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Original Article

MELK is an oncogenic kinase essential for early hepatocellular carcinoma recurrence

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ABSTRACT

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. Many kinases have been found to be intimately involved in oncogenesis and the deregulation of kinase function has emerged as a major mechanism by which cancer cells evade normal physiological constraints on growth and survival. Previously, we have performed gene expression profile analysis on HCC samples and have identified a host of kinases that are remarkably overexpressed in HCC. Among these, the Maternal Embryonic Leucine Zipper Kinase (MELK) is highly overexpressed in HCC and its overexpression strongly correlates with early recurrence and poor patients' survival. Silencing MELK inhibited cell growth, invasion, stemness and tumorigenicity of HCC cells by inducing apoptosis and mitosis. We further showed that the overexpression of MELK in HCC samples strongly correlated with the cell cycle- and mitosis-related genes which are directly regulated as part of the forkhead transcription factor FoxM1-related cell division program. Together, our data establish MELK as an oncogenic kinase involved in the pathogenesis and recurrence of HCC and could provide a promising molecular target to develop therapeutic strategies for patients with advanced HCC.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and is the second leading cause of cancer-related deaths worldwide [1,2]. Despite many attempts to develop molecularly-targeted agents and immunotherapy strategies [3,4], sorafenib remains the only currently FDA-approved molecular inhibitor for the systemic therapy of advanced HCC. Hence, it is an urgent unmet medical need to decipher the associated molecular signaling pathways in hepatocarcinogenesis to facilitate the identification of novel therapeutic targets to enable the development of novel molecularly-targeted therapeutic strategies [5].

Many kinases have been found to be intimately involved in the processes leading to tumor cell proliferation and survival and the deregulation of kinase function has emerged as a major mechanism by which cancer cells evade normal physiological constraints on growth and survival. Currently, a number of therapeutic kinase inhibitors, including antibodies or small molecules that block the interactions between kinases and substrates, and to inhibit the enzyme's adenosine triphosphate binding site inhibitors, have already been developed and approved for cancer

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treatment [6,7]. It is therefore a promising strategy to comprehensively identify and functional characterized oncogenic kinases associated with HCC to enable the development of small molecule kinase inhibitors for the molecularly-targeted treatment of HCC. In this study, the expression of over 600 kinases were studied by gene expression profile analysis on HCC samples consisting of patient samples with early recurrence (recurrent HCC disease within two years) or non-recurrence (no recurrent disease after five years) [8]. We have identified a host of HCC remarkably overexpressed oncogenic kinases. Among them, the overexpression of MELK is validated to be significantly associated with early HCC recurrence and poor patients' survival. While MELK has been studied in many human cancers, its molecular roles in HCC have not been elucidated. We have therefore further characterized the functional roles of MELK in the pathogenesis of HCC and demonstrating that MELK is a promising molecular target for the development of molecularly-targeted therapeutic strategies against HCC.

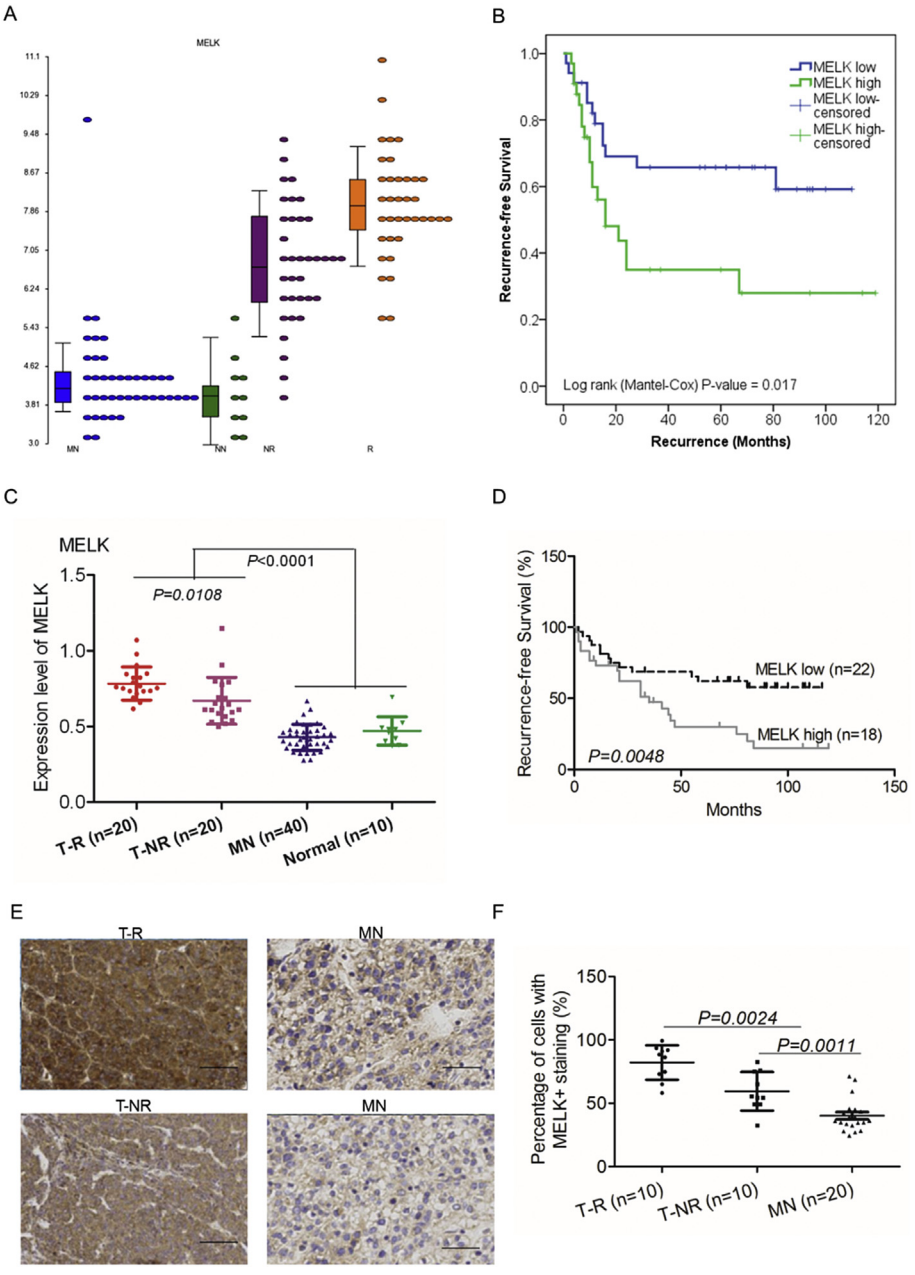

Fig. 1. MELK is highly overexpressed in HCC and its overexpression strongly correlates with early recurrence and poor patients' survival. (A) The dotplot showed that MELK was significantly up-regulated in HCC tissue samples of patients with early recurrent disease (R) compared to patients with non-recurrent disease (NR) and matched normal (MN) or histological histologically normal liver tissues (NN) (*P < 0.05 R vs NR, **P < 0.01 R + NR vs MN + NN). (B) The survival curve of 76 microarray samples showed that high expression of MELK was associated with poor recurrence-free survival of patients with HCC. (C) Validation of the expression of MELK by RT-qPCR in 20 tumor recurrence (T–R), 20 tumor non-recurrence (T-NR), 40 matched normal (MN) and 10 histological histologically normal liver tissues (NN). (D) The survival curve of 40 RT-qPCR validation samples showed that high expression of MELK was associated with poor recurrence-free survival of patients with HCC. (E) The representative imaging of IHC staining for validation of the expression of EDIL3 in another panel of HCC tumor tissues (40×). (F) The imaging analysis and quantification of IHC staining with EDIL3. The IHC images were evaluated according to the percentage of cells with positive nuclei.