cells that leads to cell death, enrichment of the remaining population of high bcl-2 expressing cells, and/or increased stability of BCL-2. In conclusion, G3139 treatment of PC-3 xenografts affected the *bcl-2-like/bax* and BCL-2/BAX at the mRNA and protein level. These *in vivo* effects observed at drug doses comparable with those used in the clinical trials (7 mg/kg/day) suggest that G3139 might be efficacious in androgen-refractory prostate cancer.

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Quantitative analysis of p53 targeted gene expression and visualization of p53 transcriptional activity following intratumoral administration of adenoviral p53 *in vivo*

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Objective: To analyze the mechanism of antitumor effects of adenoviral p53 (Ad-p53), we quantitatively assessed p53 targeted gene expression following intratumoral administration of Ad-p53 in nude mice.
Materials and Methods: 1) Human lung cancer (H1299) xenografts were established in nude mice, and treated by intratumoral administration of Ad-p53. We quantitatively assessed expression of p53 targeted gene including p21, MDM2, Noxa, and p53AIP1 by real-time quantitative RT-PCR and analyzed induction of apoptosis by TUNEL method 1, 2, 3, 7, and 14 days after the treatment. 2) We established H1299 cells expressing the GFP reporter gene under the control of p21 promoter (H1299/p53R-GFP). H1299/p53R-GFP tumors were treated with Ad-p53 as same. The transcriptional activity of exogenous p53 was visualized as intratumoral GFP expression in real-time manner by using 3CCD camera.
Results: 1) mRNA expression of p53 targeted gene (except p53AIP1) was maximum 1 day after Ad-p53 treatment, and then gradually decreased. Apoptosis was evident in situ 2 to 3 days after Ad-p53 treatment. 2) Maximal expression of GFP was achieved 3 days after the treatment, and it remarkably decreased on day 7.
Conclusions: We demonstrated that Ad-p53 treatment rapidly induced p53 targeted gene in tumors. We also succeeded to visualize p53 transcriptional activity *in vivo*. Quantitative analysis of p53 targeted gene expression and visualization of p53 transcriptional activity may be used to assess new therapeutic approaches.

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Ribozyme-PEI complex technology allows efficient down-regulation of gene expression *in vitro* and *in vivo*

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Ribozymes catalyze the sequence-specific cleavage of RNA molecules which is particularly attractive since this allows the effective abrogation of the expression of any selected protein. Therefore, ribozymes represent potentially interesting therapeutic agents in tumour therapy. Moreover, they offer powerful strategies in proteomics and target validation applications to analyse the functions of potential genes of interest, e.g. in tumour biology. So far, however, use of enzymatically active RNA molecules like hammerhead ribozymes has without chemical modification been severly hampered by ribozyme instability and poor cellular uptake. In this work, we introduce the, ribozyme-PEI complex technology' as a method for protection and cellular delivery of bioactive ribozymes. This is achieved by complexation fo ribozymes with polyethylenimines, i.e. synthetic branched polymers with high cationic charge density which form non-covalent complexes with nucleic acids and have been used so far only as DNA transfection reagents. We show that the ribozyme-PEI complex technology allows complete stabilization of ribozymes or any RNA against degradation. Upon their highly efficient cellular uptake, non-toxic PEI-complexed ribozymes display intracellular bioactivity already at low concentrations as demonstrated in tissue culture by down-regulation of two different genes in different cell lines. In particular, we describe a $\sim$ 60 % depletion of an FGF-binding protein (FGF-BP) which has been described previously as rate-limiting for tumor growth and metastasis in squamous cell carcinomas and serves as an, angiogenic switch molecule'. Likewise, the growth factor pleiotrophin (PTN) which is overexpressed in several tumors and which can be rate-limiting for tumor growth and angiogenesis, is efficiently downregulated in tissue culture upon targeting with PEI-complexed PTN ribozymes. In a mouse xenograft model, we show that upon i.p.-injection intact ribozymes are taken up by the tumor. This *in vivo* delivery of PEI-complexed anti-PTN ribozymes results in marked reduction of tumor growth and of intratumoral PTN levels. We describe the ribozyme-PEI complex technology as a novel, widely applicable method for exogenous delivery of any bioactive RNA ribozyme *in vitro* and *in vivo* without chemical modification.

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Gelsolin gene therapy using adenovirus vector for orthotopically transplanted human urinary bladder carcinoma in nude mice

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Gelsolin is an actin-regulatory protein that is undetectable or reduced in human urinary bladder tumors compared to normal epithelial cells (Tanaka, M. et al: Cancer Res, 55: 3228, 1995). Whether the over- expression of gelsolin could inhibit tumor growth was investigated in an orthotopic bladder cancer nude mouse model using recombinant adenovirus encoding wild-type gelsolin (Ad-GSN). Growth of the bladder cancer cells, KU-7 and UMUC-2 was inhibited when these cells were transduced with Ad-GSN *in vitro*. Flow cytometric analysis was conducted to examine the cell cycle after transducing the adenovirus. Over- expression of gelsolin caused these cells to arrest or delay at the G2/M phase of the cell cycle. *In vivo* cell growth was compared to control groups of these cells transduced with adenovirus containing the E. coli β-galactosidase gene (Ad-βgal). KU-7 cells were transplanted into the bladder of nude mice (Day 0) followed by three injections into the urethra (Day 2, Day 3, and Day 4) with either Ad-GSN or Ad-βgal at $1 \times 10(9)$ PFU. Eight days after initial adenovirus exposure (Day 10), each bladder was sectioned and stained then tumor mass digitally determined. In the orthotopic bladder cancer model, Ad-GSN treated animals had approximately 90 % less tumor mass compared to controls. Ad-GSN provided a significant tumor suppressive effect on human bladder cancer cells in this orthotopic nude mouse model. Adenovirus-mediated over- expression of gelsolin may be useful therapy for human bladder cancer.

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Inhibition of oncogenic RET signaling by Ad vector-mediated expression of a dominant-negative RET-mutant: implications for medullary thyroid cancer treatment

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Mutations in the RET proto-oncogene which lead to constitutive tyrosine kinase activity are the underlying cause for the development of medullary thyroid cancer (MTC). To date, treatment of MTC is primarily restricted to surgical removal of neoplastic tissue, which is often inefficient and enhances the risk of developing metastasis. To investigate an alternative strategy in MTC treatment, we took advantage of a dominant-negative RET (dn-RET) mutant, Ret51Flag, which inhibits oncogenic signal transduction by retaining the oncogenic RET protein in the endoplasmic reticulum (ER), thereby reducing the amount of oncogenic RET protein from the cell surface. We generated an adenoviral (Ad) vector expressing dn-RET51Flag protein under control of the C-cell specific synthetic calcitonin promoter (TSE2.CP1), shown to produce high levels of transgene only in thyroid C-cells (Ad-TSE2.CP1-dn-RET51Flag). Immunofluorescence microscopy in MTC-derived TT cells revealed restriction of the endogenous oncogenic RET in the ER combined with the lack of expression on the cell surface following virus infection, confirming the dominant-negative effect of dn-RET51Flag. Moreover, we observed a strong inhibition of cell viability which is partially dependent on cell growth inhibition and the induction of apoptosis. In nude mice, injection of Ad-TSE2.CP1-dn-RET51Flag treated thyroid cancer cells resulted in a significant suppression of tumor growth *in vivo*. In summary, our data indicate that succesful inhibition of oncogenic RET receptor tyrosine kinase expression by a dominant-negative RET-mutant might be a powerful approach for *in vivo* therapy of MTC. Supported by grant PU188/3-1/3-2 from the Deutsche Forschungsgemeinschaft.