Stemming CSCs

By Kai-Jye Lou, Staff Writer

A Belgian team has identified VEGF and its co-receptor neuropilin 1 as promoters of cancer stem cell proliferation.¹ The results provide additional mechanistic validation for existing VEGF-based therapeutic approaches in cancer and for neuropilin 1-targeting strategies in development. The findings also suggest the potential to combine both approaches.

VEGF is well known for its ability to drive tumor growth by promoting the formation of tumor vasculature. There are at least five marketed drugs that target VEGF or VEGF receptors in cancer.

Neuropilin 1 (NRP1) originally was identified as a mediator of axon guidance in the developing nervous system. The link to cancer

was established after multiple studies showed that this transmembrane glycoprotein is upregulated in various tumors and could act as a VEGF co-receptor.²

R7347, a recombinant human mAb that binds to NRP1, is the most advanced NRP1-targeting compound for cancer. The compound, being developed by Roche's Genentech Inc. unit, is in Phase I testing for solid tumors. Ark Therapeutics Group plc has small molecule NRP1 antagonists in preclinical development for cancer.

More recently, a number of researchers have published data suggesting VEGF also drives tumor growth directly by acting on tumor cells.^{3,4} However, it has remained unclear whether VEGF's tumorigenic activity requires the presence of a particular cell type within the tumor.

In the current study, a Belgian group led by Cédric Blanpain has shown that a cancer cell subtype is required, specifically cancer stem cells (CSCs), which have self-renewal and differentiation properties that distinguish them from other tumor cells.

Blanpain is a researcher for the National Fund for Scientific Research and a Walloon Excellence in Life Sciences and Biotechnology (WELBIO) investigator at the Institute for Interdisciplinary Research in Biology at the Free University of Brussels.

Figure 1. Model for VEGF-mediated skin tumor progression. VEGF is known to promote tumor progression by stimulating angiogenesis. As reported by Beck *et al.* in a mouse squamous skin tumor model, VEGF also may support tumor progression by promoting cancer stem cell (CSC) renewal and proliferation.

In the first role, tumor cell-derived VEGF-A signals through VEGF receptor 2 (KDR/Flk-1; VEGFR-2) on endothelial cells to promote angiogenesis and create a vascular microenvironment for CSCs [a].

In its second role, VEGF-A acts directly on cutaneous CD34⁺ CSCs themselves in a neuropilin 1 (NRP1)-dependent manner to promote their self-renewal and proliferation [b]. The researchers found that this autocrine loop contributes to CSC expansion and skin tumor initiation.

NRP1 is a VEGF co-receptor known to interact with VEGF-A.

In a mouse model of squamous skin tumors, the researchers first confirmed that a subpopulation of CD34⁺ CSCs existed in a vascular tumor microenvironment called the perivascular niche. Another European research group had previously identified CD34⁺ tumor epithelial cells as CSCs in the mouse skin tumor model,³ but their exact location was unclear.

Blanpain's group next asked whether VEGF was involved in the growth and function of those CSCs.

In the same mice, a series of studies using genetic deletion and overexpression as well as blocking antibodies showed that in addition to its proangiogenic effects on the tumor microenvironment, VEGF bound to the co-receptor NRP1 on CSCs to promote their renewal, expansion and proliferation (see Figure 1, "Model for VEGF-mediated skin tumor progression").

Results were published in *Nature*.

"Scientifically, the researchers have very clearly shown in this cancer model that this VEGF-neuropilin loop plays a role in regulating cancer stem cell function," said Chiang Li, CEO and CMO at **Boston Biomedical Inc.** "What I found to be surprising is that the cancer stem cells in this model are localized in a perivascular niche, because it is hypoxic regions that are generally thought to act as cancer stem cell repositories."

Previously, only CSCs in gliomas were known to reside in the perivascular niche.⁵

Boston Biomedical's lead compound is BBI608, an orally administered small molecule that targets both CSCs and nonstem cancer cells. It is in Phase Ib/II testing for advanced solid tumors. The company has not disclosed the molecular target.

Clinical relevance

Although researchers contacted by *SciBX* agreed that the reported results clearly link VEGF and NRP1 signaling to CSC function in the squamous skin tumor model, they noted that the clinical relevance of the findings still needs to be fleshed out.

Boston Biomedical's Li pointed out that because skin squamous cell carcinomas are easy to remove and very rarely become a problem in practice, it is unlikely that an NRP1-targeted therapeutic approach will be developed for this particular indication.

However, Blanpain noted that *NRP1* is known to be overexpressed in other malignant human carcinomas, including lung squamous cell carcinomas and breast carcinomas.

"In such cancers, it is possible that targeting NRP1 might result in therapeutic benefits," he told *SciBX*. "We are currently examining these questions in various human carcinomas."

In addition, Meenhard Herlyn, a professor and leader of the Molecular & Cellular Oncogenesis Program and director of the

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Melanoma Research Center at **The Wistar Institute**, thinks the reported findings could lead one to consider adding an NRP1-targeted therapy to the regimen of cancer patients receiving anti-VEGF therapy.

Although the reported findings do suggest a possible new mode of action for existing VEGF-based therapies, Li cautioned that current VEGF-targeting therapies have only

had limited efficacy in the tumor types in which they are approved.

"If this pathway were critical for other tumor types, then why haven't we seen a stronger effect for VEGF-targeted therapies in such tumors?" he asked.

Hence, Li thinks it will be important to characterize the relative importance of the VEGF-NRP1 loop in various types of tumors in humans.

"If this loop is found to be active in human tumors, then one can explore the translational prospects of combining compounds that target this loop with those that target cancer stem cells directly to see if there will be an improved effect," he said.

Blanpain said the group is working to validate the effect of NRP1 inhibition on tumor cell self-renewal and subsequent tumor growth.

"We need to investigate the expression of NRP1 in other types of mouse epithelial cancers and determine genetically and pharmacologically whether targeting NRP1 in these other mouse cancer models will have benefit, as it does in the skin papilloma model," said Blanpain. "We will also need to determine the expression of NRP1 using very sensitive techniques in a broad range of human cancers, develop new strategies to only inhibit NRP1 function in human tumor cells and determine the impact of NRP1 inhibition in xenograft tumor models."

The findings have not been patented.

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COMPANIES AND INSTITUTIONS MENTIONED

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