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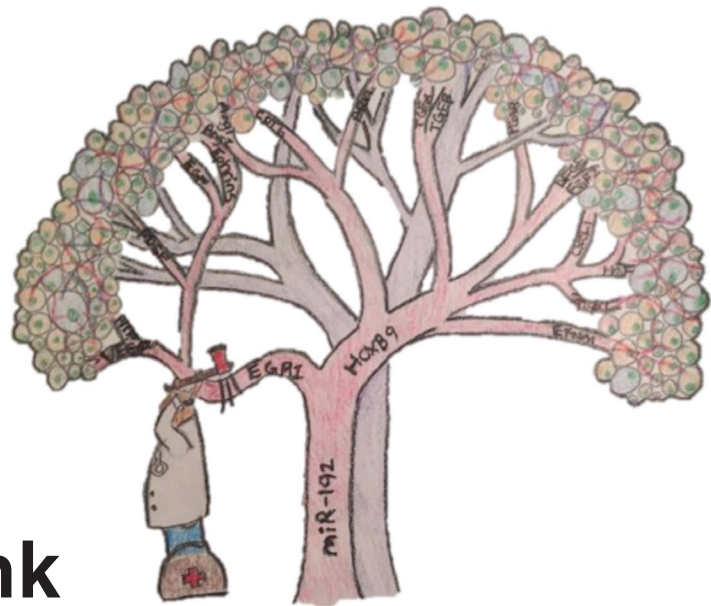
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Going for the Trunk

BY NICHOLAS NORWITZ '18

Summary

Although angiogenesis (new blood vessel formation) is an absolute requirement for tumor growth, therapies designed to treat cancer by targeting specific angiogenic factors have had limited success. One theory is that there are many different angiogenic factors that can compensate for the loss of a single factor. Therefore, a more effective strategy may be to move upstream, identify a factor that regulates the expression of many different downstream angiogenic mediators, and then measure the effect of blocking this single common factor on angiogenesis-mediated tumor growth.

What is Angiogenesis?

In 1971, Dr. Judah Folkman observed that, without new blood vessel formation, tumors arrest at a threshold size of 2-3mm (Folkman, 1971; Hanahan and Weinberg, 2000). Folkman also discovered that tumors release an angiogenic promoting paracrine factor, which he called tumor-angiogenesis factor (TAF), and hypothesized that blocking TAF signaling might serve as an effective cancer therapy (Folkman, 1971).

As angiogenesis is regulated by a balance between pro- and anti-angiogenic molecules, the basis of Folkman's hypothesis was sound. Therapies that shift the balance away from pro-angiogenic signals will impair tumor growth. However, the problem is more complicated. Scientists have discovered an abundance of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), epidermal growth factors (EGFs), transforming growth factors (TGFs), tumor necrosis factor (TNF), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), hepatocyte growth factor

(HGF), angiopoietins, and various chemokines (Ferrara, 2002; Bouis et al., 2006).

While different tumors promote angiogenesis by relying on different combinations of these factors, it is possible to develop therapies that effectively block angiogenesis by targeting specific angiogenic molecules (Fernando et al., 2008; Casanovas et al., 2005; Kopetz et al., 2010). The most studied angiogenic factor is VEGF. Many different approaches have been developed to target VEGF signaling including monoclonal antibodies and receptor blockers. While many of these therapies are initially effective, tumors are soon able to compensate by up-regulating other pro-angiogenic factors and overcoming this angiogenic blockade (Casanovas et al., 2005; Kopetz et al., 2010).

Modern Attempts to Inhibit Angiogenesis

Modern medicine has been trying to kill the cancer tree by chopping at the ends of its thinnest branches, and this strategy has had only limited success. The cancer simply learns to rely on other branches in order to grow. The work of Dr. Anil Sood et al. published in *Nature* in February 2016, concentrates on a new approach that is fundamentally different from previous therapies in that it emphasizes choking the cancer tree by chopping closer to the trunk. By identifying and targeting the molecular fork from which many angiogenic signals diverge, we might be able to develop anti-angiogenic therapies that will more successfully treat cancer.

The most obvious approach would be to inhibit transcription factors that up-regulate multiple downstream angiogenic factors. Sood's proposed therapy essentially does just this, but in a clever way that takes advantage of an

Figure 1: A novel anti-angiogenic therapy aims to target the trunk, not the branches, of this metaphorical tree of angiogenic mediators.

Source: Drawing by N. Norwitz

“While different tumors promote angiogenesis by relying on different combinations of these factors, it is possible to develop therapies that effectively block angiogenesis by targeting specific angiogenic molecules.”



Figure 2: microRNAs (miRs) are endogenously expressed noncoding RNAs that knock down the expression of target messenger RNAs (mRNAs) by silencing them before they can be translated.

Source: Wikimedia Commons
(Credit: Narayanes)

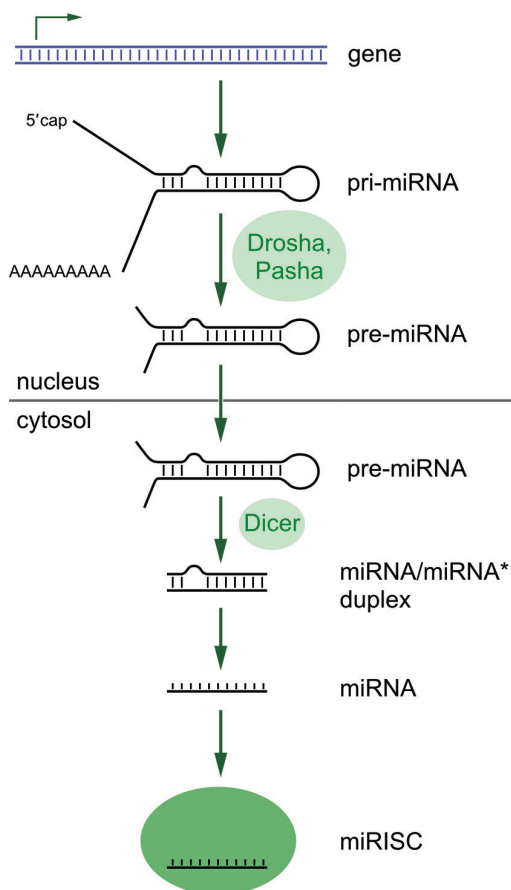
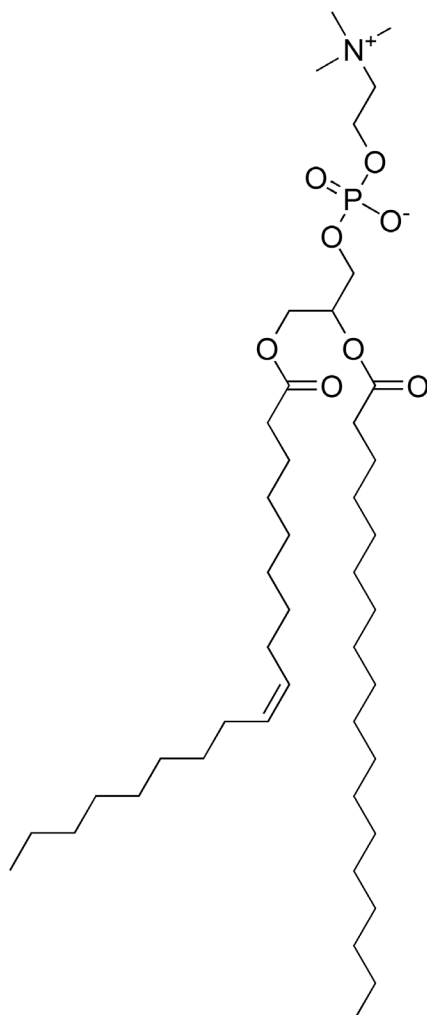


Figure 3: Nanoparticles made out of amphipathic phospholipids allow the delivery of exogenous miRs into tumor tissue.

Source: Wikimedia Commons
(Credit: Jesse)



endogenous control mechanism to amplify the breadth of its effect. In humans, microRNAs (miRNAs) are potent negative regulators of a wide array of genes. miRNAs work by binding to the 3' untranslated regions (3'UTRs) of specific mature RNA transcripts in order to prevent their translation to proteins.

By correlating the expression patterns of many miRNAs with the degree of vascularization in tumor samples, Sood's team was able to identify several miRNAs that were potential candidates for therapy. One such candidate was miR-192. The expression of miR-192 was found to correlate negatively with the expression of pro-angiogenic factors and angiogenesis, and correlate positively with patient survival.

The next goal was to discover how exactly miR-192 blocks angiogenesis. Dr. Sood and his colleagues identified 13 potential pro-angiogenic transcription factor targets of miR-192. When they measured how the levels of these transcription factors changed in cells transfected with miR-192, two transcription factors stood out--EGR1 and HOXB9.

To demonstrate that miR-192 impairs angiogenesis and tumor growth in vivo by blocking the translation of EGR1 and HOXB9, the research team devised a single elegant experiment. They transfected human ovarian cancer cells with miR-192 (or a control miRNA) before injecting the cells into mice. By monitoring tumor size, neovascularization, and circulating levels of EGR1 and HOXB9 as well as those angiogenic paracrine factors downstream of EGR1 and HOXB9, the team was able to show that miR-192 impaired tumor growth and angiogenesis, and that miR-192 overexpression decreased the expression of EGR1 and HOXB9. Superimposed on this experiment were additional studies in which miR-192 was transfected into cancer cells along with copies of the EGR1 and HOXB9 gene that lacked normal 3'UTRs. This rendered the EGR1 and HOXB9 transcripts effectively immune to miR-192 and prevented miR-192 from impairing tumor growth or angiogenesis, demonstrating that EGR1 and HOXB9 are both necessary and sufficient to account for the anti-angiogenic effect of miR-192.

The highpoint of this landmark publication was to show that exogenous treatment with miR-192 could serve as a viable and effective therapy in a murine model of ovarian cancer. The team found that injecting nanoliposomes (DOPC) filled with miR-192 into mice recapitulated the anti-angiogenic effect and blocked tumor development by over 90% ($p < 0.0001$), without any observable toxic side-effects. What's more, when DOPC miR-192 therapy was compared to treatment with an antibody against VEGF, the most studied angiogenic factor and most

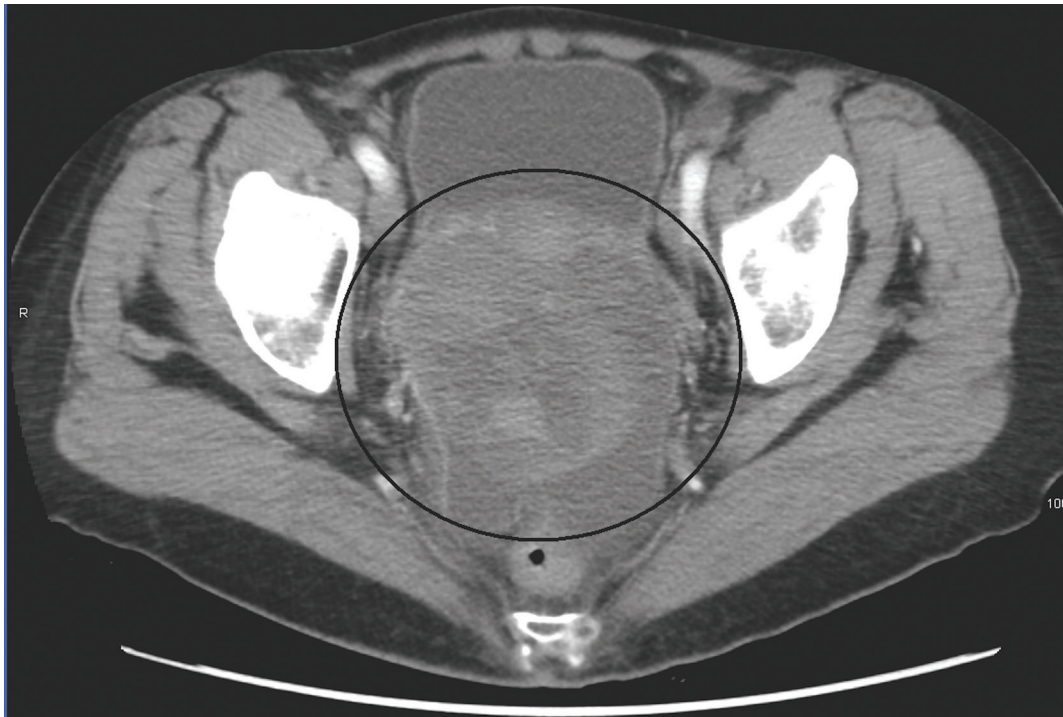


Figure 4: Certain tumors rely more on angiogenesis than others. Ovarian and kidney tumors rely heavily on the development of new blood vessels and were studied in this paper.

Source: Wikimedia Commons (Credit: James Heilman, MD)

common modern target of anti-angiogenic therapies, the DOPC treatment proved far more effective at inhibiting tumor growth over the course of three weeks ($p < 0.0001$) (Hanahan and Weinberg, 2000; Casanovas et al., 2005; Kopetz et al., 2010).

Conclusion

Although the future of miR-192 therapy appears promising, certain critical questions remain. First, it remains to be determined whether or not tumors can adapt to miR-192 therapy as they do to other anti-angiogenic therapies. Since miR-192 blocks at least two transcription factors that each block substantial and distinct arrays of angiogenic factors, it is possible that miR-192 treatment might be the first anti-angiogenic therapy that tumors cannot effectively circumnavigate. Second, there is some indication that miR-192 treatment may serve a more complex role in preventing tumorigenesis. miR-192 is a positive regulator of the tumor suppressor protein p53, and loss-of-function mutations in this gene are known to occur in more than half of all cancers (Moore et al., 2015; Lodish, 2000). Therefore, miR-192 therapy could in theory increase the robustness of the p53 DNA damage response, which may affect tumor development and growth. Moreover, p53 is a positive regulator of the potent endogenous anti-angiogenic Thrombospondin-1, adding yet another layer of complexity to the potential anti-angiogenic benefits of miR-192 treatment.

miRNA cancer therapy represents an innovative and exciting new approach for preventing angiogenesis and tumor growth and/or metastasis. Whatever the caveats and the

unknowns, the work performed by Dr. Sood and his team suggests that we shift our focus from chopping at branches and try chopping at the trunk. **D**

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