

Therapeutic genes for small cell lung cancer gene therapy

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Background

- Small Cell Lung Cancer (SCLC) is a highly aggressive cancer and SCLC patients are characterized by having a very poor prognosis.
- Novel treatment strategies are therefore in great demand.
- Gene therapy represents a novel treatment strategy for cancer, but only very few therapeutic genes have been tested for SCLC gene therapy.
- Due to the metastatic nature of SCLC, treatment must be performed systemically, requiring a high level of specific targeting to minimize non-specific toxicity.
- We have developed transcriptionally targeted SCLC gene therapy utilizing promoters highly and specifically active in SCLC^{1,2}.
- The promoter regions permit high and exclusive expression of downstream genes in SCLC cell lines and tumors and will be utilized for expression of therapeutic genes for transcriptionally targeted gene therapy.

Aim

To test therapeutic genes with very different mechanisms of action in vitro for potential for SCLC gene therapy

Materials and Methods

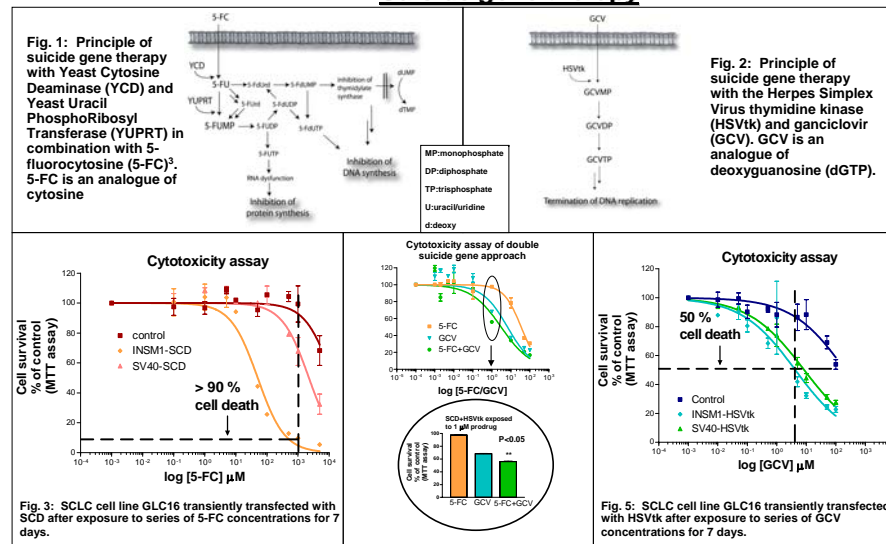
- Therapeutic genes were cloned for expression by the SCLC specific promoter, Insulinoma-associated 1 (INSM1) promoter or the unspecific promoters CMV and SV40.
- Gene constructs were transiently transfected into SCLC cell lines by Lipofectamine 2000 (Invitrogen).
- Gene expression was confirmed by western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

References:

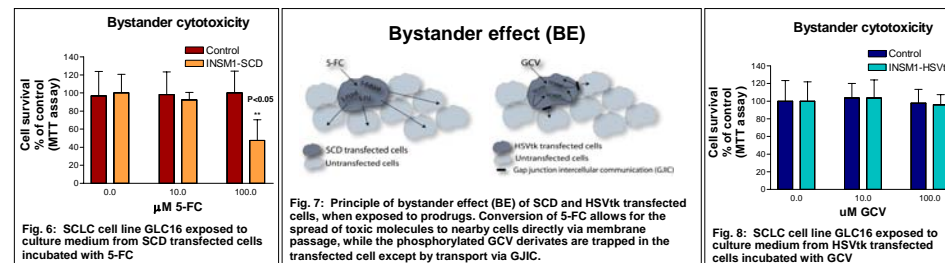
1. Pedersen, N., et al. 2003. Transcriptional gene expression profiling of small cell lung cancer cells. *Cancer Res*, 63: 1943-53
2. Pedersen, N. et al. 2006. The insulinoma-associated 1: a novel promoter for targeted cancer gene therapy for small cell lung cancer. *Cancer Gene Ther*, 13: 375-84
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Results

• Suicide gene therapy



- The YCD suicide gene strategy shows high potency for SCLC gene therapy causing more than 90% SCLC specific cell death. The fusion of YCD and YUPRT (SCD) was far superior to the YCD gene alone (Fig. 1 and 3).
- Dual transfection of HSVtk and SCD exerted synergy at specific prodrug concentrations (Fig. 4).
- The HSVtk/GCV suicide gene strategy shows potency for SCLC gene therapy mediating approximately 50% SCLC specific cell death (fig. 2 and 5). The strategy is improved by change of prodrug (Fig. 9).



- SCD/5-FC exerts a very prominent bystander effect (BE) by diffusion of the toxic metabolites to nearby cells (Fig. 6 and 7). The BE can overcome the low efficiency of gene delivery in vivo.
- HSVtk/GCV is highly dependent on gap junction intercellular communication (GJIC) for bystander effect (Fig. 7 and 8). GJIC is compromised in many cancers and only little BE can be expected. HSVtk/GCV bystander effect can only be achieved by upregulation of GJIC status in cancer cells.

• Suicide gene therapy

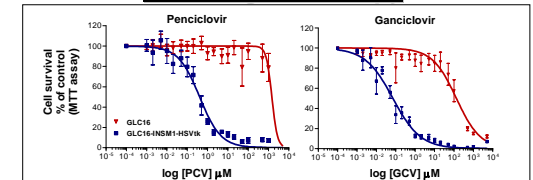


Fig. 9: SCLC stable cell clone GLC16-INSM1-HSVtk and the parental GLC16 cell exposed to series of GCV and PCV concentrations.

- The prodrug penciclovir (PCV) is less toxic to untransfected cells than GCV and greatly improves the HSVtk suicide gene therapeutic index (Fig. 9).

• Tumor suppressor restoration

- Functional loss of the tumor suppressors p53, Rb, FHIT and FUS1 (TUSC2) are observed in nearly 100% of SCLC.
- No effect was seen by the reintroduction of wild type p53, Rb, FHIT and FUS1 into SCLC cell lines.

• Inhibition of oncogene activity

- Oncogene activity of Bcl-2 was attempted abrogated by introducing a truncated form of the proapoptotic protein Bax (tBax).
- Hsp70 mediated cancer survival was attempted modulated by introducing a dominant negative Hsp40 J domain, which inhibits Hsp70 ATPase activity.
- These approaches only had minimal effect on cell survival.

Conclusion

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