Award Number: W81XWH-04-1-0316

TITLE: Prevention of the Angiogenic Switch in Human Breast Cancer

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REPORT DATE: March 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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Prevention of the Angiogenic Switch in Human Breast Cancer

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The overall goal of this research is to determine if human breast cancer can be prevented from becoming angiogenic when it is still at a microscopic size of less than approximately 1 mm³. We have made the following progress during the past year: (1) We have developed models in SCID mice of four different non-angiogenic human breast cancers, and have shown that the time to the switch to the angiogenic phenotype is predictable and reproducible for each different breast cancer type. The same is true for the percentage of tumors that become angiogenic. (2) Two angiogenesis-based biomarkers have been developed to detect the angiogenic switch when these tumors are still at a microscopic size of 1 mm³ or less. The most sensitive and accurate biomarker is the "platelet angiogenic profile," which determines and quantifies the angiogenic regulatory proteins being elaborated by a tumor. A second biomarker is a rise in circulating precursor endothelial cells exiting from the bone marrow and stimulated by a tumor that is undergoing the angiogenic switch.

Our translational goal is to treat human breast cancer, both primary and recurrent, with non-toxic angiogenesis inhibitors guided by biomarkers before tumors can be anatomically located.
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PREVENTION OF THE ANGIOGENIC SWITCH IN HUMAN BREAST CANCER.

I. INTRODUCTION:

The purpose of this research is to determine if human breast cancer (and other human tumors) can be prevented from becoming angiogenic when they are still at a microscopic size of less than approximately 1 millimeter diameter. We have proposed to develop angiogenesis-based biomarkers that will recognize the presence of such an early tumor long before it could be diagnosed by any conventional methods such as magnetic resonance imaging, CAT scan, or ultrasound. We have further proposed to study the mechanism of the switch to the angiogenic phenotype. We wish to prevent the angiogenic switch in human breast cancer by long-term administration of non-toxic angiogenesis inhibitors, or by increasing the level of one or more endogenous angiogenesis inhibitors in the circulation by oral administration of non-toxic drugs which increase the expression of these angiogenesis inhibitors.

The translational goal of this project is two-fold: (i) For women who have had a primary breast cancer surgically removed or treated by another modality, angiogenesis-based biomarkers in the blood and urine will be quantified periodically (every few months), in the post-operative period. These biomarkers will be developed during the support of this Innovator Award. They will include the platelet angiogenic profile and circulating endothelial precursor cells. (Urinary metalloproteinases will be used in conjunction with these two biomarkers and to validate them. Marsha Moses, Ph.D., in our laboratory will collaborate with Dr. Folkman on this aspect of the project). Based on what we have learned from mouse studies so far, women whose biomarkers begin to rise in the post-operative period, would have a high risk of recurrence several years before such a recurrence could be predicted with today’s technology. These women would be offered treatment by relatively non-toxic antiangiogenic therapy until the biomarkers returned to normal and for a given period beyond that time. Angiogenesis-based biomarkers would continue to be tested periodically as a surveillance measure.

(ii) Women with the mutated breast cancer gene may be monitored by periodic tests of angiogenesis-based biomarkers. Rising biomarkers could be treated as described above. If the biomarkers fell to normal, this could possibly obviate the current medical practice of advising these women to have bilateral mastectomy and oophorectomy. These angiogenesis-based biomarker blood and urine tests will need to be validated in clinical trials. Our goal during the period of support of this Innovator Award, is to complete the development of
three angiogenesis-based biomarkers and to initiate the clinical trials for validation. We estimate that approximately 2-3 years would be needed to validate these three tests in patients by using all of the Harvard Hospitals.

We also envisage that the results from these studies will apply generally to other tumors, for example colon cancer and prostate cancer.

II. BODY:

Significant progress has been made in all three tasks.

**Task 1. Median time of switching to the angiogenic phenotype for human breast cancers.** (Judah Folkman, M.D., Yuen Shing, Ph.D., George Naumov, Ph.D., Deborah Freedman, Ph.D. & Gang Liang, Ph.D.)

We have previously characterized the subcutaneous tumor growth of both angiogenic and non-angiogenic tumor cells from a human breast cancer cell line (MDA-MB-436). When SCID immunodeficient mice were inoculated subcutaneously with human breast cancer cells that were either non-angiogenic or angiogenic, tumors first became reliably palpable at approximately 50 mm$^3$. The mean time to palpation after inoculation of angiogenic breast cancer cells was 19 days (mean, 95% confidence interval [CI] =16-22 days), and in mice injected with the non-angiogenic cells was 119 days (mean, 95% CI= 53-185 days).

We have found that angiogenic tumors virtually always contain sub-populations of non-angiogenic tumor cells. We separated non-angiogenic tumor cells from angiogenic tumor cells of a human breast cancer (MDA-MB-436) and cloned a single non-angiogenic tumor cell (called clone A1). Tumor cells from clone A1 remained non-angiogenic for a mean of 234 days, (95% CI=199-269 days). Approximately 5-10% of implanted tumors from A1 switched to the angiogenic phenotype.

The non-angiogenic and angiogenic tumor cells had equivalent proliferation rates *in vitro*. Nevertheless, we found that even when a single tumor cell was cloned from an angiogenic tumor, expanded in cell culture, and inoculated into a mouse to produce an angiogenic tumor, the majority of the cells produced angiogenic tumors which grew rapidly. However, a few cells from the expanded angiogenic clone were found to be non-angiogenic.

The reversion of some angiogenic tumor cells to non-angiogenic tumor cells was only recently discovered in my laboratory during support from the Innovator Award, but the mechanism of this reversion remains unknown. It is one of the interesting problems that we will try to attack during the coming year.
At day 350 after injection of the human breast cancer cells, the cumulative incidence of palpable tumors was as follows: mice inoculated with angiogenic cells, 100%; with mice inoculated with non-angiogenic cells, 50-80%; and with non-angiogenic clone A1 cells, 10-20%.

Orthotopic inoculation of breast cancer cells into the mammary fat pad gave similar results, except that in the orthotopic site the switch to the angiogenic phenotype was delayed by approximately 30 days when compared to subcutaneously grown tumors.

We have infected all three sub-populations with Luciferase for more quantitative in vivo tumor growth monitoring. In addition, we have found that the Luciferase signal reveals a sharp increase in light flux (the angiogenic switch), approximately 20 days before the appearance of a palpable tumor.

In addition to the detailed characterization of human breast cancer cell lines that produce dormant tumors (MDA-MB-436), we are currently characterizing three other breast cancer cell lines: MDA-MB-415, MDA-MB-435S, and MDA-MB-453 (see Fig.1). When injected subcutaneously into mice MDA-MB-415 human breast cancer cells remained non-angiogenic and dormant at a microscopic size (< 1mm$^3$) for up to 280 days after injection. Currently, 2 out of 8 mice have palpable tumors which are less than 100 mm$^3$ in size. Thus, the initial observations from the MDA-MB-436 tumor cells, also appear to be reproducible for a different human breast cancer cell line (i.e., MDA-MB-415). All mice injected with the MDA-MB-453 breast (angiogenic) cancer cells formed palpable tumors.

**Figure 1:** Human breast cancer cell lines in SCID mice. 5x10$^6$ tumor cells implanted subcutaneously. Left panel: shows angiogenic cells cloned from a single cell. Middle panel: non-angiogenic cells from a different tumor line cloned from a single cell showing the angiogenic switch in approximately 70% of tumors at a median of approximately 120 days. Right panel: non-angiogenic cells from a different breast cancer cell line. Dormant tumors for up to 250 days. One tumor has begun the angiogenic switch at 275 days (this is close to the normal lifetime of these SCID mice).

Currently, 2 out of 8 mice have palpable tumors which are less than 100 mm$^3$ in size. Thus, the initial observations from the MDA-MB-436 tumor cells, also appear to be reproducible for a different human breast cancer cell line (i.e., MDA-MB-415). All mice injected with the MDA-MB-453 breast (angiogenic) cancer cells formed palpable tumors.
30 days after inoculation. However these tumors grew very slowly over the next 220 days to a final size of approximately 1700 mm$^3$. Similar slow tumor growth was observed when MDA-MB-435S cells were injected subcutaneously. All mice inoculated with these cells developed palpable tumors by day 10 and formed large tumors by day 280 after injection.

We have developed two cell lines from two MDA-MB-453 tumors. When mice were re-inoculated with the angiogenic sub-population of the MDA-MB-453 cells, palpable tumors formed by day 10 and large tumors (> 500 mm$^3$) formed within 30 days.

Isolation of the angiogenic sub-population from MDA-MB-415 tumors that switched to the angiogenic phenotype is ongoing. We have developed two new stable breast cancer models and their characterization is ongoing.

Deborah Freedman has studied one of the possible mechanisms of the switch to the angiogenic phenotype. She is working on a hypothesis that certain tumor cells may be able to induce neighboring endothelial cells to bypass senescence and become immortal. To attack this problem she has established a series of immortalized human endothelial cells that overexpress telomerase. She accomplished this by transfecting the gene for telomerase hTert. Increased hTert activity leads to stabilization of telomerase and an unlimited replicated capacity or immortality of the endothelial cells.

During the past year Dr. Freedman has found for the first time a new pathway that may be responsible for the normal senescence of endothelial cells after they have cycled through a predicted number of cell divisions. She found that senescent human endothelial cells contain extremely low or undetectable CDK2 activity which results from the dramatic reduction of CDK2 levels in senescent endothelial cells. She found that CDK2 translation declines during senescence. She showed that bypass of endothelial senescence by telomerase entails the restoration of CDK2 translation and activity. In summary, CDK2 translational downregulation turns out to be a key regulatory event in replicative senescence of endothelial cells.

Understanding the mechanisms which regulate endothelial senescence will be critical in determining the role of endothelial senescence in tumor growth, and whether tumor cells can induce neighboring endothelial cells to bypass senescence.

**Summary of progress in Task 1:**

a. During the past year we have obtained validation that the angiogenic switch is predictable for time and percentage of tumors that switch to the angiogenic phenotype.
b. We have further shown that these angiogenic switching times and percentages of the tumors which undergo the switch are reproducible for a given clone from a single cell.

c. During the next funding year we will address the following questions:

(i) Does antiangiogenic therapy permanently prevent the angiogenic switch after therapy is discontinued?

(ii) If discontinuation of antiangiogenic therapy causes dormancy, does it reset the angiogenic switch to a significantly longer time than the original switching time?

(iii) Gene array analysis will be carried out on the non-angiogenic and angiogenic pairs of each breast cancer to determine if any genes are overexpressed in the non-angiogenic tumors but down-regulated or lost in the angiogenic tumors.

(iv) If so, then genes which are significantly overexpressed only in the non-angiogenic tumors may be transfected into the angiogenic tumors to determine if the angiogenic switch can be suppressed.

Cooperative studies between Task 1 and Task 2:

In Task 1 we have ongoing studies that will quantitatively assess the pro- and anti-angiogenic factors in platelet and plasma blood compartments. These studies will elucidate the balance, dynamics, and compartmentalization of pro- and anti-angiogenic factors before, during, and after the angiogenic switch. Mice were injected with the non-angiogenic MDA-MB-436 cells and platelets and plasma were isolated for analysis. Plasma concentrations and platelet loading with pro- and anti-angiogenic factors will be quantified using the ProteinChip system (Series 40000, Ciphergen, Inc.) in collaboration with Dr. Giannoula Klement in Task 3.

We have initiated a detailed study that will dissect the steps of the angiogenic switch in breast cancer. The extent of neovascularization during the angiogenic switch will be quantitatively and qualitatively described at various stages of the angiogenic switch. The MDA-MB-436 cell dormancy model provides us with a unique opportunity for this kind of analysis. We learned the techniques for this approach by visiting Professor Donald McDonald's laboratory at the University of California, San Francisco.

We also have ongoing experiments in collaboration with Randolph Watnick (Task 2) that will assess the effect of thrombospondin-1 on the timing of the angiogenic switch in non-angiogenic tumors. We have infected non-angiogenic breast tumor cells with thrombospondin-1 antisense. We hypothesize
that this endogenous decrease in thrombospondin-1 levels will lead to an earlier switch of non-angiogenic tumor cells to the angiogenic phenotype.

**Task 2: Molecular and genetic studies of the mechanism of the stability of the non-angiogenic phenotype.** (Randolph Watnick, Ph.D.)

Previous results from our group have determined that one form of tumor dormancy is due to an inability of its tumor cells to induce angiogenesis. Our development of a set of non-angiogenic human cancers that remain dormant at a microscopic size, provides an excellent tool to study the regulation of tumor progression as well as the role of the surrounding normal tissue in this process. We hypothesize that the inability of dormant breast tumors to induce neovascularization is due to an imbalance in their production of pro- and ant angiogenic factors.

We have made the observation that aggressive angiogenic human tumor cells express little to no thrombospondin-1. Thrombospondin-1 is a potent endogenous inhibitor of angiogenesis. Furthermore, the c-Myc oncoprotein, which negatively regulates the expression of thrombospondin-1, is expressed in greater amounts in angiogenic human tumors as compared to non-angiogenic tumor cells.

During the past year we have determined that two breast cancer cell lines that form non-angiogenic, dormant tumors in mice express very high levels of thrombospondin-1 and very low levels of c-Myc or phosphorylated c-Myc. Conversely, we have found that two breast cancer cell lines that form angiogenic tumors in mice, express very low levels of thrombospondin-1 and high levels of c-Myc.

Furthermore, we have determined that angiogenic breast cancer cell lines repress the expression of thrombospondin-1 in surrounding stromal fibroblasts. In contrast, the breast cancer cell lines that produce non-angiogenic dormant tumors actually stimulate thrombospondin-1 expression in stromal fibroblasts. The latter is an unprecedented finding.

We are currently in the process of identifying and purifying the thrombospondin-1 repressing factor(s) that the angiogenic cells secrete, as well as the thrombospondin-1 stimulating factor(s) that the non-angiogenic cells secrete.

We have also found that inhibition of Myc activity in the angiogenic tumor cells results in the loss of thrombospondin-1 activity. Therefore, the expression and/or secretion of the thrombospondin-1 repressing factor(s) appears to be downstream of c-Myc.
Summary of Task 2 and Next Steps:

Our working hypothesis is that all breast tumors must pass through the non-angiogenic, dormant phase prior to becoming angiogenic and malignant tumors. Based on our current data we postulate that this transition or "angiogenic switch" is triggered by the increased expression of c-Myc, which, in turn, represses the expression of thrombospondin-1 and allows the tumor to become angiogenic and malignant.

In the next year and beyond we will determine the genetic and molecular mechanisms that regulate the expression of c-Myc in order to determine how the angiogenic switch is triggered.

Task 3: Development of a novel angiogenesis-based biomarker in platelets to detect non-angiogenic, microscopic-sized dormant tumors before or just after the angiogenic switch, but before tumors can be detected by palpation (i.e., ~50 mm³).

Platelet angiogenic profile. (Judah Folkman, M.D., Giannoula Klement, M.D., & David Cervi, Ph.D.)

Judah Folkman and Giannoula Klement reported to the American Society of Hematology in December 2004 Dr. Folkman's discovery that the proteins contained within platelets shift their concentrations to reflect the angiogenic regulatory proteins elaborated by a tumor.¹ Folkman had previously reported that of the approximately 24 proteins known to be contained within platelets, 13 were positive regulators of angiogenesis and 11 were negative regulators of angiogenesis.² He has now shown that these platelet proteins can be accurately quantified by SELDI-ToF mass spectroscopy. We have found that in immunodeficient animals bearing any one of several different types of human cancer, specific angiogenic proteins elaborated by a tumor are taken up by platelets and sequestered. The peak concentration of a given angiogenic protein elaborated by a tumor is in direct proportion to the time the tumor has been present. Therefore, the "platelet angiogenic profile" can detect an early microscopic tumor as small as 1 mm³ or less, and can indicate how long the tumor has been present. For example, if a non-angiogenic tumor is elaborating vascular endothelial growth factor (VEGF), the concentration of VEGF in platelets will increase steadily over time in a linear fashion for as long as 120 days or more. If a human tumor is elaborating more than one angiogenic regulatory molecule, i.e., VEGF, bFGF, PDGF, and endostatin, these will all reveal their increased concentrations in a given platelet sample.

We have developed a standard protocol for sample preparation of platelet lysates. We have developed platelet profile analysis based on the Protein Chip System from Ciphergen, Inc. We now have preliminary data that platelets carry both pro- and anti-angiogenic proteins but exclude other plasma proteins.
Next steps: we are currently conducting a time course experiment that will reveal changes in platelet and plasma profiles of angiogenic regulatory proteins before, during, and after the angiogenic switch. These studies will be performed in both subcutaneously and orthotopically implanted tumors.

**Circulating endothelial cells.** (John Heymach, M.D., Ph.D., Paul Beaudry, M.D., Daniela Prox, M.D.)

Growing evidence now suggests that bone marrow-derived circulating endothelial cells also contribute to tumor neovascularization and tumor growth in some tumors. In collaboration with Dr. Paul Beaudry, we have developed a standard protocol for quantification of circulating endothelial cells in whole blood.

In brief, a cocktail of antibodies is added to a citrated blood sample. These antibodies detect two separate populations of circulating endothelial cells: mature circulating endothelial cells (CECs), and circulating endothelial progenitor cells (CEPs). We have quantitatively compared the systemic mobilization of these two populations of circulating endothelial cells induced by non-angiogenic or angiogenic breast tumors.

We found that at 40 days after subcutaneous inoculation, microscopic non-angiogenic tumors caused a decrease of the CEC population, but angiogenic tumors (~600 mm³ in size) had no effect (see Fig. 2). At the same time point, angiogenic tumors increased the CEP population, but non-angiogenic microscopic tumors had no effect. We have preliminary evidence that the majority of CEC cells originate from the bone marrow, in contrast to a small percentage (~2-3%) of CEP cells. Therefore, even at a microscopic size, non-angiogenic tumors may induce a systemic effect that mobilizes bone marrow CEC cell population. We hypothesize that changes in systemic CEC and CEP populations of cells may be useful as a marker of early cancer.

![Figure 2](image-url)

**Figure 2:** Mature circulating endothelial cells (A) and circulating endothelial precursor cells (B) in whole blood analyzed by flow cytometry from mice bearing non-angiogenic and angiogenic tumor cells. (A) Non-angiogenic tumor cells suppress mature circulating endothelial cells (CEC). (B) The angiogenic tumor cells induce mobilization of circulating endothelial precursor cells (CEP) from the bone marrow.
III. KEY RESEARCH ACCOMPLISHMENTS:

- Established the non-angiogenic subpopulation of four human breast cancers in SCID mice.
- Established reproducible and predictable times to the angiogenic switch for each tumor type.
- Established predictable and reproducible percentages of non-angiogenic tumors for the angiogenic switch for each tumor type.
- Developed a novel biomarker, the “platelet angiogenic profile,” which currently may be the most sensitive and most specific biomarker for human cancer available. It can detect the presence of human tumors of less than 1 mm$^3$; it can quantify how long the tumor has been present; and, it can indicate when the angiogenic switch is occurring.
- Continued the development of circulating progenitor endothelial cells as a biomarker of early angiogenic tumors.

IV: REPORTABLE OUTCOMES:

- The “platelet angiogenic profile” was published in November 2004, and presented in December 2004 as a platform presentation to the American Society of Hematology. Of more than 3,600 papers presented at that meeting, the “platelet angiogenic profile” was among the very few which attracted science writers (see appendix, Wall Street Journal article).

V: CONCLUSIONS:

The overall goal of this Innovator Award is to prevent the angiogenic switch in women who face a possible recurrence of breast cancer, or in those at high risk of being diagnosed with the disease.

In Task 1, the experiments are designed to lead to a clinical application in which non-toxic, FDA approved angiogenesis inhibitors are administered to women before the angiogenic switch occurs in tumors that are still of microscopic size and asymptomatic.

In Task 2, our goal is to elucidate the molecular mechanism of the angiogenic switch so that the switch itself could be interrupted, possibly by novel molecules which remain to be discovered. These would be novel types of
angiogenesis inhibitors. This objective is not just wild speculation. It has recently been reported that rosiglitazone, an FDA approved orally available drug for the treatment of type II diabetes, increases the expression of thrombospondin-1 and also increases expression of CD36, the receptor for thrombospondin-1 on endothelial cells.

In Task 3, we now have developed a novel biomarker to detect angiogenic regulatory proteins, both positive and negative, elaborated from human breast cancers that are microscopic in size (<1 mm³). This new method called the "platelet angiogenic profile" can detect human cancers in SCID mice before and just after the angiogenic switch. Our next step will be to conduct a time course experiment that will reveal changes in platelet and plasma profiles of angiogenic regulatory proteins before, during, and after the angiogenic switch. These studies will be performed in both subcutaneously and orthotopically implanted tumors. Also planned are studies to determine the sensitivity of this biomarker method by adding VEGF at increasing concentrations (from femtomolar to nanomolar) to platelet rich plasma to determine the lowest concentration of added VEGF that can be detected by our mass spectroscopy.

"So What Section"
These studies advance us toward our goal of treating breast cancer at an ultra early stage before it can be located and before it is symptomatic. The goal will be achieved by administering relatively non-toxic angiogenesis inhibitors guided by sensitive and specific angiogenesis-based biomarkers. The biomarkers will be, (i) the platelet angiogenic profile; (ii) circulating endothelial precursors; and (iii) urinary metalloproteinases. Eventually, the first patients may be those women whose breast cancer has been surgically removed. The angiogenesis-based biomarkers could be used periodically (~every three months) to look for recurrence. Any microscopic recurrence may be treated by non-toxic angiogenesis inhibitors until the biomarkers return to normal. The next patients to be studied may be those at high-risk for developing a new primary breast cancer because they harbor a mutated breast cancer gene. Conventional medical practice is to offer these women bilateral mastectomy and bilateral oophorectomy. For those who refuse, we would propose that these individuals could be followed periodically by quantifying their angiogenesis-based biomarkers. Patients with rising biomarkers would be treated by antiangiogenic therapy until the biomarkers returned to normal. Patients whose biomarkers remain normal would not be treated.

As these biomarkers are validated in the clinic over the coming years, it should be possible to extend them to other cancers, such as colon cancer and prostate cancer. We have also recently proposed that it may be possible to raise the level of endogenous angiogenesis inhibitors by administration of small molecular orally available drugs as a novel treatment of ultra early cancer. The long-term goal is to treat cancer before the disease state.
VI: REFERENCES:


VII: APPENDICES:

I. Pertinent Publications


II. Personnel Report

III. Curriculum Vitaes in Biosketch Format

- David Cervi, Ph.D., Research Fellow
- Daniela Prox, Ph.D., Research Fellow
- Sandra Ryeom, Ph.D., Research Associate
- Sarah Short, Ph.D., Research Fellow
Vascular Cell Biology and Platelet Adhesion

Abstract 839

Early Tumor Detection Using Platelet Uptake of Angiogenesis

Early Warning: A New Way To Find Cancer

Blood Test Detects Beginnings Of Disease, Suggesting Possibility Of Treatment Before Tumors Grow

By Amy Dockser Marcus

A group of researchers say they have found a way to detect cancer earlier, even before the type or the location of the tumor can be known.

The method, which is expected to enter clinical trials early next year, is part of a shift in thinking under way about how early to treat cancer. Long before cancer could ever be detected by current imaging scans or blood tests, the idea is to find an ultra-early indicator of the disease: substances in blood platelets that indicate cancer is in the works. Armed with such information, the researchers involved say, doctors could potentially offer cancer therapies that would prevent tumors from developing—and save patients from having to undergo much more toxic and aggressive treatment once a cancer grows.

While such preventive measure aren't yet being used in the general population, some researchers are already studying the prophylactic use of drugs in patients at very high risk to get cancer.

The platelet substances are a so-called biomarker of cancer—characteristics in bodily fluids that can indicate the presence of disease. Probably the best-known biomarker, prostate specific antigen, or PSA, is related to prostate cancer and is already commonly used. And the search for new and better biomarkers is a hot area of research, with many companies racing to come up with new ones in saliva, urine and blood, among other fluids. The National Cancer Institute earlier this month announced a new round of funding, earmarking $9.8 million to 17 labs looking for cancer biomarkers.

But researchers involved in the platelet study, as well as other cancer experts, say this discovery pushes the issue of early detection further than ever before. In effect, it could allow cancer to be treated similarly to the way doctors now treat heart disease. Just as doctors prescribe statin drugs to help prevent heart attacks in patients with high cholesterol, oncologists could test patients for this early biomarker and prescribe drugs to help prevent cancer. And just as cardiologists don't have to wait for a heart attack in order to act, oncologists wouldn't have to wait until malignant tumors are found.

Drugs in this case would be used to stop the cancerous process itself—rather than targeting a tumor in a particular part of the body. That would be a radical departure from the current approach. "Cancer treatment has always been about location, location, location," says Judah Folkman, director of the vascular biology lab at Children's Hospital and a co-author of the abstract. "If you don't know where the cancer is—if you can't feel it, see it, locate it, or biopsy it—you can't treat it." The marker could also potentially be used to monitor patients who had already had cancer for the earliest signs of recurrence.

Details about the biomarker, developed by researchers at Children's Hospital Boston, the Dana-

Potential Treatments

Some drugs that may prevent tumors from growing blood vessels:

- Avastin
- Celebrex
- Erbitux
- Herceptin
- Iressa
- Taxol
- Velcade

Source: Judah Folkman, M.D.
Early Warning: A Way to Find Cancer

The purpose of this new idea of preventive care is to identify the process of making more blood vessels before the development is complete. In recent years, doctors who have been looking for early signs of cancer have found that certain proteins produced by the body, which are known as tumor markers, may be associated with an increased risk of developing cancer. These proteins are called tumor markers because they are elevated in the blood of people with certain cancers.

In the process of making more blood vessels, the body releases a group of proteins called cytokines. These cytokines are produced by immune cells and are involved in the immune response. When these cytokines are released into the bloodstream, they can stimulate the growth of blood vessels.

By identifying the presence of these cytokines in the blood, doctors can detect the early stages of cancer before the tumor has grown large enough to cause symptoms. This early detection is crucial because it allows for earlier treatment, which can greatly improve the chances of survival.

The process of making more blood vessels is associated with the development of new blood vessels, which can supply the tumor with nutrients and oxygen. By detecting these cytokines, doctors can identify the tumor before it has grown large enough to cause symptoms. This early detection can help doctors to develop effective treatments that can stop the growth of the tumor.

Prevention of cancer is crucial because it allows for earlier treatment, which can greatly improve the chances of survival. By identifying the presence of these cytokines in the blood, doctors can detect the early stages of cancer before the tumor has grown large enough to cause symptoms. This early detection is crucial because it allows for earlier treatment, which can greatly improve the chances of survival.
### Personnel receiving pay from the research effort:

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<thead>
<tr>
<th>Name</th>
<th>Role on Project</th>
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<tbody>
<tr>
<td>Judah Folkman, M.D.</td>
<td>Principal Investigator</td>
<td>30%</td>
</tr>
<tr>
<td>Nava Almog, Ph.D.</td>
<td>Research Fellow</td>
<td>50% (03/01/04 - 06/30/04)</td>
</tr>
<tr>
<td>Amy Birsner</td>
<td>Veterinary Technician</td>
<td>25%</td>
</tr>
<tr>
<td>David Cervi, Ph.D.</td>
<td>Research Fellow</td>
<td>100% (09/01/04 - date)</td>
</tr>
<tr>
<td>Wendy Foss</td>
<td>Administrative Coordinator</td>
<td>30%</td>
</tr>
<tr>
<td>Deborah Freedman, Ph.D.</td>
<td>Research Associate</td>
<td>100%</td>
</tr>
<tr>
<td>Lena Kikuchi</td>
<td>Research Technician II</td>
<td>100% (06/27/04 - 11/13/04)</td>
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<td></td>
<td>50% (11/14/04 - 01/22/05)</td>
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<tr>
<td>Gang Liang, Ph.D.</td>
<td>Research Fellow</td>
<td>100% (05/10/04 - date)</td>
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<tr>
<td>Meghan Lorina</td>
<td>Purchasing Coordinator</td>
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<tr>
<td>George Naumov, Ph.D.</td>
<td>Research Fellow</td>
<td>20%</td>
</tr>
<tr>
<td>Daniela Prox, M.D.</td>
<td>Research Fellow</td>
<td>50% (07/01/04 - date)</td>
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<tr>
<td>Frank Rossi</td>
<td>Lab Support Supervisor</td>
<td>45% (08/22/04 - date)</td>
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<tr>
<td>Sandra Ryeom, Ph.D.</td>
<td>Research Associate</td>
<td>100% (12/15/04 - date)</td>
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<td>Sarah Schmidt</td>
<td>Administrative Assistant</td>
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<tr>
<td>Yuen Shing, Ph.D.</td>
<td>Research Associate</td>
<td>30% (03/01/04 - 03/31/04)</td>
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<tr>
<td></td>
<td></td>
<td>25% (04/01/04 - date)</td>
</tr>
<tr>
<td>Sarah Short, Ph.D.</td>
<td>Research Fellow</td>
<td>100% (11/01/04 - date)</td>
</tr>
<tr>
<td>Dessie Stewart</td>
<td>Lab Assistant</td>
<td>20% (08/22/04 - 09/18/04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15% (09/19/04 - date)</td>
</tr>
<tr>
<td>Randolph Watnick, Ph.D.</td>
<td>Research Associate</td>
<td>50% (03/01/04 - 05/31/04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% (06/01/04 - date)</td>
</tr>
</tbody>
</table>
BIOGRAPHICAL SKETCH

NAME
Cervi, David

POSITION TITLE
Research Fellow

eRA COMMONS USER NAME

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Windsor, Windsor ON</td>
<td>B.Sc.</td>
<td>1998</td>
<td>Biological Sciences</td>
</tr>
<tr>
<td>University of Windsor, Windsor ON</td>
<td>M.Sc.</td>
<td>2000</td>
<td>Cellular Biology</td>
</tr>
<tr>
<td>University of Toronto, Toronto ON</td>
<td>Ph.D.</td>
<td>2005</td>
<td>Molecular &amp; Cellular Biology</td>
</tr>
</tbody>
</table>

A. Positions and Honors.
1998 Laboratory Technician, University of Windsor, Windsor ON
2004 Research Technician, Vascular Biology Program, Children's Hospital, Boston, MA
2005-present Research Fellow, Vascular Biology Program, Children's Hospital, Boston, MA
2005-present Research Fellow in Surgery, Harvard Medical School, Boston, MA

Honors & Awards:
1998-2000 Tuition Scholarship (University of Windsor)
1999 Dr. Joseph E. J. Habowsky Graduate Student Teaching Award
2000 Travel Grant American Society for Microbiology
2000-2001 Ontario Graduate Scholarship
2001-2002 Ontario Graduate Scholarship (declined)
2001-2004 University of Toronto Open
2001-2004 Canadian Institute of Health Research

B. Selected peer-reviewed publications (in chronological order).
BIOGRAPHICAL SKETCH

NAME
Prox, Daniela

POSITION TITLE
Research Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Vienna, Austria</td>
<td></td>
<td>1997-1998</td>
<td>Medicine, exchange program</td>
</tr>
<tr>
<td>Medical School, University of Mainz, Germany</td>
<td></td>
<td>1994-2000</td>
<td>Pre-Medicine and Medicine</td>
</tr>
<tr>
<td>Medical School, University of Munich, Germany</td>
<td>M.D.</td>
<td>2000-2001</td>
<td>Medicine</td>
</tr>
<tr>
<td>University Children's Hospital, Munich, Germany</td>
<td></td>
<td>2002-2004</td>
<td>Residency in Pediatrics</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

1999-2000  Research Technician, Surgical Research, Children's Hospital, Harvard Medical School, Boston, MA
2004-present Research Fellow, Vascular Biology Program, Children's Hospital, Harvard Medical School, Boston, MA

B. Selected peer-reviewed publications (in chronological order).


NAME: Ryeom, Sandra W.

POSITION TITLE: Instructor/Assistant Professor (paperwork pending)

eRA COMMONS USER NAME:

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
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<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wellesley College, Wellesley, MA</td>
<td>B.A.</td>
<td>1989</td>
<td>Physics</td>
</tr>
<tr>
<td>Cornell University, New York, NY</td>
<td>Ph. D.</td>
<td>1996</td>
<td>Cell Biology and</td>
</tr>
<tr>
<td>Harvard Medical School, Boston, MA</td>
<td>Post-Doc</td>
<td>1997-2004</td>
<td>Cell Biology</td>
</tr>
</tbody>
</table>

A. Positions and Honors

Positions
1989-1991: Research Assistant, Division of Infectious Diseases, Beth Israel Hospital, Boston, MA
1991-1996: Graduate Student, Dept. of Cell Biology and Genetics, Cornell University, NYC, NY
1997-2004: Postdoctoral Fellow: Dept. of Cell Biology Harvard Medical School, Boston, MA
2004-present: Research Associate, Division of Vascular Biology, Dept. of Surgery Children’s Hospital
Instructor/Assistant Professor (paperwork pending), Harvard Medical School, Boston, MA

Honors:
1994 Award of Excellence, Vincent Du Vigneaud Symposium, Cornell University, New York, NY.
1994 Trainee Investigator Award, American Federation of Clinical Research, Baltimore, MD.
1994 Selected as member of “Fundamental Issues in Vision Research” course, MBL, Woods Hole, MA.
1997 Recipient of National Research Service Award (NRSA) to support post-doctoral research
studying the signalling properties of the tight junction protein, ZO-1 in corneal epithelial cells.

B. Peer-reviewed publications (in chronological order)


BIOGRAPHICAL SKETCH

NAME
Short, Sarah

POSITION TITLE
Research Fellow

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of California, Santa Cruz, CA</td>
<td>B.A.</td>
<td>1985</td>
<td>Chemistry</td>
</tr>
<tr>
<td>University of North Carolina, Chapel Hill, NC</td>
<td>Ph.D.</td>
<td>1999</td>
<td>Pharmacology</td>
</tr>
</tbody>
</table>

A. Positions and Honors.

Professional Experience

1983-1985  Research Assistant, UC Santa Cruz, Santa Cruz, CA
2000-present Research Fellow, Children's Hospital, Harvard Medical School, Boston, MA

Awards

1989  Genetic Environmental Toxicological Association outstanding presentation award
1989  SRI outstanding performance award
1992  Genentech award for significant contribution to research project
1994  Hoechst-Celanese Scholarship award (UNC-CH research excellence award)

B. Selected peer-reviewed publications (in chronological order).


