Hepatocellular carcinoma (HCC) is one of the most prevalent tumors worldwide. Heat shock protein 17 (HSP17), which is newly identified as a liver lipid-droplet associated protein, is recently found to be over-expressed in HCC. However, the effects of HSP17 over-expression on the underlying mechanisms of HCC have not yet been fully explored. For this purpose, HSD17B13 gene knockdown with CRISPR/Cas9 technique in HCC cell line. HSD17B13 expression is detected by Flow cytometry and Western blotting with recombinant rabbit monoclonal antibody (OKI3G2) and OD450 assay. The results suggested that HSD17B13 gene knockdown cells have increased cell apoptosis and cell cycle arrest.

**Abstract**

Hepatocellular carcinoma (HCC) is one of the most prevalent tumors worldwide. Heat shock protein 17 (HSP17), which is newly identified as a liver lipid-droplet associated protein, is recently found to be over-expressed in HCC. However, the effects of HSP17 over-expression on the underlying mechanisms of HCC have not yet been fully explored. For this purpose, HSD17B13 gene knockdown with CRISPR/Cas9 technique in HCC cell line. HSD17B13 expression is detected by Flow cytometry and Western blotting with recombinant rabbit monoclonal antibody (OKI3G2) and OD450 assay. The results suggested that HSD17B13 gene knockdown cells have increased cell apoptosis and cell cycle arrest.

**Results**

**Target Selection**

**Immunization**

**Clone Selection**

**Recombinant Antibody Production and Characterization**

**Design & Methods**

**Antibody Development**

Rabbit recombinant monoclonal antibody was developed using 6BS from peripheral blood. Briefly, BS were isolated from the whole blood of immunized rabbit with HSD17B13 polypeptide. Cells from immune response positive wells were selected and identified. Rabbit IgG light chain and heavy chain variant were amplified and cloned into expression vectors. Positive antibody clones were screened to ensure its purity. Antibody purified after both light and heavy chain were our selected clones. HSD17B13 clones were screened by Westernblot/hybridization and immunoreactivity. We chose clone OTI3G2 for the study.

**Crisper/Cas9 knockdown**

To generate HSD17B13 gene knockout cells, SK-Hep-1 cells were infected with lentiviruses carrying a HSD17B13 targeting sgRNA. (2'-C)-GUGGAUUIATACACTUGGC-3' (SANGONGCATACACTUGGC3'). The infected cells were selected with puromycin. The puromycin resistant colonies were then tested to verify the efficiency of knockout in cells (HSD17B13 rabbit monoclonal antibody, clone OTI3G2).

**Conclusion**

It has been well known that following anti-angiogenic signals or DNA damage, p21 and p27 bind to cyclin-CDK complexes to inhibit their catalytic activity and induce cell-cycle arrest. MMPs family play essential roles in physiological processes such as organogenesis, angiogenesis, apoptosis, cell proliferation and metastasis. MMPs and MMPs have also been found in the nuclear of the cell which may regulate cancer-like behavior. It is important to know if the HSD17B13 knockdown clone line which can be a useful tool for discover new function in the future. Compare with wild type HCC cell line SK-Hep-1, the expression of cell cycle related protein, p21, p27, MMPs and MMPs decreases in HSD17B13 knockdown cells (ΔSK-Hep). Along with HSD17B13 knockdown, p21, p27, MMPs and MMPs expression are partially recovered but p27 expression level does not. This indicates HSD17B13 which has been identified to be a liver enriched, hepatocyte-specific, lipid droplet-associated protein play a strong relationship with p27, MMPs and MMPs. HSD17B13 play important roles in the progression of HCC lesions. HSD17B13 might be a potential immune-oncology marker for HCC.

**Reference**