

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



Thomas Tuxen Poulsen, Nina Pedersen, Hans Skovgaard Poulsen

Department of Radiation Biology, Section 6321, Finsen Center, Copenhagen University Hospital, Copenhagen, Denmark, www.radiationbiology.dk

e-mail: tuxen@rh.dk

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). 1^o antibodies: Mouse monoclonal anti human Rb (BD Pharmingen) diluted 1:1000, Rabbit polyclonal anti human GAPDH (FL-335, Santa Cruz Biotech Inc.)

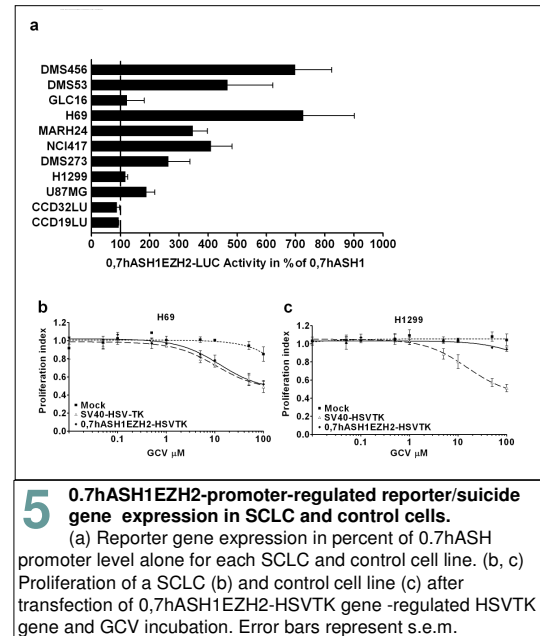
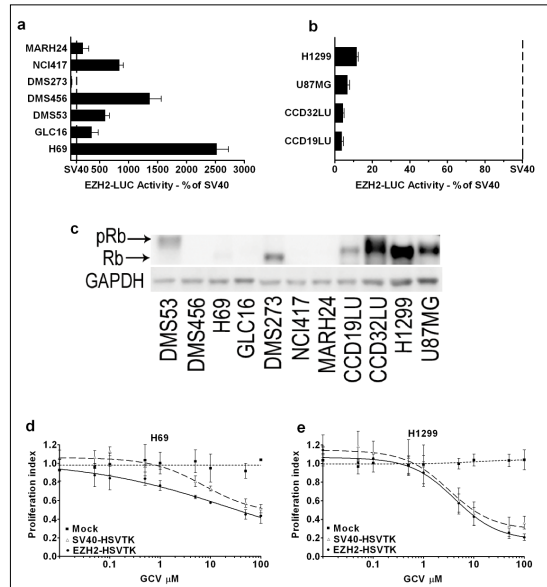
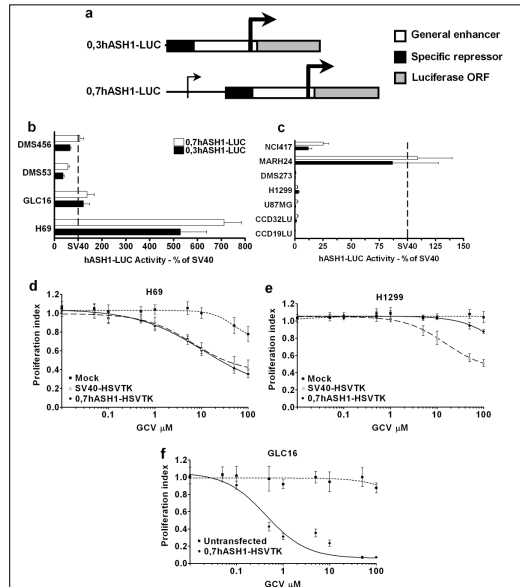
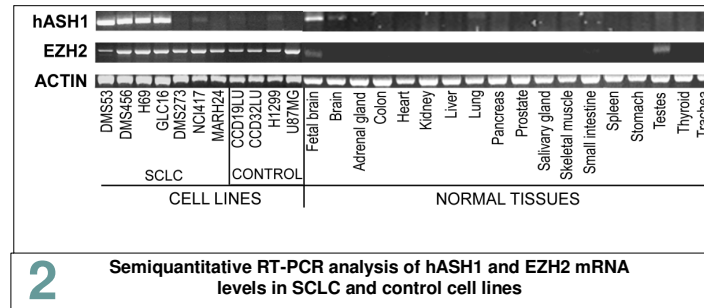
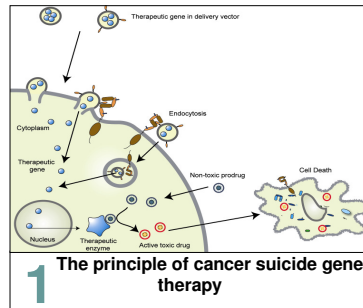
References and Acknowledgements:

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

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Results



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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