Title: Role of Ga12 gep oncogene in epithelial-mesenchymal transition of liver cancer

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Hepatocellular carcinoma (HCC) has a poor prognosis due to aggressive phenotype. Ga12 gep oncogene product couples to GPCRs, whose ligand levels are frequently increased in tumor microenvironments. Here, we report Ga12 overexpression in human HCC and the resultant induction of ZEB1 as mediated by microRNAs deregulation. Ga12 expression was higher in HCC than surrounding non-tumorous tissue. Transfection of Huh7 cell with an activated mutant of Ga12 (Ga12QL) deregulated miRNA 200b~200a~429, 194-2~192 and 194-1~215 clusters in the miRNome. cDNA microarray analyses disclosed the targets affected by Ga12 gene knockout. An integrative network of miRNAs and mRNA changes enabled us to predict ZEB1 as a key molecule governed by Ga12. Decreases of miR-200a/b, 192 and 215 by Ga12 caused ZEB1 induction. The ability of Ga12 to decrease p53 levels as a result of MDM2 induction contributed to transcriptional deregulation of the miRNAs. Ga12QL induced ZEB1 and other epithelial-mesenchymal transition markers with fibroblastoid phenotype change. Consistently, transfection with miR-200b, 192 or 215 mimic prevented the ability of Ga12QL to increase tumor cell migration/invasion. In xenograft animal studies, sustained knockdown of Ga12 decreased the overall growth rate and average volume of tumors derived from SK-Hep1 cell (mesenchymal-typed). Moreover, tumor passages from primary HCC enhanced Ga12 levels in a patient-derived HCC xenograft model, fortifying the link between Ga12 overexpression and tumor adaptation to new microenvironments. In HCC patients, miR-192, 215 and/or 200a were deregulated with microvascular invasion or growth advantage. In the HCC samples with higher Ga12 level, a correlation existed in the comparison of relative changes of Ga12 and ZEB1. CONCLUSION: Ga12 overexpressed in HCC causes ZEB1 induction by deregulating p53-responsive miRNAs, which may facilitate epithelial-mesenchymal transition of liver tumor.