# Optimization of non-viral gene delivery to the nucleus for small cell lung cancer gene therapy

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## Background

- Small cell lung cancer (SCLC) is a highly aggressive and metastasizing disease with a poor prognosis.
- 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 therapeutic genes to achieve specific cancer cell death[1,2].
- 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.
- One such strategy utilizes the NFκB transcription factor system, observed to be highly in SCLC[3]. 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 achieved by "hitching a ride" (figure 1).

## 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 was cloned for expression by the SCLC specific promoter, Insulinoma-associated 1 (INSM1).**
- **Gene constructs were transiently transfected into the DMS53 SCLC cell line by Lipofectamine 2000 (Invitrogen).**
- **Reporter gene expression was based on the luciferase reporter assay (Promega).**

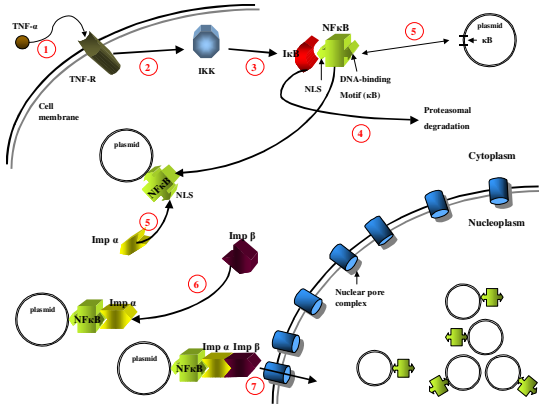


**Figure 1: The principle of the NFκB DTS nuclear translocation strategy:** The NFκB system can be activated by e.g. the inflammatory cytokine tumor necrosis factor-α (TNF-α) (1), which leads to the activation of the IκB kinase complex (IKK) (2). Activated IKK hereafter phosphorylates the inhibitor (IκB) that shields both the nuclear localization signal (NLS) and DNA binding domain of the NFκB transcription factor(3). The phosphorylation of IκB leads to its degradation (4) and unmasking of both the DNA binding domain and NLS of NFκB. This enables NFκB to recognise and bind to the NFκB-binding-site inserted into the transfected plasmid. Active NFκB is recognised by the karyopherin importin-α (5). Next, importin-β will bind to importin-α (6), commencing nuclear translocation of the complex, including the plasmid, through a nuclear pore complex (7).

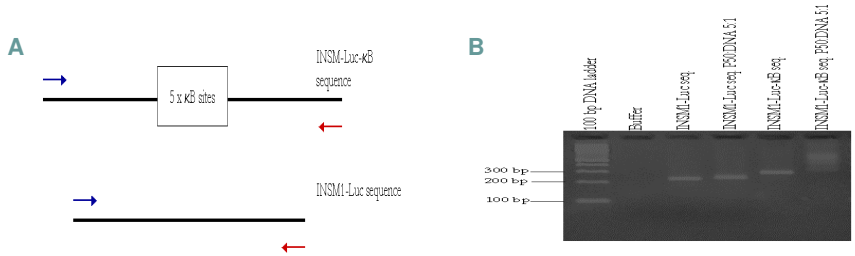


**Figure 2: Functionality verification of the NFκB binding sequence inserted into the plasmid constructs used for nuclear translocation assay. A)** In order to verify the functionality of the inserted κB sequences, two PCR products derived from the SCLC specific INSM1-promoter containing plasmid with and without the sequence containing 5 κB sites were compared. **B)** A clear gel-shift occurs only in the lane containing the INSM-Luc-κB sequence with p50, one of the most common NFκB subunits, indicating specific binding of p50 to the inserted NFκB binding sites.

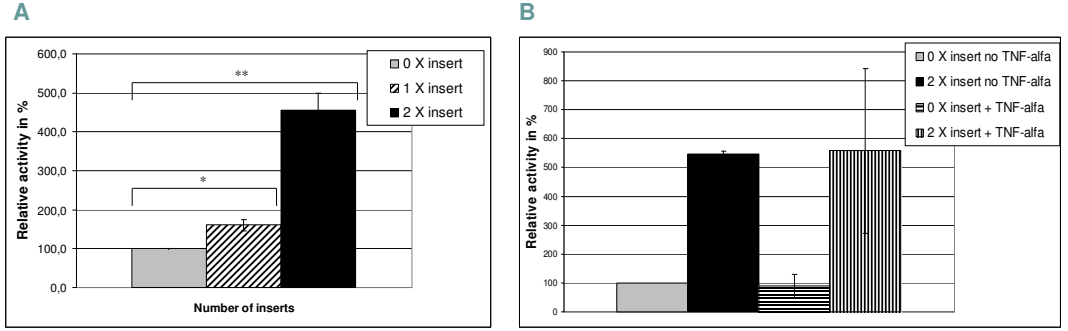


**Figure 3: Effect of NFκB-binding inserts on reporter activity (1 insert equals 5 NFκB binding sites) A)** In order to investigate if the insertion of NFκB-binding sites increased transcription, transfections were performed with plasmids containing the luciferase gene and either 0, 1 or 2 inserts. Relative activity is RLU per µg protein normalized to control (plasmid with 0 inserts set to 100 %). ∗ (comparison of 0 and 1 insert): $P < 0.05$, ∗∗ (comparison of 0 and 2 inserts: $P < 0.0005$ **B)** Addition of TNF-α did not result in any further increase of the luciferase expression from the INSM1-Luc plasmid with 2 inserts.

## Results

- For INSM1-promoter containing vectors, different numbers of NFκB-binding sequences were tested. Functionality and specificity of the NFκB-binding sequence were performed by a gelshift assay using the p50 NFκB subunit (figure 2).
- The maximum effect of the NFκB-DTS resulted in a 4-6 fold increase of reporter gene expression in the SCLC cell line DMS53 (figure 3.A).
- Furthermore, the results show a positive correlation between the number of inserts and the increase of reporter gene expression (figure 3.A).
- No further increase of reporter gene expression could be obtained by activating the classical NFκB system pathway with the addition of TNF-α (3.B).

## Conclusion

- **Implementing the NFκB DNA nuclear targeting sequence strategy in SCLC *in vitro*, resulted in a 4-6 fold increase of reporter activity.**
- **Transcriptionally targeted suicide gene therapy with an active nuclear translocation strategy could be a promising approach for treatment of SCLC.**

**References:**

1. **Pedersen, N., *et al* 2003. Transcriptional gene expression profiling of small cell lung cancer cells. *Cancer Res, 63*: 1943-53**
2. **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**
3. **Tang, X., *et al* 2006. Nuclear Factor-κB (NF- κB) is frequently expressed in lung cancer and preneoplastic lesions. *Cancer, 107*: 2637-46**