Potential of therapeutic genes for small cell lung cancer gene therapy

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Background

- Small Cell Lung Cancer (SCLC) is a highly aggressive cancer with poor prognosis.
- We have developed transcriptionally targeted gene therapy for SCLC utilizing promoters highly and specifically active in SCLC[1,2].
- This treatment protocol allows for systemic and specific treatment minimizing non-specific toxicity.
- To develop SCLC gene therapy it is apart from the developed targeting strategy, of crucial importance to identify efficient therapeutic gene candidates.
- Only very few therapeutic genes have so far been tested for SCLC gene therapy.

Aim

To test various therapeutic genes in vitro *for potential for SCLC gene therapy*

Materials and Methods

- Therapeutic genes were cloned for expression by the SCLC specific promoter, Insulinoma-associated 1 (INSM1) promoter or the unspecific promoters CMV and SV40.
- Gene constructs were transiently transfected into SCLC cell lines by Lipofectamine 2000 (Invitrogen).
- Gene expression was confirmed by western blotting.
- Cell survival was measured by a MTT assay.

Results

Tumor suppressor restoration and inhibition of oncogenes

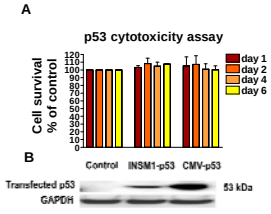

Fig. 1: A) SCLC cell line NCI-H69 transiently transfected with p53 and evaluated for cell death after 1,2, 4 and 6 days. B) Western Blotting for detection of p53 in transient transfected cells

- The tumor suppressor p53 had no therapeutic effect when transfected into SCLC cell lines (figure 1).
- Furthermore, no therapeutic effect was seen when the SCLC tumor suppressors Retinoblastoma (Rb), FHIT and FUS1 were tested (results not shown).

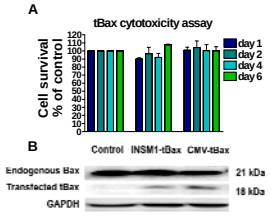

Fig. 2: A) SCLC cell line NCI-H69 transiently transfected with tBax3 and evaluated for cell death after 1,2, 4 and 6 days. B) Western Blotting for detection of tBax in transient transfected cells

- Inhibition of Bcl-2 by a truncated version of Bax (tBax) with increased apoptotic activity had no effect on cell survival (figure 2).
- Also inhibiting the protein family Hsp70 with a dominant negative version of Hsp40, normally an essential cochaperone for Hsp70, had no therapeutic potential (results not shown).

Results

Suicide gene therapy

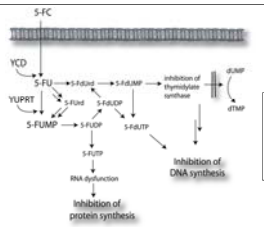

Fig. 3: Principle of suicide gene therapy with Yeast Cytosine Deaminase (YCD) and Yeast Uracil PhosphoRibosyl Transferase (YUPRT)[3] in combination with 5-fluorocytosine (5-FC). 5-FC is an analogue of cytosine.

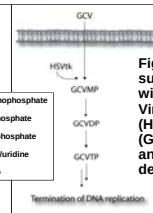

Fig. 4: Principle of suicide gene therapy with the Herpes Simplex Virus thymidine kinase (HSVtk) and ganciclovir (GCV). GCV is an analogue of deoxyguanosine.

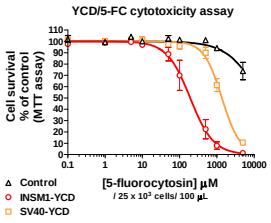

Fig. 5: SCLC cell line GLC16 transiently transfected with YCD after exposure to series of 5-FC concentrations for 7 days.

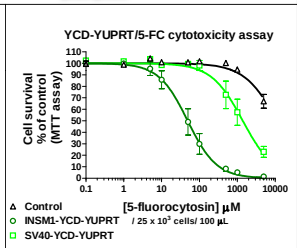

Fig. 6: SCLC cell line GLC16 transiently transfected with YCD-YUPRT after exposure to series of 5-FC concentrations for 7 days.

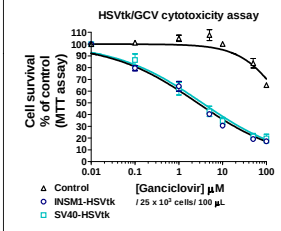

Fig. 7: SCLC cell line GLC16 transiently transfected with HSVtk after exposure to series of GCV concentrations for 7 days.

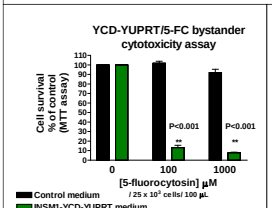

Fig. 8: SCLC cell line GLC16 exposed to culture medium from INSM1-YCD-YUPRT transfected cells incubated with 5-FC

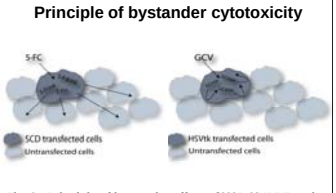

Fig. 9: Principle of bystander effect of YCD-YUPRT and HSVtk transfected cells, when exposed to prodrugs. Conversion of 5-FC allows in contrast to GCV for the spread of toxic molecules to nearby cells via diffusion

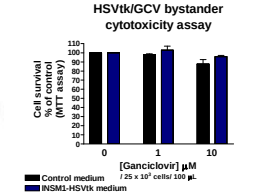

Fig. 10: SCLC cell line GLC16 exposed to culture medium from INSM1-HSVtk transfected cells incubated with GCV

- YCD/5-FC and YCD-YUPRT/5-FC demonstrate superior cytotoxicity to HSVtk/GCV in transient transfection experiments (figure 3-7).
- YCD-YUPRT/5-FC allows for the use of lower 5-FC concentrations than YCD alone without compromising therapeutic efficiency (figure 5-6).
- The pronounced therapeutic efficacy of YCD-YUPRT/5-FC is due to a strong bystander effect, killing nearby untransfected cells (figure 8-10).
- HSVtk suicide gene therapy is improved by the replacement of the prodrug GCV with another prodrug PCV, which do not display nonspecific toxicity (figure 11).

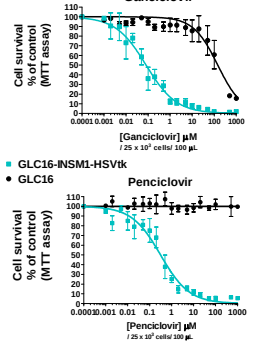

Fig. 11: SCLC stable cell clone GLC16-INSM1-HSVtk and the parental GLC16 cell exposed to series of GCV and PCV concentrations.

References:

1. Pedersen, N., *et al.* 2003. Transcriptional gene expression profiling of small cell lung cancer cells. *Cancer Res, 63*: 1943-53
2. Pedersen, N. *et al.* 2006. The insulinoma-associated 1: a novel promoter for targeted cancer gene therapy for small cell lung cancer. *Cancer Gene Ther, 13*: 375-84
3. Graepler, F.; *et al.* 2005. Bifunctional chimeric SuperCD suicide gene-YCD:YUPRT fusion is highly effective in a rat hepatoma model. *World J Gastroenterol., 11:6910-6919*

Conclusion

- *Targeting several biological aberrations of SCLC did not show potential for SCLC gene therapy*
- *Transcriptionally targeted suicide gene therapy is a very promising approach for treatment of SCLC*