

Lentiviral transduction of rat adipose-derived stem cells for stable production of TRAIL in the tumour microenvironment in an in-vitro model of breast cancer



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Rat adipose-derived stem cells (RADSC)

Adipose-derived stem cells (rADSC) play a multifaceted role in cancer metabolism and constitute a potential target for oncologic therapies.

TNF-related apoptosis inducing ligand (TRAIL)

Mediates its effect via death receptors (DR4/DR5) leaving non-tumoral cells unharmed, underlying its potential as a therapeutic candidate. In healthy and TRAIL-resistant cancer cells this pathway is blocked by decoy receptors such as soluble osteoprotegerin (OPG), through interference with caspase 8 by FLICE-inhibitory protein (FLIP).

Aim of the study

This in-vitro study aimed to evaluate an oncologic treatment based on the delivery of TRAIL-transduced RADSC to the vicinity of breast adenocarcinoma cells (RBA cell line).

Methods

TRAIL-pathway signalling

- Gene expression study of OPG, CFLIP and DR5 relative to GAPDH, LDHA, NONO in RADSC and RBA cell lines using qPCR (SYBR Green, ThermoFisher)

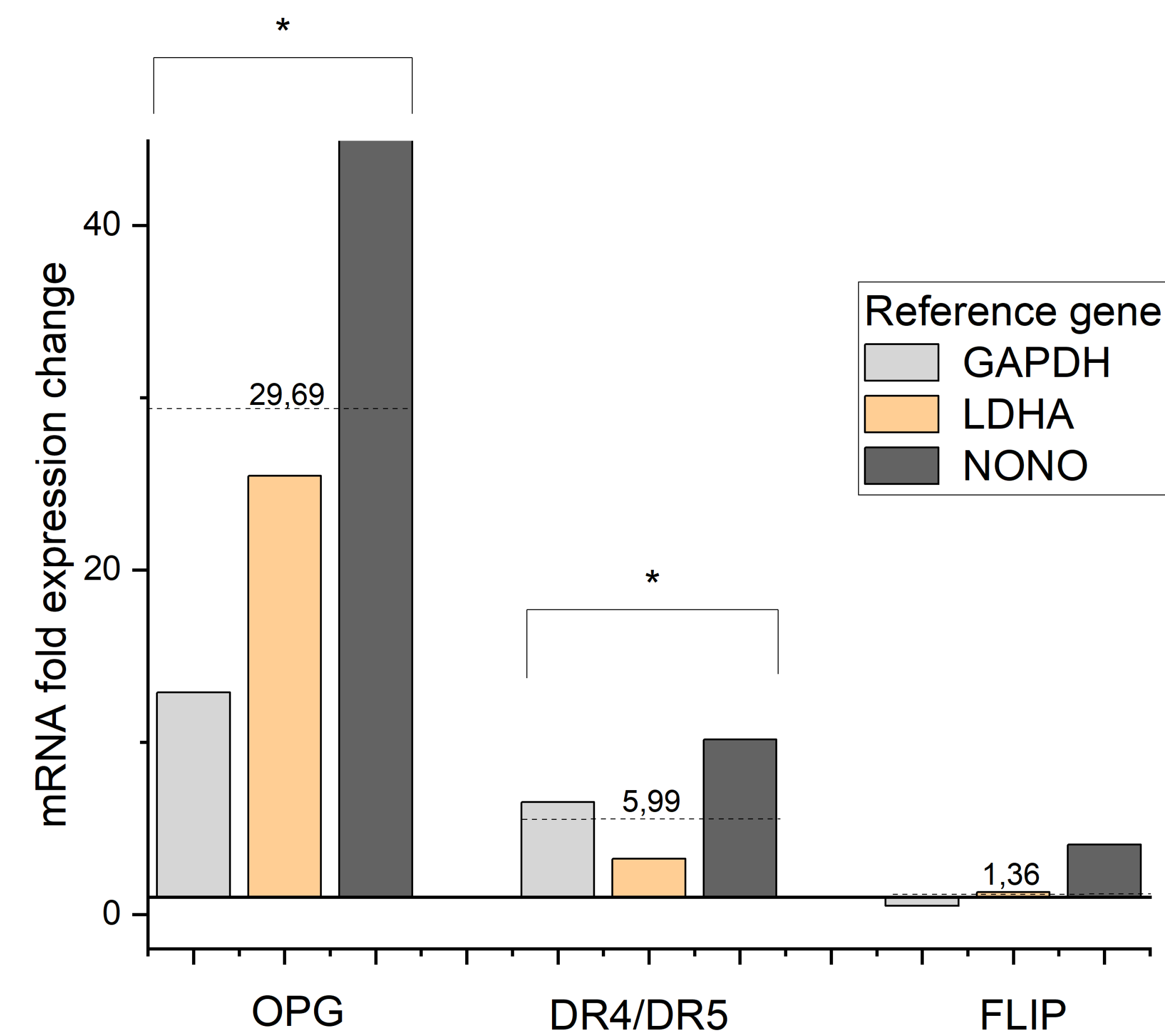
Lentiviral transduction of RADSC

- Transduction using lentiviral particles containing desired TRAIL-transgen (fourth-generation packaging system, Takara Bio)

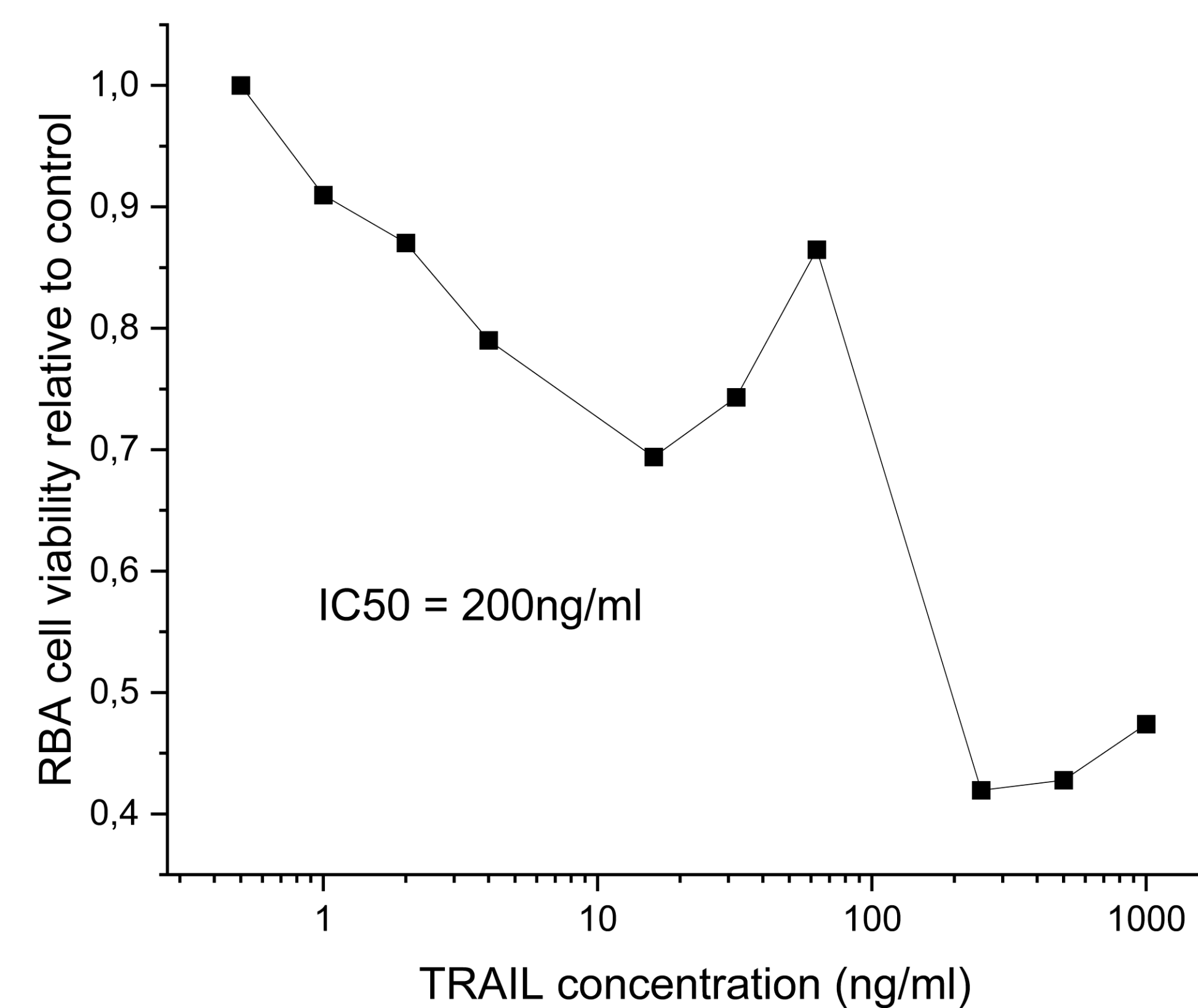
Cancer cells susceptibility to TRAIL

- Cell viability assessed after 48 hour exposure to rat recombinant TRAIL with variable concentration ranging from 0.01 to 1000 ng/ml. Measured using MTS colorimetric method (Promega)

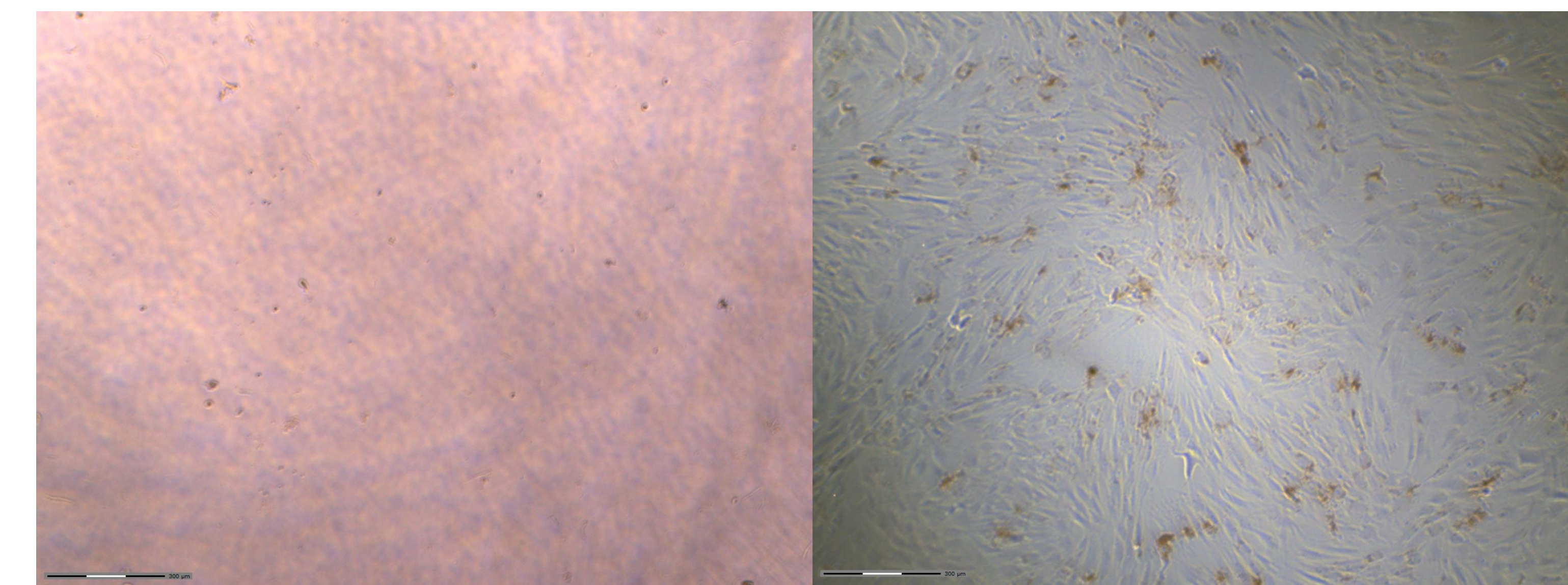
TRAIL-signalling pathway in RADSC and adenocarcinoma of mammary gland cells



TRAIL activity against adenocarcinoma of mammary gland cells



Successful transduction of RADSC



Puromycin selection (4 days), left- control, right- RADSC-TRAIL

Conclusion

- TRAIL-signalling pathway proteins are expressed at higher rate in RADSC compared to RBA
- Overexpression of osteoprotegerin may constitute the reason for TRAIL-resistance in RADSC
- Adenocarcinoma of mammary gland cells are susceptible to TRAIL after prolonged exposure (48 hours)
- RADSC can be lentivirally transduced and survive puromycin selection
- Transduced RADSC synthesise transgene for prolonged period of time