

# Cancer stem cell-associated microRNAs: searching for markers and targets in hepatocellular carcinoma

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Rational design of treatments with increasing selectivity for tumor cells has been the goal of cancer research for many decades. Advances in the knowledge of cell composition, function and regulation have been rapidly applied in the search for differences between normal and cancer cells that could provide new targets for treatment or diagnosis. Scientific and technological innovations are now opening unprecedented opportunities in this field.

The concept of cancer stem cells (CSC) has changed the way many scientist address this issue (1). Instead of studying differences between normal and tumoral tissues, the most relevant comparisons are now established between normal and CSC. The traditional stochastic model of cancer assumed that virtually all cancer cells have the capacity to sustain tumor growth, and explained the heterogeneity of cancer cells as a reflection of the genetic instability coupled with the selective pressure imposed by the host. In contrast, the CSC hypothesis proposes a hierarchical organization resembling normal tissues. According to this model, only a subset of phenotypically identifiable cancer cells (CSC) is able to sustain unlimited proliferation through asymmetrical division, resulting in self-renewal plus a different cell lineage that undergoes partial differentiation before becoming mitotically inactive. This concept, initially described in hematopoietic malignancies, was then applied to breast cancer (2), and now CSCs have been described in virtually all solid tumors, including hepatocellular carcinoma (HCC) (3). In practical terms, a dogmatic view of the stochastic and hierarchical models of cancer is not useful, because both concepts are important for the understanding of tumor biology (4). It is now clear that not all the cells have the same tumorigenic potential in a

specific moment, but the extraordinary plasticity of cancer cells can blur this hierarchy (5), and the CSC phenotype could be considered a functional state in response to stimuli from the microenvironment, rather than a lineage attribute. In agreement with this idea, the amazing field of cell reprogramming has recently illustrated the capacity of somatic cells to acquire pluripotency.

As their normal counterparts, CSCs are very resilient cells, equipped with efficient detoxifying and drug efflux systems, as well as mechanisms that protect them from pro-apoptotic stimuli and oxidative stress (1). The unlimited proliferation capacity and the resistance to conventional radio/chemotherapies point to CSCs as the main responsible for tumor relapse and metastasis formation. Therefore, identification of characteristics associated with CSC properties is crucial not only for the design of targeted therapies, but also for the development of new diagnostic and stratification algorithms to guide cancer treatment. The abundance of CSCs in tumors, based on molecular profiling, has been associated with bad prognosis and risk of relapse in a variety of cancers, including HCC (6). In addition, microarray-derived gene-expression signatures from stem cells can be used to identify single biomarkers that can be detected by clinically validated immunohistochemistry techniques, as recently described for the transcription factor CDX2 in colorectal cancer (7).

In the case of HCC, several markers have been proposed to identify CSCs, including epithelial cell adhesion molecule (EpCAM), CD13, CD24, CD47, CD90 and CD133 (reviewed in reference 8) (8). Although combinations of these markers could aid in the discrimination of normal versus cancer SCs for diagnostic purposes, definition of

therapeutic targets requires the discovery of unique features in CSC that are not shared by normal SCs or differentiated tissues. The chances of finding a particular protein or a cellular function with such specificity seem to be scarce, taking into account the similarity between physiological and carcinogenic self-renewal pathways. Encouraged by the fast development of high-throughput technologies, researchers are addressing this challenge by scrutinizing the vast diversity of cellular non-coding RNAs.

The first transcriptome analyses, carried out a decade ago, led to the amazing discovery that most of the genome is transcribed to express noncoding RNAs (9). A particularly abundant family among the non-coding RNA genes that populate the genome is involved in gene expression regulation. Regulatory non-coding genes have been divided into those that express transcripts longer than 200 nucleotides (long non-coding RNAs, lncRNAs) and those that result in short RNA molecules called microRNAs (miRs). While lncRNAs have multiple functions, the major role of miRs is to guide the RNA interference machinery to target transcripts. This results in decreased stability and reduced translation of the target gene. Interestingly, the non-coding transcriptome expressed in the cell is particularly involved in the fine tuning of cellular processes and provides new opportunities to define cell-specific patterns of expression.

In a recent issue of *Hepatology* (10), Ji and coworkers used small RNA deep sequencing to compare the miR transcriptome of EpCAM<sup>+</sup> (putative CSC) and EpCAM<sup>-</sup> HCC cells from the same patients, and then contrasted the data with normal EpCAM<sup>+</sup> hepatic SCs isolated from fetal livers and adult liver donors. Analysis of the results showed expression of 600 known miRs with a median of reads higher than 3. As many as 99 out of the 600 miRs were differentially expressed more than 2-fold between EpCAM<sup>+</sup> and EpCAM<sup>-</sup> HCC cells. The authors selected those showing drastic changes between EpCAM<sup>+</sup> and EpCAM<sup>-</sup> cells (more than 5-fold) which were not changed in normal SCs compared to hepatocytes (less than 2-fold). Among those, miR-155, miR-150 and miR-223 seemed especially relevant: they were significantly upregulated in HCCs from patients with high levels of the AFP marker and EpCAM, which correspond to patients with short survival and HCCs with strong metastatic features. Furthermore, they found a signature of 511 transcripts whose expression significantly correlated with the expression of miR-155, miR-150 and miR-223. This signature was able to discriminate EpCAM<sup>+</sup> AFP<sup>+</sup> HCCs from EpCAM<sup>-</sup> AFP<sup>-</sup> HCCs and predicted

overall survival and time to recurrence.

miR-155 was chosen for further analysis based on the strong and specific expression in EpCAM<sup>+</sup> AFP<sup>+</sup> HCCs compared with EpCAM<sup>-</sup> AFP<sup>-</sup> HCCs, adult and fetal livers, and normal hepatic stem cells. miR-155 is not the first miRNA marker described in HCC CSCs. Several authors have shown that HCC CSCs have decreased levels of miR148a, miR-142-3p, miR-150, miR-145, miR-612, miR-200a and miR-200c and increased levels of the miR-181 family and miR-21 (11-13). What seems unique for miR-155 is the exquisite specificity of expression in HCC CSCs compared to hepatocytes and normal hepatic stem cells. Similar to the other miRs deregulated in CSCs from HCC, miR-155 could be not only a marker but a driver for HCC. Supporting this possibility, silencing of miR-155 resulted in decreased levels of EpCAM<sup>+</sup> cells and inhibition of malignant features such as migration, invasion, spheroid formation or colony formation. Finally, 27 transcripts predicted to be regulated by miR-155 and downregulated in EpCAM<sup>+</sup> AFP<sup>+</sup> HCCs compared to EpCAM<sup>-</sup> AFP<sup>-</sup> HCCs served to build a signature that discriminates survival and time to recurrence in patients.

The oncogenic functions of miR-155 were described before the discovery of miRs. At that time it was identified as an oncogene called B-cell integration cluster (BIC) which induced B-cell leucosis in chickens (14). Further work demonstrated that transgenic mice overexpressing miR-155 developed lymphomas, and clinical studies found an association between miR-155 expression and bad prognosis in several human malignancies (15,16).

The findings now described by Ji and coworkers (10) pave the way for new therapies targeting HCC CSCs. As miR-155 is simultaneously a marker of CSCs and a regulatory factor, it can be used to develop new gene therapy approaches against HCC. For instance, transfer of genes encoding drug-detoxifying enzymes to the liver could protect normal hepatocytes and SCs from chemotherapeutic agents, whereas CSCs would remain sensitive to the drugs if miR-155 target sites are incorporated in the expression cassette.

Furthermore, therapies that block miR-155 should decrease cell proliferation and could be beneficial for the treatment of HCCs and other tumors whose growth is driven by miR-155 expression. However, a potential drawback of this approach stems from the physiological role of miR-155 in normal cells. Although miR-155 is absent in hepatocytes, it is expressed in the thymus and the spleen, and plays a fundamental role in immune cell functionality (17).

This includes antibody-mediated signaling in B cells and inflammatory cytokine production in macrophages and dendritic cells, where miR-155 expression increases in response to interferon and the toll-like receptor pathway (18). Therefore, therapies targeting miR-155 or other oncogenic miRs should reach most tumor or CSCs while sparing normal cells.

Another issue for the therapeutic application of miR-155 is the possibility that it only identifies a subset of HCC CSCs, specifically those expressing EpCAM. This is relevant because several biological markers of hepatic CSCs different from EpCAM have been identified, such as CD133 or CD90 (8,19,20). Due to the heterogeneity of hepatic CSCs with different CSC markers and their clinical significance, common miRNA profiles could be difficult to find. Nevertheless, progress in the characterization of HCC drivers provides new candidate targets for the development of combined therapies with increased levels of safety and efficacy.

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### Footnote

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