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Survey

Angiogenic growth factors interactome and drug discovery: The contribution of surface plasmon resonance

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ABSTRACT

Angiogenesis is implicated in several pathological conditions, including cancer, and in regenerative processes, including the formation of collateral blood vessels after stroke. Physiological angiogenesis is the outcome of a fine balance between the action of angiogenic growth factors (AGFs) and anti-angiogenic molecules, while pathological angiogenesis occurs when this balance is pushed toward AGFs. AGFs interact with multiple endothelial cell (EC) surface receptors inducing cell proliferation, migration and proteases upregulation. On the contrary, free or extracellular matrix-associated molecules inhibit angiogenesis by sequestering AGFs (thus hampering EC stimulation) or by interacting with specific EC receptors inducing apoptosis or decreasing responsiveness to AGFs. Thus, angiogenesis results from an intricate network of interactions among pro- and anti-angiogenic molecules, EC receptors and various modulators.

All these interactions represent targets for the development of pro- or anti-angiogenic therapies. These aims call for suitable technologies to study the countless interactions occurring during neovascularization. Surface plasmon resonance (SPR) is a label-free optical technique to study biomolecular interactions in real time. It has become the golden standard technology for interaction analysis in biomedical research, including angiogenesis.

From a survey of the literature it emerges that SPR has already contributed substantially to the better understanding of the neovascularization process, laying the basis for the decoding of the angiogenesis "interactome" and the identification of "hub molecules" that may represent preferential targets for an efficacious modulation of angiogenesis. Here, the still unexploited full potential of SPR is enlightened, pointing to improvements in its use for a deeper understanding of the mechanisms of neovascularization and the identification of novel anti-angiogenic drugs.

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Abbreviations: AGFs, angiogenic growth factors; Ang, angiopoietin; CBP, calcium-binding protein; CMG2, cell surface receptors capillary morphogenesis gene 2; cRGD, cyclic RGD; K_d, dissociation constant; k_{off}, dissociation rate; k_{on}, association rate; EC, endothelial cells; ECM, extracellular matrix; EFG, epidermal growth factor; FG, fibrinogen; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FN, fibronectin; GAG, glycosaminoglycan; HGF, hepatocyte growth factor; HIV, human immunodeficiency virus; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; LMW, low molecular weight; MAb, monoclonal antibody; MMP, matrix metalloproteinase; NLS, nuclear localization sequence; NRP, neuropilin; PCPE-1, procollagen C-proteinase enhancer-1; PDGF, platelet-derived growth factor; PlGF, placental growth factor; ScFv, single chain antibody; SDF-1α, stromal derived factor-1α; SPARC, secreted protein acidic and rich in cysteine; SPR, surface plasmon resonance; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of MMPs; TKR, tyrosine kinase receptor; tPA, tissue-type plasminogen activator; TSG-6, tumor necrosis factor-stimulated gene 6; TSP-1, thrombospondin-1; uPA, urokinase-type plasminogen activator; VCAM-1, V cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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1. The process of angiogenesis

Angiogenesis is the process of new blood vessel formation from existing ones that takes place in embryonic development, inflammation and angiogenesis-dependent diseases, including cancer [1,2]. It is a multi-step process that begins with the degradation of the basement membrane by activated endothelial cells (ECs) that will migrate and proliferate, leading to the formation of solid EC sprouts into the stromal space. Then, vascular loops are formed and capillary tubes develop with formation of tight junctions and deposition of new basement membrane. Physiologically, angiogenesis is tightly controlled through the balance between the production, release and action of pro- and anti-angiogenic molecules and is carried out by the action of several effectors [*e.g.* proteases, glycosidases and extracellular matrix (ECM) components] that concur to the formation of new functional blood capillaries.

To initiate the neovascularization process, angiogenic growth factors (AGFs) must engage specific tyrosine kinase receptors (TKRs) expressed on ECs [2]. However, it is common knowledge that angiogenesis does not result from a single receptor/ligand interaction, being rather stimulated by the simultaneous action of multiple AGFs [3]. Depending on the setting, AGFs act together with inflammatory cytokines [4] and viral proteins [5] as large molecular aggregates in the pericellular microenvironment with the contribution of various co-receptors, including heparan sulfate proteoglycans (HSPGs), integrins and gangliosides [2]. The complexity of these large molecular aggregates is further increased by the fact that very often AGFs and their receptors undergo oligomerization and/or coupling [6] (Fig. 1A). Once stimulated, ECs produce a wide array of effectors (proteases, glycosidases and ECM components) that create a suitable environment for new vessel formation by interacting among themselves or with AGFs and EC receptors.

Anti-angiogenic compounds are a heterogeneous group of proteins, polysaccharides and glycosphingolipids present in body fluids and ECM whose common theme is the ability to bind and sequester AGFs hampering their interaction with ECs [2]. Alternatively, some anti-angiogenic compounds act by binding specific receptors on the EC surface inducing apoptosis or causing a decrease of responsiveness of ECs to AGFs [7] (Fig. 1A).

In conclusion, neovascularization is orchestrated by a variegate and intricate network of interactions occurring among proteins, sugars and lipids, the so-called "angiogenic interactome". Among the countless molecules involved in the process, some of them can interact simultaneously with various angiogenesis regulators, thus acting as "hub molecules" that catalyze the scaffolding of the large aggregates mentioned above. The prototypic hub is represented by HSPGs, whose intricate relationships and roles in the neovascularization process are depicted in Fig. 1B.

Due to the key role in tumor growth and metastasis, angiogenesis has become a promising target for the development of novel anticancer therapies. A better knowledge of the complex network of interactions underlying neovascularization is mandatory for the development of more efficacious anti-angiogenic strategies.

The complexity of the angiogenesis interactome, as well as the necessity to screen large panels of putative anti-angiogenic compounds, call for rapid, handily and reliable technologies. Over the last two decades, surface plasmon resonance (SPR) has demonstrated to meet these requirements.

2. SPR spectroscopy

SPR is an optical-based technology developed to evaluate macromolecular interactions. A typical setup of a SPR solid-phase bioassay is schematized in Fig. 2A. Briefly, a polarized beam of visible monochromatic light passes through a prism fitted with a glass slide coated with gold and, once reflected off the gold surface, its intensity is detected at the specular angle. When the light hits the glass, an electric field intensity (evanescent wave) is generated and absorbed by the free electron cloud in the gold layer, causing a reduction of the intensity of the reflected light. The angle corresponding to the sharp intensity minimum that occurs at the SPR condition is called resonance angle that depends on the refractive index of the material present within 300 nm from the gold surface. In SPR, a molecule (ligand) is chemically immobilized onto the gold film and exposed to a sample containing a specific binder (analyte). The ligand/analyte interaction causes a change of the refractive index at the gold surface resulting in the shift of the resonance angle that is presented as a real-time graph of the response units against time (sensorgram) (Fig. 2A). For a more exhaustive description of SPR technology see [8].

In respect to conventional fluorescent-, enzyme- or radio-labeled binding assays, SPR analysis is label-free and allow the multiplexed, automatic manipulation of very small amounts of molecules covering a wide range of molecular weights and binding affinities, including weakly interacting prodrugs. Also, SPR allows the evaluation of how fast the analyte binds to and detaches from its ligand (association and dissociation rates, k_{on} and k_{off}, respectively), of the affinity of the interaction [expressed as dissociation constant (K_d), inversely proportional to binding affinity] and of its stoichiometry. Finally, it allows the study of the formation of multi-protein complexes, that, as described above, are very important in the field of angiogenesis. Due to all these advantages, SPR has been largely and successfully exploited to study different aspect of the process of neovascularization as detailed below.

3. The impact of SPR on angiogenesis research

Since its launching in 1991, the use of SPR has increased steadily in the field of angiogenesis, with more than 230 papers published to date. Almost all these works are distributed between the identification of putative anti-angiogenic drugs (up to 50% of published papers) and the basic characterization of biomolecular interactions (34%), with only few studies devoted to the detection of pro- or anti-angiogenic molecules in body fluids (6%), and the remaining 10% of the published papers dealing with other angiogenesis-related issues.

As schematized in Fig. 1A, the extracellular interactions underlying neovascularization ideally fall in 10 categories. Among these, the most studied are the interactions of AGFs with signaling receptors, with co-receptors, with free binders and with ECM