How the Right Path Can Go Wrong

Brittany Avonts
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

A novel connection in the PTEN-PI3K-AKT pathway shows AKT upregulates WNT5A, supporting glioblastoma growth. Targeted molecular therapies of WNT5A and its signaling pathway show promising treatment of glioblastomas.

Accounting for 60-70% of gliomas, glioblastomas have become a challenging area of research for the lack of a mechanism regarding the disease.1,2 Current treatments are also minimally effective because the brain is difficult to access in the presence of the blood brain barrier.3 In an attempt to further develop effective therapies for glioblastomas, Hu et. al.4 illustrate the invasive role of a protein known as WNT5A and its role in a well-known pathway that regulates the cell cycle and can be involved in cancer.

The signaling pathway in cancer, PTEN-PI3K-AKT, works because PI3K acts as a substrate for PTEN; when PI3K is increased, it recruits AKT, leading to other kinases. Some alterations in this pathway have been well studied and many mutations involved in the pathway support the growth of glioblastomas.5 Some research has reported genetic alterations in the pathway such as secretion of a protein, neuroligin 3, which alters PI3K, leading to glioblastoma growth.6 In the case of Hu et al.4, they focused on finding evidence for development of glioblastoma stem cells (GSCs) through differentiation into endothelial cells (ECs). It has previously been reported that endothelial cells support the extensive network of abnormal vasculature within glioblastomas.7 A model that could allow mechanistic insight into this pathway, a glioblastoma model (GBM), was created through the neutralization of p53, a known tumor suppressor, and through activation of AKT.

Firstly, they were interested in finding the role AKT plays in the differentiation of GSCs because of its important role for ECs. Through immunofluorescence, they found expression of CD144 and CD133 corresponded to GSC-derived endothelial progenitor cells. Thus, they considered the generation of CD144 and CD133 to be markers for finding a gene in which AKT is activated or upregulated; this would drive differentiation of GSC. In order to come up with a list of genes that could be responsible for GSC growth through AKT activation, Hu et al.4 identified 85 genes associated with AKT activation and further limited the list, only including those known to play a role in lineage commitment and cell fate determination. Of the 8 genes left, each was monitored for expression of CD144 and CD133 because expression of these cells would show differentiation of GCS. WNT5A showed a considerable amount of CD133 and CD144 and when WNT5A was knocked out, there was a substantial loss in CD133 and CD144. Considering these results, the first major findings showed AKT upregulated WNT5A, playing a pivotal role in GSC differentiation.

In addition to the role of WNT5A, a further look at the mechanistic role of regulation was explored by the researchers. They discovered two bindings motifs, PAX6 and DXL5, that work in opposing regulation of WNT5A to promote EC proliferation. Using Chromatin Immunoprecipitation coupled with PCR (ChIP-qPCR), which allows for investigation of a protein-DNA interaction at a known genomic binding site, the researchers showed PAX6 expression reduced WNT5A activation and DLX5 expression increased WNT5A activation. These results indicate the opposing actions of PAX6 and DLX5 and shows they are necessary in the mediation of WNT5A and differentiation of GSCs.

Furthermore, to address the in vivo role of WNT5A on differentiation of GSCs, they used a patient-derived GSC tumor model that better represents the human pathological condition. This model generated tumors with more rapid growth, shorter onset, and more secondary lesions formed in close proximity to the primary lesion. Thus, they concluded WNT5A drives EC differentiation and may provide a role in the invasiveness of the tumor by providing the perfect environment for growth.

Next, Hu et al.4 focused their attention on the role of ECs in the formation of secondary lesions and whether they have a role of additional invading glioma cells in the periphery. To assess the migratory response of endothelial cells, a transwell assay was used to show endothelial-like cells derived from WNT5A can stimulate recruitment and proliferation, suggesting EC acts as a host to recruit WNT5A promoting tumor growth beyond the primary tumor location. This conclusion led to their final aim which investigated recurrent tumors and the role of WNT5 on endothelial-like cells in GBM patient specimens. They found that higher levels of WNT5A were correlated with an increased number of secondary lesions and recurrence through immunohistochemistry staining of a marker for endothelial-like cells.

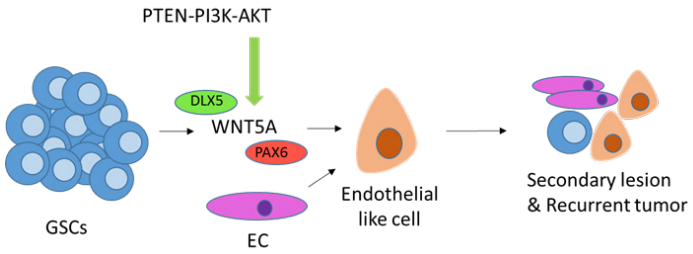

Figure 1 Activation of WNT5A contributing to glioblastoma growth. Hu et al.4 found AKT regulates WNT5A which is further regulated through activation of DLX5 and inhibition of PAX6. Differentiation of GSCs into endothelial-like cells is mediated by both WNT5A and recruitment of ECs. Clinical studies further find increased expression of WNT5A leads to secondary lesions and GBM recurrence.

These findings enabled further development of the PTEN-PI3K-AKT pathway and the mechanism of glioblastoma growth through EC differentiation. The opposing factors of PAX6 and DLX5 in the regulation of WNT5A were shown to drive differentiation of ECs and support the recurrent properties of gliomas in patients. The in vitro and in vivo findings establish the environmental niche that endothelial-like cells provide where glioblastomas establish their invasive-like properties.

The properties established regarding the supporting role of WNT5A in glioblastoma growth can contribute to important clinical therapies in the future. Some studies have already been conducted that suggest the suppression of WNT5A is a promising therapeutic target. For example, MMP-2, which normally acts to support infiltration in human glioma by WNT5A, when inhibited can suppress tumor cell infiltration by suppressing WNT5A.8 Hu et al.4 speculate further investigation into anti-vascular endothelial growth factor therapy (VEGF) and the role of WNT5A mediation of GSC will be promising because treatment with antibody bevacizumab is only slightly beneficial.9

To further consider the therapeutic role on disrupting the pathway of AKT and WNT5A, additional targets may be explored that play a role in other types of cancer. It is known the tumor type, binding of receptor, downstream efforts, and microenvironment all have an effect on the role of WNT5A in tumor metastasis.10,11 Considering the widespread role of WNT5A in cancer, it is a large undertaking to develop a therapy that focuses on specific inhibition of glioblastomas. However, continuing to find connections like the role of AKT in upregulation of WNT5A mediated by PAX6 and DLX5 can come a step closer in finding an effective therapy.

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