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REVIEW

Functional analysis of micro RNAs overexpressed in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most lethal cancers, ranking third among all cancer-related mortalities worldwide. Recent studies have shown that hundreds of microRNAs (miRNAs) were deregulated in human HCC, however their biological functions during hepatocarcinogenesis are incompletely understood. In our recent study, we found several miRNAs were upregulated in HCCs from patients and a transgenic mouse model. Using human hepatoma cells expressing individual miRNAs, we tested pro-tumorigenic function of the overexpressed miRNAs. Even though biological functions in the development of HCC are not precisely known, some miRNAs can be potentially used as a diagnostic or prognostic serum marker for HCC. In our recent study, we identified several miRNAs that were specifically upregulated in patient sera with an early and/or advanced HCC.

Keywords: hepatocellular carcinoma; microRNA; tumorigenic; migration; biomarker

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Hepatocellular carcinoma (HCC) is one of the most lethal cancers, accounting for approximately a half million deaths worldwide annually [1, 2]. Treatment modalities developed for HCC offer mostly limited success and tumors recur in approximately 70% of patients within 5 years. Understanding the molecular mechanism underlying the pathogenesis of HCC would be highly important in developing effective target therapies for this deadly disease [3, 4]. Further, there is an urgent need for molecular markers for an early diagnosis of HCC with a minimal invasiveness [5].

microRNAs (miRNAs) are 19~23 nucleotide-long non-coding RNA molecules that suppress gene expression via post-transcriptional regulation [6, 7]. Recent microarray studies have shown that hundreds of miRNAs were upregulated in human HCC [8, 9]. Although the microarray data are informative in various aspects, it is hard to know biological functions of the upregulated miRNAs in hepatocarcinogenesis. For example, of the deregulated miRNAs, some might play a major role in hepatocarcinogenesis while others are simply bystanders. For the precise determination of carcinogenic roles of miRNAs, functional tests of the miRNAs are required in HCC cells.

From a quantitative RT-PCR, we identified several miRNAs that were significantly upregulated both in human and murine HCCs, such as miR-17-5p, miR-25, miR-155-5p, miR-181b-1, and miR-221. Using HCCs from both species allowed us to identify the miRNAs that were consistently upregulated in HCCs regardless of hepatitis B virus (HBV)-infection or the presence of fibrosis because human HCC tissues used in our study were from patients with HBV infection and hepatic fibrosis while the murine HCC model had no apparent hepatic fibrosis or HBV infection. To study

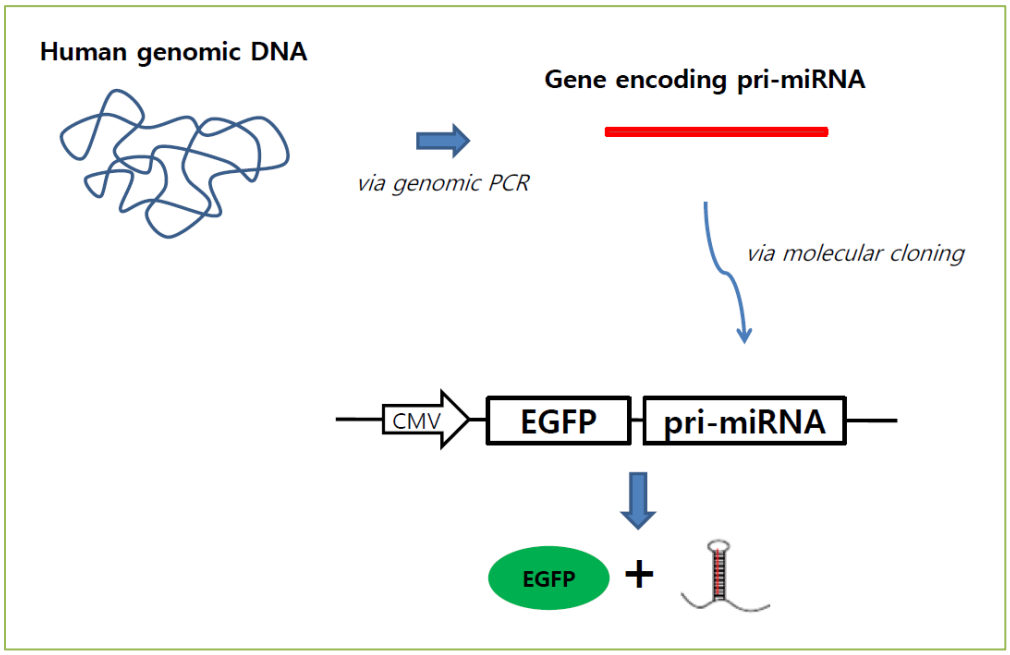

Figure 1. Construction of plasmids encoding pri-miRNAs. PCR was performed using human genomic DNA as a template to amplify the gene encoding each miRNA. Forward and reverse primers bind to locations 200–300 bp up- and downstream of pre-miRNA positions such that miRNA can be generated through the endogenous miRNA biogenesis pathway. The amplified PCR products were placed right after cDNA encoding enhanced green fluorescent protein (EGFP) under the CMV promoter.

the role of the overexpressed miRNAs in HCCs, we generated human hepatoma cells stably expressing the individual miRNAs [10]. The genomic region encoding each miRNA was amplified by PCR and subsequently located right after the enhanced green fluorescent protein (EGFP) cDNA under the cytomegalovirus (CMV) promoter, allowing expression of miRNAs to be monitored via fluorescence imaging of EGFP (Figure 1). Placing the miRNA genes under the CMV promoter also allows the genes to be transcribed by PolII, the RNA polymerase that transcribes endogenous miRNA genes. Furthermore, the genetic loci of miRNAs were amplified using forward and reverse primers binding to several hundred base pairs up- and downstream of pre-miRNA locations. The transcripts from the miRNA-expression vectors are, therefore, expected to undergo the processes by RNAse III enzymes. Thus, miRNAs produced from the expression vectors are expected to have undergone the processes of transcription and biogenesis of endogenous miRNAs.

Using stable HCC cell lines expressing miR-17-5p, miR-21-5p, or miR-221, we investigated proliferation and migration capabilities of cancer cells overexpressing each miRNA. Hepatoma cells expressing miR-221 showed increased cellular proliferation based on an MTT assay, while those expressing miR-17-5p exhibited an increased capability of migration determined by a wound-healing assay. Thus, overexpression of the miRNAs during hepatocarcinogenesis will likely increase the tumorigenic potential of neoplastic cells. Although numerous miRNAs are overexpressed in HCC, their biological functions during hepatocarcinogenesis remain largely unknown. Understanding the precise roles of individual miRNAs will expand our knowledge on the genetic mechanisms underlying HCC, leading to a better strategy for molecular targeted therapy.

Some miRNAs that were upregulated in HCCs can be potentially used as a serum biomarker for diagnosis or prognosis of HCC [11, 12]. In our pilot study, we have found that several miRNAs including miR-25 were upregulated in sera from HCC patients regardless of the stages of cancer while miRNAs such as miR-222 were upregulated only in sera from patients with an advanced HCC (Figure 2). With the increasing knowledge on miRNA biology, the potential application of miRNAs to cancer biology will likely broaden in the future.

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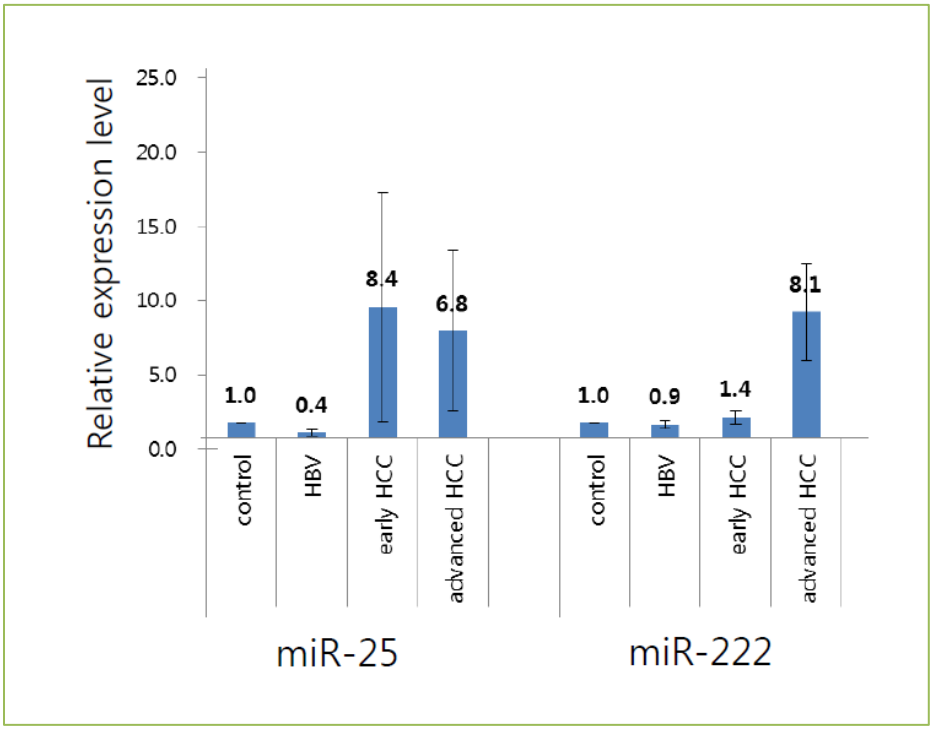

Figure 2. Expression levels of serum miRNAs in normal healthy control and patients with viral hepatitis (HBV), early HCC, or advanced HCC. miRNAs were harvested from serum and used for a quantitative RT-PCR. Note that miR-25 showed upregulation in both early and advanced HCC patients while miR-222 was elevated only in patients with an advanced HCC.

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