

## ■ CANCER AND CELL BIOLOGY

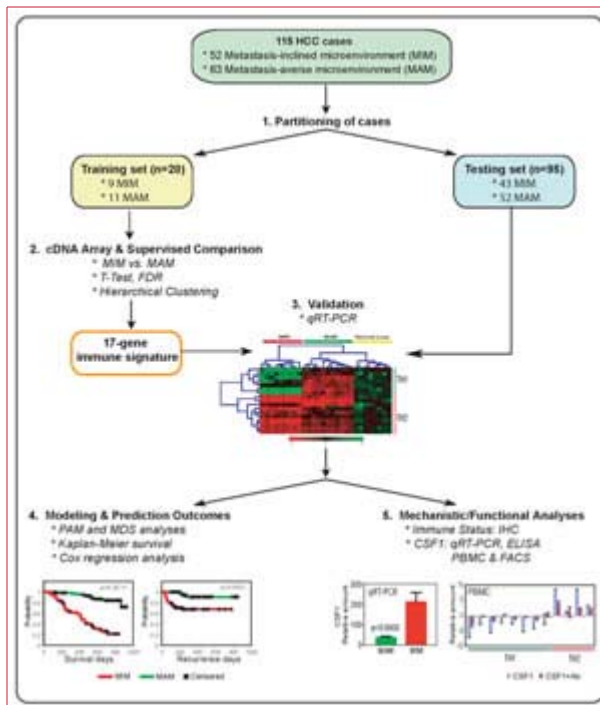
**A Unique Immune-related Metastasis Signature of the Hepatic Microenvironment**

Budhu A, Forgues M, Ye QH, Jia HL, He P, Zanetti KA, Kammula US, Chen Y, Qin LX, Tang ZY, and Wang XW. Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. *Cancer Cell* 10: 99–111, 2006.

**H**epatocellular carcinoma (HCC) is typically associated with an extremely poor prognosis. The reason for this is the highly vascular nature of HCC tumors, which increases their propensity to spread and invade neighboring or distant tissues. Intra-hepatic metastases, especially venous metastases, are a major hallmark of HCC.

Recently, we developed a gene expression signature specific to primary HCC tumor specimens that predicted prognosis and venous metastases with 78% overall accuracy (Ye QH et al. *Nat Med* 9: 416–23, 2003). Since HCC is usually present in inflamed fibrotic and/or cirrhotic liver with extensive lymphocyte infiltration due to chronic hepatitis, it is possible that HCC metastatic propensity may be determined and/or influenced by the local tissue microenvironment of the host.

To determine the role of the hepatic microenvironment in HCC metastasis, we compared the gene expression profiles of noncancerous hepatic tissue samples obtained from areas surrounding tumors in (1) patients with primary HCC accompanied by venous metastases or confirmed extra-hepatic metastases by follow-up, which we termed metastasis-inclined microenvironment (MIM) samples and (2) patients with HCC without detectable metastases, which we termed metastasis-averse microenvironment (MAM) samples (**Figure 1**). We first conducted gene expression profiling studies of a subset of MIM and MAM samples from this cohort using cDNA microarray.



**Figure 1.** Schematic of the search for a metastasis-associated signature in the hepatic microenvironment. Twenty noncancerous hepatic tissue samples from areas surrounding tumors, characterized as MIM (metastasis-inclined microenvironment) or MAM (metastasis-averse microenvironment) samples, were analyzed by cDNA microarray (Step 1). A metastasis signature composed of 17 immune-related genes, associated with T helper cell type 1 (Th1)– and Th2-like cytokines, was significantly and differentially expressed in samples with metastasis (Step 2). Following validation by quantitative real-time polymerase chain reaction (qRT-PCR) (Step 3), prediction analysis of microarrays (PAM), multi-dimensional scaling (MDS), Kaplan-Meier survival analysis, and Cox proportional hazards modeling demonstrated accurate classification of patients with metastasis and prediction of outcome based on the 17-gene signature (Step 4). The pro-inflammatory status of metastasis samples was confirmed by immunohistochemistry (IHC), and a significant increase in the abundance of macrophage colony stimulating factor type 1 (CSF1) in metastasis samples was shown by qRT-PCR and ELISA (Step 5). Peripheral blood mononuclear cells (PBMC), incubated with recombinant CSF1, recapitulated the significant Th1-Th2 cytokine shift observed in metastasis samples, indicating that CSF1 may play a role in promoting the metastatic phenotype (Step 5). In addition, fluorescence-activated cell sorting (FACS) suggested that CSF1 induced these cytokine shifts in T-cell populations (Step 5). Ab, antibody; T-Test, student’s t test; FDR, false discovery rate.

We identified a unique change in the gene expression profiles associated with a metastatic phenotype. Furthermore, using the same subset of MIM and MAM samples used in the microarray, we constructed a refined expression signature containing 17 genes (*IL1A*, *IL1B*, *IL2*, *IL12A*, *IL12B*, *IFNG*, *TNFA*, *IL15*, *IL4*, *IL5*, *IL8*, *IL10*, *HLA-DR*, *HLA-DPA*, *ANXA1*, *PRG1*, and *CSF1*), which we determined by quantitative real-time polymerase-chain-reaction analyses (qRT-PCR). This signature was validated by an independent cohort of 95 MIM and MAM samples and could successfully predict both venous metastases and extra-hepatic metastases by follow-up with greater than 92% overall accuracy. Moreover, the prognostic performance of this liver microenvironment signature was superior to and independent of

other available clinical parameters for determining patient survival or cancer recurrence. The lead signature genes were associated with the cellular immune and inflammatory responses. Consistently, predominant changes in T helper cell type 2 (Th2)–like cytokine responses, favoring a humoral anti-inflammatory/immunosuppressive microenvironmental condition, occur in MIM samples. Macrophage colony stimulating factor type 1 (CSF1) may be one of the cytokines overexpressed in the liver milieu that is responsible for this shift.

These findings suggest that the inflammatory status of the hepatic milieu, whether influenced by viral-hepatitis–mediated liver damage or individual genetic constitution, in addition to the metastatic potential of the tumor cells, plays an important role in promoting HCC tumor progression and venous metastases. In addition, this signature may be clinically useful for identifying HCC patients who may benefit from certain post-surgical treatments to prevent metastases and/or recurrence.

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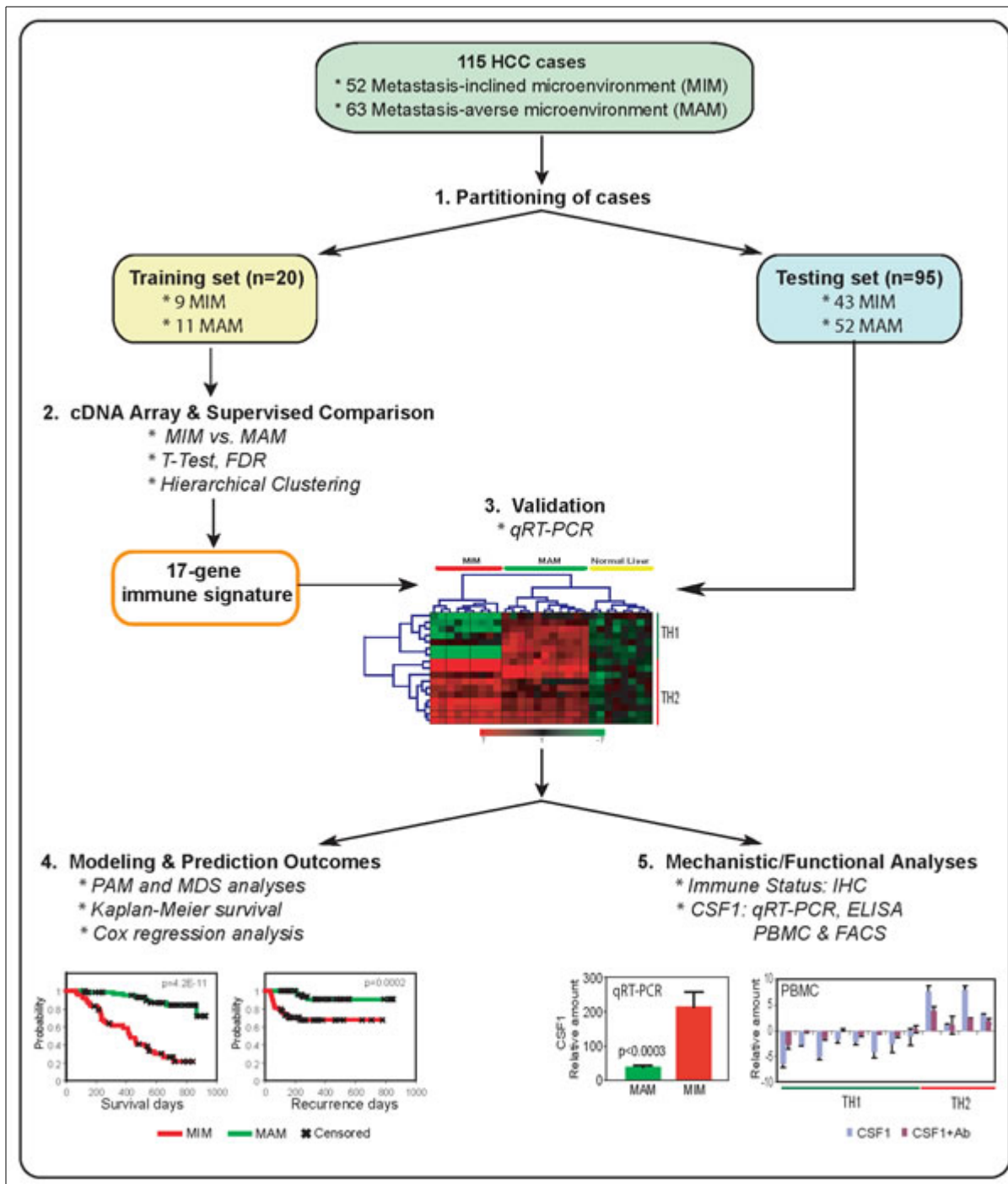
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