

Introduction:

The type 1 insulin-like growth factor receptor (IGF-IR) plays a major role in growth, differentiation and transformation of cells. Many different types of cancer cells have elevated expression of this receptor. IGF-1R signaling pathway is activated mainly through IRS1 (to Akt) and Erk (Fig. 1). Antibodies to the IGF-IR are now in phase 1 and 2 clinical trials. The liver (in animals and humans) produces large amounts of IGF-1, and provides roughly 75% of the IGF-1 levels in plasma. In addition, hepatocytes do not express IGF-IRs, but they do so in regenerating liver and in some hepatocellular carcinomas (HCC). We investigated the role of the IGF-IR and its signaling pathways in a number of HCC cell lines. We also generated stable cell lines using lentivirus-mediated shRNA targeting IGF-1R. We propose to investigate the therapeutic potential of shRNA targeting IGF-1R in human HCC.

Methods:

1. Western blot and immunoprecipitation (IP) were done according to standard protocol.
2. pLL3.7 lentiviral vector (Rubinson, D.A, et al. 2003, Nature Genetics), was used to construct the human IGF-1R shRNA. Transfection and transduction were performed according to the manufacturer's instructions.
3. Analysis of colony formation in soft agar was performed on 1,000 cells in each 6-well plate. Colonies (>125um in diameter) were counted 3 weeks after seeding.
4. Cell viability was detected by MTT method. 48 well plate (2x10⁴ cells/well) was used and 5 mg/ml MTT was added.

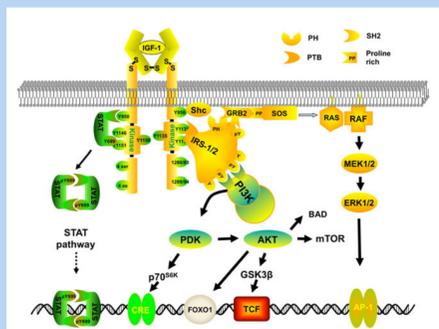


Fig. 1. Schematic diagram of IGF-1R signaling pathway

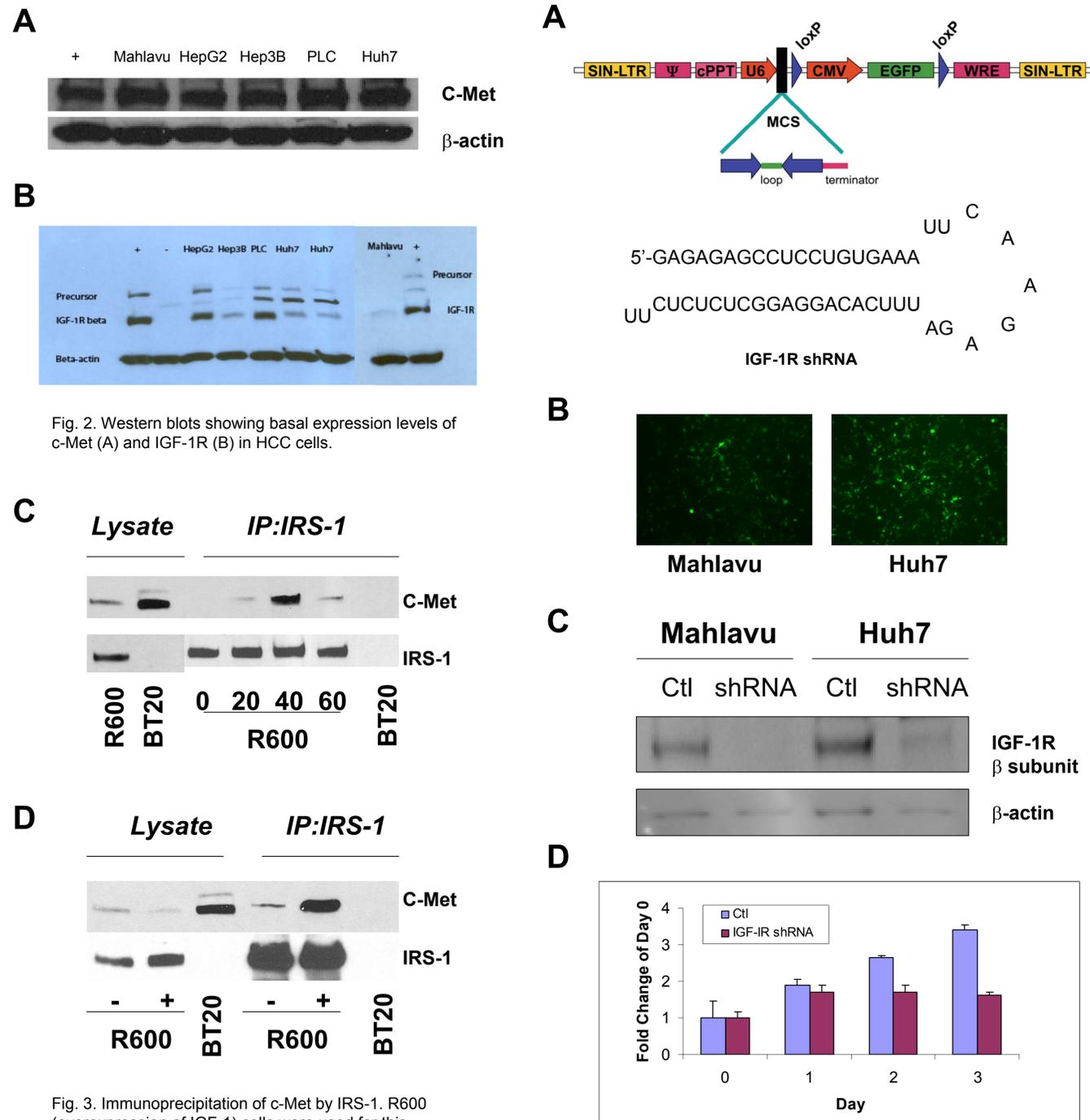


Fig. 3. Immunoprecipitation of c-Met by IRS-1. R600 (overexpression of IGF-1) cells were used for this experiment. BT20 (no IRS-1 expression) was used as negative control. Cells were starved for 24h before stimulated with IGF-1 (20 ng/ml) for 20, 40, and 60 min (C) or HGF (40 ng/ml) for 30 min. Anti-IRS-1 antibody was used to pull down c-Met from total lysate. Immunoblot was performed against c-Met and IRS1.

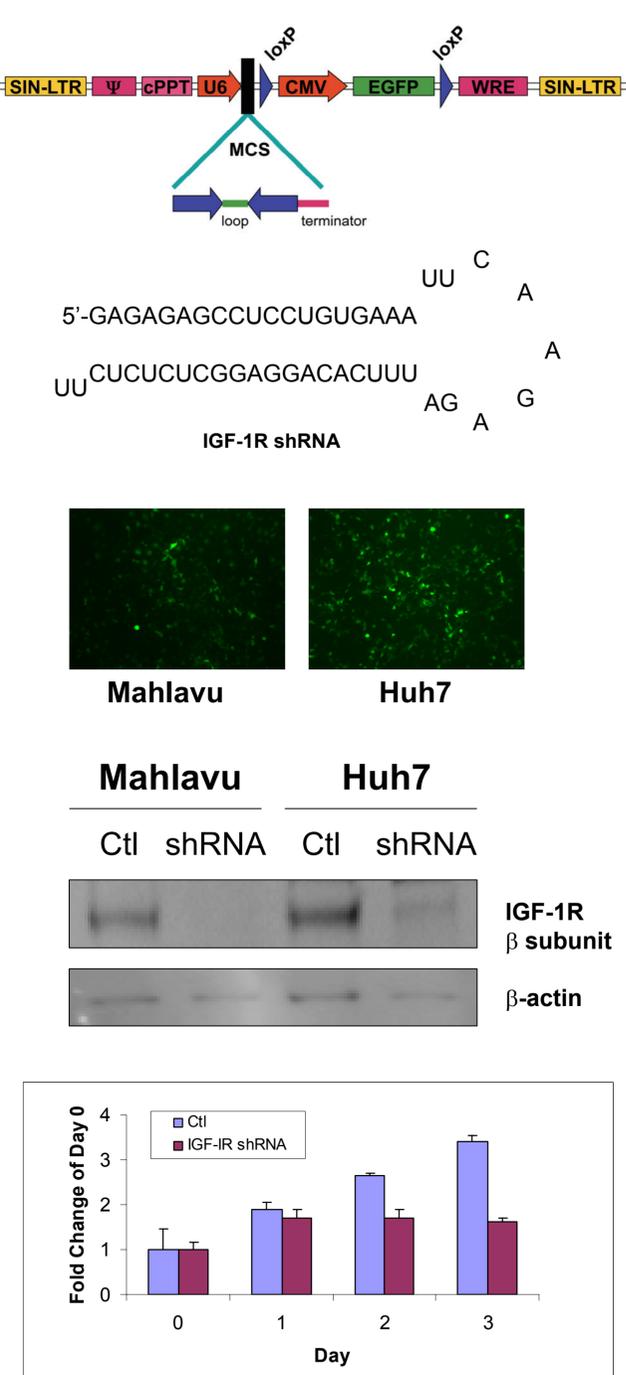


Fig. 4. Inhibitory effect on HCC cell growth by a lentiviral vector expressing IGF-1R shRNA. A. Creation of an shRNA-expressing lentiviral vector targeting IGF-1R. B. Transduction efficiencies. C. Functional silencing of IGF-1R in HCC cells. D. Reduced cell viability in Mahlavu cells. Similar result was obtained with Huh7 (not shown).

Results:

Only HepG2 and PLC expressed reasonable levels of IGF-IR, roughly between 10 and 15x10³ receptor/cell. The other 3 cell lines expressed levels below 5x10³ IGF-IRs/cell (Fig. 2B). All cell lines expressed high levels of the docking protein of the IGF-IR, the insulin receptor substrate-1 (IRS-1) (data not shown) which activates the PI3-K signaling pathway. IRS-1, however, also interacts with c-met, the receptor for the hepatocyte growth factor (HGF), which is also present in substantial amounts in the liver (Fig. 2A). IP results showed that IRS1 interacted with c-Met under both HGF and IGF-1 treatments (Fig. 3). Colony formation in soft agar was not strictly correlated with the expression level of IGF-1R (Fig. 2B and Fig. 5), indicating other factors may also involved in the determination of anchorage-independent growth of HCC cells. Lentivirus-mediated shRNA targeting IGF-1R in HCC cells showed significant knockdown of IGF-1R expression (Fig. 4C). Down-regulated IGF-1R inhibited human HCC cell growth (Fig. 4D).

Summary:

1. Human HCC cells have higher expression levels of IGF-1R, IRS1, and c-Met.
2. IRS1 interacts with c-Met. The role of IGF-1R in human HCC should be revisited.
3. Lentivirus-mediated shRNA delivery system targeting IGF-1R showed significant suppressive effects on HCC cell growth, promising a potential therapeutic treatment for human HCC.

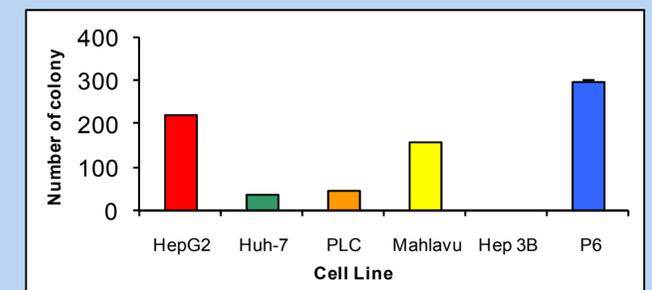


Fig. 5. Colony formation in soft agar of HCC cells. P6 is a mouse cell line over-expressing IGF-1R.