INTERLEUKIN-6 (IL-6) INHIBITS CELL GROWTH AT THE G1/M INTERPHASE IN HEPATOCELLULAR CARCINOMA

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INTRODUCTION. Interleukin-6 (IL-6) is a pleiotropic cytokine that plays a critical role in normal hepatic growth and liver regeneration following a reduction in hepatic mass. The aims of the present study were to determine the expression and function of IL-6 signaling pathways in an in vivo model of hepatocellular carcinoma (HCC) and study the effects of IL-6 signaling in the progression of HCC.

METHODS. In vivo HCC tumors were generated by the direct inoculation of H4IIE HCC cells (ATTC) into the hepatic parenchyma resulting in reproducible tumor formation 13-15 days later. HCC samples were subsequently resected and either stored for analysis or cells isolated and cultured as previously described for in vitro analysis¹. Freshly isolated hepatocytes from non-tumor burdened rats were also obtained for in vitro analysis¹. Tissue samples were then analyzed for expression and activity of components of IL-6 signaling. Isolated cultured HCC cells and hepatocytes were then treated with IL-6 and analyzed for IL-6-dependent signaling pathway activity, cell cycle progression and proliferation.

RESULTS. RT-PCR and ELISA analysis of HCC tissue and cells demonstrated HCC is associated with increased IL-6 mRNA and protein expression as compared to normal liver/hepatocytes. In contrast Western blot analysis demonstrated significantly decreased expression of gp80 and gp130 in HCC as compared to normal liver from both tumor burdened (NLTB) and sham operated animals (NLSh) (56±11% [gp80] and 34±8% [gp130] as compared to NLTB, n=4, p<0.05). No significant difference in gp80/130 expression was detected between NLSh and NLTB. Analysis of intracellular effector pathways linked to IL-6 signaling demonstrated increased MAPK (ERK) and STAT3 activity in HCC as compared to normal liver. To further investigate these findings we next examined the effect of IL-6 on signaling pathways in isolated hepatocytes and HCC cells.
These data demonstrated that both H4IIE cells and hepatocytes demonstrated similar time and dose dependent increases in STAT3 activity profiles following treatment with IL-6. In contrast IL-6 caused a monophasic increase in ERK activity in hepatocytes while causing a pronounced biphasic response in H4IIE cells. To address the potential significance of these findings we next determined the effect of IL-6 on cdk inhibitor expression. In HCC cells a significant induction of p21\(^{\text{waf1/cipl}}\) and p27\(^{\text{Kip1}}\) occurred, an effect that was not observed in normal hepatocytes. In proliferation assays IL-6 significantly inhibited serum stimulated cell proliferation in HCC cells as assessed by \([^3H]\) thymidine incorporation, Alomar Blue reduction and cell counts. Preliminary data using inhibitors of STAT3 (AG490) or ERK (PD98-095) indicate inhibition of STAT3 abrogates IL-6 induced increases in cdk inhibitor expression, an effect not observed by inhibition of an ERK pathway.

**DISCUSSION.** IL-6 has been demonstrated to be critical in the initiation phase of normal liver growth and regeneration and has been shown to act as a complete hepatic mitogen in high doses\(^2,3\). In this study we report that IL-6 acts to significantly inhibit serum stimulated HCC cell growth in vitro acting at the G1/S phase of the cell cycle. Analysis of downstream effector pathways known to be stimulated by IL-6 demonstrated different activation profiles for a MAPK signaling cascade in HCC cells as compared to normal hepatocytes. Furthermore, IL-6 causes the induction of specific cdk inhibitors in HCC cells, an effect not observed in hepatocytes. These data suggest that altered activity profiles for these two pathways is critical in determining the proliferative/anti-proliferative effects of IL-6 in normal and transformed cells. What is not currently clear is why HCC cells produce significantly higher amounts of IL-6 than normal liver when the tumor is proliferating at a much higher rate than hepatic parenchymal cells. On possible explanation is that decreased IL-6 receptor expression in HCC effectively “hides” the tumor cells from the innate immune system to avoid deletion via apoptotic processes.

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**REFERENCES**

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