Transcriptionally targeted cancer gene therapy for small cell lung cancer

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Background

- Small cell lung cancer (SCLC) is a highly malignant disease to which there currently is no satisfactory treatment.
- Treatment must be applied systemically, as the disease frequently is disseminated. Therefore treatment must be strictly targeted to the cancer cells.
- Targeted cancer gene therapy can be achieved by transcriptional regulation using cancer specific promoters controlling expression of therapeutic genes.
- Using a global gene expression analysis we have identified several genes inactive in normal adult tissues, but which are reactivated in SCLC[1]. We have cloned and tested the promoter regions from some of these genes.

Aim

To test the ability of different promoter regions to confer high and SCLC specific expression and cell death

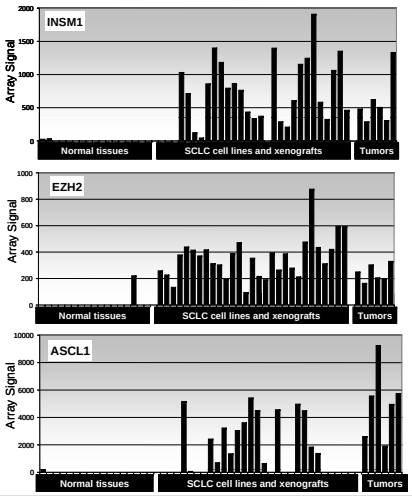

Fig. 1: Affymetrix gene expression analysis on representative normal tissues, SCLC cell lines, xenografts and tumours of 3 genes highly expressed in SCLC.

Results

- **Insulinoma associated-1 (INSM1)** is a nuclear transcriptional repressor, normally exclusively expressed transiently during early neuroendocrine development, but is upregulated in neuroendocrine cancers[2,3].

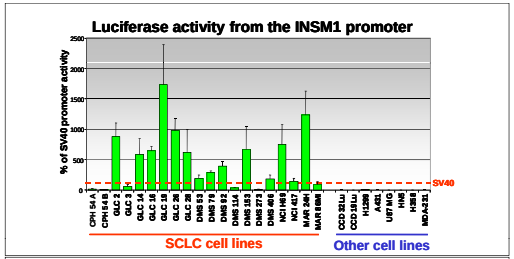

Fig. 2: Expression of a reporter gene from a 1.7 kb region of the human INSM1 promoter in SCLC and other cell lines.

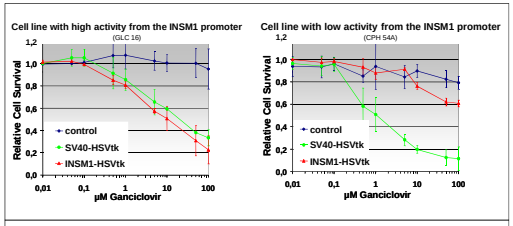

Fig. 3: Ganciclovir sensitivity of cell lines transiently transfected with HSVtk from the INSM1 promoter and the unspecific SV40 promoter.

- **The activity of the INSM1 promoter is extremely high in SCLC and absent in other normal and cancer cell lines**
- **The activity is sufficient to mediate SCLC specific cell death when regulating expression of a suicide gene[4].**

References:

1. Pedersen, N., *et al.* 2003. Transcriptional gene expression profiling of small cell lung cancer cells. *Cancer Res, 63:* 1943-53
2. Lan, M.S. *et al.* 1993. IA-1, a new marker for neuroendocrine differentiation in human lung cancer cell lines. *Cancer Res, 53,* 4169-71
3. Breslin, M.B. *et al.* 2003. NeuroD1/E47 regulates the E-box element of a novel zinc finger transcription factor, IA-1, in developing nervous system. J *Biol Chem., 278*:38991-7
4. 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
5. Bracken, A. P. *et al.* 2003. EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J, 22:* 5323-35
6. Chen, H. *et al.*1997. Tissue-specific expression of human achaete-scute homologue-1 in neuroendocrine tumors: transcriptional regulation by dual inhibitory regions. *Cell Growth Differ, 8:* 677-86

- **Enhancer of Zeste homolog 2 (EZH2)** is a member of the Polycomb group of proteins and is associated with proliferation[5]. EZH2 has been found highly overexpressed in a number of cancers, in particular prostate and breast.

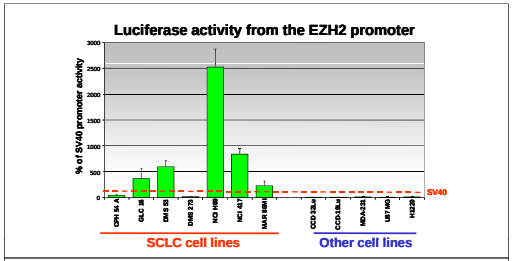

Fig. 4: Expression of a reporter gene from a 1.1 kb region of the human EZH2 promoter in SCLC and other cell lines.

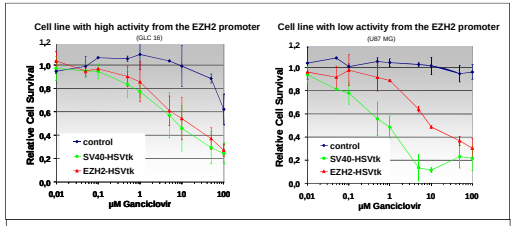

Fig. 5: Ganciclovir sensitivity of cell lines transiently transfected with HSVtk from the EZH2 promoter and the unspecific SV40 promoter.

Fig. 6: Western blot of expression of active RB and inactive ppRB in SCLC and other cell lines.

- **The activity of the 1.1 kb EZH2 promoter has been shown to be dependent on transcription factors of the E2F family.**
- **The activity of this promoter is very high in cell lines lacking functional RB (most SCLC) and low in cell lines with functional RB.**
- **The activity of the promoter is sufficient to mediate cell death, when regulating expression of a suicide gene with higher effect in SCLC cell lines.**

- **Achaete Scute Homolog 1 (ASCL1)** is a transcription factor involved in the early development of neural and neuroendocrine progenitor cells[6]. Expression of ASCL1 is highly upregulated in neuronal and neuroendocrine cancers

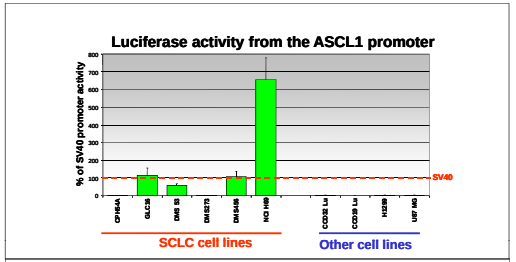

Fig. 6: Expression of a reporter gene from a 0.7 kb region of the human ASCL1 promoter in SCLC and other cell lines.

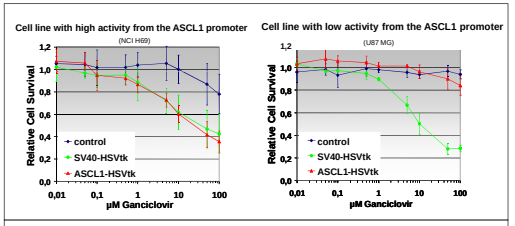

Fig. 7: Ganciclovir sensitivity of cell lines transiently transfected with HSVtk from the ASCL1 promoter and the unspecific SV40 promoter.

- **The activity of the ASCL1 promoter can confer high and SCLC specific expression and cell death.**

Conclusions

· Global gene expression analyses can identify genes from which the promoter regions can be used for high and cancer specific expression

· 3 promoters identified in this manner show potential for transcriptionally targeted cancer gene therapy