Editorial

Lnc-ing noncoding RNAs to Wnt signaling in colorectal cancer

Stacey L. Edwards, Juliet D. French

Cancer Division, QIMR Berghofer Medical Research Institute, Brisbane, Australia

Correspondence to: Juliet D. French. Cancer Division, QIMR Berghofer Medical Research Institute, Brisbane 4029, Australia.

Email: juliet.french@qimrberghofer.edu.au.

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Large-scale transcriptomic studies have shown that the majority of the genome is transcribed, however, only a small proportion codes for protein (1). The majority of noncoding transcripts produced are long noncoding RNAs (lncRNAs), loosely defined as transcripts >200 nucleotides that do not code for protein. Similar to mRNAs, the majority of lncRNAs are transcribed by RNA polymerase II and spliced using consensus splice sites (2). The exact proportion of noncoding transcripts that represent functional genes has been a hot topic for debate. However, there is no doubt that many of these transcripts are indeed functional, are key regulators of biological processes and act through an increasingly diverse array of mechanisms (3).

Multiple lncRNAs have now been shown to play important roles in cancer development and metastasis, including colorectal cancer (CRC). Notable examples include HOTAIR (HOX transcript antisense RNA), with high HOTAIR expression highly correlated with liver metastases and poor prognosis in CRC patients (4). Several studies have shown that HOTAIR promotes epithelial to mesenchymal transition (EMT) by directly binding the polycomb repressive complex 2 (PRC2) to reprogram PRC2 occupancy (5). Colon cancer associated transcript 1 (CCAT1) expression, a lncRNA transcribed from a super enhancer at 8q24, is associated with poor prognosis and can predict patient response to bromodomain and extra-terminal motif (BET) inhibitors (6). Another example is N-BLR, a primate-specific lncRNA, which promotes EMT by directly interacting with miR-141 and miR-200c-3p and acting as a miRNA sponge (7).

The recent study by Lin *et al.* (8), provides another example of a lncRNA involved in CRC invasion and metastasis. The authors first profiled lncRNA expression by microarray in six human CRC and normal adjacent tissue. They identified 2,545 lncRNAs differentially expressed between the CRC and normal samples, of which five were further validated by qPCR. Specifically, they showed that lncRNA BC032913 was markedly down regulated in CRC tissues, and associated with an advanced tumor stage, distant metastases and poor patient survival, suggesting that it may participate in tumor metastasis.

Lin and colleagues then investigated the function of BC032913 through a combination of *in vitro* assays (8). They found ectopic expression of BC032913 inhibited cell migration and invasion in HCT116 and DLD-1 cells. To further investigate the phenotype, the authors profiled metastasis-related genes in HCT116-control versus -BC032913 cells using a human tumor metastasis PCR array. They found BC032913 significantly increased the expression of several genes, including *TIMP3* (TIMP metallopeptidase inhibitor 3), a secreted protein that inhibits extracellular matrix remodelling (9). This result was further confirmed by Western blot in HCT116-BC03213 and DLD1-BC03213 cells that showed a concomitant increase in TIMP3 protein levels.

Several studies report that TIMP proteins can promote tumorigenesis through aberrant regulation of the Wnt/β-catenin pathway (10,11). Given the importance of Wnt/β-catenin signaling in CRC (12), the authors hypothesized that increased BC032913-TIMP3 levels may alter this key pathway. To explore this connection, Lin *et al.* used TOPflash reporter assays to show that BC0321913 overexpression significantly reduced luciferase activity, and that this effect was partially rescued by TIMP3 silencing.

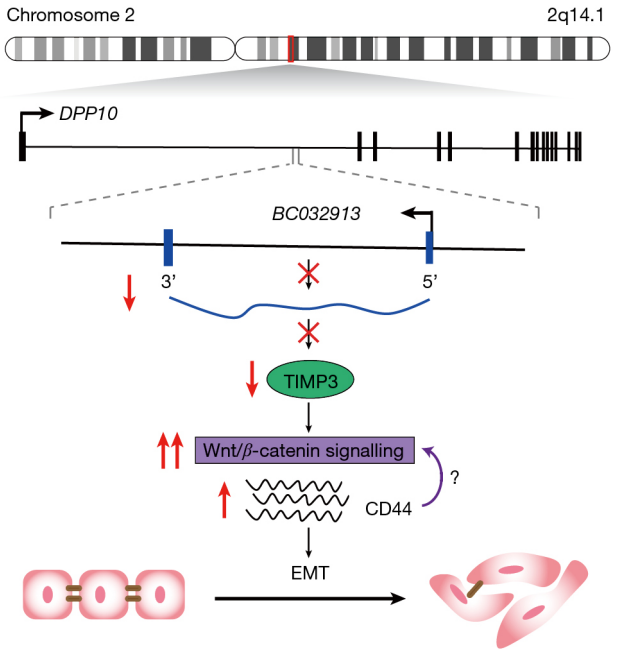

Figure 1 Proposed role of BC032913 in colorectal cancer metastasis. BC032913 (also called *DPP10-AS1*) is transcribed in the antisense direction from an intronic promoter within DPP10. Low BC032913 expression levels results in repression of TIMP3, activation of Wnt/β-catenin signaling, leading to increased CD44 (that may also provide a positive feedback loop), and promotion of EMT (epithelial-to-mesenchymal transition). DPP10, dipeptidyl peptidase like 10; EMT, epithelial to mesenchymal transition.

Western blot also revealed that BC0321913 increased E-cadherin levels, but downregulated nuclear β-catenin and CD44, which could be reversed by TIMP3 silencing. *CD44* is a downstream Wnt target gene and has previously been reported to promote EMT in CRC (13).

Finally, the authors investigated the role and potential mechanism of BC032913 on CRC metastasis *in vivo*. They subcutaneously injected HCT116-control or -BC032913 into the tail vein or spleens of nude mice and observed fewer and smaller micrometastases in the HCT116-BC032913 group. They also analysed the expression of TIMP3 and β-catenin by qPCR in metastases excised from individual mice in each group. Consistent with the *in vitro* data, the mRNA levels for TIMP3 and β-catenin were increased and decreased, respectively. While not conclusive, collectively the data presented does provide evidence that *BC032913* may directly or indirectly inhibit CRC metastasis via inactivation of the Wnt/β-catenin pathway with subsequent effects on EMT (*Figure 1*).

The exact mechanism by which *BC032913* mediates its actions is unclear. Of note, BC032913 is antisense to intron 2 of dipeptidyl peptidase like 10 (*DPP10*) and shares a bidirectional promoter with an alternative isoform of *DPP10* (*DPP10-2*). Previous studies have shown that, in a subset of neurons, *BC032913* (also known as *LOC389023*) acts as a transcriptional repressor of *DPP10-2*, likely through the recruitment of components of the PRC2 complex (14). Lin *et al.* indicated that *DPP10* mRNA levels were decreased in CRC tissue however, *DPP10* expression could not be detected in a panel of CRC cell lines making it difficult to assess the relationship between *DPP10* and BC032913. Future studies will be required to determine whether *BC032913* is chromatin associated in colorectal cells similar to what has been observed in neurons. Identifying proteins or miRNAs that interact with the lncRNA will also provide important insights into the mechanism by which BC032913 promotes cancer progression.

In summary, Lin *et al.* have characterised a novel lncRNA in CRC metastasis. This lncRNA now adds to an ever-increasing list of noncoding RNAs involved in cancer progression. The authors suggest that BC032913 may serve as a new biomarker or therapeutic target for CRC. This may prove difficult given that BC032913 is down-regulated in CRC however, targeting key regulators of BC032913 may provide an alternative avenue for boosting the levels BC032913 in CRC cells.

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Footnote

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Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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