1. Introduction to angiogenesis

The vasculature of embryonic and adult tissues is composed of a spatially organized network of blood vessels that provide adequate nutrients to all their constituting cells. Small blood capillaries essentially consist of endothelial cells, whereas larger vessels are surrounded by mural cells (pericytes in medium-sized and smooth muscle cells in large vessels) [1,2]. A major difference between embryonic and adult vessels is that the former are constantly growing and present a high mitogenic activity whereas the latter are quiescent with endothelial cells renewing every 12 to 18 months in humans. However, angiogenesis is re-activated in adult tissues under certain stress or pathological situations such as ischemia or inflammation. During development, the vascular network is formed by two distinct mechanisms termed vasculogenesis and angiogenesis. Vasculogenesis defines the differentiation of angioblasts or stem cells into a primitive vascular plexus whereas angiogenesis defines the formation of new blood vessels from pre-existing ones through sprouting, bridging and intussusceptive growth. Several key regulatory molecules controlling these processes have been identified over the last decades including vascular endothelial growth factor (VEGF), angiopoïetins and platelet-derived growth factor (PDGF). Two distinct phases are usually observed in the angiogenic process:

- activation, during which the endothelial cells of the pre-existing vessel secrete metalloproteases that degrade the basement membrane around the vessel, then migrate in the open space, proliferate and re-organize to form tubes that connect to the adjacent vessels;

- maturation, during which mesenchymal progenitor cells are recruited to the neovessel, are induced to differentiate in the muscular lineage and, once transformed into pericytes, cover and stabilize the immature neovessel [2].

There is also growing evidence that circulating bone marrow-derived endothelial progenitor cells contribute to some level to vessel growth both in the embryo and in ischemic and inflamed tissues in the adult [1].

It was observed more than a century ago that the growth of human tumors is often accompanied by an increased vascularization [3]. In 1971, Pr. J. Folkman emitted the hypothesis that tumor growth was dependent upon its vascularization and that one important step in tumor progression was the secretion of angiogenic factors by tumor cells (termed the angiogenic switch) [4]. This has opened the field of tumor angiogenesis and has led to the identification of several pro- and anti-angiogenic factors and to the development of anti-angiogenic therapies for the treatment of cancer [5]. Bevacizumab, a humanized anti-VEGF monoclonal antibody, is now on the market for the treatment of metastatic colon, breast, lung and kidney carcinomas and many other molecules are under development [6].

2. Vascularization of the normal adrenal cortex

The vascular network of endocrine glands is particularly dense. It is composed of fenestrated sinusoids which are highly permeable to fluids and small molecules, thus facilitating rapid and efficient export of synthesized steroid hormones into the blood flow. Although the protein composition of fenestrae begins to be identified, the filtration selectivity of these structures is still poorly characterized [7]. The adrenal cortex is definitely one of the most highly vascularized tissue in the adult mammalian organisms. The adrenal blood flow is centripetal with incoming blood being delivered via the ventral aorta and the
renal artery. The arteries then ramify into a dense network of capillaries which are in such a close contact with steroidogenic endocrine cells that no more than one layer of endocrine cells is present around each sinusoid [8]. Another characteristic feature of endocrine glands is their high level of VEGF expression, even at the adult stage, despite the absence of active angiogenesis in these tissues. Shweiki et al. were first to propose that the role of VEGF in this context is to maintain a high density of stable fenestrated microvessels [9].

We further documented the role of VEGF in the adrenal cortex by reporting that VEGF expression is under the positive control of ACTH, both in primary cultures of bovine steroidogenic adrenocortical cells and in vivo in a mouse model of dexamethasone-induced adrenal regression [10,11]. Interestingly, ACTH appears to stimulate VEGF mRNA levels through a post-transcriptional mechanism involving the nucleocytoplasmic translocation of the zinc finger protein HuR and its binding to specific AU-rich elements located in the 3′-untranslated region of VEGF mRNA [12]. This rapid induction (observed within 2 to 3 hours) is followed by an increase in the expression of the mRNA-stabilizing protein TIS11b. This zinc finger protein binds to adjacent AU-rich elements and brings the VEGF mRNA levels down to their control levels within 8 hours [12]. This hormonal regulation of VEGF expression was further addressed in vivo using dexamethasone-delivering osmotic mini-pumps that were subcutaneously implanted in mice. Following the total suppression of pituitary ACTH release observed after 24 h of steroid perfusion, we observed a biphasic regression of the adrenal cortex. Between day 2 and day 4, a massive apoptosis induced a rapid involution of the zona fasciculata. This was followed by a more subtle regression between day 4 and day 14 that was concomitant with a progressive decrease of VEGF mRNA and protein expression in the whole adrenal cortex and a disorganization and destabilization of the vascular network [11]. This suggested that ACTH regulates adrenocortical endocrine cell viability through a direct apoptosis-mediated control of cell survival and through an indirect VEGF- and vascularization-mediated control of cell oxygenation.

3. Angiogenesis in adrenocortical tumors

Many clinical studies have been devoted to establish a correlation between microvascular density of resected tumors and their aggressiveness (estimated by overall or recurrence-free survival of the patients) [13,14]. Although true in many tumors, this correlation suffers a number of exceptions such as pituitary tumors which are far less vascularized than the normal tissue [15]. Before describing the vascular status of the adrenocortical tumors, it is worth recalling that the density of blood vessels in the normal tissue is almost maximal as a single cell layer of endocrine cells surrounds each sinusoidal capillary [8]. It is thereby impossible to observe an increased vascular density in tumors. Three studies have reported the histological analysis of the vascularization of adrenocortical adenomas and carcinomas. Susano et al. used CD34 as a biomarker of endothelial cells and measured the vascular density and the surface covered by blood vessels [16]. They observed that the vascular density was not significantly different among normal adrenocortical tissue, adenomas and carcinomas. In contrast, the vascular area and the mean vessel diameter were higher in carcinomas than in adenomas and normal tissue whereas these latter two tissues were similar. This was largely confirmed by Díaz-Cano et al. who used CD31, rather than CD34, as a marker of endothelial cells but also observed a significant enlargement of blood vessels in adrenocortical carcinomas (n = 9) as compared to adrenocortical adenomas (n = 25) or adrenocortical multinodular hyperplasias [17]. Surprisingly, the third study by Bernini et al. reported a decrease in the vascular density of adrenocortical carcinomas but did not show enlarged vessels in these aggressive tumors [18]. The enlarged vessels observed in two out of three studies could result from enhanced proliferation of endothelial cells in a tissue context where there is no room for the formation of additional neovessels. This would be consistent with our observation that VEGF (a potent mitogen for endothelial cells) protein levels, as measured by ELISA on extracts of frozen tumors, are increased in adrenocortical carcinoma (defined in this study as tumors with a Weiss index > 3) as compared to benign adenomas [19]. Surprisingly, analysis of VEGF mRNA levels in a distinct collection of human adrenocortical tumors collected by the French COMETE network did not show significant differences between normal adrenals, adenomas and carcinomas (unpublished results). This would suggest that VEGF is up-regulated at the translational level in these tumors. Whether this is through the use of alternative translation initiation codons or through regulation by specific miRNAs, two mechanisms known to regulate VEGF translation [20,21], remains to be established. However, it is important to emphasize that VEGF is not the unique regulator of tumor angiogenesis. The transition from vascular quiescence to angiogenesis results from changes in the balance between angiogenic activators and inhibitors [22]. So far, few studies have measured the levels of anti-angiogenic factors although a number of such factors are known [22,23]. We measured by ELISA the levels of thrombospondin-1, a matricellular protein with anti-angiogenic properties, in adrenocortical tumor protein extracts and observed that they were significantly decreased in tumors with a Weiss index superior or equal to 1 as compared to benign tumors (Weiss = 0) [19]. Besides thrombospondin-1, there are certainly several other angiogenesis inhibitors whose decreased expression might contribute to the progression of angiogenesis.

4. Conclusion

Because the adult adrenal cortex is a highly vascularized organ and strongly expresses VEGF, using anti-VEGF or more generally anti-angiogenic therapies for the treatment of adrenocortical tumors may not be a good solution. Despite the characterized imbalance between pro-angiogenic and anti-angiogenic factors observed in adrenocortical carcinomas, it might be very difficult to restore a correct vascularization, which will have to remain dense enough to allow efficient steroid secretion and avoid tissue regression. The lymphatic vasculature, which is known to be stimulated during tumor progression and to contribute to metastatic dissemination [24], is probably a more pertinent target for the therapy of adrenocortical tumors. It should certainly be worth investigating, in the future,
the abundance of the lymphatic vascular network and the levels of VEGF-C and VEGF-D expression in adrenocortical tumors in order to then evaluate the potential of the recently developed anti-lymphangiogenic therapies for the treatment of adrenocortical cancer.

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