ABERRANT EXPRESSIONS OF MICRORNA-21, -155, -221, AND -222 IN HEPATOCELLULAR CARCINOMA: AN ASSAY IN CORRELATION WITH HISTOLOGICAL AND CLINICAL PARAMETERS

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Background: MicroRNAs (miRNAs) are 19 to 24 nucleotide-long RNAs, and important regulators of gene expression. MiRNAs are amplified in several types of cancer, acting as oncogenes. In fact, a number of putative oncogenic miRNAs have been proposed as cancer-related biomarkers. Mir- 21, the most commonly overexpressed oncogenic miRNA among solid tumors, is known to act as a master regulator of the metastasis.MiR-155 is upregulated in several tumors and known to be involved in cancer metastasis. MiR-221 and miR-222 contribute to cell proliferation, and their overexpression in hepatocellular carcinoma(HCC) is previously reported. Stable accessibility of miRNAs from formalin-fixed paraffin-embedded (FFPE) tissues allows miRNA expression profiling studies in the FFPE samples. Present study investigated the expression status of mir-21, mir-155, mir-221, and mir-222 in FFPE tissues of surgically resected HCC cases. Their aberrant expression was analyzed in the aspect of tumorigenesis, and clinical relevance. **Design:** 117 cases of surgically resected HCCs and paired non-neoplastic background liver tissues were analyzed. 20 cases of normal liver tissue from 9 living donors and 11 hepatolithiasis patients were used as controls. Using FFPE tissues, target area was macrodissected, RNA was extracted, and reverse transcription and quantitative real-time PCR for mir-21, mir-155, mir- 221, mir-222, and reference marker U6sn was performed. **Results and Conclusions:** mir-21, mir-155, mir-221, and mir-222 were significantly overexpressed in HCCs as compared to normal or non-neoplastic background liver($P = .001$, $P = .015$, $P < .001$, $P < .001$ in each). Within HCC groups, mir21 expression level was higher in cases with portal vein tumor thrombi than cases with no portal vein invasion (P=.004). Within non-neoplastic background liver tissues, expression level of the four miRNAs showed increasing tendency according to the extent of fibrosis. Especially, mir-222 expression level was significantly higher in cirrhosis tissues than less fibrotic liver tissues (P= .029). Extent of large cell change (dysplasia) in background liver tissue showed no difference in the expression status of the four miRNAs. In the aspect of clinical relevance, overexpression of mir-21 and mir-222 in HCC tissues was more frequently noted in cases with distant metastasis than no metastasis cases (P= .025 in each). In general, cases with amplified miRNAs showed tendency to shorter metastasis-free survival. Especially, cases with mir-222 amplified HCC displayed shorter

metastasis-free survival (P= .005). In addition, overexpression of mir-21 and mir-221 expression level in non-neoplastic background tissues was more frequent in cases with distant metastasis (P= .013, and P= .021 in each). Cases with mir-21, mir-221, or mir-222 amplification in non-neoplastic background tissues showed shorter metastasis-free survival (P = .041, P= .023, and P= .051 in each). Overexpression of mir-21, mir-155, mir-221, and mir-222 was specific to tumor, and these markers could be used as dignositic markers in differentiating HCCs from non-neoplastic lesion, although dysplastic nodule was not exmamined in this study. Elevated expression of miRNA markers in cirrhosis suggests that these miRNAs might involve in the early stage of hepatocarcinogenesis. Significant associations of the overexpressions of some miRNA with various prognostic parameters suggest their potential as prognostic biomarkers.