CANCER

CRISPRa screening in mice for melanoma's Achilles' heel

Van der Weyden, L. et al. *Commun. Biol.* **4**, 395 (2021)

Melanoma may be a rare form of skin cancer – it accounts for only about 5% of cases, trailing the incidences of the more common basal cell and squamous cell carcinomas – but it is notoriously deadly if not caught early. That's because melanoma has a particular propensity to metastasize and spread to other organs, including the lungs.

Metastasis accounts for over 90% of all cancer-related deaths, making it a critical area of research, says Louise van der Weyden, a researcher working with David Adams at the Wellcome Sanger Institute. But for a cancer to metastasize, malignant cells must make their way from their primary tumor and establish themselves in the “foreign soil” of a secondary site. The very genes that enable cancerous cells to successfully spread and grow elsewhere could however be their Achilles' heel, says van der Weyden. “If we could target those genes, then in theory the metastatic cell would no longer be able to survive,” she notes. In new work published in *Communications Biology*, van der Weyden and her colleagues struck out to find that weak point for melanoma.

In vitro proteomics approaches can whip through lists of candidate genes for their potential roles in different processes fairly quickly, but cells alone can lack a bit of biological context. “I firmly believe that you can't study metastasis in a dish. You need the microenvironment to be present – the blood/lymphatic system, the immune system, the ‘foreign soil’ that is the tissue at the secondary site, etc.,” says van der Weyden. So she and her colleagues looked to mice – including immunocompetent animals – and started in vivo screening.

CRISPR technology has been proving useful for screening gene function in vivo, though many studies have focused on knocking down or knocking out the expression of genes of interest. To study what genes help metastatic melanoma cells spread, the team needed to overexpress those on their candidate list. For this, they turned to CRISPR activation (CRISPRa), a variant of the gene editing system that increases target expression. There are a lot of genes to consider, so it made sense to narrow things down a little to focus on

A library of guide RNAs targeting various membrane proteins is mixed with melanoma cells and injected into mice to screen for genes that facilitate metastasis to the animals' lungs. Image adapted from Van der Weyden, L. (2021). Springer Nature.

those that encode proteins on the surfaces of cells – these are likely to be more accessible than ones enclosed deep within, says van der Weyden. That led them to an existing library of guide RNAs that targeted 2,195 membrane protein genes.

They took the guide library, added it to melanoma cells, and injected those into the tails of their mice, setting cancerous cells overexpressing genes for different proteins off into the bloodstream. Then they waited for what would turn up in the animals' lungs.

Black spots started appearing, and from those the team assembled their hit list. At the top they identified the gene *Leucine-Rich Repeat Neuronal 4 C-Terminal-Like (Lrrn4cl)*, which encodes a transmembrane protein of unknown function and no prior link to cancer. A look at human melanoma samples in The Cancer Genome Atlas (TCGA) database revealed a correlation between elevated human LRRN4CL expression and poor patient outcomes, while experimentally overexpressing the protein in additional human and mouse melanoma cell lines as well as mouse colon, breast, and bladder cancer cells indeed led to lung metastases in different mice.

Location, location, location, however – LRRN4CL doesn't offer a metastatic advantage to cancer just anywhere. The team didn't observe any differences in LRRN4CL-over-expressing cells relative to controls in in vitro follows up to measure metastatic mettle, nor did overexpression of the gene appear to be involved in

driving metastases to other organs in the mice, such as the liver. “Only when the LRRN4CL-over-expressing cells were in the lung did they show a phenotype,” van der Weyden stresses. “I think that speaks volumes about the critical and invaluable role that laboratory mice can play in cancer research. We never would have found the role of LRRN4CL in cancer had we done purely in vitro experiments.”

There are a few other hits to follow up on, but figuring out the mechanism by which LRRN4CL amplifies a tumor's metastatic potential is an obvious next step, says van der Weyden. “How is LRRN4CL able to regulate lung-specific metastasis of a range of cancer types? What is its binding partner in the lung – that's something I'd love to know,” she says.

The screen proved a smashing success. It was the very first time the lab had attempted a CRISPR screen in vivo, let alone a CRISPRa experiment using so many different guides RNAs, and van der Weyden was thrilled to see it work. “It provides a list of genes that are potential anti-metastatic drug targets, and knowing how accessible cell surface proteins are, I would hope these are considered as attractive targets. I think it also shows that CRISPRa can successfully be used in screens in vivo, so hopefully it will encourage other researchers to use the technology,” she says

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Published online: 19 April 2021
<https://doi.org/10.1038/s41684-021-00762-7>