Eye Pathological Angiogenesis

Subjects: Ophthalmology

Contributor: Yonathan Garfias

Angiogenesis is the new formation of vessels. Physiologically, it is essential for tissue repair; while its aberrant presentation, it may produce pathological disorders, such as Proliferative Diabetic Retinoptahy, Age Related Macular Degeneration, among many others.

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1. Ocular Immunotherapy in Pathological Angiogenesis

Novel potential therapies have been available since molecular biotechnology revolutionized the conduct of human therapeutics. In a few decades, biotechnology has surprisingly evolved. The production of therapeutic monoclonal antibodies (mAbs) was developed due to the important discoveries by Köhler and Milstein in 1975 [1]. This technique consists in repetitively immunizing mice with a specific antigen and isolating the splenic plasma cells that recognize the antigen. Antigen-specific splenocytes are then fused with myeloma cells using electrofusion or polyethylene glycol (PEG) to immortalize the antigen-specific plasma cells. Hybridomas that produce specific antibodies are isolated and cloned. To reduce xeno-immunogenic responses, mouse sequences are replaced by human constant regions (humanized mAbs) [2]. However, humanization of mouse mAbs reduces antibody affinity. In contrast, phage display technology partially substituted hybridoma technology due to the possibility of obtaining higher affinity maturation, consequently improving therapeutic clinical outcomes [3].

mAbs have been used as therapeutic agents in many diseases. Bevacizumab is a recombinant humanized mAb with an immunoglobulin G1 conformation that is 93% human and 7% rodent. It binds with high affinity and specificity to VEGF. Bevacizumab potently neutralizes VEGF by interfering with VEGF-VEGFR1 and VEGFR2 signaling pathways. It was the first Federal Drug Administration (FDA)-approved anti-VEGF mAb for therapeutic purposes in metastatic colorectal cancer [4]. It has been used for non-small cell lung cancer, renal cell carcinoma, breast cancer and gastric cancer. Studies have determined that the efficacy of bevacizumab was related to the baseline expression of VEGF-A and neuropilin-1 in gastric carcinoma [5].

Bevacizumab was originally synthetized from a specific murine mAb using hybridoma technology. Evidence indicated that VEGF-A is an important mediator of vascular leakage and pathological angiogenesis, and several isoforms of VEGF-A are generated by alternative mRNA splicing. Ranibizumab, an antigen-binding fragment (Fab) that specially blocks VEGF-A, was obtained using phage display, improving VEGF-A binding due to the affinity maturation of the antibody. The molecular weight of ranibizumab is 48.39 kDa, compared to 149 kDa of bevacizumab [6][7][8]. Aflibercept is a human recombinant fusion protein composed of the second binding domain of VEGFR1 and the third binding domain of VEGFR2, with a total molecular weight of 115 kDa. These immunoglobulin-type fragments are fused to the Fc region of a human IgG1. This fusion protein is a “trap” molecule that catches, holds and blocks different cytokines. It binds to all VEGF-A isoforms, VEGF-B and PLGF [9]. The rates of endophthalmitis following intravitreal injections of aflibercept, bevacizumab and ranibizumab are 0.100%, 0.056% and 0.047%, respectively [10].

Preclinical studies showed that trastuzumab, a full-length IgG antibody, was not capable of crossing the retinal limiting inner membrane of the retina, thus, smaller molecules were proposed for treating PDR and AMD [11][12].

Brolucizumab is a humanized single-chain Fab that blocks all isoforms of VEGF-A. The molecular weight of brolucizumab is 26 kDa, a smaller molecule compared to bevacizumab, ranibizumab and aflibercept. In a randomized clinical trial in patients with AMD, brolucizumab presented lower intraretinal and subretinal fluid compared to the patients treated with aflibercept. The patients that were treated in the aflibercept arm received twice as many unscheduled injections and presented greater tachyphylaxis phenomena compared to the patients in the brolucizumab arm. These findings suggest a more durable blockage effect with brolucizumab than the already approved aflibercept [13][14][15][16]. It is important to mention that anti-VEGF resistance conduces to refractory or recurrent neovascular AMD, and hence other molecular-

targeted therapies are being tested [17]. Moreover, systemic side effects such as thromboembolic events, hypertension, myocardial infarction, gastrointestinal perforation and cerebrovascular accident have been reported after long-term intravitreal anti-VEGF therapies, probably due to the BRB damage [6][18][19]. Anti-VEGF therapy produces local side effects that have been attributed to activation of fibrocytes, which promotes the formation of a fibrovascular membrane in the retina, producing retinal detachment and blindness [20].

2. Aptamers: Novel Oligonucleotide Therapy

Aptamers are single-stranded DNA (ssDNA) or RNA (ssRNA) molecules with variable length from 20 to 100 nucleotides. They are formed by complex and unique secondary and tertiary structures which confer enough recognition area and high range possibilities to interact with specific targets [21][22][23].

It has been widely studied that oligonucleotides recognize nucleic acid by base pairing, but surprisingly, aptamers create non-covalent bonds with multiple types of targets such as proteins, peptides, viral particles, vitamins, metal ions and even whole cells. Crystallization and structural determination of different aptamers have shown that hydrogen bonds, electrostatic interactions, hydrophobic and van der Waals forces confer high-affinity properties (ranges from µM to picoM Kd) [24][25][26]. Other types of oligonucleotide therapy are those containing immunostimulatory effects due to their CpG motifs. These CpG motifs enhance immune response by acting as agonists of Toll-like receptor 9, and therefore they could be used as vaccine adjuvants [27].

Aptamers are chemically synthesized oligonucleotides that are usually obtained from diverse nucleic acid libraries and selected by a method known as systematic evolution of ligands by exponential enrichment technology (SELEX). This method uses a random library of 10^{13}–10^{16} ssRNA or ssDNA exposed to the target to be bonded. The oligonucleotides that are not attached to the target are discarded, while the target-bonded aptamers are then amplified using RT-PCR or PCR. This selection process is repeated 6–15 times to improve their affinity and specificity binding capabilities [28][29].

Moreover, aptamer customization has several advantages over mAbs. These advantages include reproducible synthesis between batches, easy and controllable post-production modifications [30], long-term stability in solution, non-toxic, non-immunogenic, room temperature stability, low costs of production, unlimited targets and the capability to be cell-internalized [31][32]. Aptamers can be conjugated with micelle particles to facilitate the entrance into the cells, which is performed by binding a lipid tail of phosphoramidite in a polyethylene glycol located in the aptamer end [33].

Standard IgG mAbs have an average mass weight of 150–170 kDa; in contrast, nucleotide aptamers are 10-fold smaller than mAbs, with 5–15 kDa. Proteins are molecules susceptible to lose their three-dimensional (3D) conformation when denatured at high temperatures, while nucleotide aptamers are thermally stable and maintain their 3D conformation, even with repeated denaturation/renaturation cycles. Aptamer applications in medicine are diverse, and they are currently being used for flow cytometry staining purposes, can activate signaling pathways through cell surface receptor ligation, serve as drug delivery systems, block protein–protein interactions and inhibit enzyme reactions, among others [34]. Drug delivery systems have a myriad of chemical conjugation potentials to aptamers. They function as nano-sized carriers using micelles, microspheres, liposomes, polymeric nanoparticles and nanomaterial-based enzymes (nanozymes). Microspheres made from poly-*D*, *L*-lactide (PDLLA) and polylactide-co-glycolide (PLGA) have been safely used as intravitreal drug delivery systems with adequate vitreous humor biodistribution [35]. Aptamer conjugation with nanomaterials such as nanozymes have emerged in recent years for developing biosensors to detect food contaminants, pesticides, pathogens and metal ions [36]. Nanozymes have attractive properties due to their high stability, low cost of production and longer-term storage. These properties confer potential applications in countries with unfavorable meteorological conditions and warmer temperatures. Nanozymes' conjugation using affinity molecules such as aptamers and antibodies is a critical step for their production. Most usual types of conjugation are made by covalent or biotion/avidin linkages. [37].

Molecule customization has increased aptamers' biodistribution and their renal clearance. Previous studies suggested that 3′-inverted thymidine modification in aptamer molecules increased the stability and resistance to 3′-exonucleases in human serum. The biotin conjugation to 3′ carbon atom resists the catalytic activity of 3′-exonucleases and slows down the clearance rate of aptamers from the blood circulation. Chemical modifications such as 2′-O-methyl (2′-OMe) increase aptamer serum half-life with in vivo stable conformations and reduce enzyme degradation [38][39][40][41][42].

In 2004, the FDA approved the first therapeutic RNA aptamer pegaptanib for the treatment of wet AMD that is characterized by pathological choroidal neovascularization. Pegaptanib stabilized vision and reduced the risk of severe visual loss in almost all patients with AMD, because it binds and blocks the VEGF-A_{165} isoform with high affinity and

specificity. Pegaptanib intravitreally injected has a half-life of 10 ± 4 days, while ranibizumab has a half-life of 7 days, thereby pegaptanib could reduce the frequency of intravitreal administrations [43]. Studies suggested that bevacizumab can delay corneal wound healing, causing stromal thinning and affecting corneal homeostasis. Fortunately, new molecules have been found to reduce corneal neovascularization, such as a nucleolin-binding aptamer called AS1411 [44].

Molecular-targeted therapy is an emerging medical tool that has promising results in cancer and pathological angiogenesis. Encouraging results have been obtained by blocking immune checkpoints such as programmed death-ligand 1 and its receptor (PD-L1/PD-1) interactions [45]. This therapy has been used because PD-L1 plays a major role in suppressing the adaptive immune response. PD-1 is its receptor expressed on activated T cells, and the interaction with its ligand PD-L1 inhibits T cell response. PD-L1 is expressed on numerous cells, including epithelial cells, endothelial cells and immune system cells [46]. Importantly, PD-L1 is commonly upregulated on the surface of tumor cells, and this confers a tumor evasion mechanism to avoid apoptosis from lymphocytes. Thereby, poor clinical outcomes and low overall survival have been associated with the PD-1/PD-L1 pathway [47][48]. Targeted therapy that blocks the PD-1/PD-L1 immune checkpoint can enhance antitumor immunity by restoring anti-tumor cytotoxicity of activated T cells, producing a lasting clinical response and prolonging patient survival. Clinical studies indicate that therapies targeting PD-1 or PD-L1 can achieve promising results in numerous tumor types, including melanoma, prostate cancer and non-small cell lung cancer. The FDA has approved five monoclonal antibodies targeting this immune checkpoint, including atezolizumab, nivolumab, durvalumab, avelumab and pembrolizumab. Larger molecules such as antibodies led to lower tumor penetration, and therefore monoclonal antibodies could be substituted by aptamers due to their advantages. MP7 is a DNA aptamer that produces a specific antitumor response due to the inhibition of PD-1/PD-L1, diminishing tumor size in a colon carcinoma model [45][49]. Novel DNA nanostructures, named Holliday Junction (HJ), serve as carriers for drug delivery, nucleic acids and enzymes. This complex has a cross-type shape and is composed by four single-stranded DNA chains. This unique shape prevents HJ leaking out via renal clearance, augmenting its biodistribution. Recently, researchers have conjugated an aptamer with HJ (Apt-HJ) that blocks the PD-1/PD-L1 pathway. Interestingly, Apt-HJ had stronger affinity to colon cancer cells compared to the monovalent PD-L1 aptamer [46].

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