New Phenomenon

Expression of miR-224, miR-145, and their putative target ADAM17 in hepatocellular carcinoma

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MicroRNAs (miRNAs) have been demonstrated to involve in multiple biological processes, including apoptosis, proliferation, differentiation, and metastasis [1–3]. In addition, several groups have reported that abnormalities of specific miRNAs are implicated in cancer pathogenesis [4,5]. The roles of miR-224 and miR-145 in tumorigenesis have attracted the attention of many researchers. The protein disintegrin and metalloproteinase 17 (ADAM17) is over-expressed in many types of cancers, including hepatocellular carcinoma (HCC). ADAM17 expression is regulated by multiple miRNAs, which have been identified in several types of cancers [6,7]. In silico analysis suggested that ADAM17 is a common target of both miR-224 and miR-145. In the present study, we investigated the expression and distribution of miR-224, miR-145, and ADAM17 in HCC samples and analyzed the relationship between both miRNAs and ADAM17.

Forty-two pairs of primary HCC, their adjacent non-tumorous liver samples, and eight additional normal liver samples were used to perform the in situ hybridization (ISH) analysis of both miRNAs and the immunohistochemistry (IHC) analysis of ADAM17. The clinical and pathological features of HCC patients are briefly described in Supplementary Table S1. Written informed consent was provided by all patients, and all aspects of this study were approved by the Ethics Committee from Xiangya Hospital, Central South University (Changsha, China).

To detect the expression and distribution of miRNAs in formalin-fixed paraffin-embedded tissues, ISH analysis using a 5’-end single digoxigenin-labeled miRCURY LNA™ microRNA (miR-224 and miR-145) detection probe (Exiqon, Copenhagen, Denmark) was carried out. The positive cases of miRNAs were assessed according to the following criteria of ISH staining: the rate of positive cells was >10% among the cells of interest and the staining was present in either the nucleus or the cytoplasm. MiR-224 expression was readily detectable in HCC samples, and intense probe signals were observed in the cytoplasm and nucleus of tumor cells with positive cases being noted in 78.6% (33 of 42) of samples. In adjacent non-tumorous tissue, staining of miR-224 was shown in 15 cases (35.7% positive cases). Most of these positive cases exhibited weaker staining compared with the HCC samples. MiR-224 was also detected in liver bile duct epithelial cells. In addition, eight normal liver samples were tested and only one case was weakly positive in the hepatic cells. The remaining seven cases were negative (Fig. 1 and Supplementary Table S2). Statistical analysis showed that the expression of miR-224 was higher in HCC samples than that in the adjacent non-tumorous tissue and normal liver samples (P < 0.001 and P = 0.001, respectively; Fig. 1). There were no significant differences between the adjacent non-tumorous liver tissue and normal liver tissue (P = 0.381; Fig. 1).

An intense nuclear-enriched signal within HCC samples was exclusively seen after performing ISH with the miR-145 probe, and the positive rate of tumors was very high, reaching as high as 92.8% (39 of 42). The miR-145 probe also showed a strong positive reaction in adjacent non-tumorous tissue, including hepatocytes, biliary epithelial cells, fibrocytes, and vascular endothelial cells; positive cases accounted for 50% of the samples (21 of 42). However, the staining intensity and the proportion of positive cells were less than in HCC samples. MiR-145 expression was low in normal liver tissue, and the proportion of positive cases was as low as 37.5% (3 of 8) (Fig. 1 and Supplementary Table S2). Statistical analysis indicated that miR-145 expression was up-regulated in the HCC samples compared with the adjacent non-tumorous tissue and normal liver samples (P < 0.001 and P = 0.001, respectively; Fig. 1). There were no significant differences between the adjacent non-tumorous tissue and normal liver samples (P = 0.793; Fig. 1).
We attempted to correlate some clinicopathological features of HCC patients with the miR-224 and miR-145 staining results. However, no new and meaningful observations were established. MiR-224 and miR-145 expressions were not associated with clinicopathological features of HCC patients, including sex, age, histology, tumor number and size, capsule invasion, microvascular invasion, and liver cirrhosis (Supplementary Table S3).

IHC of paraffin-embedded sections was carried out according to a two-step protocol (Plink-2 plus@ Polymer HRP Detection System, GBI Labs, Bothell, USA). A mouse monoclonal antibody for ADAM17 (Abcam, Cambridge, USA) was used for this part of study. The IHC staining was classified according to the percentage of cells staining positive for ADAM17; in addition, ADAM17-positive staining was detected in the membrane and cytoplasm of HCC cells. Thirty-two of the 42 HCC samples were positive, and the positive rate was $\approx 76.2\%$ (Fig. 2 and Supplementary Table S2). In the adjacent non-tumorous tissues, however, ADAM17 expression was significantly reduced, with only 12 out of 42 showing positive expression (Fig. 2 and Supplementary Table S2). ADAM17 protein expression was absent in all eight normal liver samples. The increased ADAM17 expression in HCC samples compared with adjacent non-tumorous tissue and normal liver was statistically significant ($P < 0.001$ for both; Fig. 2), whereas no association was found with expression changes between adjacent non-tumorous tissue and normal liver samples ($P = 0.200$; Fig. 2). On combining our results with clinical analysis data for HCC patients, we found that ADAM17 expression was

![Figure 1. Expression of miR-224 and miR-145 in normal liver samples, adjacent non-tumorous tissue, and HCC samples](image1)

![Figure 2. Expression of ADAM17 in normal liver samples, adjacent non-tumorous tissue, and HCC samples](image2)
associated with microvascular invasion \((P = 0.041; \text{Supplementary Table S3})\), but not with sex, age, histology, tumor number and size, capsule invasion, or liver cirrhosis. Correlations of miR-224 and miR-145 expression with ADAM17 were further analyzed. In HCC samples, the expression levels of miR-224, miR-145, and ADAM17 were high in both staining intensity and positive rate; but in the adjacent non-tumorous tissue and normal liver samples, their expressions were low. In addition, there was no evidence that an opposite trend exists between objective miRNAs (miR-224, miR-145) and ADAM17. Statistical analysis showed that objective miRNAs (miR-224, miR-145) expression was not correlated with ADAM17 expression in HCC samples \((P = 1.000\) for both) or the adjacent non-tumorous tissue \((P = 0.203\) and \(P = 1.000\), respectively). For normal liver samples, such an inverse relation was not clear after a case-by-case analysis (Supplementary Table S4).

In conclusion, miR-224 and miR-145 were up-regulated in HCC samples compared with adjacent non-tumorous tissue and normal liver samples. The up-regulation of ADAM17 was associated with microvascular invasion of cancer cells in HCC, but the inverse expression trends have not yet been established for miR-224, miR-145, and ADAM17. Further studies are needed to explore the molecular mechanism of miRNAs in hepatocarcinogenesis, which will help to understand the etiology of liver cancer. More importantly, they may be potential biomarkers for the diagnosis and treatment of HCC, and have an impact on the existing treatment strategy for liver cancer.

**Supplementary Data**

Supplementary Data are available at *ABBS* online.

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**References**