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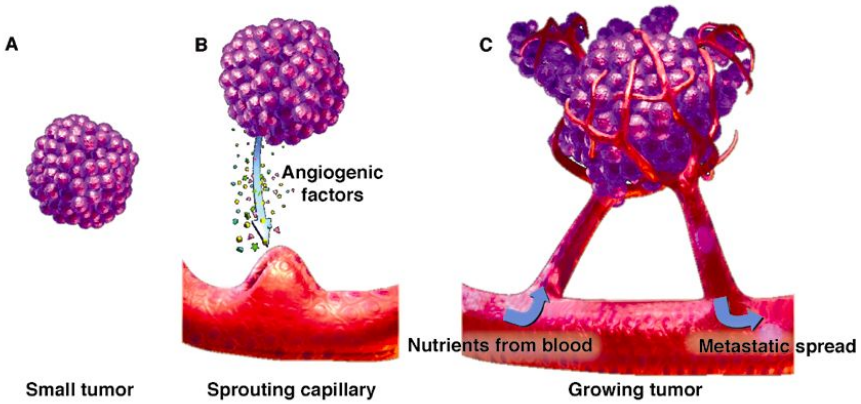
12/8/2018

Tie1 and Tie2 Dimerization and the Possible Inhibition of Angiogenesis in Tumor endothelial cells.

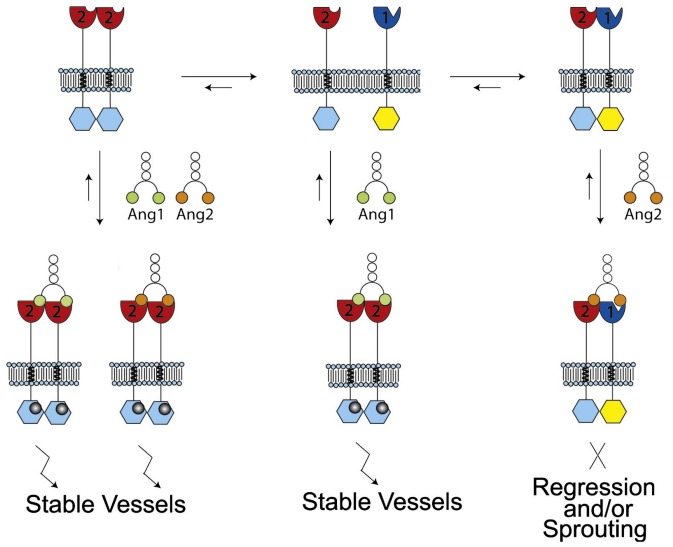
Tumors are known as some unusual mass in which cells divide uncontrollably. Tumors can be benign or malignant. In the case of malignant tumors, the tumor cells continue to grow uncontrollably and invade healthy organs and healthy cells. Oftentimes, when a tumor is identified, the main treatment is surgical removal. In 2018 alone, there will be approximately 1,735,350 new cases of malignant tumor diagnosis[4].This is an issue for many as surgery may cause injury to other areas of the healthy body. Tumor cells are able to grow and multiply due to the creation of new blood vessels. This process is known as angiogenesis(Figure 1). If there is some way to target tumor epithelial cells we could potentially manipulate the machinery involved in angiogenesis.



A scarcely visited target in tumor research is that of a family of Receptor Kinases known as the Tie receptors, in particular, Tie1 and Tie2. These two receptors are unique as the physical connection between Tie1 and Tie2 has been seen to halt the process of angiogenesis in endothelial cells[2]. This dimerization formation, or lack of, is what controls angiogenesis of their respective endothelial cells. An important thing to note is that these Tie receptors have growth factors known as Angiopoietins. Angiopoietin 1(Ang1) is especially of interest when speaking of a physical attraction between Tie1 and Tie2 because the presence of Ang1 causes Tie1 and Tie2 to split apart. As illustrated in Figure 2, when Tie1 and Tie2 are physically bound to one another, this prohibits the process of angiogenesis to take place. To prevent the growth and life of cancer cells, targeting the Tie1 and Tie2 receptors of epithelial cancer cells could provide a solution.

Tie1 has a positively charged surface while Tie2 has a negatively charged surface(Figure 3) [2]

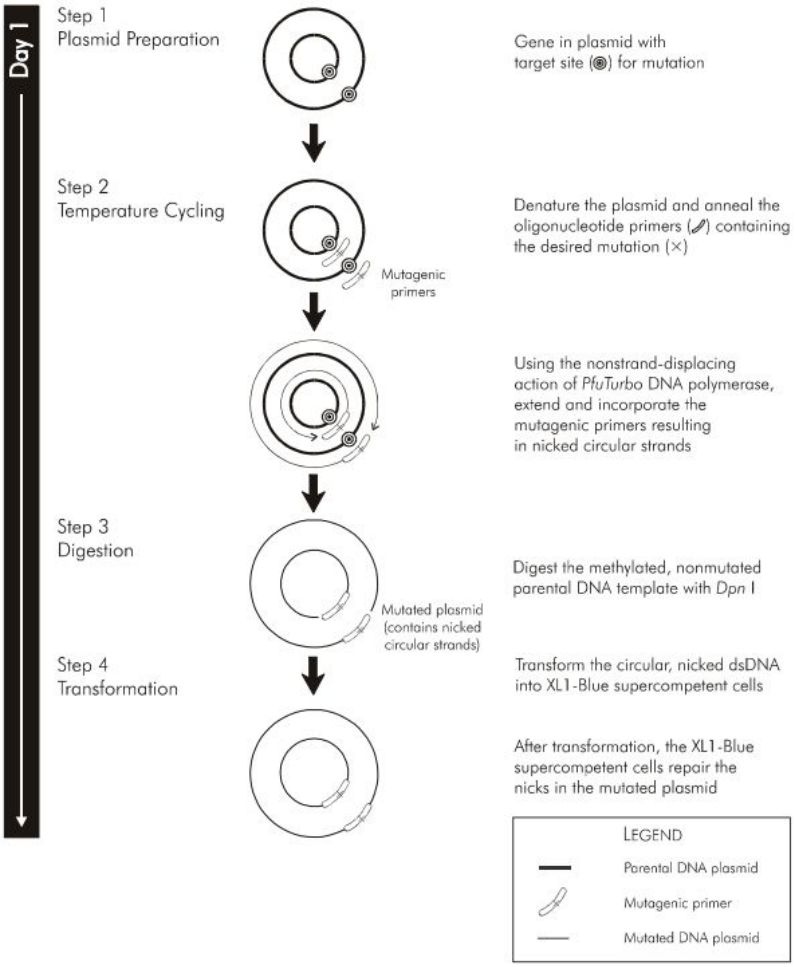
Based off of these Barton et al. findings[2] , a deduction can be made that these oppositely charged surfaces are the cause for physical interaction between Tie1 and Tie2. Likewise, these oppositely charged surfaces prohibit angiogenesis from taking place. After obtaining the crystallographic structure of both Tie1 and Tie2, oppositely charged surfaces on both Tie receptors were found and isolated. Tie2 was found to contain a large negatively charged surface which was made up of mostly glutamic acid and aspartic acid. Additionally, Tie1 was found to contain a large positively charged surface made up of mostly Arginine and Lysine[2]. These oppositely charged surfaces indicate that there is a location for physical attraction between the two receptors. Due to this attraction facilitated by oppositely charged surfaces, this may be a prime target for the prevention of angiogenesis. One possible strategy that could be utilized in order to inhibit the growth and survival of tumor epithelial cells is through the the constant physical contact, dimerization, between Tie1 and Tie2.



II.Experiment. The first step in my experiment would be to identify susceptible locations of amino acids on both Tie1 and Tie2 which could possibly be changed to increase the charge of that particular charged surface. This may be performed using a molecular visualization system knows as PyMOL. This system allows a user to find the exact location of specific amino acids of a desired protein.

After isolating the desired amino acids that are between Tie1 and Tie2 when physically bound to one another, Quickchange site-directed mutagenesis will be used to cause desired mutations to both Tie1 and Tie2 in hopes of increasing the charge density between the two receptors. Two primers per site of intended amino acid changes will be created and utilized based off of a ~20 nucleotide sequence which includes the amino acid mutation. Each of these primers anneal to a different strand of the DNA and subsequently creates two mutated strands which are complementary to one another. The parental DNA will be digested and the remaining mutated plasmid DNA will be sealed by DNA Ligase. From there, the mutated DNA plasmid will be transferred into a host cell to be cloned. If this charged interaction between Tie1 and Tie2 does in fact impact angiogenesis, this increased charged density should cause a constant physical

affinity between the two receptor kinases. One would expect this constant interaction to prohibit the transfer of nutrients and supplies to tumor cells which would soon after lead to cellular



death.

In order to measure if the experiment was successful, FRET(fluorescence resonance energy transfer) may be used in order to measure the attraction between these two receptors when both receptors are labeled with fluorescence[6]. Additionally, the introduction of Ang1 while capturing FRET would be helpful to see how strong the connection between Tie1 and Tie2 are. The introduction into Ang1 and its ability or inability to break apart Tie1 and Tie2 would decipher if the experiment was successful or not.

Although Site-directed mutagenesis is a very useful tool, the outcome may not be as intended due to the delicacy of protein structure. When selecting the specific amino acids we wish to mutate, it is imperative to **avoid hydrophobic areas** as this could cause issues with protein folding and function.

This experiment proposal was intended to be a basis for further research to potentially

build upon. If these two Tie receptors and the way that they interact with one another can be understood further, this may be a future target for tumor suppression and later cancer research.

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