Editorial

LncRNA *miR503HG* is a new player in hepatocellular carcinoma metastasis

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Recently, lncRNA have been found to be involved in many biological processes (1-4). They have been discovered in all of the hallmarks of cancer (5,6) and many are used as biomarkers and therapeutic targets in specific cancers. Therefore, it is not surprising that their dysregulation has been found to lead to disease and progression of diseases such as cancer (6-9). As the third-leading cause of cancer death worldwide, it is important to study the underlying mechanisms related to hepatocellular carcinoma (HCC) progression (10). Though surgery is an effective treatment option for HCC, many patients succumb to the disease due to recurrence and metastasis (11,12). This is why prognostic biomarkers are so important to the treatment of cancer. They give doctors a way to determine and select the best path of treatment and what can provide (13,14).

Recently Wang *et al.* reported that a lncRNA known as miR503HG is responsible for inhibiting metastasis in HCC through its regulation of the heterogeneous nuclear protein (hnRNP) hnRNPA2B1 mediated NF-κB pathway (15). In their assessment of a large group of HCC patient samples compared to paracancer controls, 713 lncRNA were found to be dysregulated. The authors then narrowed the search to differentially regulated lncRNA that were transcribed from host genes to microRNA and found five lncRNA that fit this criterion. Only one of these lncRNA were found to be significantly dysregulated (downregulated) in HCC, miR503HG. To support the importance of this lncRNA in HCC, a Kaplan-Meier analysis of patients was performed and indicated that miR503HG is an important factor for survival after surgery as well as decreasing the probability of recurrence after surgery. In order to understand the relationship between patient prognosis and miR503HG, Wang *et al.* selected two HCC cell lines for migration and metastasis experiments *in vitro* and *in vivo*. The two cell lines are known as SMMC-7721 and Huh7 and have decreased expression of miR503HG lncRNA, but still much lower than a normal liver cell line. These cell lines allowed for the knock down of miR503HG, which exacerbated migration and invasion severity indicating miR503HG plays a role in HCC metastasis. Overexpression of the lncRNA led to a drastic decrease in both *in vitro* and *in vivo* migration and invasion demonstrating that miR503HG is involved in the suppression of metastasis. Also of note, it was discovered that the microRNA, miR503, which is also expressed from the host gene of *miR503HG*, can work synergistically with miR503HG to mediate the suppression of cell migration and invasion. However, the two noncoding RNAs use independent pathways to suppress metastasis. This microRNA has been studied in HCC previously and it has been implicated as a player in the inhibition of proliferation and induction of apoptosis in HCC (16). This likely means that miR503 could be involved in multiple pathways to aid in the regulation of cancer progression. Since miR503HG is also transcribed from the same gene, it may also play a role in other cancer suppression pathways as well. In total, these experiments revealed the important overall biological function of miR503HG to be suppressing metastasis in HCC.

After uncovering the overall functional importance of miR503HG, Wang *et al.* investigated the mechanism

through which miR503HG acts on metastasis in HCC. First, the group wanted to identify any direct protein interactions with the lncRNA. In an RNA pull-down assay using HCC cell extracts and mass spectrometry, it was discovered that 25 proteins potentially interacted with miR503HG. It has been shown previously that lncRNA can interact with hnRNPs in signaling pathways and to effect chromatin regulation (17,18). Therefore, the hnRNP, hnRNPA2B1, was selected from among the 25 interacting proteins and further investigated. This hnRNP was confirmed as a specific binding partner for miR503HG through western blot analysis and RNA immunoprecipitation (RIP). The 493–644 nt region of miR503HG and the RRM region of hnRNPA2B1 were deemed responsible for this interaction. These regions were discovered through a series of deletion experiments that removed either highly structured portions of the lncRNA or known functional domains of the protein. Such deletion mutants have been very useful for the discovery of RNA binding domains in proteins since it seems that, especially in the case of lncRNA, sequence tends to not be critical for specific binding, but structure (19). Patient overall survival was shown to be better with low presence of the hnRNPA2B1 protein indicating an inverse relationship between miR503HG lncRNA expression and hnRNPA2B1 protein presence and potentially suppressive role for miR503HG against hnRNPA2B1. When assessing 22 HCC tissues, this relationship was confirmed. Wang *et al.* hypothesized that miR503HG is causing the destabilization of the hnRNPA2B1 protein and to test this hypothesis, performed an experiment overexpressing miR503HG and using a proteasome inhibitor, MG132, to prevent any potential degradation of hnRNPA2B1 by miR503HG through proteasomes. It was revealed that in the presence of MG132, hnRNPA2B1 protein was present at wild-type levels even though miR503HG was present in high levels. Overexpression of miR503HG also led to an increase in the ubiquitination of hnRNPA2B1 providing further evidence that miR503HG was downregulating hnRNPA2B1. These experiments demonstrated that miR503HG and hnRNPA2B1 form a complex, and through this complex miR503HG is able to trigger the ubiquitination of hnRNPA2B1 and destabilize the protein for downregulation.

Finally, Wang *et al.* chose to go one-step further and deciphered through which signaling pathway lncRNA miR503HG and hnRNPA2B1 were involved in suppressing HCC metastasis. Taking advantage of signaling pathway reporter vectors, it was discovered that when hnRNPA2B1 is knocked-down, the NF-κB pathway was the most significantly downregulated pathway. After thoroughly investigating an exhaustive set of combinations of knockdown and overexpression of miR503HG and hnRNPA2B1, it was established that miR503HG was mediating HCC cell migration through hnRNPA2B1 and the NF-κB pathway. These sets of experiments went so far as to uncover the precise mRNA that hnRNPA2B1 were acting upon (p52 and p65) and downstream genes (*c-myc*, *EZH2*, *cox2*, and *VCAM1*) that are affected when miR503HG is present. These genes are all key factors in the NF-κB pathway leading to metastasis. The mRNA of the NF-κB subunits, p52 and p65, and the downstream genes are downregulated in the presence of miR503HG. It was reasonably speculated that formation of the hnRNPA2B1-miR503HG complex prevents the attachment of hnRNPA2B1 to the mRNA of p52 and p65 causing the mRNA destabilization and inhibition of the NF-κB pathway (*Figure 1*). Regulation of mRNA through lncRNA-protein complexes have been proposed previously (18). In a study by Lan *et al.*, it was demonstrated that the lncRNA lnc-HC forms a complex with hnRNPA2B1 to bind and downregulate the mRNA of *Cyp7a1* and *Abca1*. This posttranscriptional regulation aids in the negative regulation of cholesterol metabolism in hepatocytes. This is a slightly different mechanism from the model proposed by Wang *et al.*; however, it is another example of lncRNA-mediated regulation of hnRNPA2B1. It would be also informative to see if the mRNA of p52 and p65 precipitate with the hnRNPA2B1 protein in a RIP or RNA pull-down assay.

As a whole, Wang *et al.* were able to demonstrate that miR503HG suppresses metastasis in HCC through binding to the heterogeneous nuclear ribonuclear protein hnRNPA2B1. This study provides a step-step experimental procedure to follow when studying lncRNA in cancer. Wang *et al.* conducted a comprehensive investigation starting from discovery of lncRNA dysregulation all the way to working out its mechanism of influence in HCC progression. This information can now be fully taken advantage of in the clinic to study how miR503HG can be exploited in the treatment of HCC. Another recent study by Huang *et al.* in large-cell lymphoma also investigated miR503HG lncRNA and revealed its involvement in the mechanism of proliferation in this cancer (20). This study provides even further support of the important role miR503HG plays in cancer progression not only in HCC, but in other cancers as well. The lncRNA miR503HG

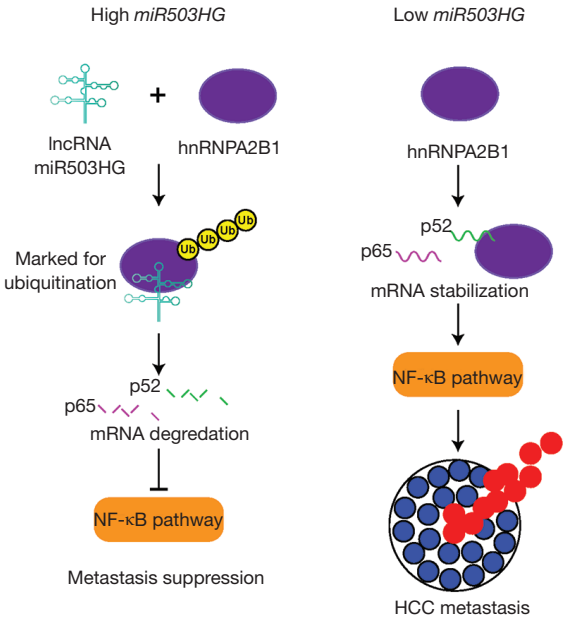

Figure 1 Metastasis suppression by lncRNA-protein complex mi503HG-hnRNPA2B1 in HCC. The presence of lncRNA miR503HG and its binding with hnRNPA2B1 marked the lncRNA-protein complex for ubiquitination. This destabilization of hnRNPA2B1 prevents the protein from participating in the metastasis pathway in which it would stabilize *p52* and *p65* mRNA. Instead, these mRNA are degraded and prevented from a functional role in the NF-κB pathway for activating metastasis in HCC. HCC, hepatocellular carcinoma. (Illustration based on conclusions in Wang *et al.* 2018).

potentially could be involved in the metastasis of other cancers through the same mechanism presented by Wang *et al.* and should be investigated. It would also be prudent to study whether miR503HG can predict the effectiveness of certain treatment modalities in patients. Since Wang *et al.* already demonstrated its usefulness as a biomarker to predict probability of recurrence after surgery and overall survival (15). Therefore, there is a good chance that it may be a helpful predictor for other treatments such as sorafenib, lenvatinib, and regorafenib (12,14).

This comprehensive study by Wang *et al.* is a strong case for the involvement of miR503HG in HCC metastasis and for its use as a biomarker for overall survival and recurrence. It has also provided a platform for how lncRNA function can be investigated. Further studies of miR503HG and other lncRNA mechanisms in cancer can help to provide a better picture of cancer genetics. With a better understanding of cancer genetics, treatments that can take advantage of lncRNA mechanisms can be developed.

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