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Anti-Angiogenesis and its Applications to Cancer Treatment

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Angiogenesis is the process by which blood vessels branch out to deliver oxygen and nutrients to ischemic tissues within the body[1]. By its very nature, angiogenesis is an essential component in the growth and eventual spread of cancerous tumors in the body. Scientists have recently discovered several of the biochemical mechanisms by which angiogenesis occurs both naturally, and under the influence of a cancerous environment, and with that knowledge have begun to propose methods for the treatment of cancers such as microRNA manipulation and vascular endothelial growth factor suppression.

Introduction

The process of angiogenesis begins when a cell outside the blood vessel enters a state of hypoxia and sends out a chemical "distress signal" to the cells that make up the blood vessel. There are many of these chemical distress signals, like Semaphorin 4D (SEMA4D), which is usually associated with the regulation of motility in many different cell types[6], but the most notable of these signals is the vascular endothelial growth factor (VEGF). Endothelial cells sheathed by pericytes and bound together by a substrate initially form the blood vessel, but when the VEGF reaches the vessel, the pericytes detach from the substrate exposing the endothelial cells to the VEGF. The endothelial cells loosen their connections to each other, dilating the vessel, and the VEGF increases the permeability of the cell membrane allowing plasma proteins to escape the vessel and form an Extra Cellular Matrix (ECM)[1] onto which the endothelial cells then migrate. In order to keep all of the endothelial cells from migrating toward the source of the VEGF, one of the endothelial cells becomes a "tip cell." This is possible because the VEGF activates the VEGF receptor-2 (VEGFR-2), which increases the expression and subsequent release of the protein DLL4 from the affected cell. DLL4 is a ligand of the NOTCH receptor, which decreases the expression of the VEGFR-2 receptor in the endothelial cells adjacent to the new tip cell. In effect, the endothelial cells are jockeying for position as the tip cell until one of them gets ahead enough to begin releasing more DLL4 proteins than its neighbors. The endothelial cells that do not become the tip cell begin expressing WNT ligands (also expressed due to the activation of the NOTCH receptor) which in turn begin a chemical cascade that effects gene transcription in such a way that the cells begin to divide[2], allowing the new branch of the vessel to extend toward the source of the VEGF with the aid of SEMA4D[6]. In order for blood to flow, the vessel joins with another vessel at the site of the hypoxia with the aid of myeloid bridge cells, and matures by attracting pericytes with chemical signals such as platelet-derived growth factor B (PDGF-B) or transforming growth factor-β (TGF-β)[1].

Angiogenesis as Affected by Cancer

Cancerous cells, by their very nature, are constantly dividing. Because of this, the tumor cells must take advantage of the process of angiogenesis in order to obtain the necessary nutrients and oxygen required for them to grow. Once a tumor reaches a certain size, the interior cells are no longer able to readily obtain nutrients and oxygen, and therefore, enter a state of hypoxia[3]. The tumor cells then begin emitting VEGF and other chemical distress signals, and the process of angiogenesis begins. However, because the tumor cells are constantly dividing, more and more of the chemical distress signals are released into the environment, causing more and more branching of the vessel, eventually leading to an abnormal and even "torturous[3]" vasculature. In effect, because there

are so many chemical distress signals like VEGF in the environment, in the jockeying for position of the tip cell that the endothelial cells go through, there is never a clear winner. Added to that is the fact that the stroma of the tumor is studded with tumor related fibroblasts that "aberrantly deposit extracellular matrix (ECM) proteins and release stimulatory factors,[3]" and it is clear that the tumor environment creates a confusing soup of pro-angiogenic factors that lead to an aberrant and constantly changing vasculature.

MicroRNA Treatment and its Drawbacks

Recent studies have shown that microRNA molecules (miRNAs) play a pivotal role in the expression and regulation of the various signals related to angiogenesis[3]. These miRNAs accomplish this by binding to the 3' end of untranslated mRNA and either block translation or recruit a silencing complex, a conglomerate of proteins bound to the miRNA that, when attached to the mRNA, cleaves the mRNA, also preventing translation[4]. Several miRNAs both promoting and prohibiting angiogenesis, termed angiomiRs, have been discovered[3]. The main method of anti-angiogenic treatment currently being explored is to deliver the opposing sequence of miRNA, termed the anti-miRNA to the target cells, thus inhibiting the function of the miRNA and allowing the gene that it would have repressed to be expressed. One prominent study showed that the switch in endothelial cells to the angiogenic mode of proliferation mainly involved the suppression of different genes. This suppression involved the angiomiR miR-126. It is suggested that treatment with the corresponding anti-miR would prevent the suppression of anti-angiogenic genes, and hopefully prevent angiogenesis from occurring. The opposite also proved to be effective in a recent study in the suppression of the pro-angiogenic receptor of SEMA4D. Instead of preventing suppression of anti-angiogenic genes, the receptor of a pro-angiogenic gene was suppressed instead, causing a marked decrease in cell motility, thereby inhibiting angiogenesis[7]. The main drawback with this method is that current gene delivery techniques are not yet able to produce the precision with which the miRNAs or anti-miRs would need to be delivered to their desired products. Progress has been made with liver cancers and cancers that metastasize to the liver due to its proclivity to take up systemically derived miRNA agents, and methods of adeno-associated virus transmission have shown promise in other parts of the body, but these and other gene transfer methods must be improved upon before the miRNA method of treatment can be moved into clinical trials[3].

VEGF Suppression Treatment and its Drawbacks

The pivotal role that VEGF plays in angiogenesis both naturally and under the influence of a cancerous environment makes it a tempting target for anti-angiogenesis treatments. There has even been some success in the treatment of early stage cancers and even some non-malignant diseases such as macular degeneration in elderly patients[1]. The current methods for anti-VEGF treatment are the VEGF-neutralizing antibody bevacizumab (Avastin), and several multi-target tyrosine-kinase inhibitors (TKIs) that disrupt the signaling pathways of VEGF. Note that the success with macular degeneration was achieved with a different antibody, ranibizumab (Lucentis), and an intravenous injection of the VEGF aptamer pegaptanib (Macugen)[1]. Despite the success that has been achieved, recent studies have shown that many angiogenic factors increase in tumors when treated with anti-VEGF techniques[5]. For example, recent studies show that during VEGF suppression treatment, tumors in later stages of development adopt a more rigorous and metastatic method of initiating angiogenesis that revolves around different chemical distress signals such as Semaphorin 4D (SEMA4D), ultimately leading to the failure of the treatment. The same study experimented with anti-SEMA4D components in conjunction with anti-VEGF components and found that tumors treated with both displayed the least amount of vascularization at the end of trials, suggesting a joint treatment between these pro-angiogenic factors and others to help suppress other factors may be a probable anti-angiogenic treatment.

Discussion

The method of anti-angiogenic treatment of cancerous tumors promises to be a perfectly viable method for, if not the eradication, at the very least the suppression of malignant cells. There is, however, still quite a ways to go. For the method of miRNA suppression, a method of accurate gene transfer to the target cells as of yet needs to be developed, and the same could also be said for VEGF suppression as current methods provide no method of localization. The main problem with VEGF suppression is that VEGF is not the only pro-angiogenic factor involved in the process. As shown with the experiments with SEMA4D, a joint treatment that represses all of the angiogenic factors will be required for VEGF suppression (which at that point would actually be angiogenic suppression) to be a viable method of cancer treatment.

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