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 RNA-seq 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 IncRNAs reported to directly or indirectly regulate HIF expression, it is reasonable to assume that hypoxia-responsive IncRNA alterations may improve targeted strategies against metastatic breast cancer.

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Footnote

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