Discovery of the First Stimulating Factors of Blood Vessels

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The work of Judah Folkman indicated that one or more factors produced by a tumor could stimulate angiogenesis. These factors were called Tumor Angiogenesis Factors (TAFs), and various laboratories set out to identify these TAFs. The problem was that even if the culture medium or the extracts of the tumor cells or of other tissues showed an important stimulatory activity for endothelial cells, the responsible factors could not be efficiently purified. This changed with the discovery that these factors had a strong affinity for heparin. Using heparin-sepharose chromatography, Michael Klagsbrun and Jay Shin from the Judah Folkman laboratory, and researchers from several other laboratories, were able to isolate these factors—which were called heparin-binding growth factors—and to determine their amino acid sequences [114–116] (Fig. 6.1).

Chromatography is a method of separating proteins using columns containing a sepharose gel at various densities, on which a tissue extract or a culture medium to be separated is deposited. In the case of heparin-sepharose, which has a high capacity for binding to basic proteins, heparin is fixed irreversibly to sepharose. A chromatography column is then filled with the functionalized sepharose. The heparin-binding proteins are thus retained in the column in a first step. In a second step, these proteins are then detached from the column (eluted) by a buffer with a high ionic strength (elution with high concentration of salt) and collected.

Many teams then began to purify these factors from many normal or pathological tissues. This was the case with Denis Gospodarowicz, Roger Guillemin (winner of the Nobel Prize for Medicine), and Peter Böhlen, all localized at the west coast of the United States. These factors were first called according to the organ from which they were identified. For example, Yves Courtois's team, he being, at that time, director of an eye research laboratory in Paris, had identified in the eye a factor called eye-derived growth factor. It was therefore important to reach a consensus and to establish an appropriate nomenclature. This was first done in 1985, and these factors were called acidic or basic Fibroblast Growth Factors (FGF) according to their amino acid sequences [117] and then named by assigning them a number for the factor type. Thus, acidic FGF was designated FGF-1 and basic FGF-2.

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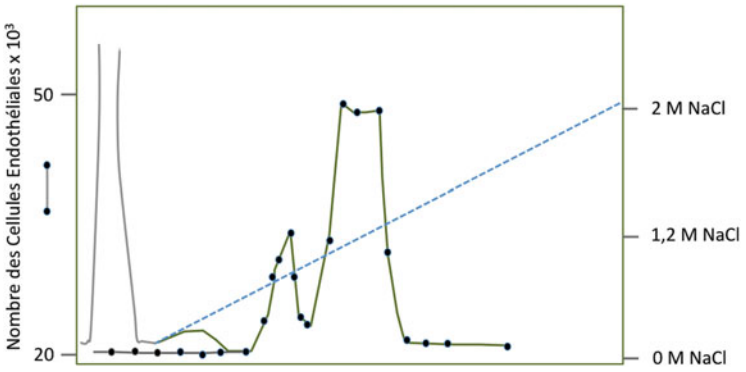

Fig. 6.1 Isolation of endothelial cell growth factors by heparin-sepharose chromatography. The tissue extract is homogenized and deposited on a heparin-sepharose column. A gradient containing increasing concentrations of NaCl is then applied and elution is performed. The various fractions eluted are then tested for their ability to stimulate the proliferation of endothelial cells. It can be seen that the most stimulatory fractions are eluted from 1.2 M NaCl. (Figure redrawn and modified by the author of Klagsbrun and Shing, Proc Natl Acad Sci USA, 1985, 82: 805–809) [121]

The list of these factors has grown considerably, now comprising 23 members (FGF-1 to FGF-23).

However, were FGFs the long sought-after TAFs? I still remember the animated discussions at a scientific meeting in Paris in 1995, with Werner Risau, director at the time of the Max Planck Institute on angiogenesis in Bad Nauheim, Germany. He was barely 40 years old and was then one of the most respected scientists in the field. One of the articles he published was entitled "What if anything is an angiogenic factor?" [118] to relativize the fact that any supposed factor may represent an angiogenic factor, even if it stimulated the development of blood vessels when applied externally. He always said that the expression profile of FGF did not fit with a role in vascular development and that FGF (at least some of them) did not have a so-called "signal" sequence that allows them to be efficiently exported from the inside of the cell into the extracellular medium, which is a property absolutely required for an angiogenic factor. The feeling shared by many researchers at that time was that FGFs were not the long sought-after TAFs.

If the FGF was not the TAF, as it appeared at the time, what then was the biochemical nature of this activity? Many teams continued their research, including the team of Judah Folkman.

In Boston, Judah Folkman contacted the famous Boston chemist Bert Vallée and obtained a very important funding to identify this factor. Bert Vallée was a celebrity in Boston and a highly respected chemist. Nevertheless, this collaboration was not fruitful because Bert Vallée was determined to pursue this research all by himself. A molecule called angiogenin was isolated and characterized [119, 120]. However, this molecule proved to be a ribonuclease. It had limited activity on vascular cell proliferation and lacked an adequate temporo-spatial expression to attribute angiogenin to a significant role in tumor angiogenesis. Exit therefore angiogenin.

In the 1990s, the idea emerged that there must exist vascular-specific factors that had the ability only to stimulate angiogenesis. This(these) factor(s) would have a role in embryonic development and during vascularization of tumors and other pathologies. The era of Vascular Endothelial Growth Factor (VEGF) was about to begin!