Transfection of 3T3-L1 cells with METAFECTENE si

siRNA mediated suppression of beta2-syntrophin (SNTB2) in 3T3-L1 cells

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Introduction:

SNTB2 is a cellular adaptor protein affecting several receptors. The aim of the study was to analyse whether suppression of SNTB2 in immature adipocytes and subsequent differentation affects the phenotype of mature cells.

Materials and methods:

METAFECTENE si, 3T3-L1 adipocytes, X-treme Gene siRNA Transfection (Roche)
Cultivation medium: DMEM (4.5g glucose/l) 10% NCS (*Newborn Calf Serum*), 1% Penicillin/ Streptomycin
Differentiation medium: DMEM/Ham's F, 12.5% NCS (*Newborn Calf Serum*), 1% Penicillin/ Streptomycin, 2 ng/ml apotransferrin, 17 nM panthotenic acid, 10 nM biotin, 30 ng/ml fetuin, 100 nM insulin, 1 nM corticosteron, 200 nM ascorbat, 2.5 nM IBMX

Experimental procedures / transfection protocol:

- 3T3-L1 cells (50,000 cells per well) were seeded in 6-well plates and cultivated over night
- Medium was removed and 800 µl differentiation medium was added.
- Transfection mix was prepared as indicated in the protocol (180µl 1x si-buffer + 12µl Metafectene si + 10µl siRNA (10µM) = 202µl) and was incubated for 15 min at room temparature
- Transfection mix was added drop-wise to the adherent cells while gently swirling the plate
- After 72 h medium was changed and cells were differentiated for 4 additional days to obtain mature adipocytes

Results and discussion:

When X-treme Gene siRNA Transfection (Roche) was used SNTB2 was reduced by about 50%.

Using METAFECTENE si transfection reagent we observed highly efficient knock-down of SNTB2. Toxicity was only observed in SNTB2 siRNA treated cells.

Conclusion / summary:

We suggest that METAFECTENE si is a highly efficient transfection reagent for 3T3-L1 cells.

Appendix:

Cell code	**Primary**	**Class**	**Species**	**Organ**	**Type**	**Identifi-cation**	**Reagent**	**Growth Properties**	**Genetic Material**	**Efficiency**	**Toxicity**
3T3-L1	no	mammalian	mice	Fat	Adipose tissue	Fibroblast Adipocyte	METAFECTENE si, X-treme Gene siRNA Transfection	adherent	siRNA	Not determined	No

X-treme Gene siRNA Transfection

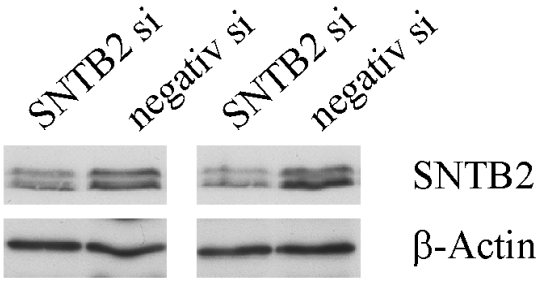

Fig. 1: SNTB2 knock-down using X-treme Gene siRNA Transfection reagent. Cell viability was not affected (not shown).

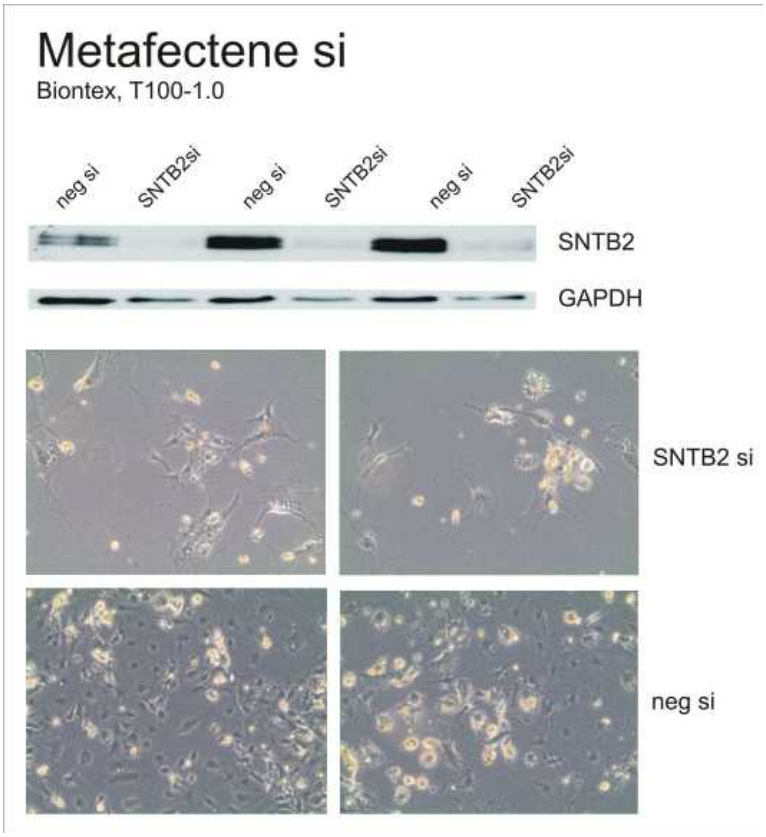

Fig. 2: SNTB2 knock-down and cell morphology of differentiated cells using METAFECTENE si transfection reagent.