6 Summary

This study aims to analyse the cause of the leaky activity of the tumor- and tissue-specific promoters (ttsPs) after its integration in an adenoviral vector genome and how this affects the viral replication. We demonstrate which impact cause several viral and nonviral factors. Based on these findings, we were able to optimize the specificity of the virus replication.
Furthermore the development of an replication deficient adenoviral vector (rdAdV) which express a proapoptotic Dox-controlled gene was proved to be a trend-setting for a new strategy approach in tumor therapy.

1. By the insertion of the ttsPs (CEA Promoter, SPB Promoter, hTyr2E/P Promoter or [HRE]AFP Promoter) into a luciferase-expressing rdAdV shown the ttsP just a conditional cell specifity (expression also in non target cells) while its endogenous homologue kept the specificity. The analysed elements listed below influenced the ttsP activity:
The 5´-terminal adenoviral E1A enhancers fused to the ttsP affected their leakiness.
The insertion of transcriptional regulator elements in the ttsP region carried in part to a modification on the promoter activity. On the one hand increased the murine tyrosinase enhancer (2mE-Tyr) the tyrosinase promoter activity, on the other hand didn´t show the human tyrosinase enhancer (2hE-Tyr) any effect. The transcriptional element [HRE] inhibited the AFP promoter activity. All these effects affected to the same degree the ttsP-AdV replication.
The restricted replication competent adenovirus (Ad5tetO$_7$CEA-E1A$_{\Delta pRB}$ and Ad5tetO$_7$SPB-E1A$_{\Delta pRB}$) expressed through the leaky ttsP activity the E1A(13S) protein. This protein mediate the transactivation of the ttsP and induce via autoactivation feedback loop an increased E1A(13S) expression and virus replication.

2. Based on these cognitions was introduced a tetracyclin-controlled system in the ttsP-AdV which enables a extrinsic transgene expression regulation. Through this system succeeded the control of the ttsP activity and ttsP-RRCA replication. Substantial findings of this work consist in the confirmation that the doxycyclin (Dox)-

controlled transcriptional silencer (tTS) can inhibit the ttsP-RRCA replication through the blocking of the leaky ttsP activity and through the arresting of the E1A(13S) transactivation. The tTS showed itself as an universal inhibitor of the ttsP activity. As long as is not possible a complete blocking of the leaky ttsP-RRCA replication, offers the use of a tetracyclin system in the ttsP-RRCA only a limited security for its use in tumor therapy.

3. Another therapy approach was tested by the insertion of the proapoptotic FasL gene in an adenoviral genome. Through the modified Dox-controlled gene expression system (Tight-system) succeeded an optimized regulation in the FasL expression. The new developed replication deficient adenoviral vector Ad5Tight-FasL caused effective apoptose via FasL expression under Dox induction in the cell line HeLa, whereas was not detect in non induction conditions, in contrast to the original TRE-system.
By the trials in lung cancer cells (H441, BEN and DMS53) it was possible to detect apoptose by the application of Ad5Tight-FasL, however was the effectivity 200 to 400 fold lower than in HeLa cells. In this respect possess lung cancer cells a relativ resistance against FasL-induced apoptose. The insertion of the FasL gene in an oncolytic adenovector may remedy this problem. Further research on the basis of FasL-expressed oncolytic adenovectors could demonstrate its applicability for cancer therapy.