

5- SUMMARY

Hepatitis B virus (HBV) is a hepatotropic, non-cytopathic DNA virus that causes acute and chronic necroinflammatory liver disease and hepatocellular carcinoma. It has been shown that HBV DNA replication is suppressed by interferon (IFN) α/β , IFN γ and other cytokines.

The aim of my thesis was to establish a liver directed gene transfer and to characterize the effect of local expression of interferon on HBV replication. Two sets of adenoviral vectors were established: the first using the CMV promoter (P_{CMV}) (AdmIFN γ and AdmIFN β) and the second using a bidirectional tetracycline (tet)-regulated promoter (P_{bi-1}) (Adbiluc vectors) to locally express mouse interferon (mIFN) α , β and γ .

In mouse hepatocyte cultures, a 5- to 10-fold decrease of intracellular HBV DNA was observed following infection with AdmIFN β or AdmIFN γ . The antiviral effect was enhanced in HBV transgenic mice where 95% of intrahepatic and serum HBV DNA disappeared 2 weeks after intravenous injection. However, the effect was transient and HBV replication was restored. This was partially due to a down regulation of the CMV promoter.

Using the Adbiluc vectors, expression of the reverse tet-transactivator (rtTA) under liver specific promoters (e.g. in transgenic mice or with a second vector) ensured tissue specificity of gene expression. Co-expression of luciferase with the bidirectional promoter P_{bi-1} allowed monitoring. Simultaneous, strong expression of both genes dependent on the dose of tetracycline *in vitro* and tight regulation in living mice were shown. However, in one direction, gene expression was slightly leaky. This was exploited to avoid separate expression of the tet-transactivator: rtTA was cloned under control of P_{bi-1} to establish a tet-regulated one-vector system.

Using the Adbiluc-IFN vectors, rapid induction of cytokine expression in the livers of transduced mice was fatal, but moderate expression was well tolerated. Due to these difficulties in the first experiments in mouse models for chronic and acute HBV infection, the antiviral effect of regulated IFN-expression could not yet be demonstrated.

Taken together, local expression of cytokines was successfully established following adenoviral gene transfer and an antiviral effect was demonstrated. A set of novel adenoviral vector allowing tet-regulated and monitored cytokine expression will allow characterizing the antiviral pathways. This approach can also be used to develop gene therapy of chronic HBV and hepatitis C virus infection and other diseases like malaria or liver cancer.