Hepatic angiomyolipoma and hepatic stellate cells share a similar gene expression profile

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Summary
Background and Aims: Angiomyolipomas (AMLs) of the liver are rare neoplasms composed of large epithelioid cells with intermixed fat and blood vessels. Hepatic AMLs have no clear normal-cell counterpart in the liver. However, AMLs and stellate cells both are positive for neural crest–derived markers including HMB-45 antigen.
Methods: To further explore the similarities between hepatic AMLs and stellate cells, gene expression of a hepatic AML was studied by cDNA microarray. Real-time polymerase chain reaction was used to confirm gene expression. Hepatic stellate cells can be quiescent, activated, or have a myofibroblastic phenotype depending on their state of activation. Expression of known markers of activated stellate cells was compared between the AML, activated primary mouse stellate cells, and stellate cell lines with activated and myofibroblastic phenotypes. Next, 5 novel genes from the AML were selected because they were not previously known to be markers of stellate cells and mRNA expression measured in the activated mouse stellate cells and in myofibroblastic stellate cell lines. Finally, expression levels of 10 novel genes were determined in 5 cirrhotic and 5 noncirrhotic human livers.
Results: Overexpression of known markers of activated stellate cells including transforming growth factor β (TGF-β), smooth muscle actin, and collagen was found in the hepatic AML. Three of 5 novel markers that were identified in the AML, RRAD (Ras-related associated with diabetes), CTSK (cathepsin K), and NIBAN were also found to be overexpressed in activated stellate cells compared with quiescent or myofibroblastic stellate cells. In addition, 9 of 10 novel genes overexpressed in AML were also overexpressed in cirrhotic human livers versus noncirrhotic livers.
Conclusions: Hepatic AMLs share a similar gene expression profile and may differentiate toward activated stellate cells.
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1. Background

Most neoplasms exhibit morphological and protein immunophenotypic similarities to a recognizable benign cell type. This basic observation is the foundation of the modern medical classification of tumors, where most tumors are classified by their similarity to benign tissues based on morphology and protein expression as detected by immunohistochemistry. In the liver, tumors that differentiate toward hepatocytes, bile ducts, and blood vessels are well recognized. However, a few primary liver tumors, such as hepatic angiomyolipomas (AMLs), do not have a clearly defined normal-cell counterpart.

In studying the processes of hepatic fibrosis, we noted a number of interesting similarities between AML and hepatic stellate cells that suggested they shared a similar gene expression profile including that (A) both stellate cells and AML express proteins found in neural crest–derived cells, including HMB-45 and MelA (Melan-A) [1] and (B) both express smooth muscle cell markers [2,3].

Identifying the neoplastic counterpart of hepatic stellate cells may have important implications because overexpressed genes may serve as excellent markers of stellate cell activation and as surrogate markers for liver fibrosis. In this study, we performed gene expression profiling of a hepatic AML and demonstrate that many of the genes expressed in stellate cells are overexpressed in the hepatic AML, and conversely, report a number of new markers identified in the AML that are also overexpressed by activated stellate cells. Because stellate cells can be quiescent, activated, or have a myofibroblastic phenotype, we also examined mouse stellate cells that had been chemically activated as well as a rat cell line with a myofibroblastic phenotype.

2. Materials and methods

2.1. Human liver tissues

All studies were performed after obtaining approval from the Johns Hopkins institutional review board. A hepatic AML that has been previously described in detail [4] was harvested at the time of surgery and frozen in liquid nitrogen. Additional liver tissues were obtained at the time of surgery for primary liver neoplasms to evaluate cirrhotic and noncirrhotic livers. Nonneoplastic liver tissues away from the tumor masses were harvested, snap-frozen in liquid nitrogen, and stored at −80 C before use. The histological diagnoses were confirmed in all cases by routine light microscopy.

2.2. Primary mouse liver stellate cells

All experiments conformed to institutional and National Institutes of Health guidelines for humane animal care. C57BL/6 mice (Jackson Laboratory, Bar Harbor, Me) were fed a half choline-deficient diet (0.5 MCDE) supplemented with ethionine (0.15%) to enhance oxidative liver injury and stellate cell activation. Both the MCDE diet and a methionine-choline–deficient control diet (MCDC) were from ICN (Aurora, Ohio). Livers were harvested from 14- to 16-week-old mice fed MCDC or 0.5 MCD-deficient diets. Pronase-collagenase digestion was used to isolate stellate
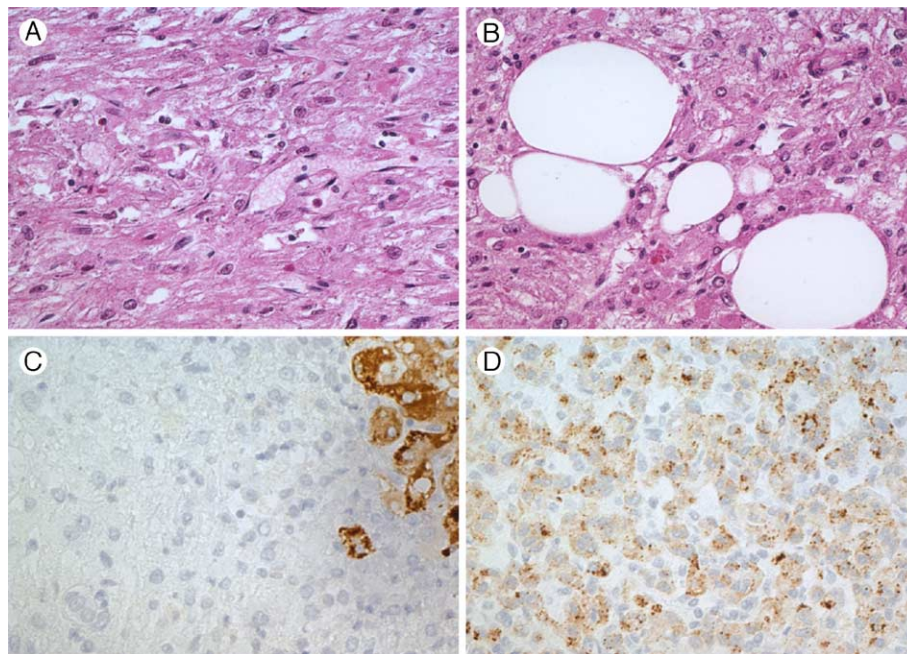

Fig. 1 Most of the AML was composed of epithelioid to spindly cells (A, ×100). Focal fatty change was present in some sections (B, ×100). The AML was negative for Hep-Par, a marker of hepatocyte differentiation. A small portion of normal liver is present in the upper right corner (C, ×100). The AML was positive for HMB-45 (D, ×100).