# ImmunoTools *special* Award 2014

**Prafull Kumar Singh,** PhD student

Supervisor: Prof. Dr. Jürgen Borlak

Institute for Pharmaco and Toxicogenomics, Hannover
Medical School, Feodor-Lynen-Straße 35, 30625 Hannover

## Deveolpment of Molecularly targeted therapeutics against hepatocellular carcinoma

Hepatocellular carcinoma (HCC), a multifactorial disease is one of the leading causes of cancer related death worldwide. The potential risk factor for the disease includes infections with hepatitis B/C virus, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH). Different immune cell subsets, cytokines and chemokines have been linked to HCC development and treatment outcome. Our laboratory is particularly interested in studying the altered pathways of tumorigenesis, NASH and development of improved therapeutics to curb the mortalities caused by this aggressive disease. Recent studies suggest that angiogenesis and signaling through the RAF/MEK/ERK cascade play crucial role in the development of HCC. The multikinase inhibitors have been shown to block tumor cell proliferation and angiogenesis by inhibiting serine/threonine kinases as well as the receptor tyrosine kinases (VEGFR, PDGFR, FLT3, Ret, and c-KIT). However the full therapeutic potential of these drugs can not be harnessed due to the significant off target effects. Development of liver specific delivery strategies will help in overcoming these adverse effects and will enhance the therapeutic efficacy of drug by increasing the bioavailability at the tumor site. In this regard, the surface proteins of certain viruses have been shown to primarily involve in their hepatic tropism. Furthermore, studies demonstrated that the hepatic cell lines can be rendered non-infectable to hepatitis virus by competitive inhibition by using putative domain of such viral proteins and thus can be exploited as cargo peptide for delivery of novel therapeutic

molecules against HCC. We have developed such a peptide based delivery agent and linked novel therapeutic molecules to it. Fluorescent microscopy experiments demonstrated the specificity of the combined molecule in different hepatic cell lines (HUH7, HepG2 and PLC/PRF5) and primary human hepatocytes with significant cell binding and uptake as compared to controls and non liver origin cells. The therapeutic efficacy of the developed molecule will be evaluated in transgenic (EGF2B) and xenograft mouse models of HCC. These models will be treated with therapeutic and above doses of the developed molecule and tumor growth inhibition will be assessed by a series of molecular imaging (µCT and µPET), histopathological, immunological, immunohistochemistry, immunoblotting, flow cytometry and expression profiling experiments. As mentioned above, the profiling of mouse immune cells ($CD4^+$ T cells, $CD8^+$ T cells, $CD16^+$ NK cells, Treg cells, Th17, dendritic cells and monocytes, CD80/CD86) cytokine and chemokines (IL-1α, IL-2, IL-3, IL-6 $CD1c^+Lin^-$, IL-8, IL-12p40, CCL27, CXCL1, CXCL10, CXCL12, IFN- γ, IFN-α2, M-CSF, GM-CSF, CXCL9, β-NGF, SCF, SCGF-β, TNF-α/β, sCD25, TGF-ß, PDGF; VEGFR, p-ERK, Flt3) at different stages of tumor progression and treatment will add to better understanding of underlying molecular events and therapeutic efficacy of the drug under test. Interestingly, the antibodies and cytokines mentioned in the ImmunoTools mouse reagent covers most of the required ones that I am planning to use for my research. I strongly believe that the support provided by ImmunoTools will contribute greatly to my research project.