THYROID RECEPTORS ANTAGONIZE TGFβ ACTIONS IN VIVO AND IN CULTURE CELLS

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ABSTRACT

Transforming growth factor beta (TGFβ), which plays a key role in cancer and fibrotic disorders, mediates its actions mainly through activation of Smad transcription factors, which bind to Smad Binding Elements (SBEs) in target genes. We have previously observed that the thyroid hormone T3 blocks transactivation of SBE-containing reporter plasmids by TGFβ, and represses transcription of endogenous TGFβ target genes. We have now analysed the thyroid hormone receptor (TR) isoform as well as the receptor domains responsible for this transcriptional antagonism. In GH4C1 pituitary cells, the TRβ specific agonist GC1 is as potent as T3 to repress SMAD-dependent transactivation, showing that this isoform mediates the antagonism. However, expression of TRα in other cell types also mediates repression of TGFβ-dependent transactivation by T3. Therefore, both receptor isoforms can antagonize TFGβ actions. Using TRα and TRβ mutants we observed that the receptor DNA binding domain (DBD) is essential to carry out the repressive effect, whereas the ligand-dependent transcriptional activation domain responsible for coactivators binding appears to be dispensable. We also detected a direct interaction of TRα and TRβ with Smad2/3 and Smad4. The DBD plays an important role in this interaction, which is reversed by T3. In chromatin immunoprecipitation (ChIP) assays with SBE-containing promoters, T3 inhibits TGFβ–dependent recruitment of Smads. TRs bind to the SBE-containing regions and this interaction is also released by T3. We had observed that hyperthyroidism alleviates the fibrotic response induced by bleomycin in mice skin. We have now examined if TR signalling could also impact liver fibrogenesis. Indeed, 18-month-old knock-out mice lacking TRα and TRβ exhibited a spontaneous injury phenotype with increased collagen deposition, demonstrating that the endogenous receptors play a role in vivo as inhibitors of this TGFβ-dependent response.

T3 blocks SMAD-dependent transactivation and represses TGFβ–induced endogenous gene expression

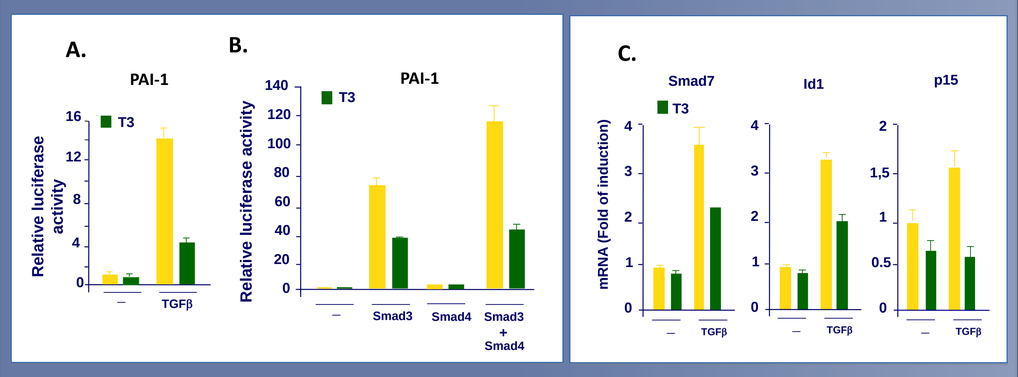

Figure 1. A. Luciferase activity in pituitary GH4C1 cells transfected with the PAI-1 promoter treated with T3 (5nM) for 40h and with TGFβ (10ng/mL) for the last 5h. **B.** Cells were transfected with the reporter plasmid and Smad3 and Smad4 expression vectors and treated with T3 for 40h. **C.** Smad7, Id1 and P15 mRNA levels were determined in GH4C1 cells treated with T3 and/or TGFβ.

Both thyroid receptors isoforms, TRα and TRβ, can mediate transrepression

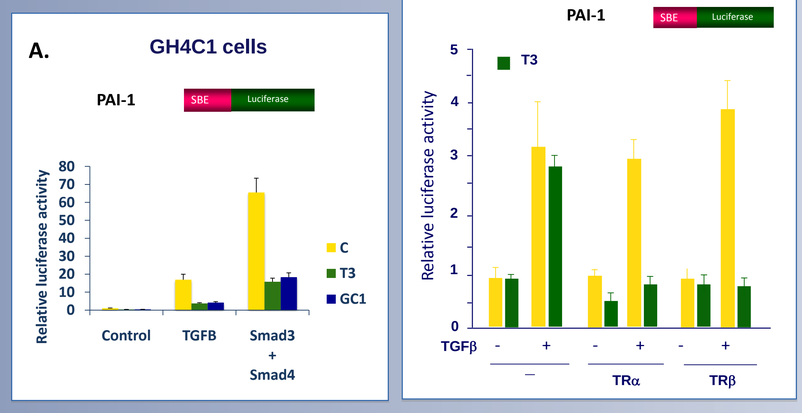

Figure 2. A. Luciferase activity in GH4C1 cells treated with T3 or the thyroid receptor β specific agonist GC1 (5nM) for 40h, and with TGFβ for the last 5h. **B.** HaCaT cells were cotransfected with the reporter plasmid and expression vectors for TRα or TRβ. Cells were treated with T3 and TGFβ as indicated.

T3 releases TRs binding to SBE-containing promoters and reduces TGFβ–dependent recruitment of Smads

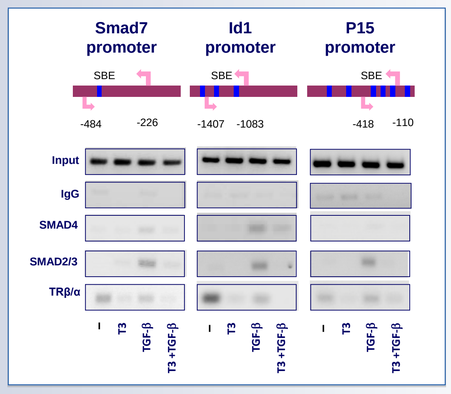

Figure 3. GH4C1 cells were treated with T3 and/or TGFβ for 1h. Chromatin immunoprecipitation (ChIP) assays were carried out with non-specific serum (IgG), Smad4, Smad2/3 and TRα/β antibodies. Primers amplifying the SBE-containing regions of the Smad7, Id1 and P15 promoters indicated at the top of the figure were used.

The TRα/β DNA Binding Domain (DBD) is involved in transrepression by T3

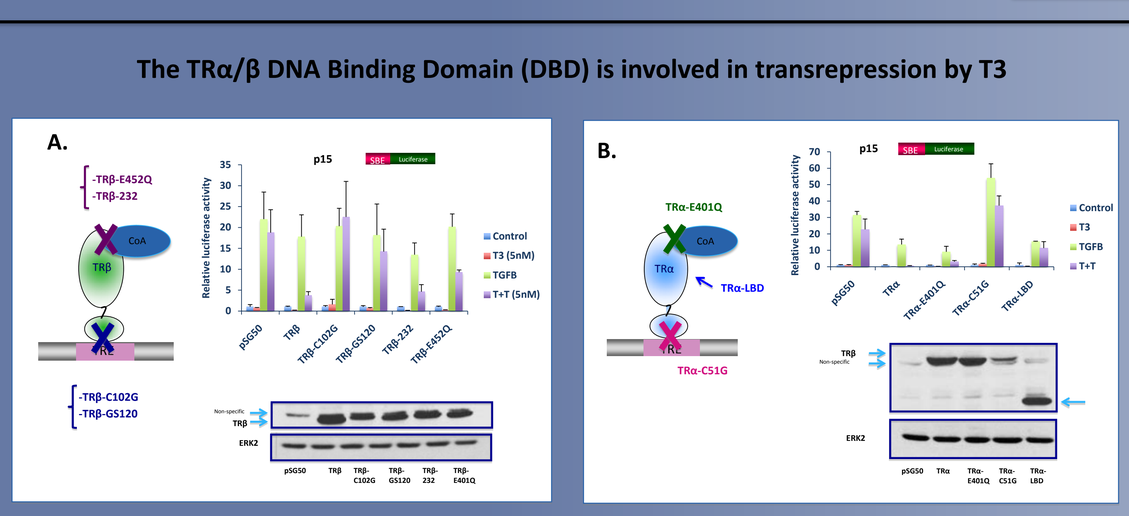

Figure 4. A. MvLu cells, widely used to study TGFβ actions, were transfected with a reporter plasmid, which contains the p15 promoter, and expression vectors for wild-type TRβ , the DBD mutants TRβ-C102G and TRβ-GS120, and TRβ-E452Q and TRβ-232 mutants that are not able to recruit coactivators. Cells were treated with T3 for 40h and with TGFβ for the last 5h. **B.** The same experiment with the TRα mutants, TRα-C51G, TRα-E401Q, and the TRα-LBD, which lacks the N-terminal region including the DBD.

The DBD is involved in direct protein to protein interactions between TRs and Smad transcription factors

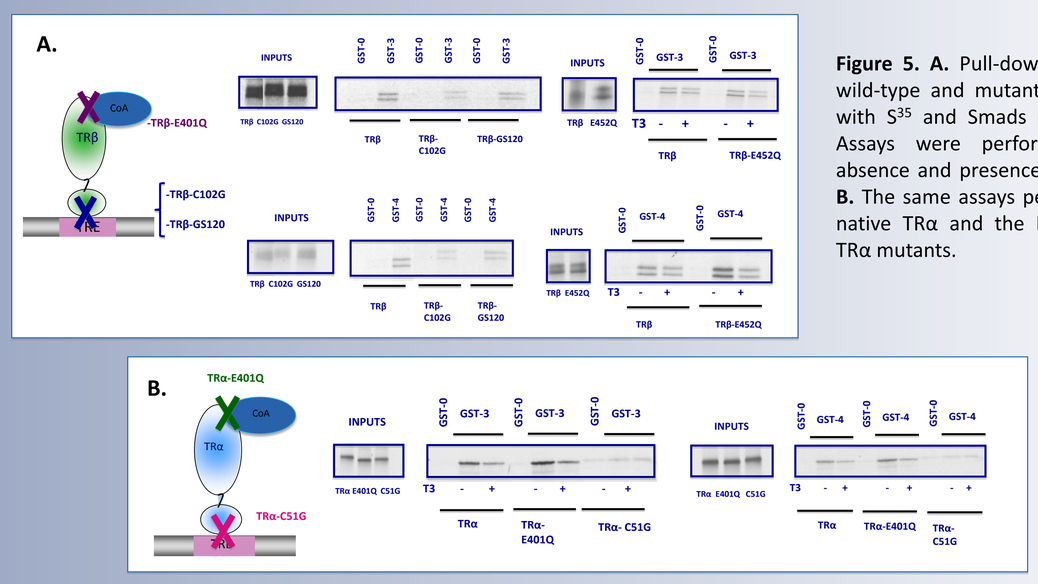

Figure 5. A. Pull-down assays with wild-type and mutant TRβ labelled with S^{35} and Smads fused to GST. Assays were performed in the absence and presence of T3 (1μM). **B.** The same assays performed with native TRα and the DBD and LBD TRα mutants.

Hyperthyroidism reverses the fibrotic response in mice skin

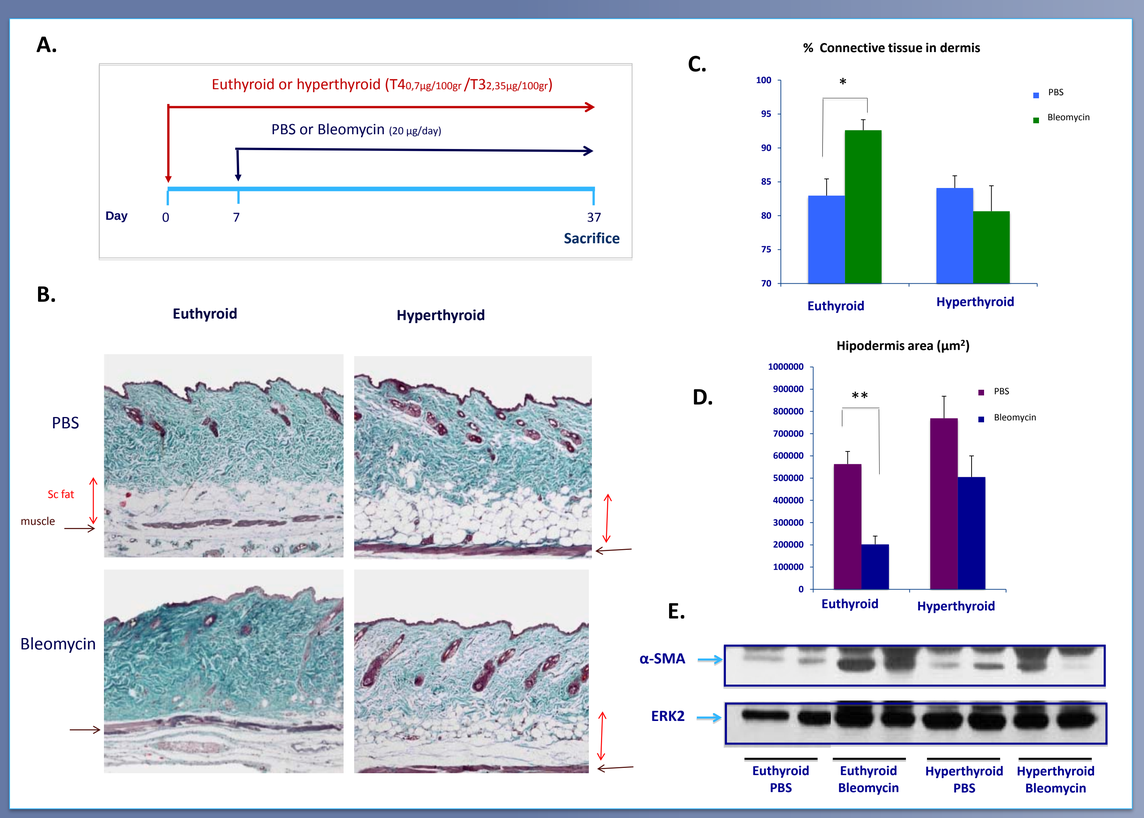

Figure 6. A. Scheme of the experimental design. T3 and T4 were added to the drinking water of mice and bleomycin or PBS were injected daily subcutaneously in the dorsal skin. **B.** Masson trichrome staining showing infiltration of subcutaneous (Sc) fat by collagen fibers in euthyroid (but not hyperthyroid) animals treated with bleomycin. **C.** The percentage of the connective tissue in the dermis is increased in euthyroid, but not in the hyperthyroid animals treated with bleomycin. **D.** Quantification of the hipodermis area, which is not reduced in bleomycin–treated hyperthyroid mice. **E.** Western blot with α-SMA (α-Smooth muscle actin) in the skin of the different groups.

Old knock-out mice lacking TRα1 and TRβ exhibit a spontaneous liver injury phenotype with increased collagen deposition

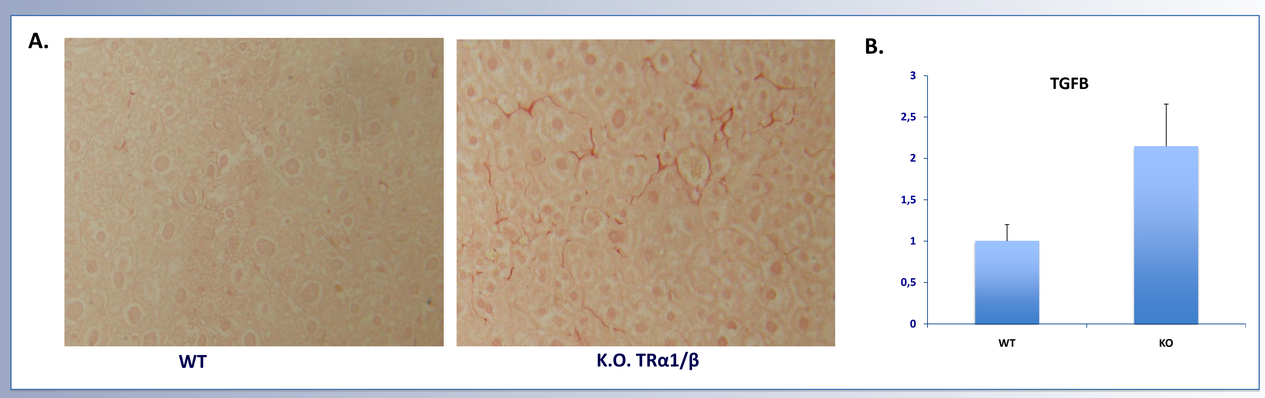

Figure 7. A. Picrosirus red staining (collagen staining) in livers of 18 months-old wild type and TRα1/β knock-out mice. Collagen fibers are only observed K.Os. **B.** Liver TGFβ mRNA levels in both groups.

CONCLUSIONS

- **Both TRβ and TRα can antagonize TGFβ dependent transcription, in a ligand dependent manner.**
- **Thyroid receptors are bound to SBE-containing TGFβ target promoters. Binding is released by T3. T3 also reduces Smad2/3 and Smad4 recruitment induced by TGFβ.**
- **The TRβ/α DNA Binding Domain is involved in the repression of TGFβ-induced transcription by T3, while the interaction with coactivators appears to be dispensable.**
- **There is a direct protein-protein interaction between the receptors and SMADs, in which the receptor DNA binding domain plays an important role. This interaction is reduced by T3.**
- **Hyperthyroidism alleviates fibrosis development in mice skin, while there is spontaneous collagen deposition in livers of old mice lacking TRα1 and TRβ. Therefore, hormone-bound TRs can antagonize TGFβ-dependent actions *in vivo*.**