A review of Judah Folkman's remarkable achievements in biomedicine

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On January 14th of this year, the biomedical research community lost Judah Folkman, the father of angiogenesis research. Folkman's warm and humble personality, inspirational teaching, unlimited creativity, and vast clinical experience have been described elsewhere (1). Here, we focus on Folkman's ineffaceable scientific achievements in angiogenesis research, which revolutionized biomedical research and clinical drug development.

Folkman founded an entirely new field of basic and clinical research and discovered a previously unknown family of angiogenesis regulatory molecules. He showed experimentally how these molecules provide a fundamental mechanism that controls the growth of virtually all tumors. He showed that expansion of tissue mass, whether neoplastic or nonneoplastic, critically depends on continuous endothelial replication and neovascularization.

Those discoveries paved new avenues for the development of a new class of drugs, angiogenesis inhibitors, which have already provided novel therapies for human cancer and age-related macular degeneration.

Professor Donald S. Coffey of The Johns Hopkins University (Baltimore, MD) recently said about professor Folkman: "Few ever get to see their lifetime thoughts, insights, creativity, and long effort explode across science and into the clinics as [his] many contributions have."

Fortunately, we are among those few who had the privilege to see and work with Dr. Folkman, who changed not only our lives but those of millions of others.

Judah Folkman. (Image courtesy of Jon Chase/Harvard News Office.)

Early Evidence of Tumor Angiogenesis

In 1971, Folkman proposed a hypothesis that tumor growth is angiogenesis-dependent (2). In that report, Folkman showed preliminary evidence that tumors could not enlarge beyond millimeter diameters without recruiting new capillary blood vessels (microvessels). His hypothesis stated that tumors secreted a diffusible substance that could stimulate endothelial cell proliferation in host capillary blood vessels.

This 1971 report was the first to introduce the concept of a novel form of tumor dormancy caused by blockage of angiogenesis. In that paper, Folkman also introduced the concept of "anti-angiogenesis" as a potential novel anticancer therapy.

Folkman wrote in 1971, "If a tumor could be held indefinitely in the nonvascularized dormant state . . . it is possible that metastases will not arise." He also predicted that a therapeutic agent could be made against a putative tumor-derived angiogenic factor. He concluded, "It has not been appreciated that the population of tumor cells and the population of capillary endothelial cells within a neoplasm may constitute a highly integrated ecosystem. In this ecosystem, the mitotic index of the two cell populations may depend on each other."

Folkman followed his 1971 hypothesis with supporting evidence indicating that tumors secreted diffusible endothelial mitogens *in vivo* (3), which could induce angiogenesis in neighboring microvessels. He and his colleagues then showed that tumor growth could be inhibited by blocking angiogenesis, and they proved that diffusible angiogenesis inhibitory factors existed (4).

Folkman and colleagues also reported that macromolecules and proteins can be released from polymers implanted in the cornea, a methodology that was critical for proof of angiogenic bioactivity of a given molecule *in vivo* (5). His research also identified the existence of a family of angiogenic peptides (6), and he showed that removing an angiogenic stimulus leads to regression of neovascularization (7).

Folkman's 1971 supposition initiated a field of angiogenesis research that has produced >35,000 reports on angiogenesis in peer-reviewed journals, and >70 monographs and books.

This single hypothesis has been remarkably fruitful. During the subsequent 37 years, it led to an almost uninterrupted series of new findings and discoveries, not only in the Folkman laboratory but in hundreds of laboratories worldwide.

Molecular Basis of the Discovery

Folkman showed that human and animal tumors produce a defined set of proangiogenic and antiangiogenic proteins. When the expression, secretion, or generation of these proteins is in equilibrium so that their proangiogenic and

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antiangiogenic activities are balanced, or oppose each other, tumor mass cannot expand beyond the limited distance that oxygen can diffuse from the nearest open capillary blood vessel (<250 microns). As a result of these metabolic restrictions, the development of most human cancer is usually arrested at a microscopic, dormant mass of 1–2 mm^3 or less. This early stage is also called *in situ* cancer.

Many humans harbor these microscopic tumors in various organs throughout life (8), and the vast majority of such tumors never expand further. They remain nonangiogenic, dormant, and harmless (9). They may contain up to ≈1 million tumor cells that are proliferating and undergoing apoptosis, but the microscopic tumors do not invade locally and do not metastasize.

In the rare event that a shift in the balance of proangiogenic and antiangiogenic proteins occurs, so that the total output of these proteins renders the tumor mass proangiogenic, the tumor is then enabled to "switch to an angiogenic phenotype" (10). As first elucidated by Folkman, the tumor itself expresses and secretes this excess of proangiogenic proteins or, alternatively, it enzymatically mobilizes proangiogenic peptides from host proteins in the circulation or the supportive framework of an organ or gland (stroma).

The angiogenic switch can also be modulated by host cells in the stroma or the circulation. After the angiogenic switch, both benign and malignant tumors can expand their tumor mass. Malignant angiogenic tumors, however, invade locally, metastasize to remote sites, and are potentially lethal. In the absence of the angiogenic switch, even tumors that are malignant by all other criteria may be harmless to the host (11).

Beginning in 1980, Folkman was the first to completely purify angiogenesis regulatory molecules. This identification of angiogenesis regulatory molecules became possible because of three novel bioassays that he had developed in the 1970s: cloned capillary endothelial cells *in vitro* (12); neovascularization induced in the rabbit and mouse corneas by angiogenic proteins undergoing sustained release from implanted polymers (4, 5); and the *ex vivo* chicken embryo chorioallantoic membrane (13). Today, at least 28 endogenous angiogenesis inhibitory molecules, and >10 proangiogenic molecules, have been discovered in the body. Many others are synthetic molecules.

Preclinical Applications of Angiogenesis

By the late 1980s, the scientific community had begun to accept some of the general principles of tumor angiogenesis: naturally occurring angiogenic and antiangiogenic molecules existed, a new biology of microvascular endothelial cells was emerging, a new form of tumor dormancy was caused by angiogenic blockade, and tumor growth and metastases were angiogenesis-dependent.

Around that time, Folkman and his associates began experiments that laid the groundwork for translation of these molecules and mechanisms to clinical application. He demonstrated that the rapid growth of metastases after surgical removal or radiation therapy of certain primary tumors, a phenomenon previously called "concomitant immunity" or "concomitant resistance" is in fact caused by withdrawal of angiostasis (14).

He also reported that human leukemia is angiogenic, and he showed in animal models that leukemia is angiogenesis-dependent (15). Before Folkman and colleagues' reports, physicians believed that because leukemic cells circulated in blood, the growth of leukemia cells did not need neovascularization. Folkman showed that neovascularization in the bone marrow was critical for expansion of leukemic mass in the marrow.

"Angiogenesis research will probably change the face of medicine in the next decades."

Folkman and colleagues (16) also showed for the first time that certain conventional cytotoxic chemotherapeutic agents, when administered frequently and at low doses, are endothelial-dependent. He named this "antiangiogenic chemotherapy." Others (17) subsequently called it "metronomic therapy."

His work showed that tumors that had become completely resistant to conventional cytotoxic therapy administered at maximum tolerated doses followed by off-therapy intervals could, in contrast, be significantly inhibited or permanently regressed by antiangiogenic chemotherapy (18).

Along with his collaborators, Folkman showed that platelets sequester angiogenesis regulatory proteins and segregate them into two sets of alpha granules, according to proangiogenic and antiangiogenic bioactivity (19).

Their analysis of the "platelet angiogenesis proteome" in tumor-bearing mice revealed that the presence of human tumors can be detected at a microscopic size that is below the resolution of any conventional imaging methods. This platelet angiogenesis proteome is now being studied in clinical trials at Harvard (Cambridge, MA) in collaboration with St. Jude's Cancer Center (Memphis, TN) for ultra-early detection of recurrent cancer years before it is symptomatic or can be anatomically located.

Folkman and his associates (20) also showed that infants with a life-threatening condition called hemangiomatosis, which results from an abnormal proliferation of blood vessels in any vascularized tissue, could be successfully treated by antiangiogenic therapy, and that experimental endometriosis could also be inhibited by antiangiogenic therapy (21).

A Beautiful Approach

Folkman hypothesized that certain nonneoplastic diseases such as psoriasis, rheumatoid arthritis, and ocular neovascularization could also be angiogenesis-dependent. He suggested that angiogenesis inhibitors developed for cancer therapy could possibly be used to treat nonneoplastic angiogenesis-dependent diseases (22).

A beautiful example of this translational approach is the concerted effort launched in 1992 by the Folkman laboratory with their collaborators to find the molecular target for choroidal and retinal neovascularization in humans. This target was unknown before the mid-1990s.

Ophthalmologists had previously used the term "X-factor" to indicate a putative diffusible molecule that mediated neovascular age-related macular degeneration. In the mid-1990s, Folkman and colleagues published a series of papers (e.g., see ref. 23) demonstrating that vascular endothelial growth factor (VEGF) is a critical mediator of retinal neovascularization, so it would therefore be an appropriate target for antiangiogenic therapy of age-related neovascular macular degeneration.

Those studies led directly to the clinical testing and eventual U.S. Food and Drug Administrative (FDA) approval of Pegaptanib (Macugen) which is an aptamer of VEGF, and ranibizumub (Lucentis), which is an anti-VEGF antibody.

These inhibitors are also approved in many countries, including those in the European Union, India, and Switzerland. With Lucentis, nearly 40% of patients who are blind (visual acuity of 20/300) regain their eyesight within a few months of initiation of local therapy (24).

Clinical Drug Delivery

Folkman's discovery that tumor growth is angiogenesis-dependent led to a profound paradigm shift in cancer therapy and cancer biology. Before his 1971 hypothesis,

the cancer cell *per se* was the only target of cancer therapies. Now it is accepted that the microvascular endothelial cell recruited by tumors is a second target.

Before its FDA approval in 2003 for multiple myeloma, bortezomib (Velcade) was reported to have potent antiangiogenic activity (25). Since 2003, 10 drugs that are pure angiogenesis inhibitors or that have potent antiangiogenic activity have been approved by the FDA and similar regulatory agencies in >40 other countries. Eight of these are being used to treat cancer, and two are used for the treatment of age-related macular degeneration (26).

In February 2004, FDA Commissioner Mark McClellan announced that, in addition to surgery, radiotherapy, and chemotherapy, "antiangiogenic therapy can now be considered the fourth modality of cancer treatment."

The FDA subsequently approved bevacizumab (Avastin) as a first-line treatment for patients with metastatic colorectal cancer. Three months later, Andrew C. von Eschenbach, the director of the National Cancer Institute, and Allen M. Spiegel, the director of the National Institute of Diabetes and Digestive and Kidney Diseases, wrote that "the approval marked the arrival of an intervention in which the primary mechanism of action is angiogenesis inhibition. We now can unequivocally say that angiogenesis is not only a critical factor for cancer, but for a host of other diseases."

Significant increases in survival have already been reported for patients with colon cancer and lung cancer treated with the angiogenesis inhibitor bevacizumab.

In 2006, ≈1.25 million patients received a prescription for one of these antiangiogenic drugs, and during the first 11 months of 2007, >1.5 million patients received prescriptions for antiangiogenic therapy (from a search of worldwide databases by "info2go," Thomas Pharma, Chemical Market Reporter and Business Communications). At the end of 2007, ≈23 drugs with antiangiogenic activity were in phase III clinical trials, and >30 were in phase II clinical trials.

In December 2005, *Nature* published a major review of the field of angiogenesis research and predicted that "angiogenesis research will probably change the face of medicine in the next decades" with more than 500 million people worldwide predicted to benefit from pro- or antiangiogenesis treatments (27).

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