Unlike neuropilin (NRP) 2a, NRP2b uniquely supports TGF-beta-mediated lung cancer progression

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Dr. Robert Gemmill (front, seated), Melvyn Berlinsky Endowed Chair for Cancer Research at the Medical University of South Carolina (MUSC), and Dr. Patrick Nasarre, an assistant professor in the College of Medicine at MUSC, are first and second authors on the Jan. 17, 2017 *Science Signaling* article showing that NRP2b uniquely supports TGF-beta-mediated lung cancer progression. Credit: Medical University of South Carolina.

Medical University of South Carolina (MUSC) investigators report preclinical research showing that the tumor-promoting properties of neuropilin (NRP)-2 reside predominantly on isoform NRP2b, while NRP2a has the opposite effects in non-small cell lung cancer (NSCLC), in the January 17, 2017 issue of *Science Signaling*.

In mouse models, NRP2a inhibited tumor cell proliferation, while NRP2b promoted metastasis and progression. This new understanding may lead to improved therapies that specifically target NRP2b, while sparing the tumor-inhibiting functions of NRP2a.

Lung cancers are highly invasive, metastatic, and drug resistant—accounting for one fifth of cancer deaths worldwide each year. In part, the malignant properties of lung cancer are driven by the epithelial-mesenchymal transition (EMT), which is primarily induced by transforming growth factor beta (TGF-beta), and results in the proliferation of cancer stem cells.

It is known that the two human NRPs—NRP1 and NRP2—are often up-regulated in tumors and associated with poor patient prognosis. Previous work by MUSC Hollings Cancer Center researchers Robert Gemmill, Ph.D. and Harry Drabkin, M.D., first and senior authors on the *Science Signaling* article, demonstrated that NRP2, in particular, was up-regulated in cultivated NSCLC lines during TGF beta-mediated EMT. Furthermore, inhibiting NRP2 was found to reduce TGF-beta-mediated responses, including invasive tumor growth.

However, to date, nearly all studies of NRP2 have focused on the 2a isoform. Until now, NRP2b, the alternatively spliced isoform, has been largely uninvestigated and poorly understood.

"Most research has been focused on understanding the major effects of NRP2 overall and wasn't really concerned with breaking down the roles of its component parts—the 2a and 2b isoforms," explained Gemmill, who holds the Melvyn Berlinsky Endowed Chair for Cancer Research at MUSC. "So, we know that NRP2a and NRP2b are nearly identical - only about the last 100 amino acids at the C terminus are different. There was some speculation that they might have different functions, but most of us assumed those differences were minor."

"It's turning out that there are lots of molecule variants that people just haven't looked at before and that we've only recently been discovering are important,"said Drabkin, who holds the Mary Gilbreth Endowed Chair in Clinical Oncology at MUSC. "We usually find the big stuff first and then work down into the deeper layers and that takes time. As they say, 'the devil is in the details' and there's lots of details."

The team was drawn into their investigation of NRP2b by the results of experiments they were conducting on a potent tumor suppressor, semaphorin 3F (SEMA3F), which uses NRP2 as its receptor. When analyzing NRP2 expression, they unexpectedly observed a double band and that induction appeared to affect one band more than the other. This led them to question why one band was altered more than the other. In addition, they found that, during the progressive changes that lead to tumor metastasis, SEMA3F was lost.

"So, we asked, what happens to it? Where does it go? And we found that, during lung cancer progression, SEMA3F is lost and NRP2b is induced," explained Gemmill. "That led us to investigate the 2b isoform to see what it does because no one knew."

To this end, the team designed a series of experiments. Real-time, reverse transcription polymerase chain reaction assays revealed that TGF-beta stimulation substantially increased NRP2b but not NRP2a. This was

the first time that NRP2 up-regulation by TGF-beta had been shown to preferentially involve the uninvestigated 2b isoform. They then looked at tumor cell migration and invasion patterns and found that cancer cell migration across Matrigel-coated membranes was inhibited in NRP2b knockdown models and enhanced by NRP2a knockdown.

Repeated experiments confirmed that TGF-beta-mediated cancer cell migration and invasion were dependent on NRP2b. That's when the team realized that the two isoforms had very different functions in terms of cancer progression, leading them to extend their studies to in vivo animal models.

"As soon as we saw the migration results, we knew we had to put it in an animal model using cancer cell lines where we could control the isoform expression. We thought, 'this is just too good to be true,' but it was true," explained Gemmill. "We got the same results time after time. Whenever we expressed NRP2b the cancer metastasized, and whenever we expressed NRP2a progression and metastasis were suppressed. Clearly, with the 2b isoform, we have found something that promotes metastasis."

Harry Drabkin, M.D. (left) of the Medical University of South Carolina (MUSC) is the senior author on the Jan. 17, 2017 *Science Signaling* article. Dr. Drabkin holds the Mary Gilbreth Endowed Chair in Clinical Oncology at MUSC. Credit: Medical University of South Carolina

"Other people had looked at NRPs as co-receptors but never got into the details of which isoform was playing what role," said Drabkin. "It's sort of like detective work. You follow leads and ask questions. Sometimes you follow a false lead—but in this case we found something new that turned out to be important."

Additional experiments showed that cancer stem cell tumor-spheres, which are highly tumorigenic and resistant to chemo- and radiation-therapies, were substantially reduced in NRP2b knockdown models.

Specifically, significantly fewer cells developed gefitinib chemotherapy resistance in models that knocked out NRP2b (i.e., tumor environments with very low levels of 2b) and significantly more cells developed resistance in NRP2a knockout (i.e., tumor environments with very low levels of 2a).

Finally, the researchers looked at human tissue samples and found that NRP2b abundance in human lung tumors was correlated with a higher cancer stage and more advanced progression.

"EMT produces a wholesale change in the repertoire of growth factors and receptors. In tumors where EMT is underway, there's more resistance to treatment," explained Drabkin. "What we found was that, in these epithelial tumors, if we block NRP2—especially the 2b protein—on the cell surface, they just did not respond in any way like control cells in terms of their ability to take on the EMT phenotype and migrate. By inhibiting that receptor we'd made a big dent in their ability to become resistant."

"Honestly, I was very, very surprised at how distinctly different the two isoforms were," said Gemmill. "When we first realized that there was differential expression, I thought we would have to look really hard to find some very minor differences. The fact that they were opposites—one inhibiting and one promoting cancer cell migration—and that this difference fell out so quickly was astounding because there's such a short list of things that are different about these 2 isoforms."

Although there is still a lot to learn about how NRP2a and NRP2b function in both normal tissue as well as cancers, these discoveries open new avenues for potential therapies. Possibilities range from developing monoclonal antibodies to target the 2b isoform, to immunotherapies, to using NRP2b as a biomarker for predicting patients' responses to particular therapies.

Gemmill anticipates that, in the wake of their findings, researchers will start looking at the role of NRP2b in other disease areas.

"I think a lot of people are going to sit up when they read this article and say, 'I wonder what it does in my system?'" said Gemmill. "For example, fibrosis is often associated with a TGF-beta response and we now know that TGF-beta induces NRP2b. So, maybe, NRP2b plays a role in fibrosis that affects the kidneys, liver, and lungs."

Both researchers agree that this work exemplifies the importance of not dismissing small but bothersome findings.

Gemmill notes that many of the biggest breakthroughs in science start with one person sitting in a laboratory, pursuing a single, seemingly minor, phenomenon.

"If you see something that isn't quite right, don't dismiss it," said Gemmill. "This is an exciting finding that came from not letting a detail slip by."

"That's how we find out what's really going on in a system," said Drabkin. "If you work out enough of the details, you start to see how things interact. The more you learn, the more you see how things connect and where the pivot points are that can have biologic consequences. Progress happens little-by-little until we find a weakness where we can direct therapy."

More information: Robert M. Gemmill et al, The neuropilin 2 isoform NRP2b uniquely supports TGFβ-mediated progression in lung cancer, *Science Signaling* (2017). DOI: 10.1126/scisignal.aag0528