



Commentary

Biological and Clinical Relevance of H19 in Colorectal Cancer Patients



Heidi Schwarzenbach

Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany

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Numerous studies have reported H19, the first discovered long non-coding RNA (lncRNA), to have key regulatory functions in tumor development and progression (Brannan et al., 1990). This paternally imprinted gene is located close to the telomeric region of chromosome 11p15.5 that frequently harbors genetic alterations in tumors. The activities of H19 are diverse, and include genetic and epigenetic mechanisms. Despite the recent disputes and discrepancies showing the involvement of H19 in opposed processes, e.g., in cell proliferation and differentiation processes, as well as in epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET), emerging evidence supports its oncogenic properties (Raveh et al., 2015). The association of H19 with tumorigenesis and invasion is partially owed to its regulation of carcinogenic miR-675. H19 is the precursor-RNA of this microRNA (miRNA) that is located in the first exon of the H19 gene (Tsang et al., 2010). It is actually demanding to ascribe the oncogenic functions of both molecules either to H19 or to miR-675. Tsang et al. showed that both, H19 and miR-675 were upregulated in human colorectal cancer (CRC) cell lines and primary tissues, but H19-derived miR-675 regulated CRC development by downregulating the tumor suppressor retinoblastoma 1 (RB1). The fact that RB1 is a direct target of miR-675 was demonstrated using a luciferase assay; miR-675 suppressed the activity of the reporter plasmid carrying the 3'-untranslated region (3'UTR) of RB1 mRNA with the miR-675 binding site (Tsang et al., 2010). In this case, the data emphasize that H19 functions are performed by miR-675. However, Calin and coworkers (Ohtsuka et al., 2016–in this issue) found a direct interaction of H19, without the involvement of miR-675, with RB1 protein, using RNA immunoprecipitation experiments. This excellent study also showed that H19 silencing dramatically reduced RB1 phosphorylation by reducing both RNA and protein expression of cyclin-dependent kinase (CDK) 4 and cyclin D1 (two essential upstream factors controlling RB1 phosphorylation). Thus, their findings indicate that H19 regulates G1-S transition in RB1/E2F

signaling to control cell proliferation. They also investigated miR-675 (both miR-675-5p and miR-675-3p) expression in clinical samples. MiR-675-5p was undetectable in TCGA samples, while miR-675-3p expression correlated with H19 levels, but was no predictor either for overall or disease-free survival. At least in their model system that Calin and coworkers used, H19 effect could hardly be attributed to miR-675 function (Ohtsuka et al., 2016–in this issue).

Moreover, Calin and coworkers also showed that H19 is able to regulate β -catenin activity via modulating CDK8 expression (Ohtsuka et al., 2016–in this issue). In CRC, CDK8 is an essential oncogenic driver with multiple functions. Together with its partners cyclin C, MED12, and MED13, CDK8 forms a subcomplex that controls mediator-polymerase II interaction to initiate gene transcription (Allen and Taatjes, 2015). Thus, as a part of the transcription machinery, CDK8 plays a crucial role in regulating gene expression. Accordingly, the findings by Calin and coworkers (Ohtsuka et al., 2016–in this issue) show, that apart from the detection of the H19 action on CDK8/ β -catenin, as well as on RB1/E2F signaling, H19 may be involved in further regulation networks that control CRC malignancies.

Compared to previous studies (Raveh et al., 2015), the strength of the study by Calin and coworkers lies in the detailed investigation on the involvement of H19 in RB1/E2F and CDK8/ β -catenin signaling pathways using effectively combined bioinformatics (TCGA database) and in vitro experimental models (Ohtsuka et al., 2016–in this issue). It is also of note that H19 was reported to affect the Wnt/ β -catenin signaling pathway (Wang et al., 2016), and additionally, to be involved in the repression of E-Cadherin (CDH1) (Luo et al., 2013). In this regard Luo et al. demonstrated that H19 repressed CDH1 expression via interaction with the member of the polycomb group of proteins EZH2 in bladder cancer (Luo et al., 2013), whereas Calin and coworkers suggested another mechanism in CRC by revealing that CDH1 as well as CSRP2 are direct H19 targets, and thus, identified H19 as the most significant lncRNA associated with CRC patient survival (Ohtsuka et al., 2016–in this issue).

In recent years, elucidating the role of lncRNAs in tumors has become a hot research topic. Their potential applications in the clinical diagnosis and treatment of cancer are promising. Knowledge of aberrant signal transduction pathways underlying tumor progression, metastasis and drug resistance have provoked development of targeted therapies which inhibit oncogenic signals. However, the potential mechanisms of how lncRNAs affect signaling pathways remain largely undefined, and most of lncRNA functions remain unclear. The excellent study by Calin and coworkers (Ohtsuka et al., 2016–in this issue) may contribute to advancing our knowledge on lncRNAs, in particular for H19 in CRC. In

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view of its intrinsic role in cancer and its function in regulating a series of cell-cycle genes and signaling pathways (Raveh et al., 2015; Ohtsuka et al., 2016—in this issue; Han et al., 2016), H19 should be considered more profoundly as a therapeutic target and a cancer marker in the fight against cancer. Accordingly, the detailed investigations by Calin and coworkers could help H19 to become a targeted molecule for precision and personalized medicine. To achieve a more effective antitumor therapeutic approach, future lncRNA-based methods are anticipated to improve the treatment and prognosis of CRC. However, it should be kept in mind that a signaling pathway can be affected by several lncRNAs or miRNAs, and that a single lncRNA and miRNA can affect several pathways. Thus, it is possible that silencing of only H19 cannot stop CRC tumorigenesis and progression. Therefore, prior to starting targeted therapy with lncRNAs, their multiple behaviors should be investigated. Their expression patterns in the temporal and local context also need to be taken into contextual evaluation. Finally, the recent doubts on the oncogenic properties of H19 should be eliminated, to definitely demonstrate whether H19 as targeted molecules may be beneficial in CRC patients.

Besides, increasing evidence has indicated that lncRNAs and miRNAs can regulate each other and affect their activity and function. On the one hand, lncRNAs may be the precursor of miRNAs, whereas on the other hand, lncRNAs may be the targets of miRNAs. Accordingly, Salmena et al. (2011) introduced the competing endogenous RNA (ceRNA) hypothesis, and suggested that miRNA response elements (MREs) can build a bridge for the communication among various types of RNAs. Acting as ceRNAs, lncRNAs may regulate posttranscriptionally the levels of miRNA targets. Notably, an example for these cross talks between lncRNAs and miRNAs is H19 and miR-675, that interaction should further be analyzed.

So far, H19 has not been measured in the blood circulation of CRC patients. To date, quantifications of circulating H19 have been carried particularly in plasma of gastric and breast cancer patients, and its role as a potential non-invasive diagnostic biomarker has been demonstrated in both cancer types (Hashad et al., 2016; Zhang et al., 2016). Considering that during chemotherapy no or little tumor tissue is available, the possibility of monitoring the changes in circulating nucleic acid profiles induced by treatment becomes increasingly important. Therefore, it would also be interesting to perform such non-invasive analyses in CRC patients and to examine whether plasma H19 can serve as a potential “liquid biopsy” biomarker for CRC diagnosis or prognosis. Additionally, the quantification of H19 levels in the blood circulation of CRC

patients could also help to monitor their response to therapy, and identify those patients who can benefit from adjuvant chemotherapy.

In conclusion, Calin and coworkers (Ohtsuka et al., 2016—in this issue) have impressively analyzed and described the complex molecular mechanisms of H19 in CRC. Although the diverse functions of H19 need to be further clarified, as well as its downstream targets, the role of H19 in RB1/E2F and CDK8/ β -catenin signaling may not only help us to elucidate the pathogenesis of CRC, but also offer H19 as promising target molecule for lncRNA-based clinical strategies.

Disclosure

The author declares no conflict of interest.

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