity of chemotherapy. This will also explain why in some patients the imaging findings are of only a small tumor nidus but with a large amount of vasogenic edema or necrosis (Loeffler et al., 1997). The existence of intravascular initiation of brain metastasis makes early detection and treatment clinically important and more practical.

Though we hypothesized that the unique feature of richness of tight junctions among endothelia in BBB would prevent the metastatic cells from extravasation, it was obviously not the case, because in the very early stage single GFP tumor cell was quite often found outside the vessels. The function of the BBB is an important issue with regard to drug treatment of brain tumors. For the first time we demonstrated the temporal and spatial heterogeneity of the BBB function in the same brain metastatic setting, whether the difference in BBB function in small brain metastases in early stage was temporal, or due to the structural difference in BBB, or biologic properties in tumor cells remain important issues. Our results will not only explain the controversy between the evidence that BBB function is not intact and the lack of efficiency of chemotherapy but also suggest the necessity of drug therapy to prolong patient life.

More importantly, all the observations were made in the natural brain metastasis settings (with all the multiple cell types, the cytokines, etc. from the blood and the brain in place to interact with the metastatic cells) and from a large number of mice with different stages of brain metastases, the results should have more accurately reflected the clinical situations.

In conclusion, our results provide a framework about how breast cancer brain metastases initiate and progress and the heterogeneity of BBB function in brain metastases. They are important for us to better understand clinical presentations and develop new approaches to detecting and treatment of brain metastases.
**Figure Legends**

Fig. 1. Extravascular growth of breast cancer brain metastases. MDA-MB-435GFP breast cancer cells were injected into the left heart of female Nude mice. On day 14, mice were sacrificed and brain metastases examined with confocal microscope 1 hr after i.v. injection of rhodamine-albumin. The perivascular growth of metastatic cells indicates the completion of extravasation.

Fig 2. Perivascular distant migration of metastatic breast cancer cells. MDA-MB-435GFP cells were injected into the left heart of nude mice. Eight weeks later, the brain metastases were examined under fluorescence stereoscope. The micrographs show that tumor cells could travel far away from their primary sites (arrow) along the vascular network. Images were taken under fluorescence alone (a) or under dual fluorescence and bright illumination (b). c: H/E staining shows tumor cells completely or partially surrounded vessels.

Fig. 3. Intravascular growth of breast cancer brain metastasis at early stage. MDA-MB-435GFPBr cells were injected into the left heart of nude mice, one week later, the brain metastasis was examined under fluorescence stereoscope (a, b). The micrographs showed that the tumor cells were confined in vessels as strings. c shows immunostaining of the brain metastasis with CD31 antibody. Arrow indicates that tumor cells were wrapped by vascular wall.

Fig. 4. Intravascular proliferation of breast cancer brain metastasis and cerebral infarction. MDA-MB-435GFP cells were injected in the left heart of nude mice. Two weeks later, the brain metastases were examined by fluorescence stereoscope. Confinement of green tumor cells in the vessel under low (a) and high (b) magnification. c: H/E staining showed necrosis areas (indicated by white arrows) from cutting 1. d: Red arrow indicated tumor cells in vessel from cutting 2.

Fig. 5. Intravascular spread of brain metastasis under green fluorescence only (a) and with slight bright illumination (b). 4T1GFP cells were injected into the left heart of nude mice, two wks later, brain metastases were examined using fluorescence stereoscope. The arrow indicates the direction through which intravascular brain metastasis released tumor cells into downstream of vascular network.

Fig. 6. Vessel rupture by intravascular growth of brain metastasis. MDA-MB-435GFP breast cancer cells were injected into the left heart of female Nude mice. Four weeks later, mice were sacrificed and brain metastases examined with confocal microscope 1 hr after i.v. injection of rhodamine-albumin. The arrow points to the rupture site of vessel wall where tumor cells leaked out.

Fig. 7. Intact BBB in brain metastasis of early stage. MDA-MB-435GFP breast cancer cells were injected into the left heart of female Nude mice. One week later, mice were sacrificed and brain metastases examined with confocal microscope 1 hr after i.v. injection of rhodamine-albumin. The green tumor cells were confined in the vessels without the increase of BBB permeability.

Fig. 8. Increased permeability of BBB in brain metastasis of early stage. MDA-MB-435GFP breast cancer cells were injected into the left heart of female Nude mice. Two weeks later, mice were sacrificed and brain metastases examined with confocal microscope 1 hr after i.v. injection of rhodamine-albumin. The arrow points to the area of increased permeability for rhodamine-albumin.
Fig. 9. Heterogeneity of the BBB function induced by perivascular growth of brain metastasis. MDA-MB-435 GFP cells were injected into the left heart of nude mice, when the mice were moribund, their brain metastases were examined under fluorescence stereoscope 1 hrs after i.v. injection of Texas red-albumin. Note: the white arrow indicates increased vascular permeability but most vessels did not show increased permeability despite engulfed by tumor cells. a. GFP tumor. b. Rhodamine-albumin penetration.
Fig. 3
Fig 4
Fig. 6
Fig. 7
Fig. 9
Key Research Accomplishments:

- Abstract for Era of Hope, Breast Cancer Program Meeting 2005: Pathogenesis and blood-brain barrier heterogeneity of breast cancer brain metastasis
- Manuscript for publication: Pathogenesis and blood-brain barrier heterogeneity of breast cancer brain metastasis. In preparation
Reportable Outcomes:

The experiences obtained from the grant contributed to the application for the new idea award in DOD program: **Breast cancer growth and metastasis vascularize by tumor cell adaptive migration into and replacement of normal tissue.**
Conclusions:

The data from this proposal revealed the complexity of mechanisms by which brain metastases were developed. Breast cancer brain metastases could be established by tumor cells proliferation after extravasation or direct intravascular growth. The latter mechanism makes the brain metastasis extremely aggressive in that it serves as a consistent source to spread tumor cells to other areas of the brain and the block of blood supply by tumor growth in vessel damages brain tissue. The results showed the temporal and spatial heterogeneity of the BBB function in brain metastases. These results will significantly improve our understanding of the mechanisms behind the clinical presentation of patients with brain metastases, and help clinicians to optimize the therapeutic strategies addressing the complexities of the mechanisms and BBB function in brain metastases.
References:


between Rho, NHE, and p65/NFκB. Identification of this central signaling link may help us ultimately in the design of new anti-metastatic drugs directed at inhibiting the p65/NFκB pathway, or on breast cancer cell surfaces, thereby preventing adhesion to lung endothelium/OECFV and reducing the risk of lung metastasis.

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P66-8: INVESTIGATION OF THE ROLE OF THE ARHGAP5 GENE IN REGULATION OF BREAST TUMOR CELL MIGRATION, INVASION, AND EPITHELIAL-TO-MESenchymal TRANSITION

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Recent work in our laboratory has focused on the identification of a positive tumor suppressor gene (TSG) within 1q Megakaryocytes (MK) isolated on chromosome 22q7.3 in breast cancer. We report here the discovery of the DecamGAP-similar TSG (ARHGAP5) which was cloned by expression analysis on the basis of homology to the known TSGs MM1 and AIP50. A PCR fragment containing a full ARHGAP5 cDNA was identified and was recently shown to be a functional tumour suppressor (TSG) gene. The product of the ESRH 1 gene encodes a protein of 577 amino acids with a high degree of similarity to the Cdc25A kinase family. Analysis of DNA methylation and histone deacetylation in the silencing of ARHGAP5 expression in breast cancer cells.

The involvement of DNA methylation and histone deacetylation in the silencing of ARHGAP5 expression in breast cancer cells was examined. Tumors expressing the breast cancer model (MDA-MB-231 and MDA-MB-435) were examined for DNA methylation and histone deacetylation. DNA methylation and histone modifications were found to be key regulators of ARHGAP5 expression in breast cancer.

P66-19: PATHOGENESIS AND BLOOD-BRAIN BARRIER HETEROGENEITY OF BREAST CANCER BRAIN METASTASIS

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A large number of young breast cancer patients die of brain metastasis. Our knowledge, which brain metastases develop after metastatic cells extravasate and localize and how these metastatic cells evade the blood-brain barrier (BBB) to enter the brain, is largely based on uncontrolled experiments in other systems and the existence of blood-brain barrier (BBB) has been the major reason for the inefficiency of current chemotherapeutic regimens. Development of effective therapies will depend on a better understanding of the pathogenesis of brain metastases and the role of BBB in its development.

By using a selected, green fluorescent protein expressing breast cancer cell line (MDA-MB-231-GFP), we found that metastatic breast tumor cells extravasate is regulated by the BBB in a complex manner that involves several different steps. The fluorescence of the tumor cells increased with time, and the BBB remained intact. The BBB was disrupted in the late stages of tumor invasion, and the BBB was disrupted in the late stages of tumor invasion.

The results up to the abstract submission were as follows: First, the very early stage breast cancer cells exhibited a red fluorescent signal at the tumor site. Second, the breast cancer cells extravasated from the blood vessels into the brain. Third, the breast cancer cells invaded the brain. Fourth, the breast cancer cells invaded the brain. Fifth, the breast cancer cells invaded the brain. Sixth, the breast cancer cells invaded the brain. The results up to the abstract submission were as follows: First, the very early stage breast cancer cells exhibited a red fluorescent signal at the tumor site. Second, the breast cancer cells extravasated from the blood vessels into the brain. Third, the breast cancer cells invaded the brain. Fourth, the breast cancer cells invaded the brain. Fifth, the breast cancer cells invaded the brain. Sixth, the breast cancer cells invaded the brain.

We conclude that metastatic breast cancer cells extravasate into the brain by a complex process involving the BBB, which provides a unique opportunity for the early detection and treatment of breast cancer brain metastasis.
P60-11: A MODEL OF COX2-MEDIATED BONE METASTASIS IN HUMAN BREAST CANCER

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Invasion and Metastasis III

P60-15: TRAFFICKING OF BREAST CANCER METASTATIC CELLS IN BONE

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Breast cancer cells frequently metastasize to bone where they cause osteolytic lesions. It is not known whether bone loss that the lesions become visible by X-ray. At that time the tumor masses are mostly visible in the ends of the long bones, areas of active hematopoiesis. However, very little is known about the early infiltrating patterns of the metastatic cells. The purpose of this study is to determine the movement of breast cancer cells in the long bones over time.

Method: MDA-MB-231 human breast cancer cells, engineered to express green fluorescent protein (GFP) were inoculated into the left femoral head of syngeneic mice. The femurs were harvested at 1, 2, 4, 6, and 24 weeks, after the inoculation. Intraosseous metastatic foci were examined by fluorescence microscopy. Some foci were then fixed and demineralized for histology. In the latter case, other femurs were sent out for routine histology and immunohistochemistry. At each time, other femurs were sent out for routine histology and immunohistochemistry. At each time, other femurs were sent out for routine histology and immunohistochemistry.

Results: A few GFP expressing cancer cells could be clearly seen in the intact bone as early as one week. Using fluorescence microscopy to examine 25-mm sections of the femur, we detected 126 foci of GFP cells in the bone sections. Interestingly, the distal ends of the femur were populated by cancer cells first. Then the cancer spread to the proximal end. Almost never were cancer cells seen in the cortical shaft of the bone. These general patterns also were seen by flow cytometry and by presence of the cancer cell DNA. Based on previous in vitro data, we also measured apoptotic events in breast cancer cells using a TUNEL assay. The number of apoptotic cancer cells increased as the number of breast cancer cells increased. Moreover, apoptotic cancer cells were seen in proximal to prevent distant metastasis early in the process.

Conclusions: These results taken together suggest that the tumor cells enter the bone macrophages to phagocyte directly, to the hematopoietic stem at the ends of the bone. It is likely that the macrophage are in contact with the tumor cells and provide a favorable environment for their growth. As the cancer cells multiply, local osteoclasts undergo apoptosis indicating that the bone cells are not easily expanded even if the cancer cells are blocked. This information is used to plan strategies to prevent distant metastasis early in the process.

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Patents Granted and Pending
Patents pending:

1. Therapy of cancer by insect cells containing recombinant baculovirus encoding genes. Isaiah J. Fodler, Zhongyun Dong, and Weixin Lu. International patent application No. PCT/US00/07200 and U.S. Serial No. 09/271,01


PUBLICATIONS

28


Lu, W., Dong, Z., Donawho, C., Fidler, I. J. Specific immunotherapy against occult cancer metastases. Int J Cancer 100: 480-5, 2002


Lu, W., Schroit, A. J. Vascularization of melanoma by mobilization and remodeling of preexisting latent vessels to patency. Cancer Res, 65(3): , 2005

Abstracts (Last five years only)


Suppression of orthotopic murine colon cancer and liver metastasis by a baculoviral vector system-mediated interferon-β gene therapy. Shutaro Ozawa, Weixin Lu, Zhongyun Dong,


EDITORIAL AND REVIEW ACTIVITIES
N/A

TEACHING
N/A

PROFESSIONAL MEMBERSHIPS/ACTIVITIES
Professional Society Activities, with Offices Held
Associate Member, AACR