Insertion of transcription factor nuclear factor kappa B binding sites into plasmid DNA expressing a suicide gene facilitates increased small cell lung cancer cell death *in vitro* due to increased nuclear uptake

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Background

- Small cell lung cancer (SCLC) is a highly aggressive and metastasizing disease with a current 2-year survival rate below 15 %.
- New treatments are therefore in high demand and a promising strategy could be systemically delivered gene therapy.
- We have therefore developed a strategy based on transcriptional targeting of suicide genes to achieve specific cancer cell death[1,2,3].
- For systemic and repeated delivery of therapeutic DNA we aim to use a non-viral delivery vector.
- The nuclear membrane has been shown to constitute a major barrier when using non-viral delivery vectors and many strategies have therefore been developed to circumvent this problem. None of these strategies have however, been tested in relation to SCLC and suicide gene therapy.
- One such strategy utilizes the NFκB transcription factor system, observed to be highly active in SCLC[4]. The NFκB transcription factor will upon activation, be translocated to the nucleus due to its nuclear localization signal. By inserting NFκB binding sequences into plasmids, increased therapeutic gene nuclear translocation could potentially be

Aim

To investigate the potential of the NFκB DNA nuclear targeting sequence strategy for increasing nuclear translocation of therapeutic DNA in SCLC

Materials and Methods

- **Luciferase reporter gene (LUC) or a dual suicide gene system (SCD) was cloned for expression by the SCLC specific promoter, Insulinoma-associated 1 (INSM1).**
- **Gene constructs were transiently transfected by Lipofectamine 2000 (Invitrogen) *in vitro* or with DOTAP:Cholesterol *in vivo*.**
- **The MTT assay (Sigma) was used as an measurement of cell viability.**

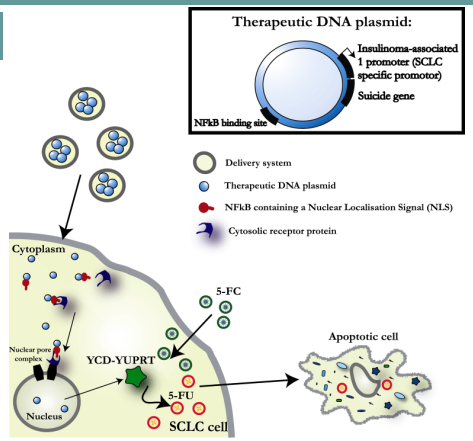

Figure 1: The principle of the implementation of the NFκB nuclear translocation strategy in a suicide gene therapy system: The sucide system consist of the yeast cytosine deaminase (YCD) fused to yeast uracil phosphoribosyltransferase (YUPRT). Both genes are under the regulation of the SCLC specific promoter INSM1. Once inside the cytoplams the therapeutic plasmids will be recognized and bound by the NFkB transcription factor. The NFkB transcription factor will in turn, due to its NLS signal, be recognized and bound by cytosolic receptor proteins. Hereafter, nuclear translocation will commence, resulting in an increase of therapeutic plasmids getting "dragged" into the nucleus. Once inside the nucleus, the transcription of the YCD and YUPRT gene will result in the production of "suicide" enzymes that converts the non-toxic prodrug, 5-flurocytosin (5-FC) into the toxic drug, 5-flurouracil (5-FU). The production of 5-FU results in SCLC cell death due to blocking of both DNA and RNA synthesis.

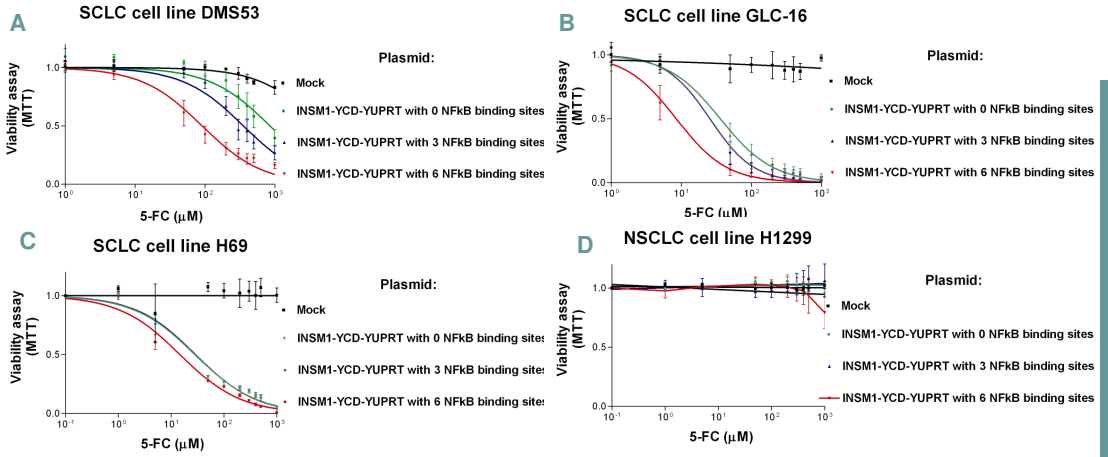

Figure 2: Effect of NFκB-binding inserts in a suicide gene expressing system. A-C) To further investigate if this strategy could improve the efficacy of non-viral suicide gene therapy, NFκB DNA binding sites were cloned into an expression plasmid containing the YCD-YUPRT suicide system and either 0 (green), 3 (blue) or 6 (red) NFκB binding site insertions. Cells were exposed to increased concentrations of the prodrug 5-flurocytosin (5-FC). A significant larger proportion of the SCLC cells died when treated with the suicide gene plasmids utilizing the nuclear translocation strategy compared to the original system. **D)** To investigate whether the insertion of NFκB binding sites compromised the SCLC specificity of the INSM1 promoter, the system was tested in the non-SCLC cell line H1299. The viability remained unchanged regardless of NFκB binding insertions, proving that the nuclear translocation strategy does not compromise the SCLC specificity of the suicide system.

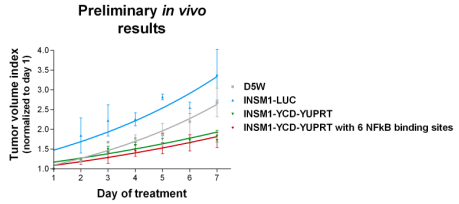

Figure 3: Preliminary *in vivo* results. A SCLC xenograft model was established in nude NMRI mice with the SCLC cell line DMS53. The 4 treatment groups were treated for 4 consecutives days with intratumoral injection (i.t) injections of 100 µl of either: 5 % glucose (D5W), INSM1-LUC (mock), INSM1-YCD-YUPRT (original suicide system) or with INSM1-YCD-YUPRT with 6 NFκB binding sites (original suicide system with the implementation of the NFκB translocation system). From day 1 of i.t injections 350 mg/kg of 5-FC were injected intraperitoneally twice a day. Tumor size were measured daily with caliper.

Results

- For INSM1-promoter containing vectors, different numbers of NFκB-binding sequences were tested. The maximum effect of the NFκB-DTS resulted in an approximately 10 X decrease in 5-FC IC50 value when implemented in a suicide gene system (figure 2.A)
- Furthermore, the results show a positive correlation between the number of inserts and the increase of gene expression (figure 2.A-C)
- The SCLC specificity of the INSM1-promoter was not compromised by the insertions of NFκB-binding sites (figure 2.D).
- Preliminary *in vivo* results, indicate that the suicide system results in DMS53 tumor growth delay and that the implementation of the NFκB strategy may increase the efficacy of the gene therapy treatment (figure 3).

Conclusion

Implementing the NFκB DNA nuclear targeting sequence in a suicide gene strategy resulted in a significant increase of SCLC cell death *in vitro*.

Transcriptionally targeted suicide gene therapy with an active nuclear translocation strategy could be a promising approach for treatment of SCLC.

References:

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