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Tipping the balance for angiogenic disorders ▼

Uncontrolled angiogenesis is the cause of many human disorders, including cancer and ocular disorders. All of the gene therapies for cancer that are currently in clinical trials target the tumour cell. However, it might be more effective for all these disorders if gene therapies targeted angiogenic factors.

It is believed that angiogenesis is regulated by two counter-balancing systems: angiogenic stimulators and angiogenic inhibitors. The balance between these two systems is crucial for the regulation of angiogenesis [1,2]. Vigorous vessel growth is absolutely necessary for the early stage of life when angiogenic stimulators, such as vascular endothelial growth factors (VEGFs), are dominant in the balance (Fig. 1a). Angiogenesis decreases, or stops entirely, except for specialized cases, such as wound repair in the adult. By contrast, angiogenic inhibitors, such as pigment epithelium-derived factor (PEDF), predominate in the balance at this stage (Fig. 1b). The disruption of the balance has an essential role in the development of a variety of diseases, from cancer to proliferative diabetic retinopathy [2,3]. In these pathological conditions, the ratio of angiogenic stimulators to inhibitors increases again, which breaks the dormancy of angiogenesis (Fig. 1c).

The pathological growth of blood vessels (neovascularization) in the eye is responsible for most cases of vision loss in industrialized countries. Gene delivery of either angiogenic inhibitors or stimulator antagonists represents a potential solution for long-term suppression of ocular disorders of neovascularization. In addition, it is much easier and less expensive to manufacture gene therapy vectors than to produce huge amounts of purified proteins.

VEGF is a potent endothelial cell-specific mitogen that has a crucial role in angiogenesis. Antagonists of VEGF, such as anti-VEGF antibody, soluble VEGF-receptor chimeric proteins and antisense oligodeoxynucleotides, have been shown to inhibit retinal and iris neovascularization [4]. Unpublished data also show that gene transfer of soluble VEGF receptor (sFLT1) inhibits retinal neovascularization in a murine model.

By contrast, gene therapies that deliver genes encoding various endogenous angiogenic inhibitors, including angiostatin, endostatin and PEDF, have been shown to suppress ocular neovascularization in pre-clinical studies [4]. PEDF is more appealing because it is

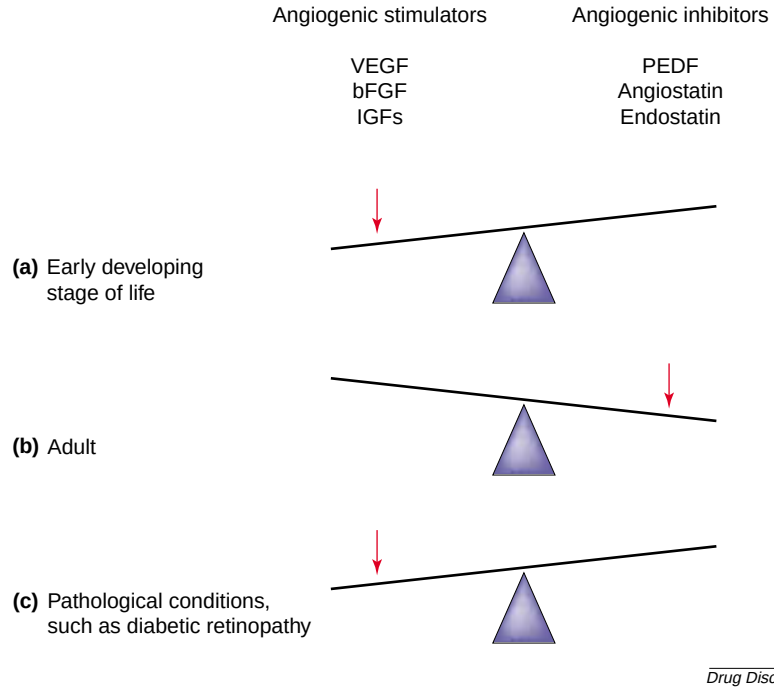

Figure 1. Schematic drawing of different stages of angiogenesis balance. Abbreviations: bFGF, basic fibroblast growth factor; IGF, insulin-like growth factor; PEDF, pigment epithelium-derived factor; VEGF, vascular endothelial growth factor.

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a natural product of the eye and because it has potent anti-angiogenic activity. PEDF is a major angiogenic inhibitor in the vitreous and is probably responsible for the normal avascularity of several of the ocular tissues. Loss of PEDF has a 'permissive role' in ischemia-induced neovascular growth [5]. Therefore, gene therapy by delivery of PEDF to suppress ocular neovascularization could be more promising.

Although several different gene products can cause inhibition of ocular neovascularization, only partial suppression was observed in most of these cases. For example, blocking VEGF inhibits ~50% of retinal neovascularization. Restoration of the balance by provision of endogenous inhibitors, such as PEDF, and blocking of stimulators, such as VEGF, at the same time to arrest the progression of ocular neovascularization, might have synergistic effects.

Anti-angiogenic gene therapy has not been tested in clinical trials. All of the gene therapies in clinical trials against cancer target the tumour cell; the major obstacles include heterogeneity and drug resistance of tumour cells. Anti-angiogenic gene therapy directly targets the genetically stable endothelial cells, does not encounter drug-resistant tumour cells, and inhibits tumour growth independently of tumour cell heterogeneity in addition to its prolonged effect [6]. Therefore, it might have potential as a feasible and effective strategy in the treatment of cancer, ocular disorders and other angiogenesis-related diseases.

References

1 Bussolino, F. *et al.* (1997) Molecular mechanisms of blood vessel formation. *Trends Biochem. Sci.* 22, 251–256
2 Jimenez, B. and Voipert, O.V. (2001) Mechanistic insights on the inhibition of tumor angiogenesis. *J. Mol. Med.* 78, 663–672
3 Gao, G. *et al.* (2001) Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. *FEBS Lett.* 489, 270–276
4 Rasmussen, H.S. *et al.* (2001) Looking into anti-angiogenic gene therapies for disorders of the eye. *Drug Discov. Today* 6, 1171–1175
5 Chader, G.J. (2001) PEDF: Raising both hopes and questions in controlling angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2122–2124
6 Cao, Y. (2001) Endogenous angiogenesis inhibitors and their therapeutic implications. *Int. J. Biochem. Cell. B.* 33, 357–369

Guoquan Gao and Jianxing Ma
Department of Ophthalmology,
Medical University of South Carolina
167 Ashley Ave, Charleston, SC 29403, USA
tel: +1 843 792 9180
fax: +1 843 792 1723

Taking the complexity out of protein sequence analysis ▼

The importance of revealing the function and properties of novel protein sequences has recently become evident to the international scientific community [1]. Both *in vitro* and *in silico* techniques are required for discovering these new protein sequences, their roles in the cell and their potential as drug targets. Eventually, a synergistic effect between *in silico* and *in vitro* approaches will facilitate the synthesis of novel and more specific drugs, leading to increased longevity and better health for the human population.

The availability of interactive sequence databases on the Internet has become important during the past decade. Sequence databases, such as Entrez GeneBank [2] and SWISSPROT [3], provide genomic sequences for many organisms, such as *Homo sapiens*, *Drosophilia melanogaster*, *Caenorhabditis elegans* and *Mus musculus*. To discover the properties of this vast number of protein sequences, from a phylogenetic perspective, as well as a proteomic one, several interactive tools have gradually emerged. Examples of these can be found at http://www.expasy.org, which provides and cross-links a large collection of tools for predicting translation, secondary structure, transmembrane regions, glycolysis patterns and other protein properties. Discovering what is most and least relevant for determining the crucial properties of an unknown protein sequence is the goal. However, it can be quite difficult because of the large number of interactive tools. Courses, personal preferences or advice from colleagues are usually the way we 'get used to' using the same engines time and again for analyzing protein sequences. However, this population of engines grows every year, and keeping updated with the various possibilities is important.

Therefore, an easy step-by-step procedure has been supplied (Figs 1 and 2), to delineate the possibilities and availabilities of different types of proteomic engines that are available on the Internet. Many of the listed tools are based on BLAST algorithms [4], such as ProDom [5], the Protein Information engine [6] and the Protein Fingerprint Database [7]. Others additionally apply Hidden Markov Models statistical analysis to sequence–structure correlations [8], as used in the SAM-99 engine [9] and HMM Superfamily engine [10]. Additionally, an interactive interface of the CLUSTALW package [11] has been suggested because of its frequent use in making multiple sequence alignments. Furthermore, the Prosal-interface, which connects several secondary structure prediction-, homology modeling-, threading- and sequence alignment-interfaces into one submission form, is proposed.

The order for the use of these engines is presented to facilitate the decomposition of pre/pro-regions (functionally superfluous regions removed after secretion from Golgi apparatus) down to catalytic domains, hormone-binding domains and other core parts of important physiological function. Once these sub-components of the full sequences have been identified, and superfluous regions have been removed, the suggested modeling procedures become much easier to perform and the results are more reliable

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