Abstract: The intrinsic oncolytic specificity of vesicular stomatitis virus (VSV) is currently being exploited to develop alternative therapeutic strategies for hepatocellular carcinoma (HCC). We have observed earlier that, in contrast to cultured human HCC cells, primary human hepatocytes (PHHs) are refractory to VSV infection. Impairment of the type I interferon (IFN) pathway in HCC cells has been suggested to be the mechanism by which these cells become susceptible to VSV infection. The goal of this study was to elucidate the nature of the IFN defect in human HCC. We demonstrate here that the defect in IFN-beta signaling in HCC cells results from a deregulated IFN regulatory factor-3 (IRF3) pathway. Expression of IRF3-spliced variant (IRF3-nirs3) was constitutively observed in HCC cells and, importantly, also in primary HCC samples. In contrast, IRF3 was readily activated in PHHs after stimulation with dsRNA or infection with VSV. In addition, overexpression of IRF3-nirs3 significantly abrogated the IFN-beta response to VSV infection and improved viral growth. Our data provide evidence that aberrant splicing of IRF3 in HCC contributes to the defect in IFN-mediated antiviral defenses. This work may provide a potential molecular basis for selecting HCC patients for oncolytic VSV therapy in future clinical trials.