(1) Final/Final es on IBM 5
(2) also add M.K's correction

p20 omit homogeneize, by use 1. highly purified

p17 "of a serum-derived factor now known to be PDGF

For PERSPECTIVES IN BIOLOGY AND MEDICINE. [Invited Review]

Draft #9 MARCH 30, 1985.

TOWARDS AN UNDERSTANDING OF ANGIOGENESIS: SEARCH AND DISCOVERY

Use This for Corrections of Galley's

Judah Folkman, M.D.

Fortuitous Failure of Neovascularization.

I was in the Navy, assigned not to a ship, but to the Naval Medical Research Institute in Bethesda. Frederick Becker* and David Long** were there also. The three of us had been invited to interrupt our respective residencies to fulfill two years of military obligation. It was 1960 and the Navy was curious about the feasibility of blood substitutes for use in its hospitals aboard aircraft carriers.

We ~~began a study of~~ experimented with hemoglobin solutions. Dog thyroid glands were perfused ~~kept~~ in ~~an~~ isolated glass chambers with hemoglobin solution for ~~more than~~ a week ~~by perfusing hemoglobin solutions through an artery~~. The perfused organs appeared to be viable, but could they support growth? ~~Tumor~~ cells from a mouse melanoma were injected ~~were injected~~ into the thyroid glands. Tiny tumors developed and grew rapidly, but stopped enlarging at diameters of 1 to 2 mm (1). ~~Immune rejection was an unlikely~~ cause of the tumor's arrested growth. These isolated organs were incapable of an immune response, because they were perfused with acellular fluids. Furthermore when tumors were transplanted to a fresh isolated thyroid gland, the size

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limit held. However, when reimplanted into their donor mice, these tumors grew rapidly, beyond 1 cm^3, and killed their host. In the mice the tumors were vascularized; in the isolated perfused organs, they were not. (FIGURE 1). What we had witnessed, although we did not realize it at the time, was a fortuitous failure of neovascularization.

The remarkable outcome of this experiment was that a tumor implant was prevented from becoming vascularized just long enough so that the subsequent inhibition of tumor growth could be measured. In conventional studies of transplantable tumors in animals, the avascular phase is brief and usually imperceptible. Once a tumor can be detected, it is already well vascularized. Needless to say, our interest was drawn to the study of tumor growth, rather than to hemoglobin perfusates.

During the next three years of the completion of surgical residency (at the Massachusetts General Hospital in Boston), this experiment fermented in my subconscious. I knew that we did not understand its full significance. When I operated on patients with cancer, their tumors were already large and well vascularized, like the mice. But, on occasion I glimpsed tiny tumors in the prevascular stage, waiting for capillary blood vessels to connect to them, analgous to the melanomas in the isolated perfused organs. For example, there was a woman with a large, vascularized carcinoma of the ovary; metastases had implanted on the peritoneal lining. The implants were white, avascular, and uniformly less than 2-3 mm diameter. There was a man with colon cancer which had seeded the omentum. Some of the tumor implants were already vascularized, and as large as 1 cm^3, but many others were tiny, white, avascular nodules of a few millimeters diameter.

History of growth factor purification

1972	Cold Spring Harbor Course on Viruses-learn about 3T3-SV3T3 Meet Chick Scher-Reccommends me for job with Folkman
1973	Begin angiogenesis work with CAM assays,find 3T3 angiogenic (Klagsbrun et al, Cancer res ,1975) Begin endothelial cell work with Haudenschild(Haudenschild et al,Exp Cell Res ,1976)
1974	Writes successful fellowship application on cartilage inhibitors of angiogenesis Share lab with Chich Scher,get interested in growth factors (discuss serum growth factor work,almost get involved in PDGF purification,learn 3T3 assay from Scher and refine it for large scale work)
1975	Use 3T3 assay to purify cartilage inhibitors but find growth factor instead,becomes major resaerch interest
1976-1978	partial purification of CDGF(Klagsbrun et al Exp Cell Res 1977,Klagsbrun and Smith J Biol Chem,1980) Discover milk -derived growth factors(Klagsbrun,PNAS,1980) Discover Sertoli-derived growth factor(Feig et al, PNAS,1980)
1978-1979	Zetter comes to lab ,develops CE cell migration,proliferation assays,In survey of growth factors,we find that CDGF is mitogenic for CE and stimulates migration(Zetter et al Plasma and cellular Modulatory Proteins ,1981)
1979	Decide that cartilage not feasible source of CDGF,go to George Martin and bring back rat chondrosarcoma for CDGF purification
1979-1981	Jane Azizkhan finds that CDGF,ChDGF are chromatin associated ChDGF is stimulator of CE migration(Azizkhan et al,Can Res,183)
1981	Develop BioRex step from histone literature Develop collagenase tissue digest technique
1981	Shing arrives to work on milk growth factors
1981-1982	Klagsbrun,Sullivan and then later Shing work on CDGF,ChDGF purification,develop HPLC techniques,get high degree of purification but poor yields
1982	Heparin breakthrough-Murray uses heparin column to purify cartilage inhibitor, has 2 cuts(0.1M-0.5M NaCl and 0.5M-2.0 M NaCl),Inhibitor is in first cut and wants to know if contaminated with CDGF.Using 3T3 assay Shing or Rob finds that GF is in 0.5M-2M NaCl cut M.Klagsbrun realizes possible significance only if activity is in higher range of 0.5M-2 M NaCl cut and suggests gradient.Rob(CDGF) and Shing(ChDGF)both find activity at high salt suggesting affinity and high purity.
1982-1983	Purification of ChDGF is acheived using 3T3 as guide to CE assay (Shing et al,Science 1984)CDGF is purified(Sullivan and Klagsbrun J Biol Chem,1985
1984	first good quantitative angiogensis results(Shing et al,J Cell Biochem,1985 submitted)