GP115

Loss of cells expressing the T-box transcription factor TBX1 might be associated with a quiescent phenotype in parathyroid tumours.

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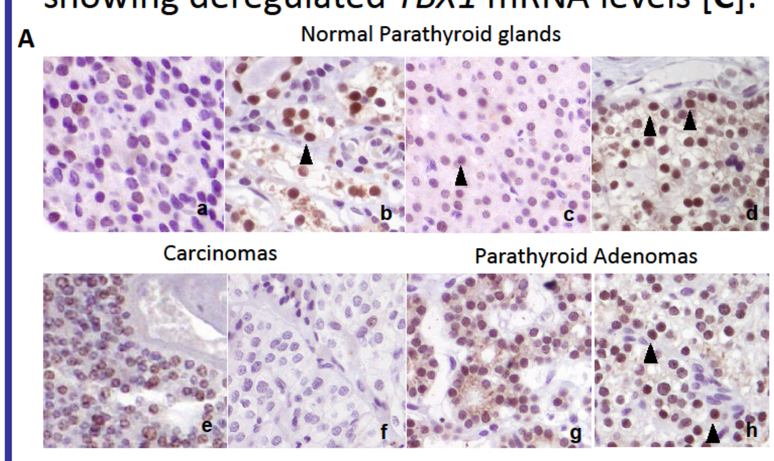
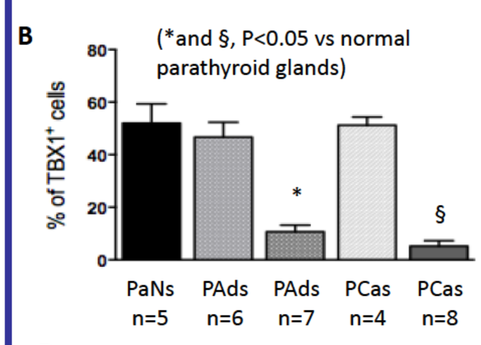
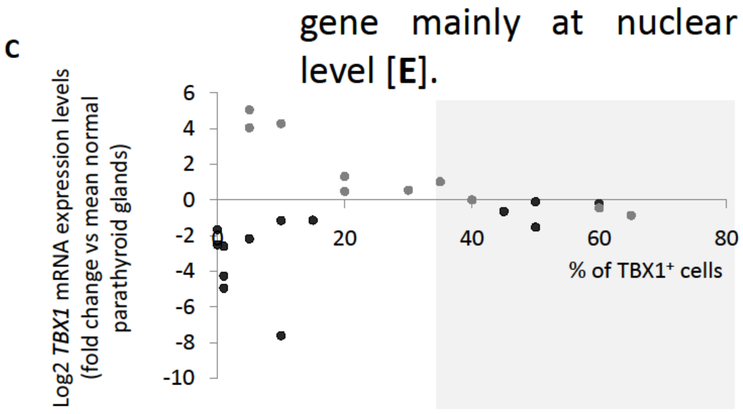
Introduction: Embryonic transcription factors have been involved in tumourigenesis. The transcription factor TBX1 regulates the embryonic parathyroid cells fate. Indeed, it has never been investigated in human adult parathyroids.

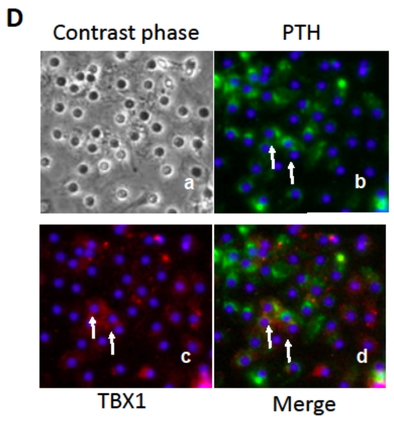
Aim: Expression, function and regulation of the *TBX1* gene were analysed in adult normal and tumour parathyroid tissues.

RESULTS: Expression of the embryonic transcription factor TBX1 in human parathyroid tissues: Immunohistochemistry identified 30-70% (mean±SEM, 52.0±7.3%) of cells expressing TBX1 at nuclear levels in normal parathyroid glands (n=5). Parathyroid adenomas (Pads) showed a highly variable proportion of TBX-1 expressing cells ranging from 5 to 65% [**A**,**B**]. About a half of parathyroid tumours [12 parathyroid carcinomas and 13 adenomas (PAds)] had reduced TBX1$^+$ cells (0-15%), showing deregulated *TBX1* mRNA levels [**C**]. Immunofluorescence on PAds-derived cells identified a subset of cells co-expressing TBX1 and PTH [**D**]. Western blot analysis on fractioned proteins from PAds (n=11) showed that PAds expressed the 53 kDa isoform C of the TBX1 gene mainly at nuclear level [**E**].



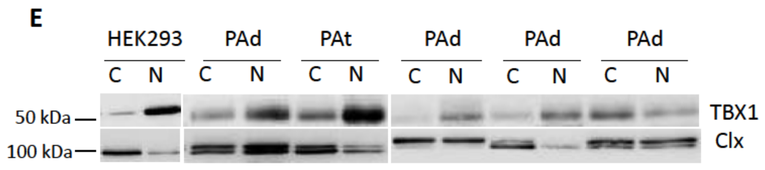




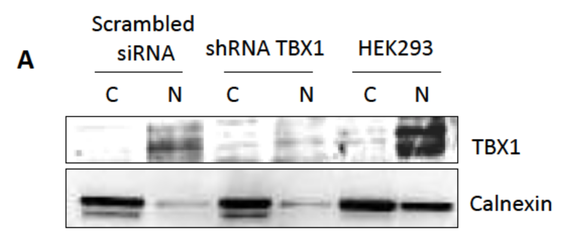


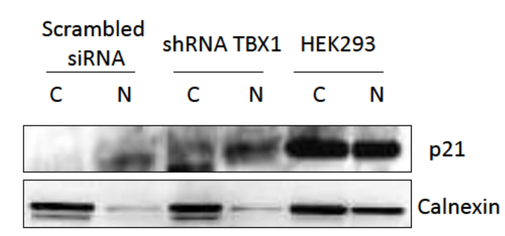
PaNs - normal parathyroids
PAds – parathyroid adenomas
PAt - atypical parathyroid adenomas
PCas – parathyroid carcinomas

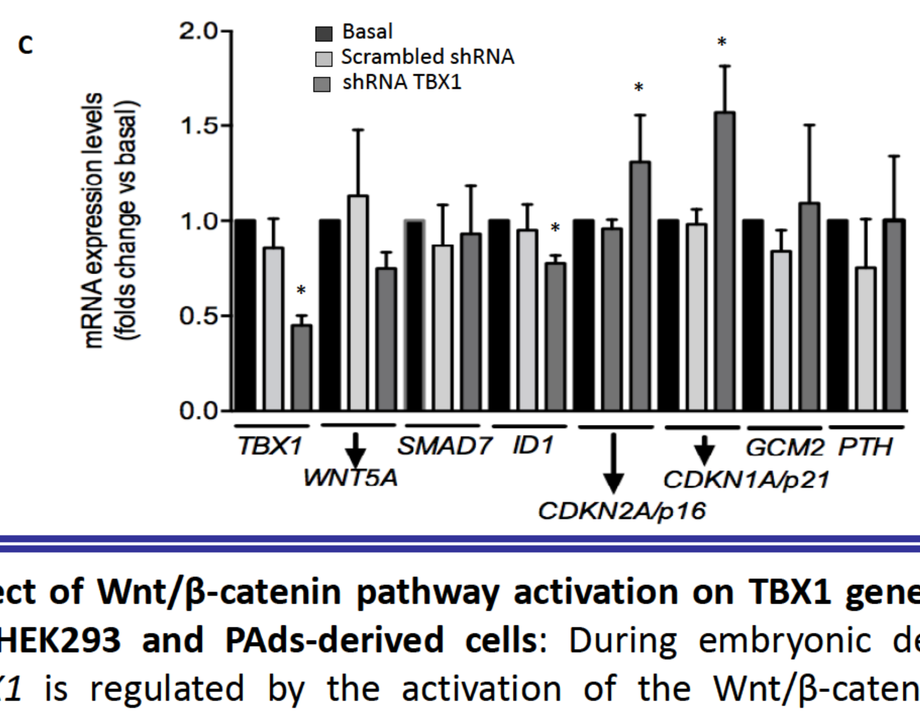
C – Cytoplasm
N - Nucleus



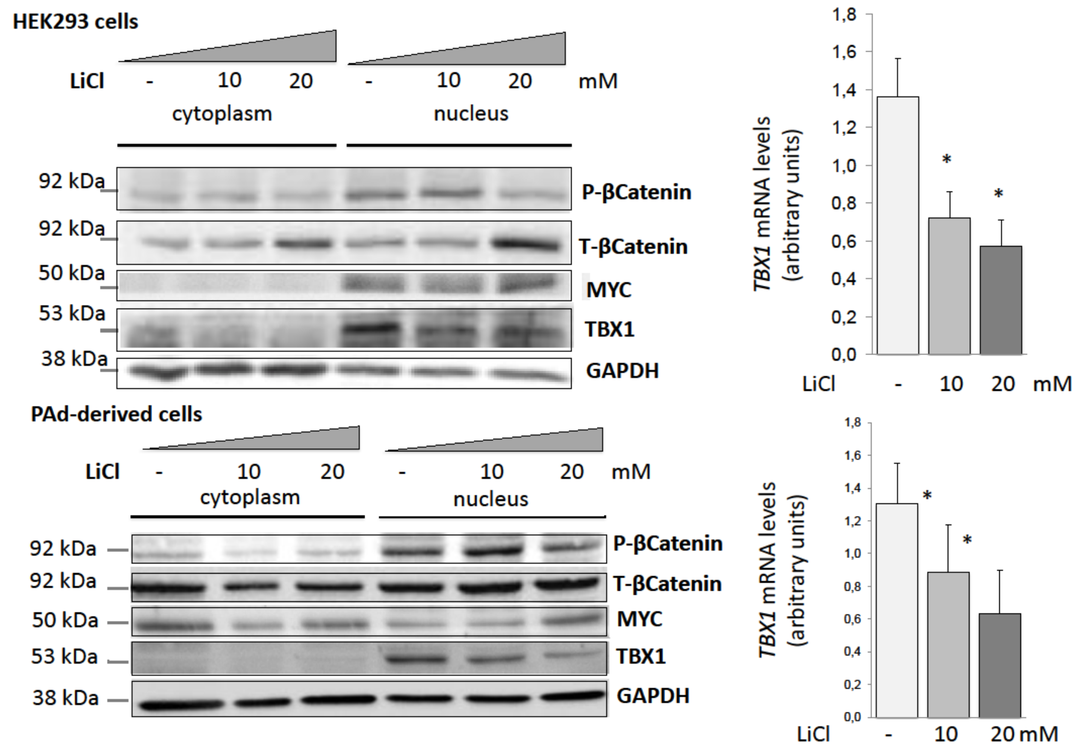
Effect of TBX1 reduced levels in PAds-derived cells: consistent with the promotion of cell cycle arrest, *TBX1* silencing [**A**] increased *CDKN2A/p16* mRNA levels , *CDKN1A/p21* mRNA and protein levels and decreased *ID1* (*inhibitor of DNA binding 1*) levels in PAds where any significant change in both *GCM2* and *PTH* levels could be detected by TBX1 silencing[**B**,**C**].





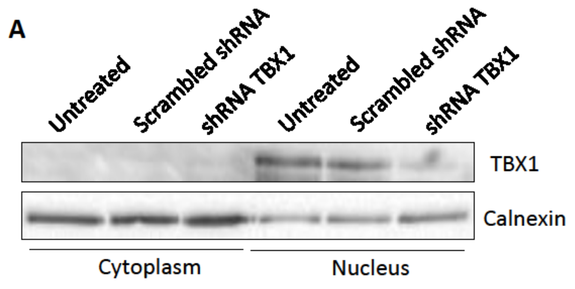


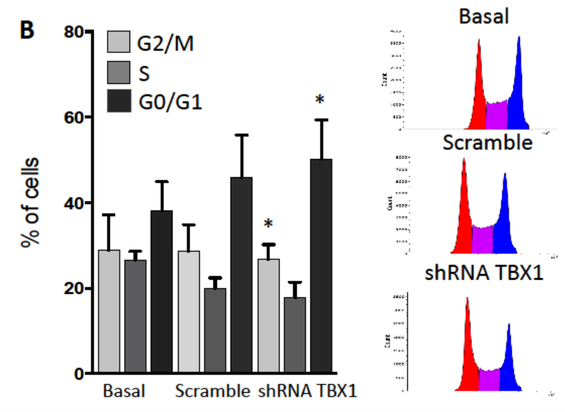
Effect of Wnt/β-catenin pathway activation on TBX1 gene expression in HEK293 and PAds-derived cells: During embryonic development, *TBX1* is regulated by the activation of the Wnt/β-catenin pathway. Short-term (8 hours) 10-20 mM lithium chloride treatment induced β-catenin nuclear accumulation and inhibited *TBX1* mRNA levels in 5 out of 7 PAds cell preparations.

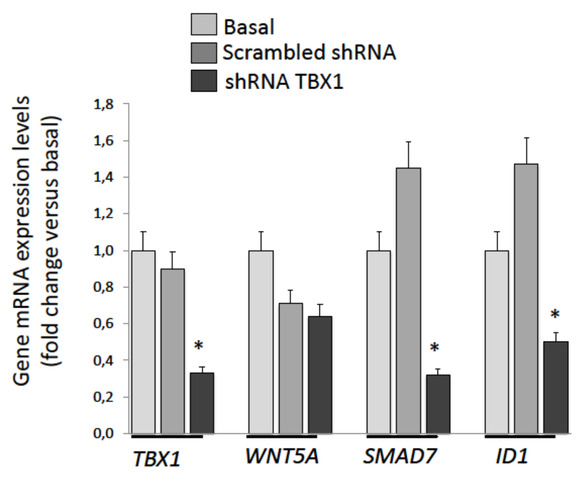


PAds samples with reduced TBX1 protein levels showed significantly higher *AXIN2* mRNA levels, a marker of β-catenin transcriptional activity (median, IQR; 0.16, 0.05-0.65 *vs* 1.57, 0.38-2.93; P=0.03, n=11).

Effects of TBX1 reduced levels in HEK293 cells: TBX1 function was investigated in HEK293 cells, which express the gene. Stable silencing of *TBX1* gene in HEK293 cells reduced nuclear TBX1 protein to 30% of basal levels [**A**] and increased the proportion of cells in the G0/G1 phase (from 38.2±6.7% to 50.2±9.1%, P=0.04), suggesting that loss of TBX1 induced cell cycle arrest [**B**]. TBX1 silencing in HEK293 was associated with a significant downregulation of the target genes *SMAD7* and *ID1* [**C**].







In conclusion, the embryonic transcription factor TBX1 is expressed in a subpopulation of adult parathyroid cells, which is reduced in half of tumours. Reduction of TBX1 expression is associated with cell quiescence, a feature that might be in line with the extremely low cell proliferation rate described in parathyroid tumours.