

Transcriptionally targeted cancer gene therapy for small cell lung cancer

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Background

- Small cell lung cancer (SCLC) is a 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.
- Cancer specific promoters are often associated with genes active during early development, silenced in the adulthood, but re-activated in cancers.
- Using a global gene expression analysis we have identified several genes re-activated in SCLC¹ and cloned their promoter regions.

Aim

- To test the ability of different promoter regions to confer SCLC specific expression.
- To test the capacity of the promoters to confer SCLC specific cell death.

Conclusions

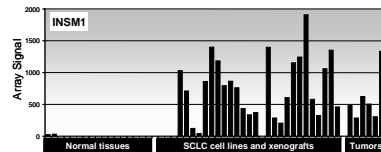
- We have demonstrated "proof of principle" for using global gene expression analyses for identification of cancer specific promoters.
- The INSM1 promoter is a novel candidate for transcriptionally targeted cancer gene therapy for SCLC.
- Other promoters show high activity, but specificity must be determined.

References

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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. 2005. The insulinoma-associated 1: a novel promoter for targeted cancer gene therapy for small cell lung cancer. *Cancer Gene Ther.* Jul 29. [Epub ahead of print]
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
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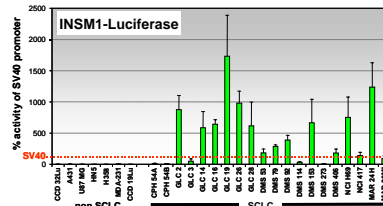
Results

- **Insulinoma associated-1 (INSM1)** is a nuclear transcriptional repressor, normally exclusively expressed transiently during early neuroendocrine development.
- The expression of INSM1 is regulated by tissue and developmentally regulated neuroendocrine transcription factors.
- INSM1 mRNA is highly re-expressed in SCLC cell lines and tumors with no expression in adult tissues².



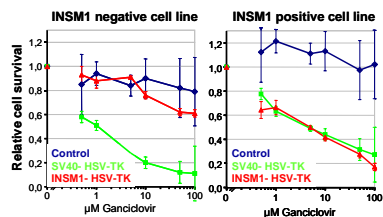
Affymetrix analysis of INSM1 expression

- A 1.7 kb region of the human INSM1 promoter has been shown to mediate correct spatial and temporal expression in transgenic mice³.
- Expression of reporter genes from this INSM1 promoter region showed very high expression in SCLC cell lines and no expression in other cell lines.



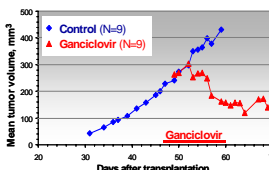
Luciferase assay after transient transfection

- The INSM1 promoter is sufficiently active to confer cell death in INSM1 positive cell lines, when regulating expression of a suicide gene (HSV-TK)⁴.



Transient transfection of cell lines with HSV-TK expressed from SV40 or INSM1 promoters and treatment with ganciclovir.

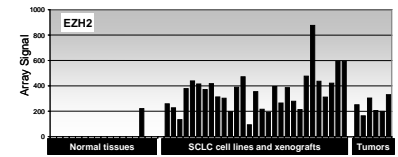
- The INSM1 promoter controlling expression of a suicide gene can cause tumor regression of xenografted tumors.



Tumor growth of xenografts of an INSM1 positive cell line stably transfected with INSM1-HSV-TK and treated with ganciclovir

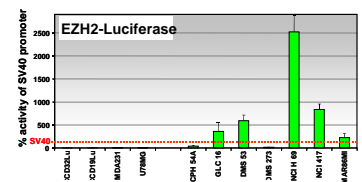
Results

- **Enhancer of zeste homolog 2 (EZH2)** is a member of the Polycomb group of proteins important for maintaining the silenced state of homeotic genes in the adult.
- EZH2 has been found highly overexpressed in a number of cancers, in particular prostate and breast.
- EZH2 mRNA is also highly expressed in SCLC and only in testes in the adult.



Affymetrix analysis of EZH2 expression

- A 1.1 kb region of the EZH2 promoter has been shown to be activated by members of the E2F family⁵.
- Expression of a reporter gene from this EZH2 promoter region showed very high expression in several SCLC cell lines with no expression in normal fibroblast cell lines. No expression was observed in the breast cancer cell line MDA 231.

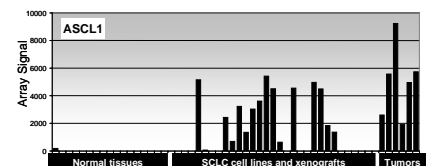


Luciferase assay after transient transfection

- This region of the EZH2 promoter is a potential alternative SCLC specific promoter, if further studies verify the initial observations

- **Achaete scute homologous protein (ASCL1)** is a transcription factor involved in the early development of neural and neuroendocrine progenitor cells.

- ASCL1 is highly upregulated in many cancers of neuronal and neuroendocrine origin.
- ASCL1 mRNA is highly expressed in SCLC, but only in the "classical" type with neuroendocrine features.



Affymetrix analysis of ASCL1 expression

- A 0.7 kb promoter region containing a proximal repressor⁶ gave high expression of a reporter gene in both "classical" and "variant" SCLC cell lines. Inclusion of a distal repressor region is currently being tested for activity and specificity.