7 Summary

The hepatitis C Virus (HCV) is an enveloped positive-strand RNA virus which belongs to the family of the *Flaviviridea.* The genome codes for the structural proteins core, E1 and E2 and the nonstructural proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. For the majority of these proteins the function is at least partially known. Some of them are able to affect central signal cascades of the cell controlling i.e. apoptosis, cell growth and differentiation, but the different observations are sometimes controversial. The nonstructural protein 5A is able to deregulate the Raf-1/ERK-dependent MAP kinase signalling pathway. Based on this and other investigations, which showed the ability of NS5A to influence important signalling cascades, the protein is thought to play a major role in the development of HCV-associated HCC progression.

In the first part of this work the influence of two subgenomic HCV replicons, huh9-13 and huh ET cells, on death receptor-mediated and intrinsic apoptosis was investigated. Based on Western blot experiments for detection of PARP cleavage and FACS analysis for quantification of Annexin-V staining an increased sensitivity of huh9-13 but not of huh ET replicon cells towards TNFα- and TRAIL-mediated apoptosis compared to huh7 control cells was observed. This difference in the sensitivity to death receptor-mediated apoptosis is reflected in a variety of differentially regulated genes involved in apoptosis induction as determined by a membrane array. After elimination of the replicon RNA by IFNα treatment, the increased sensitivity of huh9-13 cells was abolished elucidating an influence of the HCV replicon on receptor mediated apoptosis for huh9-13 cells. In the case of huh ET cells the interferon treatment did not restore the sensitivity to TNFα-dependent apoptosis. Using etoposide and actinomycin D for the induction of apoptosis, no effect of the replicon RNA on the intrinsic apoptotic pathway could be detected, however strong effects of the cellular background have been detected. These data provide a basis for further studies of the influence of HCV towards apoptosis. Furthermore these data point up the necessity of well controlled conditions and accurately selected model systems for such investigations.

In the second part of this work the effect of NS5A on MAPK signalling was examined. Using phophospecific antibodies an increased Raf-1 phosphorylation at serin 338 was observed, which was caused by NS5A expression. Unexpectedly, the increased Raf-1 phosphorylation had no effect on MEK1/2 phosphorylation at serin 217/221. Investigating different NS5A deletion mutants, a region between amino acid 302-449 was identified to be sufficient for increased Raf-1 phosphorylation. Thereby a correlation of NS5A mutant localisation and

measured effect on Raf-1 phosphorylation has been observed. Inhibition of different kinases involved in MAPK signalling revealed no participation of PKC. Based on the observation that NS5A-dependent activation of Raf-1 does not result in activation of MEK1/2 further experiments could elucidate alternative functions of Raf-1 beyond the MAPK-pathway.

In the third part of this work a new NS5A transgenic mouse was characterized to elucidate the potential role of NS5A for HCV-associated liver damage. The mice were genotyped using PCR. Robust, liver specific and long term transgene expression was monitored by Western blot analysis showing an equivalent NS5A expression in two different founder strains. In transgenic mice no tumour formation or liver damage was detected not even after irradiation of mice with sub-lethal doses of 3 Gy gamma radiations. To investigate alterations in gene expression triggered by NS5A, transgenic mice and wild type littermates were subjected to microarray and proteome analysis detecting several genes altered on mRNA or protein level. To control the postulated effect of NS5A on the interferon response the mice were infected with LCMV. In the transgenic mice a reduced virus elimination and elevated GPT-activity in the sera was observed. On the molecular level an impaired induction of 2´,5`-OAS and of PKR was observed in transgenic liver samples. These results argue for an inhibitory effect of NS5A on interferon-mediated antiviral response which could mediate a chronic progression in case of HCV infection. Based on these data further studies can lead to a new model of NS5A function in the context of chronic HCV infection and associated liver damage.