

## Pietro M. Gullino and Angiogenesis

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### BIOGRAPHICAL NOTES

Pietro M. Gullino died 2 years ago. His work represents a pioneering contribution in tumor pathophysiology and angiogenesis. He received his M.D. degree at the University of Turin, Italy, and, after receiving the diploma from the Italian Board of Pathology, he spent two years training in biochemistry at the Technische Hochschule in Munich with Professor Waldschmidt-Leitz. He then joined the National Cancer Institute in the United States, first as a grantee of the National Cancer Institute (1954–1955) and later as a staff member in 1957. He was extensively involved (1971–1979) with the U.S.A. Breast Cancer Program and served as chairman of the Breast Cancer Task Force (1975–1979). After retirement from the National Cancer Institute, he moved back to Italy where he was Professor of Pathology at the University of Turin.

Pietro M. Gullino was author of many communications, lectures, courses, and seminars at various European and American academic institutions. His pioneer work introduced several novel, now widely accepted, concepts in the angiogenesis field. Among these are (1) the relationship between acquisition of angiogenic capacity and neoplastic transformation of a cell population; (2) the concept of high tumor interstitial pressure (of pivotal importance for a better understanding of the mechanisms of tumor cell dissemination during metastasis and of antineoplastic drug delivery); (3) the modifications of tissue composition at the onset of angiogenesis; and (4) the role of microenvironment in mediating tumor angiogenesis.

This brief report, far from being exhaustive, is a testament to the novelty that Gullino's work represented for his contemporary scientific community and will summarize the ma-

ior contributions of Pietro M. Gullino to tumor angiogenesis research.

### TUMOR PATHOPHYSIOLOGY: INTERSTITIAL PRESSURE AND BLOOD DISSEMINATION

The study of tumor microenvironment and its relationship with tumor angiogenesis represented a major task in Gullino's work. In the early 1960s, Gullino studied in vivo tumor microenvironment by developing a novel tissue-isolated tumor preparation separated from the host. The procedure required (1) preparation of a tumor connected to the host by a single artery and vein by injecting tumor cells into the rat ovary; (2) transfer of the tumor from the host to an ex vivo perfusion system; (3) constitution of the perfusion circuit; (4) analysis of the composition of the perfusates; and (5) control of vascular and metabolic parameters during perfusion (Gullino 1980).

Ten years later he wrote, "Increased hydrostatic pressure in tumor interstitial space was a consistent finding. Micropore chambers embedded in transplanted tumors drained 4 to 5 times more interstitial fluid than did identical chambers in the s.c. tissue. It is concluded that: (a) convective currents are present within the interstitial spaces of tumors; (b) the magnitude of fluid transfer can be measured by the difference in hemoconcentration between afferent and efferent tumor blood; and (c) the volume of this fluid transfer is not altered by hormone-induced tumor regression. The increased hydrostatic pressure of tumor interstitial spaces is interpreted as being due to absence of an anatomically well-developed lymphatic network. The bulk transfer of fluid within interstitial spaces is comparable to lymphatic drainage and should be considered in assessing drug concentration and distribution in solid tumors" (Butler et al. 1975). Also, Butler and Gullino found that tumors shed approximately  $10^6$  cells per 24 h per gram of tissue in the efferent venous blood. "Thus, a 2-g MTW9 carcinoma pours enough cells into the host circulation to transplant the tumor every 24 h" (Butler and Gullino 1975).

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Received 8 July 2002; accepted 22 July 2002.

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## ANGIOGENESIS AS A MARKER FOR NEOPLASTIC TRANSFORMATION

In the 1970s, using the rodent mammary gland as a model, Gullino and coworkers (Gimbrone and Gullino 1976a; 1976b; Brem et al. 1977, 1978) observed that adult resting mammary gland has limited, if any, angiogenic capacity. However, angiogenic capacity is consistently acquired by mammary carcinomas. Interestingly, lesions with high frequency of neoplastic transformation induced angiogenesis at a much higher rate than did lesions with low frequency of transformation. This elevated angiogenic capacity was observed long before any morphological sign of neoplastic transformation (Gimbrone and Gullino 1976a; 1976b; Brem et al. 1977, 1978). Hyperplastic lesions of the human mammary gland showed a similar behavior (Brem et al. 1977). Thus, angiogenesis may represent an early marker for neoplastic transformation. After several years, the established role of oncogene activation and oncosuppressor gene inactivation in modulating the expression of pro- and antiangiogenic factors has confirmed Gullino's observations at a molecular level (Rak et al. 2002).

## NEOPLASTIC CELL POPULATIONS RELEASE MOLECULES ABLE TO STIMULATE ANGIOGENESIS INTO THE SURROUNDINGS: THE ROLE OF PROSTAGLANDIN E-1

Gullino built a micropore chamber by first cutting a 3- to 4-mm-thick ring from a standard polystyrene tubing. Two Millipore filters were then sealed to the opposite surfaces of the ring to form a round chamber with a cavity containing about 100  $\mu$ l of fluid. A catheter forced into a hole drilled through the polystyrene ring permitted withdrawal of the fluid from the chamber. The chamber was placed in a subcutaneous pouch and small tumor fragments were seeded around it. A tumor

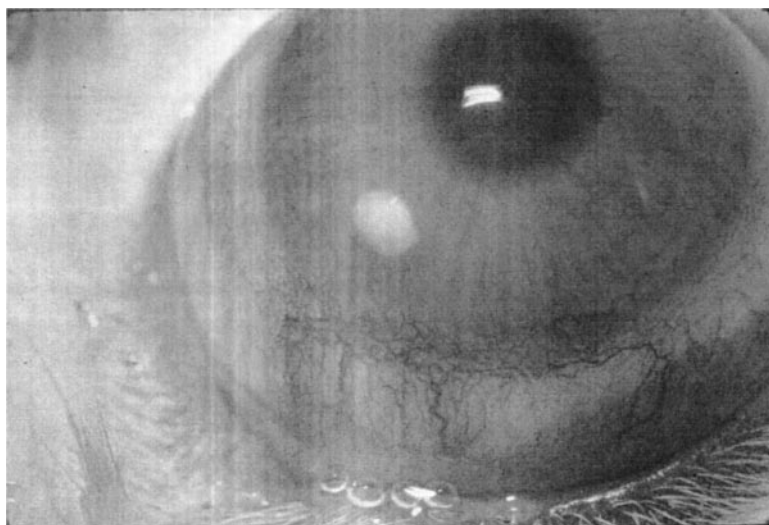
developed that incorporated the chamber. The neoplastic cells grew against the filter but could not penetrate into the capsule cavity, whereas the fluid surrounding the cells did penetrate and was sampled. The tumor interstitial fluid (TIF) was lyophilized and the residue was compressed into 2-mm pellets and implanted into the rabbit cornea, where they induced an angiogenic response (Gullino et al. 1964).

In the search for tumor-released proangiogenic molecule(s), Gullino observed that the concentrations of type E prostaglandin (PGE) were two- to threefold more abundant in TIF than in the subcutaneous fluid sampled by a Millipore chamber located in the same rat (Ziche et al. 1982). On this basis, several prostaglandins were tested for their angiogenic capacity and PGE-1 was active at very low doses (Ziche et al. 1982). PGE-1 was incorporated into a pellet of ethylene-vinylacetate copolymer (ELVAX) and implanted into a pocket of a rabbit cornea. When the pocket was 1 to 2 mm distant from the edge of the cornea, capillary sprouts were formed from the vessels of the limbus. Four days after pellet implantation, capillaries began to penetrate into the cornea and a dense brush of vessels surrounded the PGE-1 pellet by day 7 (Fig. 1) (Ziche et al. 1982). Thus, Gullino's studies allowed the identification of PGE-1 as a potent proangiogenic compound and represented a molecular link between tumor angiogenesis and inflammation.

## MICROENVIRONMENT AND TUMOR ANGIOGENESIS: THE ROLE OF COPPER AND GANGLIOSIDES

During angiogenesis *in vivo*, before any vessel is formed, the tissue to be invaded by the newly formed capillaries modifies its composition so as to favor growth and motility of capillary endothelium.

Gullino's studies led to the observation that the concentration of copper ions was more elevated in corneas treated with the



**FIG. 1.** Elvax pellet containing 1.0  $\mu$ g PGE-1 implanted in the cornea 5 days earlier. Newly formed vessels coming from the limbus had penetrated the cornea. This original picture was a gift of Pietro M. Gullino to Dr. Ribatti.

proangiogenic PGE-1 than in untreated corneas or in corneas treated with the nonangiogenic PGE-2 or PGI-2. These data were interpreted as an indication that an increment in copper ion concentration occurs in the cornea at the onset of angiogenesis and may favor blood vessel growth.

On this basis, angiogenesis was tested in copper-deficient rabbits (Ziche et al. 1982). In agreement with the hypothesized role of copper in angiogenesis, copper-deficient animals were unable to mount an angiogenic response to effectors active in rabbits with normal levels of plasma copper (Ziche et al. 1982).

Then, Gullino hypothesized that copper-carrying molecules might be endowed with angiogenic activity. The obvious choice was ceruloplasmin, the natural copper-carrier in plasma. As anticipated, native ceruloplasmin was angiogenic when implanted in the rabbit cornea, whereas copper-free apoceruloplasmin was not (Raju et al. 1982). Moreover, Gullino and coworkers showed that heparin/copper complex was able to stimulate the motility of capillary endothelial cells in vitro without affecting the migration of fetal calf fibroblasts or aortic endothelium. The effect of heparin/copper complex was significantly more potent than that exerted by heparin alone (Alessandri et al. 1983, 1984). Since then, several studies have confirmed the role of copper in angiogenesis and led to the hypothesis that copper chelating agents may represent the basis for the development of antiangiogenic therapies (Brem 1999; Brewer 2001).

The role of microenvironment in tumor neovascularization was investigated further by studying the in vitro and in vivo consequences of the modulation of extracellular ganglioside concentrations on angiogenesis. Gullino and coworkers found that ganglioside preincubation increased the capacity of capillary endothelial cells to bind fibronectin and to respond to a chemotactic stimulus (Alessandri et al. 1986). In vivo, gangliosides could modulate the angiogenic response exerted by PGE-1 in the rabbit cornea (Ziche et al. 1989). Also, the monosialogangliosides GM<sub>2</sub> and GM<sub>3</sub> inhibit endothelial cell proliferation mediated by the prototypic angiogenic fibroblast growth factor-2 (FGF2), whereas gangliosides (GD<sub>3</sub>) restores optimal levels of cell growth (Alessandri et al. 1992; Ziche et al. 1992). In contrast, GD<sub>3</sub> enhances the chemotactic activity exerted by FGF2 on endothelial cells, which is counteracted by GM<sub>3</sub> (Ziche et al. 1992). Moreover, GM<sub>1</sub>, GD<sub>1b</sub>, and gangliosides GT<sub>1b</sub> act synergistically with FGF2 in favoring survival, growth, and motility of capillary endothelial cells (De Cristian et al. 1990). Finally, angiogenesis induced by FGF2 in the rabbit cornea assay can be stimulated or repressed by modulating the GM<sub>3</sub>:GD<sub>3</sub> molar ratio (Ziche et al. 1989). The relationship among gangliosides, copper, and tumor angiogenesis was reviewed by Gullino and coworkers (Gullino et al. 1990).

These observations prompted us to attempt to identify the molecular bases of the modulation of angiogenesis by gangliosides. We observed that free sialogangliosides can bind directly to FGF2 (Rusnati et al. 1999). In contrast, cell-associated gangliosides may act as functional FGF2 coreceptors in endothelial cells (Rusnati et al. 2002).

## CONCLUDING REMARKS

There is abundant evidence that angiogenesis plays a significant role in the biology of solid and blood malignancies. Although angiogenesis is becoming established as a useful prognostic indicator, there are increasing preclinical data that antiangiogenic strategies may be of therapeutic benefit. Indeed, various angiogenesis inhibitors have been developed so far, their efficacy has been evaluated in different in vitro and in vivo assays, and their clinical evaluation in cancer patients is in progress (see the National Cancer Institute [NCI] web site: <http://cancertrials.nci.nih.gov>). The hypothesis that antiangiogenic compounds can be used in combination with cytotoxic drugs for tumor therapy has been advanced. Also, chemotherapeutic agents have shown antiangiogenic properties in vitro and in vivo, leading to the concept of antiangiogenic scheduling of chemotherapy. Further understanding of the molecular and cell biology of angiogenesis in cancer will offer important directions for estimation of patient outcome and treatment.

Pietro M. Gullino contributed significantly to set the basis for a better understanding of the angiogenic process, essential for the development of efficacious novel antineoplastic therapies. He was always generous with helpful suggestions, especially to younger colleagues, including us, stimulating critical thinking and careful experimental planning. He was a true and admirable person of science—in other words a “Maestro.”

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