A chimeric fusion of the human Achaete Scute Homolog 1 and Enhancer of Zeste Homolog 2 promoters is a promising regulator of suicide gene therapy for small cell lung cancer

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Background

- **Small Cell Lung Cancer (SCLC) is a highly malignant disease, with a poor prognosis and an urgent requirement for more effective treatments**
- **Suicide gene therapy by systemic delivery (Fig. 1) constitutes an experimental therapeutic strategy for SCLC**
- **To minimize non-specific toxicity it is of vital importance that the suicide gene is highly activated *exclusively* in the cancer cells, rendering normal cells resistant to treatment**
- **Cancer specificity can be accomplished by placing the therapeutic gene under the control of a gene regulatory region exclusively active in cancer cells: The cancer specific promoter**
- **The human Achaete-Scute Homolog 1 (hASH1) and Enhancer of Zeste Homolog 2 (EZH2) genes are highly and specifically active in SCLC, compared to normal tissues**
- **The hASH1- and EZH2 promoters constitute potential regulators for systemic SCLC gene therapy**

Aim

To investigate the use of hASH1- and /or EZH2 promoter regions for regulating suicide gene therapy of SCLC

Materials and Methods

Semiquantitative RT-PCR analysis: 25 cycles of amplification were performed using the Superscript RT III® and platinum Taq® polymerase systems (Invitrogen)

Promoter activity Luciferase assay: Promoter regions were cloned in front of the Luciferase reporter gene in pGL3Basic® (Promega), cells were transiently transfected and gene activity (Relative Light Units) was measured after 24 hours of incubation.

GCV-HSV-TK MTT cell proliferation assay: Transiently transfected cells were exposed to ganciclovir (GCV) for 5 days, cell proliferation was measured by MTT-assay (Sigma).

Western blot: was performed on 10µg total protein using the NuPage system (Invitrogen). 1º antibodies: Mouse monoclonal anti human Rb (BD Pharmingen) diluted 1:1000, Rabbit polyclonal anti human GAPDH (FL-335, Santa Cruz Biotech Inc.)

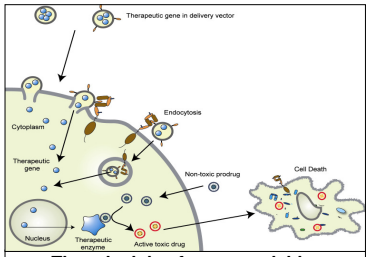
References and Acknowledgements:

[1] Chen H. *et al.* Cell Growth Differ. 1997;8:677-86

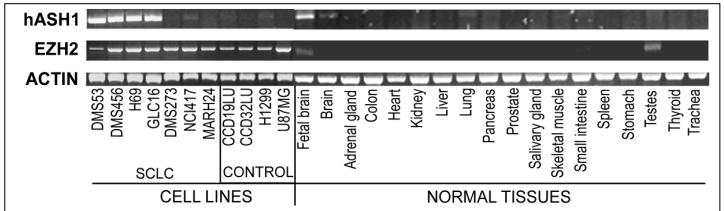
We thank Dr. K. Helin, BRIC, Copenhagen, Denmark for kindly providing the 1,1kb EZH2-promoter construct.

Selected results from this work were recently published:
Poulsen TT *et al.* Cancer Gene Ther. 2008; DOI:10.1038/cgt.2008.24

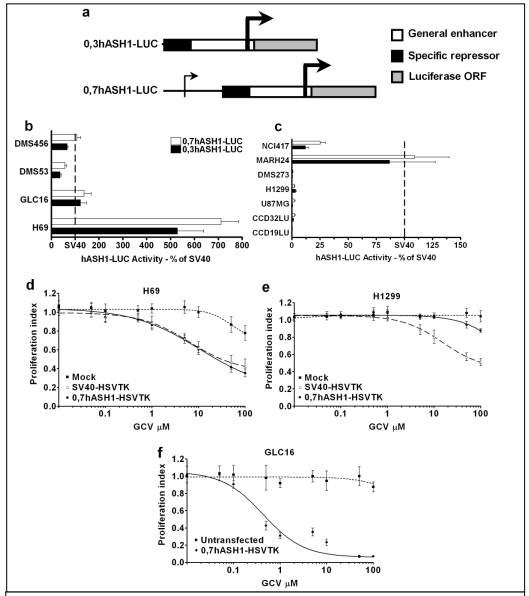
Results



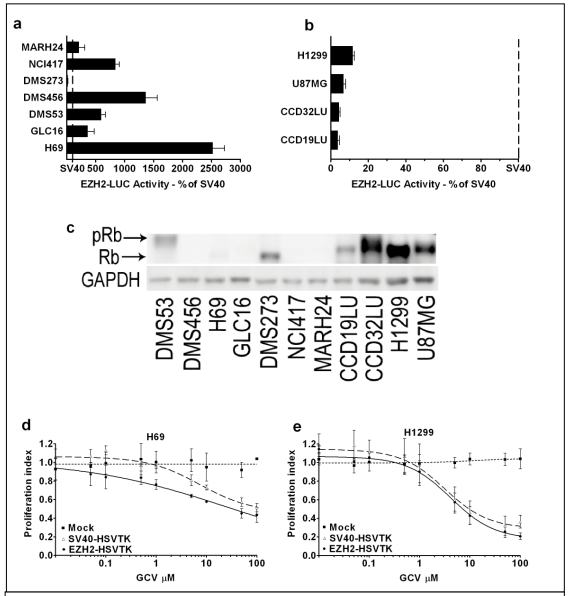
1 **The principle of cancer suicide gene therapy**



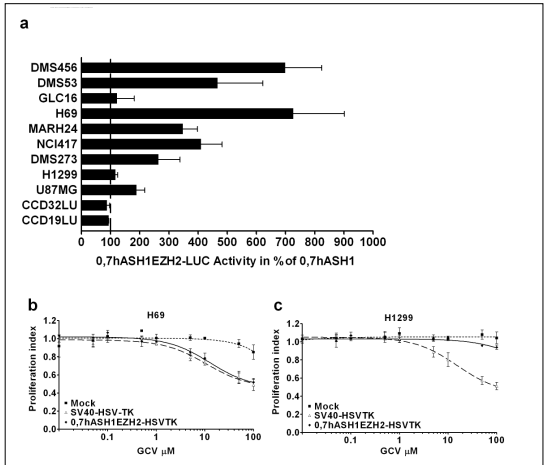
2 **Semiquantitative RT-PCR analysis of hASH1 and EZH2 mRNA levels in SCLC and control cell lines**



3 **hASH1-promoter-regulated reporter/suicide gene expression in SCLC and control cells.** (a) Overview of 0.3 and 0.7kb hASH1-LUC constructs with transcription start sites and regulatory elements as previously published[1]. (b, c) Reporter gene expression in percent of SV40-expression level in hASH1 positive SCLC (b) or control cell lines (c). (d, e) Proliferation of a SCLC (d) and control cell line (e) after transfection with 0.7hASH1-HSVTK gene and GCV incubation. (f) Proliferation of SCLC cells stably transfected with 0.7hASH1-HSVTK after incubation with GCV.Error bars represent s.e.m.



4 **EZH2-promoter-regulated reporter/suicide gene expression in SCLC and control cells.** (a, b) Reporter gene expression in percent of SV40-promoter expression level in SCLC (a) and control cell lines (b). (c) Western blot detection of inactive hyperphosphorylated Rb (pRB) and active hypophosphorylated Rb (Rb) in SCLC and control cell lines. GAPDH was used as loading control. (d, e) Proliferation of a SCLC (d) and control cell line (e) after transfection of EZH2-HSVTK gene and GCV incubation.
Error bars represent s.e.m.



5 **0.7hASH1EZH2-promoter-regulated reporter/suicide gene expression in SCLC and control cells.** (a) Reporter gene expression in percent of 0.7hASH promoter level alone for each SCLC and control cell line. (b, c) Proliferation of a SCLC (b) and control cell line (c) after transfection of 0,7hASH1EZH2-HSVTK gene -regulated HSVTK gene and GCV incubation. Error bars represent s.e.m.

Conclusions

- *A 0,7kb promoter region from the hASH1-gene is specifically active in SCLC*
- *A 1,1kb EZH2 promoter region is highly active in SCLC but not completely SCLC specific*
- *A hASH1-EZH2 chimeric promoter construct is highly and specifically active in SCLC and a promising regulator for SCLC gene therapy*

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