Sox9 is expressed in thyroid follicular cells and regulates the expression of thyroid differentiation genes.

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INTRODUCTION

Sox9 is a transcription factor of the HMG box DNA binding family essential for chondrocyte differentiation, sexual determination and the maintenance of the stem/progenitor cells in several tissues. Furthermore, Sox9 is involved in the development of endoderm-derived-organs. Due to the endodermic origin of the thyroid, we planned to study the expression of Sox9 in thyroid follicular cells and its regulation by TSH , TGF β 1 and by thyroid transcription factors. Furthermore, we studied its role in the regulation of the expression of thyroid differentiation genes.

MATERIALS AND METHODS

-<u>Cell systems</u>: Rat thyroid follicular cells PCCl3 maintained in Coon's medium 6H (5% Donor serum, +TSH, +IGF-1), Coon's 4H (0.2% Donor serum, -TSH, -IGF1). Hela cells grown in DMEM medium supplemented with Fetal Bovine Serum at 10%. TSH, IGF1, Forskolin and TGFβ were added to the medium. H89, the PKA specific inhibitor, was added 1.5 hours before stimulous.

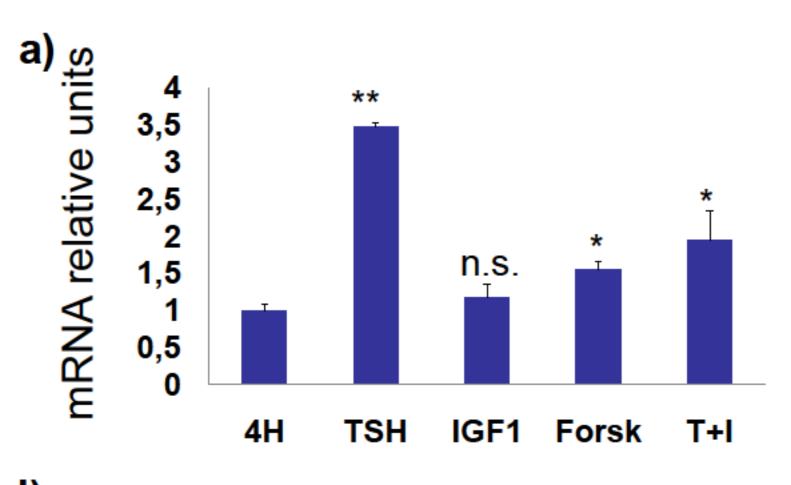
-DNA binding Activity: Electrophoretic Mobility Shift Assay

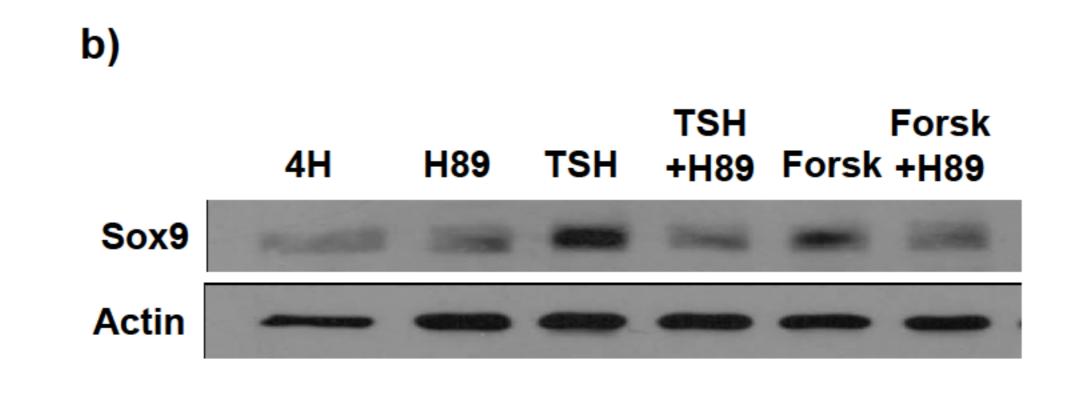
-Protein Detection: Western-Blot -mRNA detection: RT-qPCR

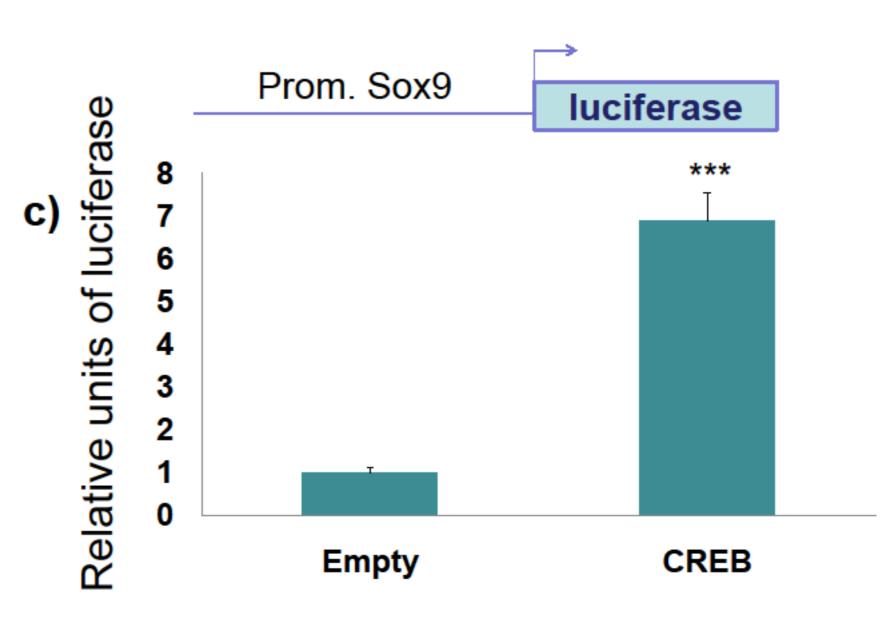
-<u>Transfection assays:</u> Determination of the luciferase activity of the construction pGL3-promRatSox9 transfected in PCCI3. Co-transfections assays were performed with different promoter constructions and expression vector of CREB, Pax8, Nkx2-1, FoxE1,Smads and Sox9.

RESULTS

1) TSH induces Sox9 expression throught cAMP/CREB signalling pathway







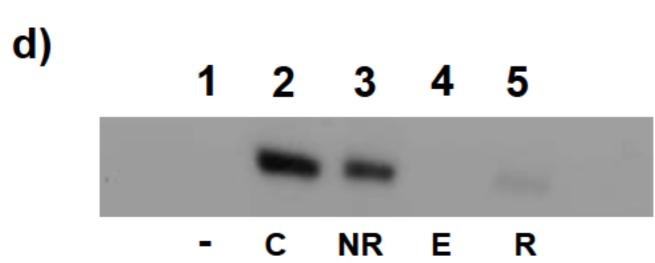
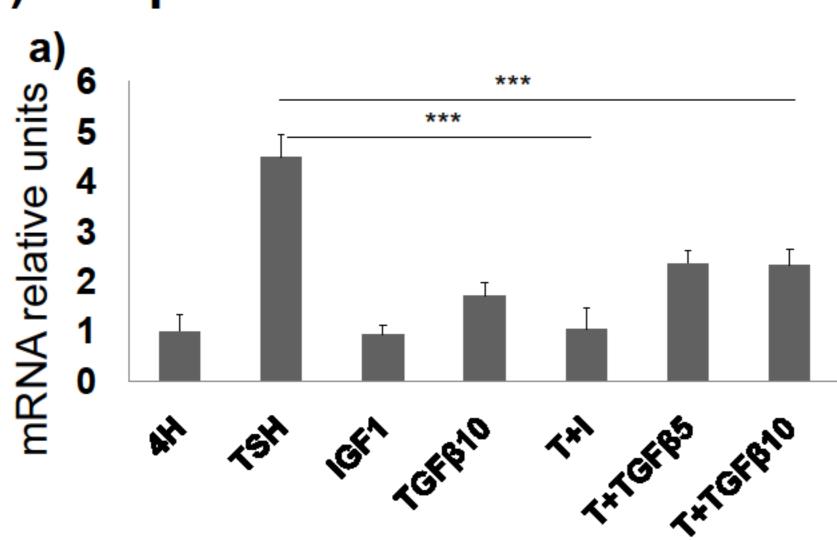
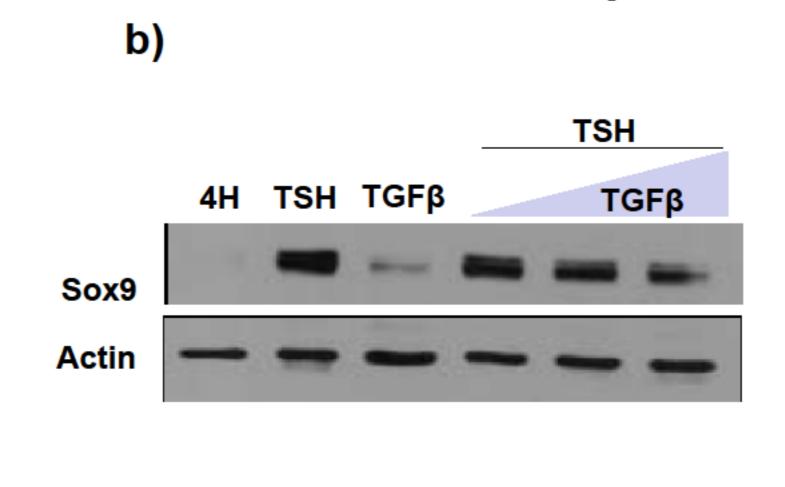


Fig.1: (a) RT-qPCR of Sox9 mRNA in PCCL3 maintained 48 hours in 4H medium and treated during 2 hours with different stimuli. (b) Western-Blot of Sox9 protein in PCCl3 cells maintained 48 hours in 4H medium and treated during 24 hours with different stimuli. (c) Co-transfection in Hela cells with pGL3-promSox with/without CREB expression vector. (d) CREB binding activity to the Sox9 rat promoter was determined by EMSA. The probe was incubated without (lane 1) or with 5 µg of CREB purified protein (lanes 2–5). Competition was performed with a100-fold molar excess of unlabeled unrelated (NR; lane 3) or related (R;lane 5) oligonucleotide.

2) TGFβ1 and IGF1 inhibit the inductive effect of TSH in Sox9 expression





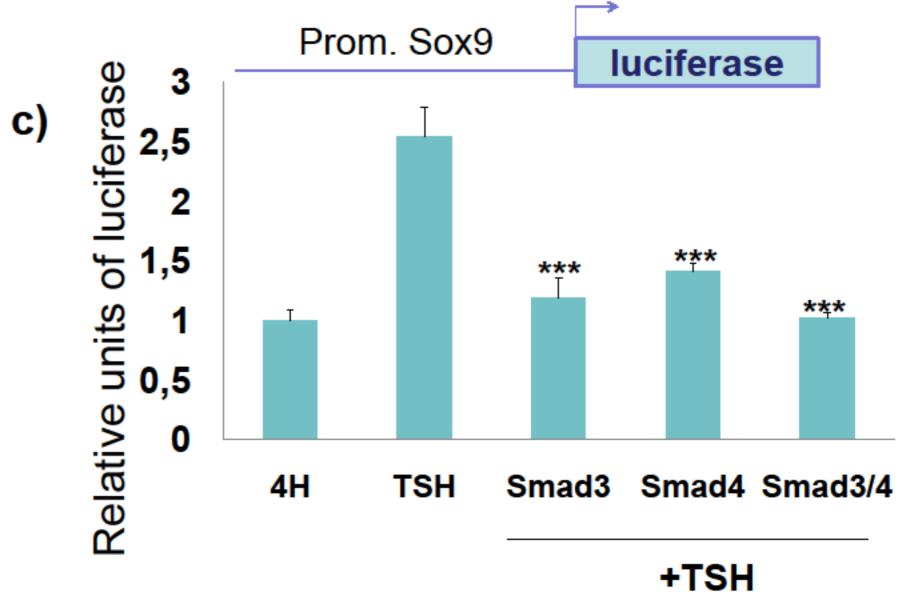
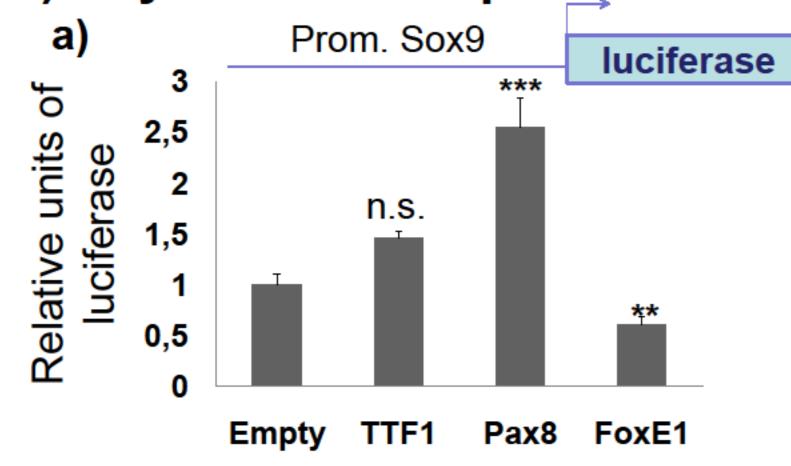
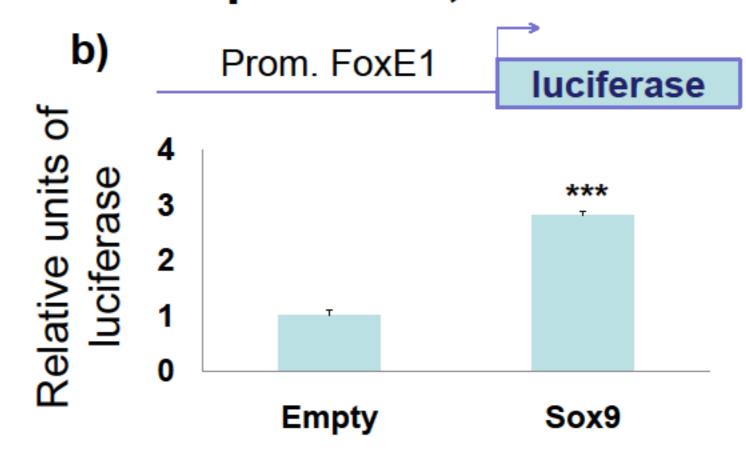


Fig.2: (a) RT-qPCR of Sox9 mRNA levels of PCCl3 cells maintained 48 hours in 4H medium and treated during 2 hours with different stimuli. (b) Western-Blot of Sox9 protein in PCCl3 cells maintained 48 hours in 4H medium and treated during 24 hours with different stimuli (c) Co-transfection in PCCl3 cells with pGL3-promRatSox9 and Smad expression vectors. Cells were treated with TSH 24 hours.

3) Thyroid transcription factors regulates Sox9 expression, which also regulates FoxE1 and Pax8 expression





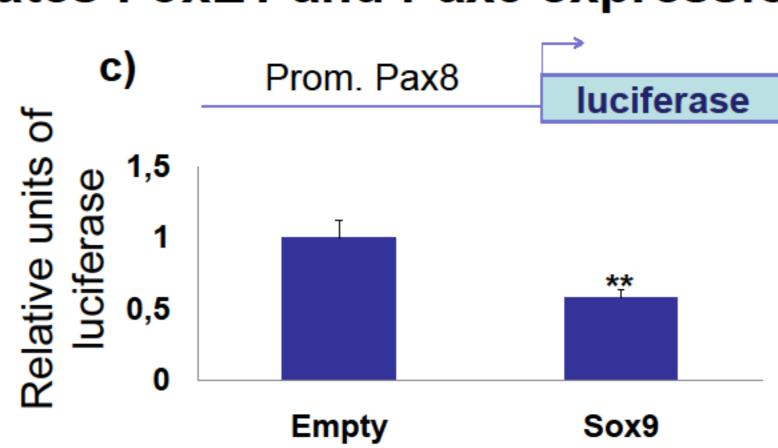


Fig.3: (a)Co-transfection assay in Hela cells with pGL3-promRatSox9 and TTF1, Pax8 and FoxE1 expression vectors. Co-transfection assays in Hela cells with pGl3-promRatPax8 (c) and Sox9 expression vector.

CONCLUSIONS

- 1. TSH induces Sox9 expression through cAMP/PKA signalling pathway in PCCI3 cells.
- 2. TGFβ and IGF1 represses the inductive effect of TSH.
- 3. Sox9 is involved in the expression control of thyroid trancriptions factors, wich also regulates its expression.

DISCUSSION

These results demonstrate the involvement of Sox9 in the pathophysiology of the thyroid, something hitherto unknown, and open a new field of study in the development and differentiation of the thyroid gland.



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