Editorial Commentary

A new hypoxia-responsive lncRNA in metastatic breast cancer

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Metastatic breast cancer, refers to the spread of the disease from the breast to other parts of the body, most often to bone, brain, liver or lungs. Despite advances in breast cancer management, most cancer deaths result from metastases that are resistant to systemic therapies (1). Hypoxia (or reduced oxygen availability) is a hallmark of the breast tumor microenvironment and plays an important role in metastatic progression. Breast tumor cells adapt to hypoxia by increasing the activity of the hypoxia-inducible transcription factors (HIF1 and HIF2), which regulate the expression of target genes involved in cancer progression (2). Recent studies have implicated long non-coding RNAs (lncRNAs) in hypoxia/HIF-associated breast cancer metastasis, through various mechanisms. Notable examples include the nuclear lncRNA *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1), which is widely reported as a metastasis-promoting lncRNA, however a recent study provided strong evidence that *MALAT1* suppresses breast cancer metastasis through inactivation of the TEAD transcription factor (3). *HOTAIR* (HOX transcript antisense RNA) is highly expressed in primary breast tumors and metastases and associated with poor prognosis. *HOTAIR* promotes epithelial to mesenchymal transition (EMT) by recruiting the polycomb repressive complex-2 (PRC2) to epigenetically silence target gene promoters (4). Furthermore, hypoxic induction of *NEAT1* (nuclear paraspeckle assembly transcript 1) induces the formation of nuclear structures called paraspeckles and retention of F11R (also known as junctional adhesion molecule 1) mRNA in the nucleus (5). Induction of *NEAT1* in hypoxia also leads to hallmarks of increased tumorigenesis including acceleration of tumor cell proliferation and inhibition of apoptosis (5).

The recent study by Niu *et al.* (6), provides another example of a hypoxia-responsive lncRNA involved in metastatic breast cancer. The authors initially used RNAseq to identify an hypoxia-inducible antisense lncRNA, called *RAB11B-AS1* from MDA-MB-231 breast cancer cells under hypoxic conditions (7). Subsequent ChIP-seq and qPCR showed that HIF2, but not HIF1, was enriched at the *RAB11B-AS1* promoter and responsible for hypoxia-induced lncRNA expression. Nui and colleagues then investigated the oncogenic role of *RAB11B-AS1 in vitro* and *in vivo* through gain- and loss-of function studies. They found ectopic expression of *RAB11B-AS1* promoted cell migration and invasion in MDA-MB-231 and SUM159 breast cancer cells, whereas *RAB11B-AS1* depletion caused the opposite effect. Orthotopic injection of MDA-MB-231 cells, ectopically expressing *RAB11B-AS1*, into the mammary fat pads of NSG mice, did not affect primary tumor growth, but showed increased expression of the endomucin capillary marker and extensive metastases to the lungs and liver as compared to the control group. Collectively, these results suggest that hypoxia-induced *RAB11B-AS1* promotes metastasis of breast cancer cells to distant tissues.

To investigate the mechanisms by which *RAB11B-AS1* contributes to angiogenesis, the authors analysed RNA-seq data from *RAB11B-AS1*-overexpressed MDA-MB-231 under hypoxia. Gene ontology analysis indicated multiple biological process likely contribute to the phenotype, although it was difficult to rank the results as no value for enrichment or fold-change was provided. RT-qPCR in *RAB11B-AS1*-overexpressed MDA-MB-231 was used to validate the RNAseq results, but it is unclear how many genes from the pathway analysis were assessed. The authors

chose to highlight increased *VEGFA* and *ANGPTL4* mRNA levels (both genes encode pro-angiogenic factors) in hypoxic breast cancer cells, although this result would be strengthened by confirming a concomitant increase in protein levels. Of note, *RAB11B-AS1* and *ANGPTL4* map to chromosome 19, whereas *VEGFA* locates to chromosome 6, suggesting *RAB11B-AS1* operates either *in trans* to directly regulate these two genes or indirectly through other mechanisms. Notably, ectopic expression or silencing of *RAB11B-AS1* did not affect the mRNA levels of the *RAB11B* sense transcript or other key angiogenic factors such as *FGF*, *ANGPT2* and *CXCR4*, suggesting some level of trans-acting target-specificity.

The gene ontology results identified pol II gene regulation as the top biological function, suggesting *RNA11B-AS1* may alter pol II regulation or function. RNA pulldown assays detected strong binding of pol II to the *RNA11B-AS1* transcript in MDA-MB-231 cells under both normoxic and hypoxic conditions. ChIP-PCR then showed that pol II was enriched at the promoters of *VEGFA* and *ANGPTL4*, and this enrichment was significantly increased in hypoxic MDA-MB-231 cells. Importantly, *RNA11B-AS1* depletion reduced pol II occupancy at the *VEGFA* and *ANGPTL4* promoters, which correlated with decreased gene expression in hypoxic MDA-MB-231 cells. It is possible that *RNA11B-AS1* regulates the recruitment of pol II to a subset of genes involved in hypoxia.

Of note, it is established that HIF binding also activates *VEGFA* and *ANGPTL4* transcription in hypoxic breast cells (8,9). To explore any connection to *RNA11B-AS1*, Niu et al used RNA pull down assays to show that neither HIF1 or HIF2 bound to the *RNA11B-AS1* transcript. In addition, ectopic expression of *RNA11B-AS1* had no effect on HIF transcriptional activity in reporter assays in normoxic and hypoxic cells. However, it is not clear if a reporter construct can adequately recapitulate the chromatin environment. It will be therefore be important to show that overexpression and/or depletion of *RNA11B-AS1* does not affect the recruitment of HIF to hypoxia-responsive promoters such as *VEGFA* and *ANGPTL4*.

In summary, Niu et al have characterised a hypoxia-responsive metastasis-associated lncRNA, which adds to the ever increasing list of lncRNAs involved in breast cancer progression. Mechanistically, *RNA11B-AS1* likely contributes to metastasis through pol II activation of a subset of angiogenic factors including VEGFA and ANGPTL4, suggesting it functions as an oncogene. However, a recent study provided evidence that *RNA11B-AS1* prevents osteosarcoma progression via suppression of *RAB11B*, suggesting in this context *RNA11B-AS1* acts as a tumor suppressor (10). The authors suggest that *RNA11B-AS1* may serve as a new therapeutic target for breast cancer. Therapeutic targeting of hypoxia and HIFs in cancer is a very active research area, however developing specific inhibitors has been challenging (11). Given the large number of lncRNAs reported to directly or indirectly regulate HIF expression (12), it is reasonable to assume that hypoxia-responsive lncRNA alterations may improve targeted strategies against metastatic breast cancer.

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