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A revisited concept. Tumors: Wounds that do not heal

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ABSTRACT

In 1986, Harold F. Dvorak, Professor of Pathology at Harvard Medical School Boston, published an essay in the New England Journal of Medicine entitled "Tumors: Wounds that do not heal" pointed out that similarities exist between tumor stroma generation and wound healing. Cancers share many features in common with tissue regeneration, including immune response, cell proliferation, cell migration, tissue remodeling, and cell death. In this review article, we analyze the importance and the limits of this concept, which confirm the close relationship between two apparently different biological processes.

1. Background

In 1863, Rudolf Virchow had already recognized that "chronic irritation and inflammatory hyperplasia are predispositions for cancer development" (Virchow, 1863). In 1974, Alexander Haddow suggested that "tumor production is a possible overhealing" (Haddow, 1974). Development of cancer in fibrotic tissue is a frequent event (Dunham, 1972), taking place in lung and breast tissue, through the disruption of cell polarity, stimulation of angiogenesis and inflammation. In the other hand, tumor development at the wound site was prevented by anti-inflammatory therapy (Martins-Green et al., 1994).

Tumor stroma and granulation tissue in skin wounds are both characterized by the presence of a fibrin clot, inflammatory cells, including neutrophils, macrophages, mast cells and lymphocytes, newly formed blood vessels, and a large number of fibroblasts and myofibroblasts. In addition, migrating and proliferating keratinocytes are present in the wound and enhanced migration and proliferation of transformed epithelial cells are principal features of carcinomas.

Among the regulators of re-epithelization and tumor growth, the stromal cell derived factor-1 (SDF-1), which is mitogen for keratinocytes and is expressed in endothelial cells and fibroblasts of normal and wounded skin (Avniel et al., 2006) and in tumors (Orimo et al., 2005).

The main difference between migration of normal keratinocytes and of transformed epithelial cells is the epithelial mesenchymal transition (EMT) seen in cancer cells (Ribatti, 2017). EMT is characterized by the breakdown of adherens junctions and loss of epithelial markers, including cytokeratins and E-cadherin, and by the overexpression of mesenchymal markers, including fibronectin, N-cadherin, and vimentin, as well as the acquisition of an invasive fibroblastoid phenotype. Furthermore, myofibroblasts might also derive from epithelial cells through EMT (Radisky et al., 2007).

2. The contribution of Harold D. Dvorak

In 1986, Harold F. Dvorak (Fig. 1) Professor of Pathology at Harvard Medical School Boston, published an essay in the New England Journal of Medicine entitled "Tumors: Wounds that do not heal" pointed out that similarities existing between tumor stroma generation and wound healing. Cancers share many features in common with tissue regeneration, including immune response, cell proliferation, cell migration, tissue remodeling, and cell death.

As Dvorak pointed out in 2015: "The novelty of my 1986 article was based on two, at the time, recent findings, namely the discovery of VPF as tumor product and the recognition that the chronic vascular hyperpermeability induced by VPF likely accounted for the fibrin deposited in solid tumors and in early stages of wound healing. Together, these findings anticipated that tumors and wound healing could be linked together in a fundamental way at the molecular level." (Dvorak, 2015)

Dvorak noted that wounds, like tumors, secrete vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), causing blood vessels to leak plasma fibrinogen, which stimulates blood vessel growth and provides a matrix on which they can spread. The endothelial cells in the vasculature of VPF/VEGF transfected tumors are fenestrated and contain open interendothelial junctions (Roberts and Palade, 1997). VEGF promotes vascular permeability by disruption of adherens juctions and tight junctions, resulting in transient opening of endothelial cell-cell contacts (Dejana, 2004). Indeed, VEGF promotes

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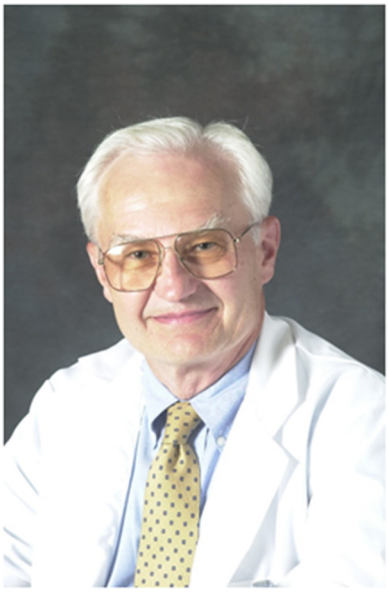

Fig. 1. A port trait of Harold D. Dvorak. Reproduced from (Ribatti, 2010).

tyrosine phosphorylation of VE-cadherin and of its binding partner β-catenin, plakoglobin, and p120, in a Src-dependent mechanism (Eliceiri et al., 1999). Following transfection with VPF/VEGF cDNA, human melanoma cells that otherwise expressed only low levels of VPF/VEGF induced hyperpermeable vessels when transplanted into immunodeficient mice (Claffey et al., 1996).

VPF/VEGF is among the most potent vascular permeabilizing agents known, with a potency some 50,000 times that of histamine and it is effective at concentrations well below 1 nM in the Miles assay which provides a sensitive measure of increased microvascular permeability to plasma proteins (Dvorak et al., 1992).Vascular hyperpermeability becomes evident within a minute or two of VPF/VEGF injection into normal skin or other tissues and persist for 20 min. As Dvorak remembered: “In the Miles assay (Fig. 2), Evan’s blue dye is injected

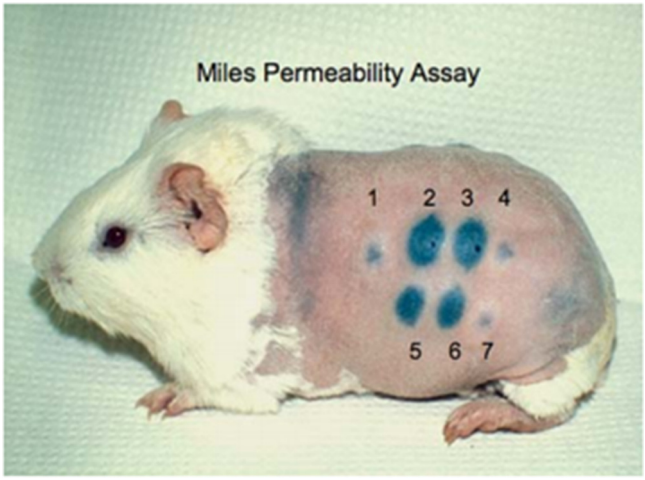

Fig. 2. Miles permeability assay. Various test substances were injected intradermal into the shaved and depilated flank skin of a Hartley guinea pig, followed immediately by an intravenous injection of Evan’s blue dye. Animal was photographed 30 min later. Injected materials were as follows: 1, Neutralizing antibody against VEGF-A; 2 and 5, ascites tumor-associated VEGF-A; 3 and 6, ascites tumor associated VEGF-A plus control immunoglobulin; 4 and 7, ascites tumor-associated VEGF-A plus specific VEGF-A neutralizing antibody. Reproduced from (Nagy et al., 2008) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

intravenously into experimental animals, and test substances such as histamine are injected intradermally into the depilated flank skin. Testing cell-free culture supernatants from a variety of human and animal tumor cells in the Miles assay, I found, to my delight, that supernanants from early all generated intense blue spots, whereas those from several normal cells did not. I called this tumor supernatant permeabilizing activity vascular permeability factor or VPF.” (Dvorak, 2006).

Dvorak found that cultured tumor cells secreted VPF/VEGF and characterized it to be a protein whose activity was not inhibited by antihistamines and other classic inhibitors of vascular permeability (Dvorak et al., 1979a). Unlike wounds that turn off VPF/VEGF production after healing, tumors did not turn-off their VPF/VEGF production and instead continued to make large amounts of VPF/VEGF, allowing malignant cells to continue to induce new blood vessels and so to grow and spread. Thus, tumors behave as wounds that fail to heal (Dvorak, 1986).

3. Anti-permeability factors

The most important anti-permeability factors are angiopoietin-1 (Ang-1) and its cognate receptor Tie-2. Ang-1 is produced by pericytes and smooth muscle cells, activates endothelial Tie-2, maximizes interactions between endothelial cells and pericytes and is expressed behind the leading edge of angiogenic vessels, a position consistent with vessel maturation (Sundberg et al., 2002). Mice deficient for either Ang- 1 or Tie-2 die during embryonic development with vascular defects similar to those observed for platelelet-derived growth factor beta (PDGF-B) deficient mice (Jones et al., 2001). Ultrastructural analysis suggests that Tie-2- knock out blood vessels lack mural cells (Patan, 1998). In PDGF-B deficient mice, recombinant Ang-1 restored the vascular structure and permeability in the growing retinal vasculature (Uemura et al., 2002). Moreover, Ang-1 also counteracts VEGF-induced endothelial leakiness (Thurston, 1999).

4. Normalization agents

The concept of “normalization” of tumor blood vessels by anti-angiogenic drugs was introduced in 2001 by R.K. Jain in a pivotal paper published in “Nature Medicine” (Jain, 2001). The state of normalization is probably transient and dependent on the dose and duration of the treatment. VEGF inhibition could temporarily restore or normalize the function of tumor-associated vasculature, decreasing vascular permeability in conjunction with restoration of sustained pressure gradients, as demonstrated by intravital imaging studies in preclinical models and in cancer patients (Fukumura et al., 2010), thereby enhancing systemic delivery of oxygen or perfusion of cytotoxic agents to intratumoral sites (Tong et al., 2004). Moreover, abrogation of VEGF signaling increases collagenase IV activity, leading to restoration of normal basement membrane (Winkler et al., 2004), which generally in tumors has an abnormally thickness (Baluk et al., 2003).

5. Microvascular hyperpermeability is a feature of wound healing

Wounds in rodent skin, like tumors, secrete VPF/VEGF: within 24 h of wounding VPF/VEGF mRNA expression increases in epidermal keratinocytes at the wound edge (Brown, 1992). VPF/VEGF overexpression reaches a peak at 2–3 days and persists at an elevated level for about 1 week, the time required for granulation tissue to form and migrating keratinocytes to cover the wound defect. In contrast to tumors, VPF/VEGF expression was downregulated as healing progressed and, parallel with the decreased expression of VPF/VEGF, vascular permeability returned to normal.

In contrast to normal mice, congenitally diabetic db/db mice have elevated endogenous levels of VPF/VEGF mRNA in their nude skin, which increase transiently after wounding. However, the rise of VPF/VEGF is not sustained and, as granulation tissue forms, VPF/VEGF