Focal loss of long non-coding RNA-PRAL, as determinant of cell function and phenotype of hepatocellular carcinoma

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Submitted Mar 14, 2016. Accepted for publication Mar 22, 2016.

doi: 10.21037/atm.2016.03.47

View this article at: http://dx.doi.org/10.21037/atm.2016.03.47

Hepatocarcinogenesis is a long process characterized by the progressive development of preneoplastic and neoplastic lesions, and the acquisition of multiple genetic and epigenetic events contributing to the biochemical and molecular heterogeneity of the disease (1,2). Hepatocellular carcinoma (HCC) constitutes the second/third cause of cancer-related deaths in the world (3). Curative strategies of HCC, such as liver transplantation, radiofrequency ablation, alcoholization, or sorafenib (a multikinase inhibitor) are available (4). Unfortunately, the majority of patients are not candidates for these therapies, due to the delay of HCC diagnosis. In addition, the survival benefit of sorafenib is still modest. Therefore, there is an urgent need to develop molecular tools and guarantee patients stratification on the basis of the molecular and clinical features, and to identify new strategies to prevent relapse and prolong patient survival (5). Although significant progress has been made in the knowledge of HCC pathogenesis, the information on molecular mechanisms underlying HCC development and progression is still incomplete (1,2).

Copy number changes, in a part or entire chromosomes, or of specific genes can have a dramatic impact on the fitness of an organism (6). Cancer is an excellent example of amplifications and deletions driving disease (7). Chromosome rearrangements are a hallmark of most solid tumors. Cytogenetic studies allowed the identification of Rb suppressor gene as a consequence of chromosome deletion del[13] (q14) in retinoblastoma, and the translocation t(8;14) of the proto-oncogene c-myc in human Burkett's lymphoma (8). Additionally, cytogenetic studies associated to molecular analyses of recurring chromosome changes has greatly improved the identification of oncogenes and tumor suppressor genes that could play a critical role in tumor development.

Genes located in chromosomal regions frequently amplified or deleted are often not expressed, either in normal or tumors tissues, making unclear the functional role of these alterations. The identification of chromosomal regions containing DNA copy number or specific genes alterations, consequently associated to transcriptional deregulation, might offer a promising strategy to identify driver genes accounting for cancers aggressivity and/or functioning as biomarkers (9). Second generation sequencing, and comparative genomic hybridization array allow the characterization of somatic copy number alterations/variations (SCNA/SCNV) in cancer samples (10,11).

In addition to small regulatory RNAs (e.g., microRNA and siRNA), mammalian genome transcribes other non-coding RNAs as long non-coding RNA (lncRNA) (12). LncRNA have been implicated in embryogenesis, gene dosage compensation, invasiveness, metastasis, and other biological processes (13). LncRNA regulate gene expression serving as repressor or activator of transcription process, lncRNA-p21 acts as repressor of p53. LncRNA are recently associated to a variety of diseases, for example neurodegenerative diseases and cancers (14,15).

Recently Dr. Zhou and collaborators published an interesting paper in which they consider the opportunity to evaluate the outcome of HCC based on the loss of the lncRNA-PRAL, a p53 regulation associated lncRNA (16).

SCNA may lead to the identification of new cancer-causing genes suggesting specific therapeutic approaches. Using the genome-wide chromosomal copy number analysis, it was found that more than 80% of pancreatic intraepithelial neoplasms and pancreatic intraductular papillary mucinous neoplasms, from patients with a familial

history of pancreatic cancer, do not show detectable SCNA. Approximately 95% of familial pancreatic precancerous lesions harbored K-RAS codon 12/13 mutations. However, a small percentage of pancreatic preneoplastic lesions showed SCNA and, in some samples, the SCNA preceded the K-RAS mutations (17).

Zhou and Coworkers underline the consistent challenge to identify oncogenes and tumor suppressor genes targeted by SCNA and to elucidate SCNA functions affecting HCC phenotype. They refer to several sophisticated methodologies, cytogenetic studies, array-based profiling and the more recently targeted exome capture, as tools to identify recurrent SCNA associated to HCC. They emphasize, however, the difficulty to link directly SCNA (localized often in intergenic regions) to proteins content and/or function.

The authors suggest the possible role of lncRNA as tumor suppressors or oncogenes drivers, involved in HCC and other cancers development. The observations that lncRNA are located in genomic fragile sites or in genomic abnormal regions, associated to cancer phenotype, strongly supports Zhou and Coworkers hypothesis. To better understanding cancer pathogenesis, the authors focus their attention to the link of SCNA and lncRNA. Zhou and Coworkers performed a data mining process on published data (GSE38323), and evaluated the frequency of DNA amplifications or deletions on HCCs samples and matched non-tumor liver tissues. By integrating SCNA profiles with lncRNA expression signatures, 11 lncRNA within SCNA regions, up to 73 lncRNA previously isolated, were identified by the authors. Among the 11 lncRNA, lncRNA-PRAL was significantly underexpressed and recurrently deleted in HCC. The genomic lncRNA-PRAL alteration was highly correlated with poor prognosis of HCC bearing patients. Markedly, lncRNA-PRAL exhibited greatest reductions of both DNA copy number and RNA transcript levels, and Kaplan-Meier analysis demonstrated that the low genomic level of lncRNA-PRAL in HCC was significantly correlated with reduced tumor-free survival and overall survival of HCC bearing patients. With respect to the etiology, the presence of SCNA and genome instability was significantly more remarkable in HBV- than in HCV- or alcohol-related HCC. In all these HCC subgroups, more than 43% of SCNA were located in human genome intergenic regions.

By functional experiments, Zhou and Coworkers show that lncRNA-PRAL was localized into both cytoplasmic and nuclear compartments and that its expression was significantly lower in several hepatoma cell lines compared to immortalized hepatocytes. LncRNA-PRAL knock-down by siRNA, led to increase in cell proliferation of hepatoma cell lines and to lower apoptosis, compared to control cells. In contrast, HCCLME and SMMC-7721 cells, forced to overexpress lncRNA-PRAL, showed lower proliferation and higher apoptosis.

The potential biological therapeutic relevance of lncRNA-PRAL was evaluated by delivering adenovirus vector-lncRNA-PRAL (AV-PRAL) in nude mice, pre-injected with human HCC cell lines. This treatment induced a significant inhibition of tumor growth. This experiment supports the suggestion that AV-PRAL may have considerable potential as HCC gene therapy.

The molecular basis linking lncRNA-PRAL deletion with HCC poor prognosis is explained by Zhou and coworkers showing that lncRNA-PRAL enhances p53 stability, *in vitro* and *in vivo*, favoring the formation of HSP90-p53 complex and apoptosis, and inhibiting MDM2-dependent p53 ubiquitination and degradation. In addition, lncRNA-PRAL directly binds to HSP-90.

The experiments published by Zhou and coworkers, clearly demonstrate that lncRNA-PRAL down-regulation may be responsible for p53 inactivation in p53-wild type HCC. Indeed, the Authors state that apoptosis is absent in p53-deficient (Hep3B) or p53-mutant (Huh7) cells cultured in presence of lncRNA-PRAL.

It must be noted, however, that HSP90 is a chaperone molecule for over 100 client proteins, several of which are involved in signaling pathways (18), and contributes to modify chromatin conformation and to the expression of numerous genes. HSP90 favors also the stability and function of HMGA2 (19), a non-histone protein acting as a transcriptional regulating factor. Zhou and coworkers suggest a more complex role of HSP90 in modulating cancer development and progression, beyond to be a simple apoptosis inhibitor. These considerations are in line with the proposal that HSP90-associated lncRNAs may provide new and ideal cancer therapeutic tools (19).

The results and conclusions published by Zhou and coworkers on *Hepatology* paper (16) may help planning future actions in the fight against liver cancer, either through the detection of early cancer lesions or improving diagnosis and therapy.

Acknowledgements

None.

Footnote

Provenance: This is a Guest Commentary commissioned by Guest Editor Haitao Zhao, MD, PhD (Department of Liver Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China).
Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Zhou CC, Yang F, Yuan SX, *et al.* Systemic genome screening identifies the outcome associated focal loss of long noncoding RNA PRAL in hepatocellular carcinoma. Hepatology 2016;63:850-63.

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Cite this article as: Feo F, Simile MM, Pascale RM. Focal loss of long non-coding RNA-PRAL, as determinant of cell function and phenotype of hepatocellular carcinoma. Ann Transl Med 2016;4(9):183. doi: 10.21037/atm.2016.03.47

