Emerging role of lncRNA in cancer: a potential avenue in molecular medicine

Nidhi Jariwala[1], Devanand Sarkar[1,2,3]

[1]Department of Human and Molecular Genetics, [2]Massey Cancer Center, [3]VCU Institute of Molecular Medicine (VIMM), Virginia Commonwealth University, Richmond, VA 23298, USA

Correspondence to: Devanand Sarkar. Department of Human and Molecular Genetics, Virginia Commonwealth University, 1220 East Broad St, PO Box 980035, Richmond, VA 23298, USA. Email: devanand.sarkar@vcuhealth.org.

Abstract: Hepatocellular carcinoma (HCC) accounts for the second largest number of cancer related deaths globally with limited management options for the advanced disease. Although substantial research has identified molecular targets, with strong validation in pre-clinical *in vivo* studies, translation of therapeutics to clinics has shown modest success. In a recent manuscript in *Hepatology*, Zhou and Yang *et al.* unravel a novel p53 associated long non-coding RNA (PRAL) as a potential prognostic marker and molecular target in HCC. Their work provides a promising approach at capitalizing the tumor suppressive role of p53 protein in fighting HCC. More importantly, it emphasizes the evolving significance of long non-coding RNAs (lncRNA) in molecular medicine. Current research trends focus on identifying and understanding roles of lncRNA in regulation of gene expression relevant to multiple disease pathophysiologies thereby presenting a new avenue of research in molecular and translational medicine.

Keywords: Long non-coding RNA (lncRNA); p53 associated long non-coding RNA (PRAL); p53; hepatocellular carcinoma (HCC); biomarker

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Hepatocellular carcinoma (HCC): current scenario

HCC is a highly aggressive form of primary liver malignancy arising in hepatocytes. Most cases of HCC occur secondary to a pre-existing liver condition. A range of primary conditions are known to lead to this aggressive neoplasm, including viral hepatitis, cirrhosis (alcoholic or non-alcoholic) and fatty liver disease (1,2). A primary pathological event causes hepatocyte injury, which initiates a cascade of inflammatory events and eventually progresses into neoplastic transformation (1). Diversity in primary conditions that predispose patients to HCC has resulted in a highly variable and evolving form of cancer, proven to be very challenging to curtail with current management options. Surgical resection of tumor, percutaneous ethanol injections and chemoembolization are some of the common options presented to HCC patients, subjective to the health of organ and inflammation grade. Sorafenib, a multikinase inhibitor targeting VEGF and RAF/MEK/MAP signaling, is the only FDA approved molecular therapeutic in clinical use. However, clinical trials with sorafenib in combination with available management options show only moderate increase in overall survival of patients (3-6). SHARP trial demonstrated an average of two months increase in overall survival and 3 months increase in radiologic disease progression in HCC patients receiving sorafenib in comparison to the placebo group (3). Similar phase II and phase III trials have shown increase in overall survival period up to 9 months and approximately 4 months to disease progression (5,6). Sorafenib also failed to show clinically significant results in combination with transarterial chemoembolization (4). Thus, there is heavy emphasis on identifying and developing high efficacy targeted therapeutics for HCC.

Emerging evidence on the role of long non-coding RNAs (lncRNAs) in cancer

Chromosomal instability (CIN), also described as somatic copy number variations (SCNVs) or DNA copy number alterations (CNAs), has long been established as a crucial hallmark of cancer genome and cancer evolution (7). High level of heterogeneity is observed in CIN within cancer types, although a substantially higher rate of CIN is observed in epithelial cancers in comparison to hematologic cancers (7). Recent studies indicate association of CIN with disease prognosis and management as well as therapy resistance (7). Genome sequencing and targeted exome capture sequencing have provided substantial evidence of increased DNA alterations in human cancers (8-10).

Though identification of unstable foci has led to identification of novel oncogenic drivers, it has been noted that a vast majority of copy number variations have been observed to lie within the intergenic regions of human genome. Little was known about the role of intergenic DNA in cellular transcriptome until recent studies identifying lncRNA. These RNA elements, initially identified as non-protein coding nucleic acids, are now associated with regulation of expression of several genes, with emphasis in tumor biology.

From poor prognosis to promoting hallmarks of cancer, lncRNAs have been shown to play a versatile role in carcinogenesis making them a potentially high efficacy therapeutic target. While some are oncogenic in function, driving angiogenesis, invasion, metastasis and proliferation, others are tumor suppressive promoting cellular apoptosis and chemo-sensitivity. Metastasis associated lung adenocarcinoma transcript (MALAT-1) and HOTAIR are the most extensively studied lncRNA in association with cancer phenotype, till date. MALAT-1 is overexpressed in pancreatic, prostate as well as triple negative breast cancers, affecting cell proliferation and apoptosis (11-14). In HCC, overexpression of MALAT-1 is associated with poor prognosis and tumor recurrence in patients (14,15). Anti-sense oligonucleotides targeting MALAT-1 have shown promising results, both *in vitro* as well as *in vivo*. lncRNA HOTAIR is identified as an oncogene in breast, gastric, colorectal and ovarian cancers, HCC, and non-small cell as well as small cell lung cancer (16-21). It is often linked with tumor recurrence and chemo-resistance as in non-small cell lung cancer and ovarian cancer (20,21). Serum levels of HOTAIR have been validated as a potential biomarker for colorectal cancer (22). It is reported to promote tumorigenesis in small cell lung cancer by primarily affecting proliferation, migration and invasion (19). lncRNAs can modulate expression of microRNAs as seen in HCC wherein HOTAIR promotes proliferation via regulating expression of miR-218 (18).

Several molecular studies have been carried out investigating the role of lncRNAs as potential therapeutic targets in carcinogenesis. Common observations from these studies indicate that lncRNAs play an important role in modulating expression of genes thus far identified to play a crucial role in tumor development, progression and therapy resistance. Subsequent studies employing lncRNAs as therapeutic targets show promising results in animal models, although number of such studies is low. It would be naïve to claim relevance of lncRNAs in molecular medicine without supporting clinical data and further validation in orthologous models. Thus, these oligonucleotides are making a mark not only as potential targets but also as high efficacy tools for disease diagnosis and progression.

Role of lncRNAs in liver pathology

HCC is a highly aggressive disease and the third leading cause of cancer related mortality worldwide (23). Five-year survival rates of patients diagnosed with HCC are bleak, with 28% for localized disease, 7% for regional disease and mere 2% for distant metastasized disease (24). As such there is an ever-increasing demand to diversify the approaches employed at targeting this disease. lncRNAs have proven to be promising in understanding and identifying a key regulatory element that can be employed as a molecular target to achieve a combined block of multiple signaling cascades. Wang *et al*. performed a genome wide analysis to map frequent CNAs in HCC tumor tissue (10). Their research developed a composite approach aimed at identifying key oncogenic drivers, based on identification of the most frequently altered chromosomal regions. This approach promises to not only identify novel genes present in those genomic areas but also recognize some well-studied genes with available therapies. They present an overview of the pathways affected by major CNAs enriched in genome-wide analysis, which includes the critical PI3K signaling pathway, TGFβ signaling, MAPK as well as Wnt signaling pathways (10). More recent efforts have helped elucidate the role of lncRNAs in liver pathology. Expression of ICAM-1, shown to be associated with occurrence of portal vein tumor thrombus, is positively regulated by ICAM-1 related lncRNA (ICR) (25). It was shown to positively

correlate with ICAM-1 expression and maintain the stemness of ICAM-1 positive liver cancer stem cells (25). Relative expression of ICR also correlated with portal vein tumor thrombus (25). Similarly, expression of lncRNA down regulated in liver cancer stem cells (DILC) inversely correlated with early tumor recurrence and shorter disease free survival (26). DILC expression negatively correlated with that of EpCAM and CD24, markers for cancer stemness. DILC, thus acts as a tumor suppressor lncRNA for HCC, as it inhibits STAT-3 activating IL-6 signaling and prohibits liver cancer stem cell expansion. Linc00152 can activate mTORC signaling via its interaction with EpCAM (26). Plasma levels of lncRNAs have also been proposed at biomarkers for HCC disease, metastasis and recurrence.

Apart from carcinogenic activity, lncRNAs also modulate other functions of liver. Highly upregulated lncRNA of liver cancer (HULC) increases expression of transcription factor PPARA by inhibiting mir-9 activity (27). This induction in PPARA results in increased accumulation of intracellular triglycerides and cholesterol. Elevated cholesterol level causes upregulation of HULC expression via positive feedback loop involving RXRA, thus resulting in abnormal lipogenesis (27). Thus, increasing numbers of molecular studies are accentuating the role of lncRNAs in liver homeostasis and present a wide avenue for translational research.

Clinical evidence on p53 targeting in cancer

p53 has been investigated in-depth at a molecular level since its discovery in late nineties (28). Its potential as a tumor suppressor is promising in molecular and *in vivo* studies employing animal models. However, clinical trials employing adenoviral delivery of p53 in cancer patients have exhibited only modest results. Vaccine therapy in metastatic breast cancer patients increased disease free survival by a meager ~6 weeks (29). Similar approach in lung cancer patient population showed a promising 1 year disease free survival, however, the sample size was too small to establish statistical significance (29). T-cell receptor (TCR) gene engineered lymphocytes transduced with p53 were employed as a therapeutic in metastatic cancer patients (29). This phase II clinical trial failed as all patients manifested adverse events described by low bone marrow/blood leucocyte, lymphocyte and platelet count, while only 10% of the recruited patients manifesting tumor regression (29). In an independent attempt at employing p53 in clinical research, patients with metastatic renal cell carcinoma or melanoma were treated with p53 transduced TCR gene engineered lymphoblasts in combination with IL-2 (29). None of the patients manifested disease regression and were reported with adverse events. There are some on-going clinical studies combining p53 gene therapy with trans-catheter arterial embolization or trans-catheter chemo embolization in patients with advanced HCC (29). Meanwhile, efforts are channeled to identify novel approaches, utilizing tumor suppressive properties of p53 in targeting HCC.

p53 associated long non-coding RNA (PRAL) as a potential therapeutic in HCC

Identification of lncRNA regulating p53 function shows a promising potential in molecular targeting of HCC via restoration of p53 activity (30). The authors present a composite study involving identification of PRAL, elucidating its structure, delineating its molecular function and providing evidence for its tumor suppressive response *in vivo*. Validation of PRAL copy number and expression in human HCC cohorts along with the molecular observations make significant evidence in favor of PRAL's important role in cellular proliferation and apoptosis in HCC. With a significant proportion of literature proving role of p53 in regulation of cell cycle and cell proliferation, this data only underlines the specificity of function of PRAL. Authors present a detailed overview about structure and function of PRAL, also deducing the molecular mechanism. This study confirmed that PRAL binds to Heat shock protein 90 (Hsp90) via a 1,180 nucleotide stem structure (30). This interaction was validated *in vitro* and found to correlate with increased p53 pro-apoptotic activity, while the p53 expression remained unaltered (30). It is hypothesized that PRAL interaction with Hsp90 augments its interaction with p53; thereby decreasing the rate of MDM2 induced p53 ubiquitination (30). Although it is exciting to observe significant reduction in tumor growth in xenograft model, it remains unobserved how this therapeutic approach might prove in an orthotopic model or a syngeneic animal model.

Importantly, the authors report that low genomic copy number of PRAL is also concurrent with disease free survival in HCC patients, making this lncRNA a potential prognostic marker. Overall, this study presents promising findings, coupled with significant literature on role of p53 in HCC, giving substantial confidence to subsequent observation in clinical and translational research.

Clinical research has just ventured into exploring role of lncRNA as therapeutic agents. There is an on-going trial, validating role of specific lncRNA as prognostic marker for triple negative breast cancer as well as cardiovascular morbidities in chronic renal disease (29). In such scenario, potential of PRAL in molecular medicine seems encouraging. However, one caveat to this approach is that PRAL will only work in a WT p53 state and considering the fact that p53 mutation is the most common molecular abnormality in HCC, the therapeutic utility of PRAL might be limited. Liver is an ideal organ for oligonucleotide-based therapies, given the high efficiency at portal absorption of a therapeutic via the hepatic artery (31). Being the largest internal organ, and crucially involved in metabolism, it is predisposed to absorb therapeutics with higher efficacy (31). It is also involved in secretion of several metabolites into blood that play critical role in molecular signaling. Thus, a therapeutic specifically targeting single molecule is expected to yield higher efficiency when targeted towards liver pathology and must be pursued enthusiastically.

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Footnote

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