

Effect of transcriptionally targeted suicide gene therapy for small cell lung cancer *in vitro* and *in vivo* by DOTAP/Cholesterol- and adenoviral-mediated delivery

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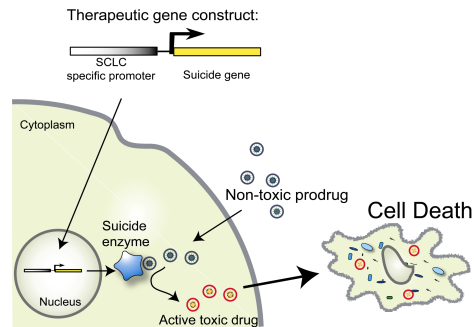


Aim

To develop an efficient gene therapeutic drug based on cancer specific promoters and suicide genes for the treatment of small cell lung cancer

Background

- Small cell lung cancer (SCLC) is characterized by aggressive progression, widespread metastasis and poor patient prognosis
- An efficient gene therapeutic drug for SCLC should be administered systemically in order to target metastasized tissue – however this requires a high level of targeting of the drug to avoid toxic side-effects to normal tissues
- We have identified SCLC promoter regions which confer highly specific expression of suicide genes in SCLC cells
- For transcriptional regulation of suicide genes we have chosen to use the Insulinoma-associated 1 (INSM1) promoter and the chimer promoter of the human achaete-scute homolog 1 and the enhancer of zeste homolog 2 (hASH1-EZH2) promoter



References:

- Pedersen et al, Cancer Research, 2003
 Pedersen et al, Cancer Gene Therapy, 2006
 Poulsen et al, Cancer Gene Therapy, 2008
 Christensen et al, Clinical Cancer Research, 2010

Results

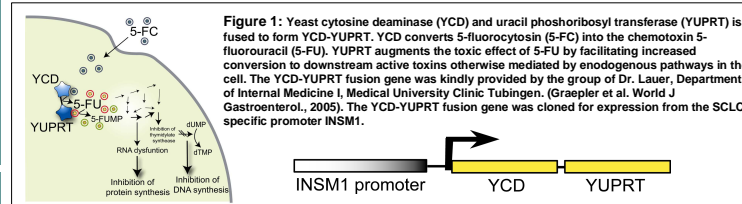


Figure 1: Yeast cytosine deaminase (YCD) and uracil phosphoribosyl transferase (YUPRT) is fused to form YCD-YUPRT. YCD converts 5-fluorocytosin (5-FC) into the chemotoxin 5-fluorouracil (5-FU). YUPRT augments the toxic effect of 5-FU by facilitating increased conversion to downstream active toxins otherwise mediated by endogenous pathways in the cell. The YCD-YUPRT fusion gene was kindly provided by the group of Dr. Laurer, Department of Internal Medicine I, Medical University Clinic Tubingen. (Graeppler et al. World J Gastroenterol., 2005). The YCD-YUPRT fusion gene was cloned for expression from the SCLC-specific promoter INSM1.

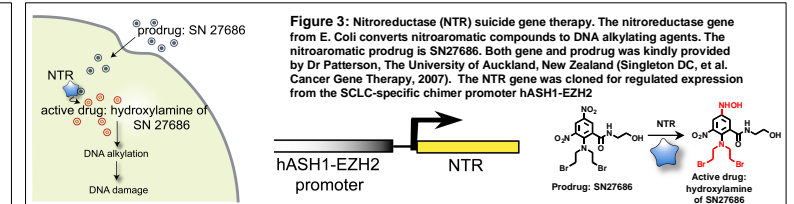
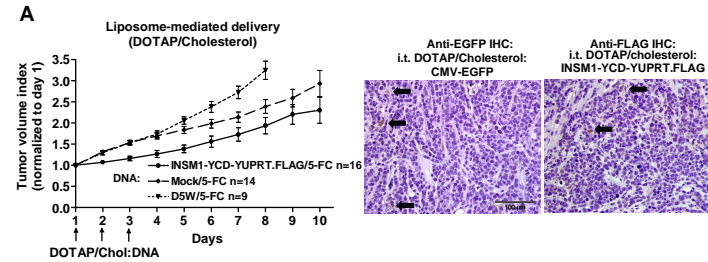


Figure 3: Nitroreductase (NTR) suicide gene therapy. The nitroreductase gene from *E. Coli* converts nitroaromatic compounds to DNA alkylating agents. The nitroaromatic prodrug is SN27686. Both gene and prodrug was kindly provided by Dr. Flaterson, The University of Auckland, New Zealand (Singleton DC, et al. Cancer Gene Therapy, 2007). The NTR gene was cloned for regulated expression from the SCLC-specific chimer promoter hASH1-EZH2

INSM1 promoter-driven YCD-YUPRT/5-FC therapy induces significant anti-tumor effect *in vivo* using a A) liposomal or B) adenoviral vector



TREATMENT PROTOCOL	TUMOR DOUBLING TIME (DAYS)	ANIMALS SURVIVING AFTER 10 DAYS
DOTAP/Cholesterol: INSM1-YCD-YUPRT/5-FC	8.5 (7.8;9.4)	6/12 - 50 %
DOTAP/Cholesterol: Mock/5-FC	6.3 (6.6;6)	3/11 - 27 %
DSW	4.8 (4.6;5)	0/7 - 0 %

Christensen et al, Clinical Cancer Research, 2010

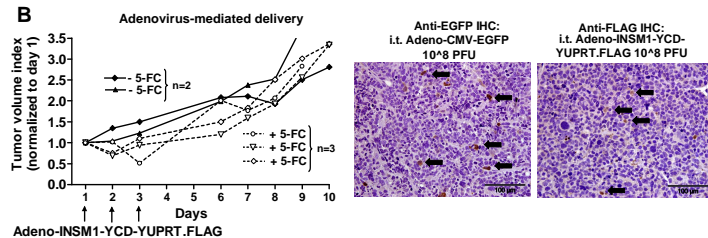


Figure 2: Xenografts were established from the SCLC cell line NCI-H69 by transplanting NCI-H69 xenograft tissue on nude NMR1 mice. When tumor volume reached 200-600 mm³ intratumoral (i.t.) injections were performed with INSM1-YCD-YUPRT.FLAG or mock (INSM1-LUC or CMV-EGFP) DNA either A) in plasmid form encapsulated in DOTAP:Cholesterol liposome or B) incorporated and packed in replication-deficient adenovirus. For both A) and B) treatment was performed for 3 consecutive days and from day 1 of treatment intraperitoneal (i.p.) injections of 500 mg/kg 5-FC was given daily until day 10 where animals were sacrificed due to tumor size. Tumor volume was measured by caliper. In A) a control group of mice received i.t. injections with DSW (isotonic glucose alone). In both A) and B) right panels show immunohistochemical (IHC) analysis of representative tissue. IHC was performed with anti-FLAG (i.t. INSM1-YCD-YUPRT.FLAG) and anti-EGFP (i.t. CMV-EGFP). Since CMV is a constitutive active promoter, anti-EGFP IHC served as transfection/transduction control.

NTR suicide gene therapy induces significant cytotoxicity in a panel of SCLC cell lines *in vitro*

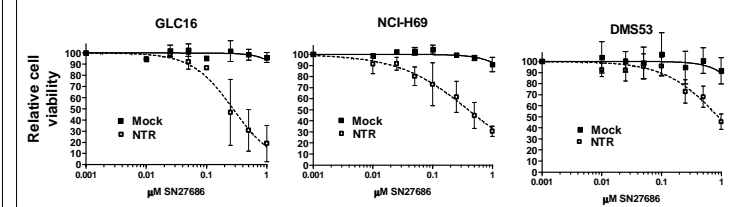


Figure 4: SCLC cells were transiently transfected (lipofectamine) with mock- or CMV-NTR plasmid and exposed to increasing doses of the prodrug SN27686. Cell viability was measured by MTT assay and data was normalized to control (0 µM prodrug) set to 100 %.

Combination of suicide gene therapy systems and conventional chemotherapy causes additive effects in SCLC cells *in vitro*

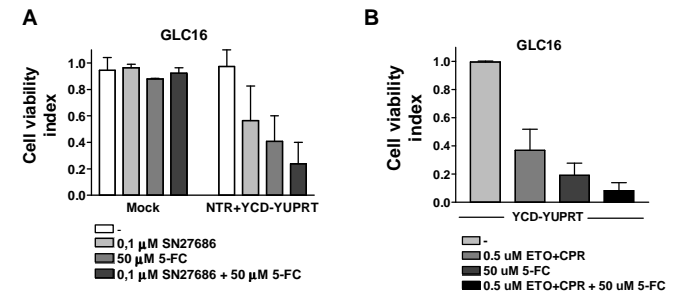


Figure 5: A) The SCLC cell line GLC16 was transfected (lipofectamine) with mock plasmid or co-transfected with CMV-NTR and INSM1-YCD-YUPRT plasmid and exposed to either SN27686, 5-FC or both in indicated doses. B) The SCLC cell line GLC16 was transfected (lipofectamine) with INSM1-YCD-YUPRT plasmid and exposed to etoposid and cisplatin (ETO+CPR), 5-FC or both in indicated doses. In both A) and B) Cell viability was measured by MTT assay read by 570 nm absorbance.

Conclusion

Transcriptionally targeted suicide gene therapy is a highly promising strategy for the treatment of SCLC