Where It All Began

4

Algire had observed changes in blood vessels when treating fibroblasts with a chemical methyl cholanthrene [98]. This suggests that factors (after stimulation) could be produced from the cells surrounding the blood vessels, such as fibroblasts that stimulate vascular growth.

In 1948, Michaelson [99] published his observations concerning the vascular system of the retina. He had developed a technique, which consisted of injecting India ink into the arterial system, thereby allowing him to visualize the retinal vascularization. Using this method, he studied the development of the retinal vessels and showed that these vessels emerged through the optic nerve to colonize the surface and the interior of the retina. On the basis of these observations, Michaelson postulated that a factor, which he named factor X, was present and induced the budding of new vessels, and that this factor was regulated by hypoxia. Furthermore, in 1951, Campbell [100] observed that the number of capillaries in the retina increased in an oxygen-deficient environment. These observations strongly suggested the presence of mediators of angiogenesis.

However, the answer as to the identity of this factor came from a completely different field, tumor biology. Indeed, tumors are characterized by abundant angiogenesis, and it is the study of tumor angiogenesis that provided the long-awaited answer to the identity of factor(s) X. It is clear that modern research on angiogenesis has really begun with Judah Folkman (Fig. 4.1, Table 2.2) because (1) he provided indisputable experimental evidence that the process of angiogenesis is crucial for in vivo development of tumors and (2) he showed that this was because of a diffusible factor he called "tumor angiogenesis factor" or TAF.

A famous experiment conducted by Michael Gimbrone, a post-doctoral fellow, and by Judah Folkman in 1972 is illustrated in Fig. 4.2 [101]. In this experiment, pieces of a rabbit tumor, called "Brown-Pearce epithelioma," were implanted either at the iris, which is richly vascularized, or in the anterior chamber, a non-vascularized part of the eye. Gimbrone and Folkman observed that the tumors implanted at the iris had an exponential growth whereas the tumors implanted in the avascular region did not grow but nonetheless remained viable. In the words of

A. Bikfalvi, *A Brief History of Blood and Lymphatic Vessels*,
https://doi.org/10.1007/978-3-319-74376-9_4

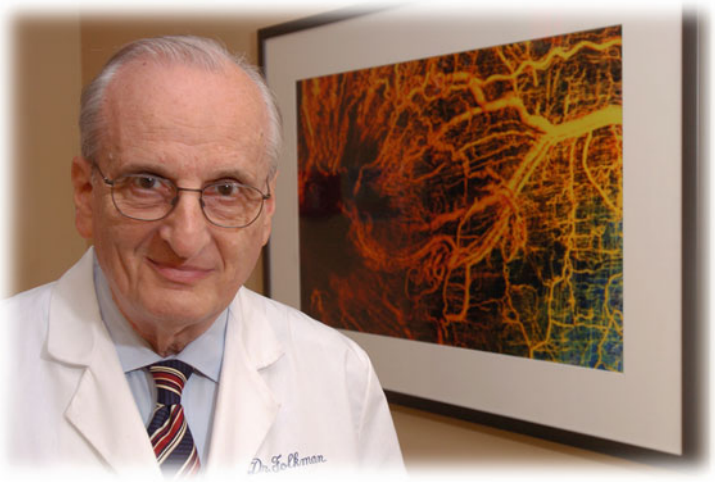

Fig. 4.1 Judah Folkman, pioneer of the tumor angiogenesis field and discoverer of Tumor angiogenesis factor (TAF). Image courtesy of Harvard University Photo Service courtesy of Harvard Public Affairs and Communication (Jon Chase/Harvard Staff Photographer)

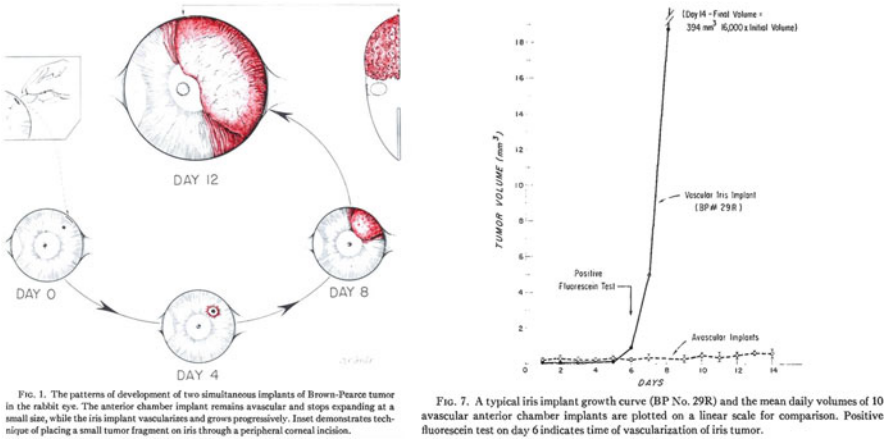

FIG. 1. The patterns of development of two simultaneous implants of Brown-Pearce tumor in the rabbit eye. The anterior chamber implant remains avascular and stops expanding at a small size, while the iris implant vascularizes and grows progressively. Inset demonstrates technique of placing a small tumor fragment on iris through a peripheral corneal incision.

FIG. 7. A typical iris implant growth curve (BP No. 29R) and the mean daily volumes of 10 avascular anterior chamber implants are plotted on a linear scale for comparison. Positive fluorescein test on day 6 indicates time of vascularization of iris tumor.

Fig. 4.2 Illustration of the experiments carried out by de Gimbrone and Folkman. Implantation of tumor tissue either at the level of the avascular cornea or near the iris. Implantation technique (*left*). Quantification of tumor growth of these tumors after implantation (*right*). It is seen that tumors implanted near the iris grow exponentially because it is vascularized whereas avascular tumors implanted in the cornea do not develop. (Figure from Gimbrone MA Jr, Leapman SB, Cotran RS, Folkman J (1972) J Exp Med, 136(2):261–276 [101]. Permission to reproduce: Rockefeller University Press)

Michael Gimbrone and Judah Folkman: "*These experiments provide in vivo evidence that prevention of neovascularization can block the growth of a solid tumor at an early stage. It appears that vascularization permits an implant to enter exponential growth, while avascularity forces it to remain dormant at a small size*" and further

"*Population dormancy, therefore, appears to be the fate of avascular solid tumors. If neovascularization can be prevented, local malignant growth and perhaps distant metastasis will not occur. These observations thus suggest that specific blockade of tumor-induced angiogenesis would be effective in controlling neoplastic growth*".

What has just been written is the fundamental paradigm of angiogenesis and represents the framework in which much research has been conducted in the following decades. However, in the light of current knowledge, this concept/dogma is in some need of revision. This is discussed later.

A factor called TAF (Tumor angiogenesis factor) was identified by Judah Folkman from the melanoma tumor known as "Walker 256" implanted in rats [102]. Tumors were chemically fractionated and extracts were tested using the method of rat dorsal air sac to show their ability to induce angiogenesis (Fig. 4.3). For Folkman this

ISOLATION OF A TUMOR FACTOR RESPONSIBLE FOR ANGIOGENESIS*

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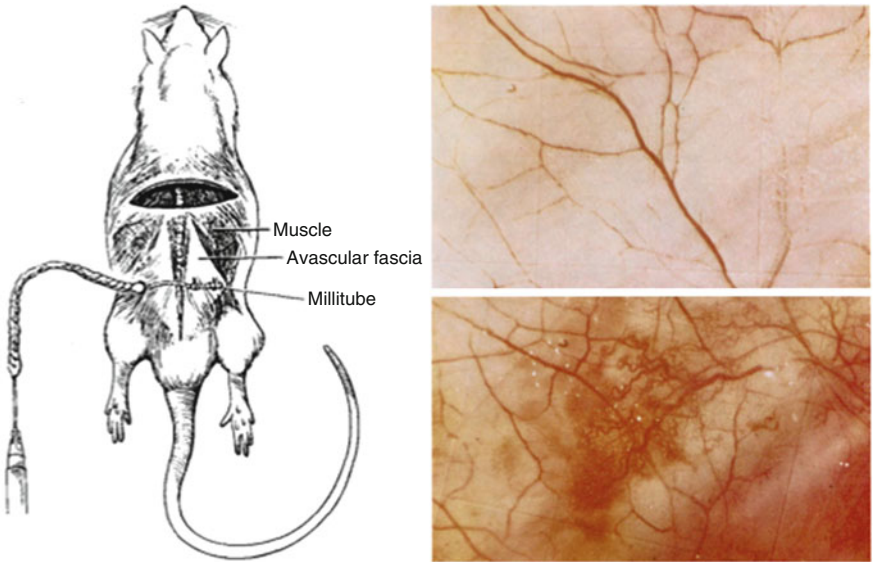

Fig. 4.3 Title of the article by Folkman (*top*) and illustration of the experimental setting that made it possible to evidence the existence of the Tumor angiogenesis factor (TAF) using the dorsal chamber in the rat. The two micrographs on the *right* show that TAF strongly stimulates angiogenesis (*bottom*) compared to control (absence of TAF) (Folkman J, Merler E, Abernathy C, Williams G. Journal of Experimental Medicine 1971, 133(2):275–288 [101]. Permission to reproduce: Rockefeller University Press)

method was more reliable than other tests used at that time, such as using the chorioallantoic membrane of chicken or implantation into the anterior chamber of the eye. Indeed, it was assumed at the time that the angiogenic effects were caused by nonspecific reactions such as tissue irritation or inflammation that appeared in some of the tests. In the same article, Folkman made a hand drawing where he drew the non-growing tumor and a growing vascularized tumor that was induced by the TAF (Fig. 4.4).

In the words of Judah Folkman: "*The presence of a tumor-angiogenesis factor suggests a transfer of information from tumor cells to capillary endothelial cells. The relationship between tumor cells and endothelial cells may be interdependent. Tannock has shown that the nutritional environment of tumor cells becomes poorer as the spacing between blood vessels increases, and this leads to a decreased rate of proliferation and cell death. His work implies that the rate of proliferation of endothelial cells may limit indirectly the rate of tumor growth*".

The pivotal moment that marks the initiation of modern research on angiogenesis is the article published in 1971 in the Journal of Experimental Medicine [102]. Folkman wrote in the introduction: "*The growth of solid neoplasms is always accompanied by neovascularization. This new capillary growth is even more vigorous and continuous than a similar outgrowth of capillary sprouts observed in fresh wounds or in inflammation. Many workers have described the association between growing solid malignant tumors and new vessel growth. However, it has not been appreciated until the past few years that the population of tumor cells and the population of capillary endothelial cells within a neoplasm may constitute a highly-integrated ecosystem.*"

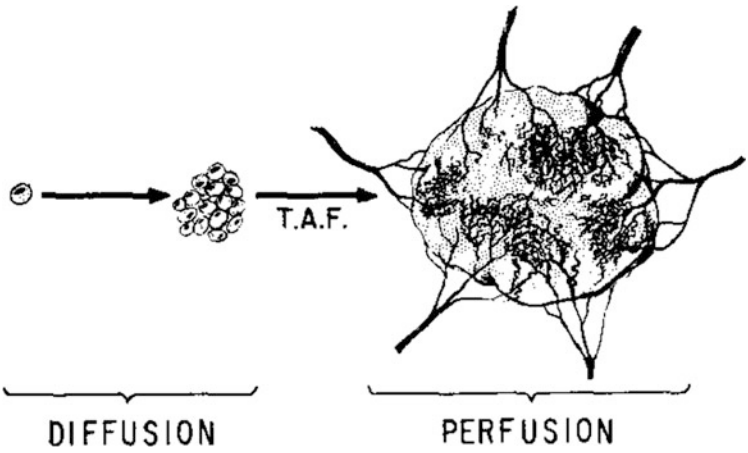

Fig. 4.4 Original drawing by Folkman, from the same article, describing the Tumor angiogenesis factor (TAF). A tumor, having acquired a certain size, has the ability to produce TAF. TAF vascularizes the tumor, which then develops rapidly (Folkman J, Merler E, Abernathy C, Williams G. Journal of Experimental Medicine 1971, 133(2):275–288 [102]. Permission to reproduce: Rockefeller University Press)

It should be noted that Folkman uses the term "highly integrated ecosystem." This implies the existence of intercellular and intracellular signaling systems with mechanisms of amplification and inhibition between different cell populations. This can also be seen as a complete control system with numerous control and feedback loops between tumor cells and blood vessels. The reader cannot escape the view that these interactions and loops may constitute potential therapeutic targets. A more detailed discussion of this notion is provided in the last part of this book.

Folkman formulates the paradigm of angiogenesis in a detailed way in an article published on November 18, 1971 in the New England Journal of Medicine [103]. In his famous article, Folkman put forward very detailed arguments derived from experimental observations, which led him to the following conclusions:

1. Blood vessels in tumors are new, and the tumor actively induces them
2. Induction is caused by tumor-derived factors (TAF) that are diffusible (diffusion means that it can penetrate the tissues to act more remotely)
3. These diffusible proteins actively attract the blood vessels
4. If this process could be blocked or inhibited, tumors should remain in this case of small size, or even regress

This is a research program for several decades! We see in subsequent chapters how this program has been implemented by the different researcher teams. These conclusions are generally correct, but must be modulated or revised in the light of current knowledge.