P505
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The Wnt/beta-catenin pathway regulates the expression of early embryonic stem cell genes in human parathyroid tumors.

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Introduction

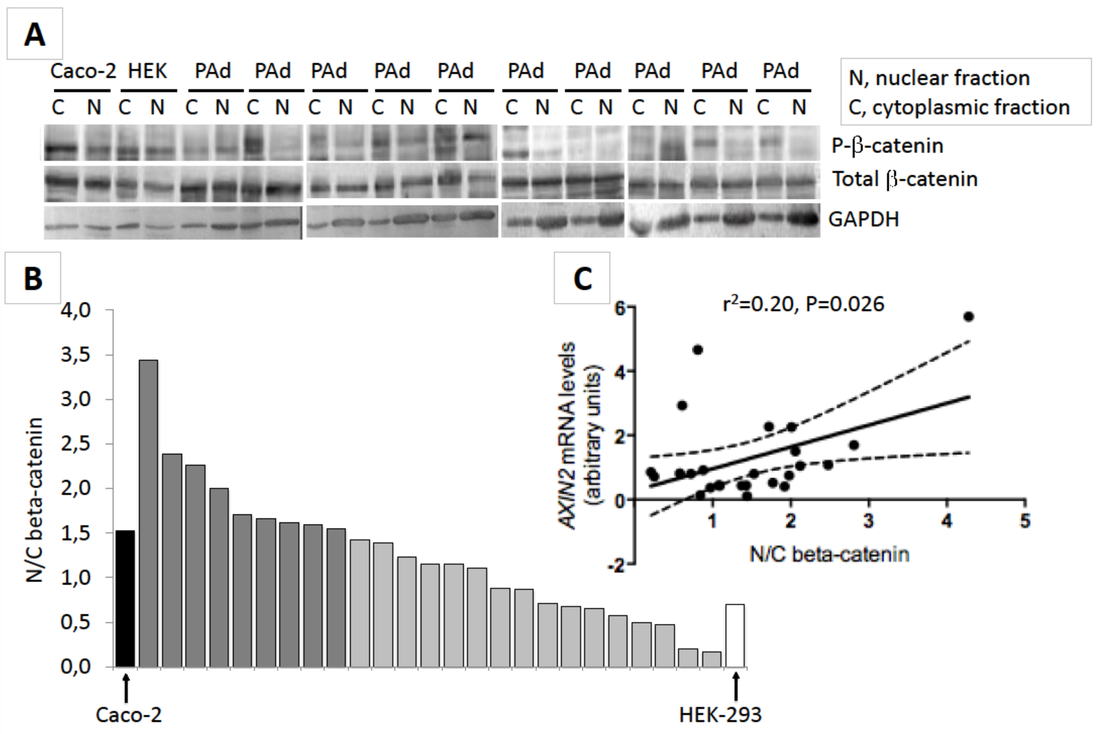
Evidence suggested an embryonic epigenetic signature in parathyroid tumors, with deregulated miRNAs and gene methylation. In embryonic stem cells, the Wnt/β-catenin signaling regulates the expression of the core stemness genes, namely NANOG, OCT4 and SOX2. Though constitutive nuclear accumulation of β-catenin has not been detected, the Wnt/β-catenin pathway might be deregulated in parathyroid tumors, as Wnt signaling inhibitors have been found reduced.

Aim of the study To investigate the embryonic signaling Wnt/β-catenin – core stem cells genes in adult human tumoral parathyroids.

Results

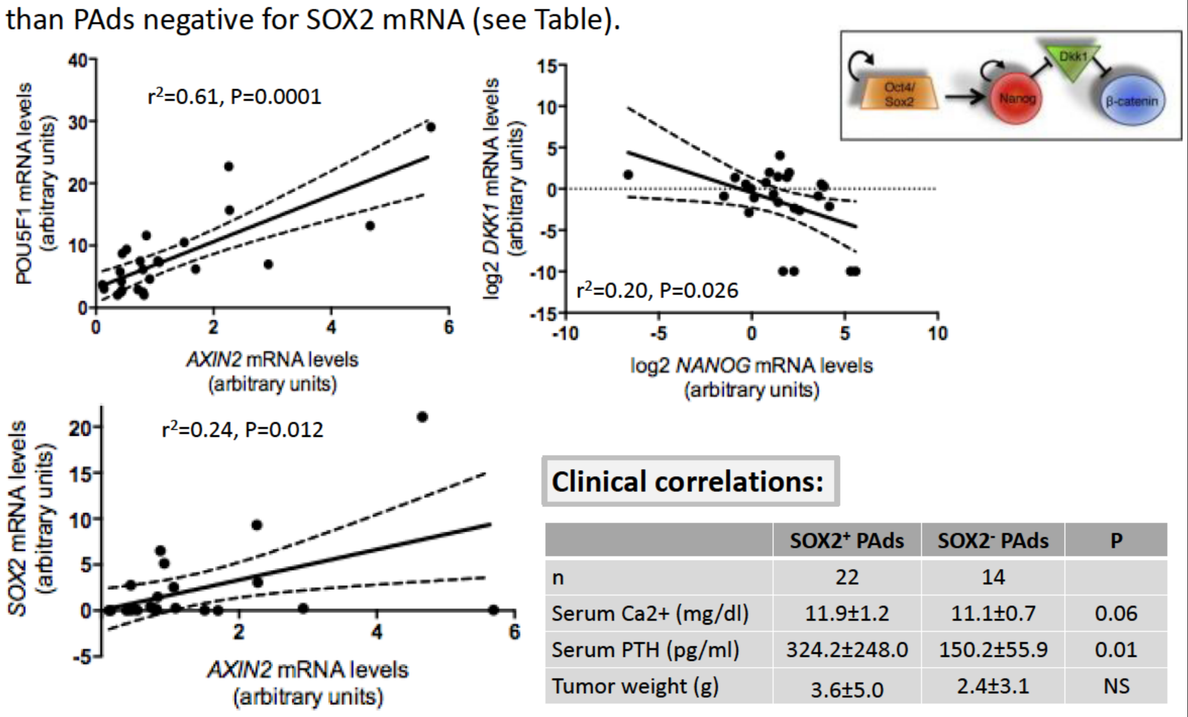
Nuclear active β-catenin levels in parathyroid adenomas:

We investigated unphosphorylated active β-catenin distribution by western blot in 25 typical parathyroid adenomas (PAds)(**Panel A**): β-catenin accumulation in the nuclear protein fractions varied from the levels detected in Caco-2 cells with constitutively active Wnt signaling (9 PAds) to the levels measured in HEK293 cells with intact Wnt signaling (6 PAds)(**Panel B**) and positively correlated with *AXIN2* mRNA levels (r=0.445, P=0.026)(**Panel C**).



AXIN2 mRNA expression correlated with *POU5F1* and *SOX2* mRNA expression levels:

the mRNA levels of *POU5F1* and *SOX2* genes in 25 PAds positively correlated with *AXIN2* mRNA levels, in agreement with the effect of LiCl in dispersed cells.
NANOG mRNA levels negatively correlated with *DKK1* mRNA levels.
PAds expressing SOX2 transcripts were associated with more severe hyperparathyroidism than PAds negative for SOX2 mRNA (see Table).

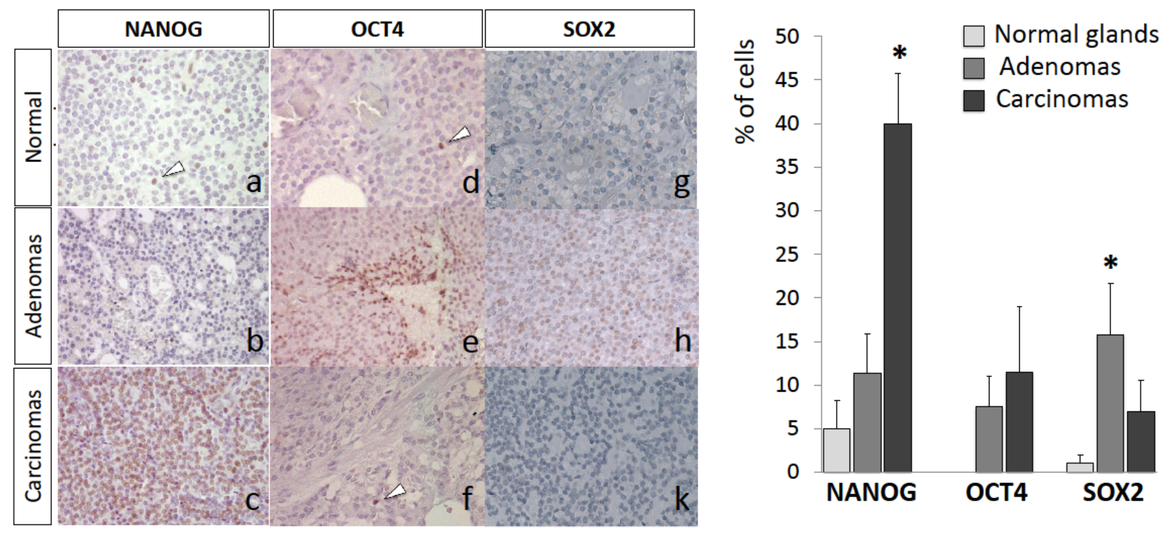


Clinical correlations:

	SOX2⁺ PAds	SOX2⁻ PAds	P
n	22	14	
Serum Ca2+ (mg/dl)	11.9±1.2	11.1±0.7	0.06
Serum PTH (pg/ml)	324.2±248.0	150.2±55.9	0.01
Tumor weight (g)	3.6±5.0	2.4±3.1	NS

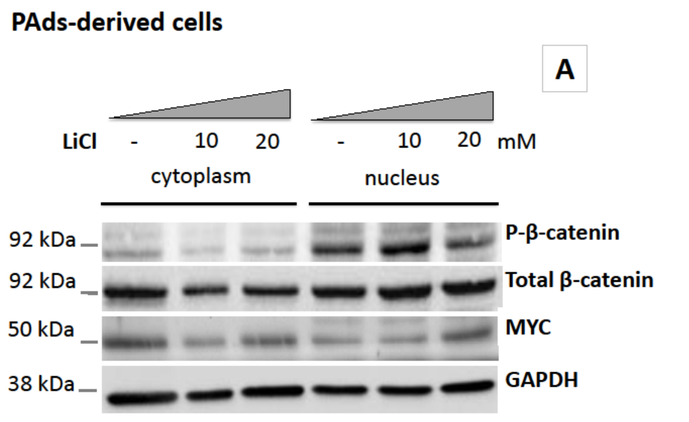
Core stem genes analysis by immunohistochemistry in parathyroid tumors:

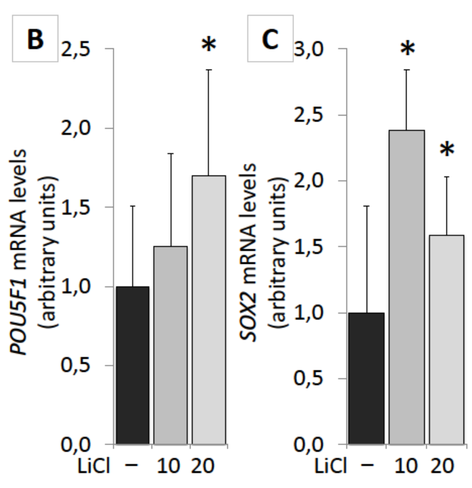
Immunohistochemistry of tumor sections [11 PAds, 8 carcinomas (PCas)] identified cells expressing at nuclear level the core stem cell genes:

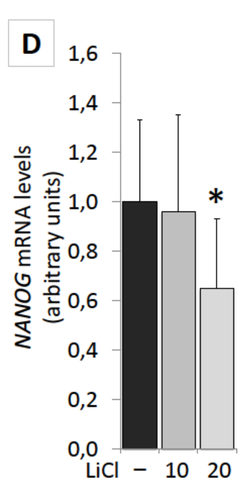


Effects of Lithium Chloride (LiCl) treatment on stem cell markers in parathyroid adenomas (Pads)-derived cells:

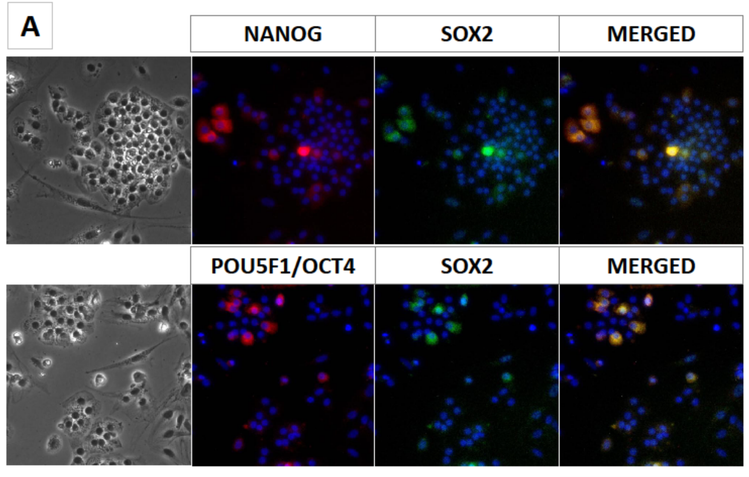
The Wnt/β-catenin pathway is intimately connected to the embryonic pluripotent core circuitry. Treatment of PAds-derived cells (n=5) with 10-20 mM lithium chloride (LiCl) for 8 hours induced nuclear accumulation of β-catenin (**Panel A**) and concomitant increases in mRNA levels of *POU5F1* (**Panel B andC**) and *SOX2* genes, as reported in embryonic stem cells. Nonetheless, at variance with embryonic stem cells, β-catenin accumulation induced a decrease in *NANOG* mRNA levels (**Panel D**).
* P<0.05



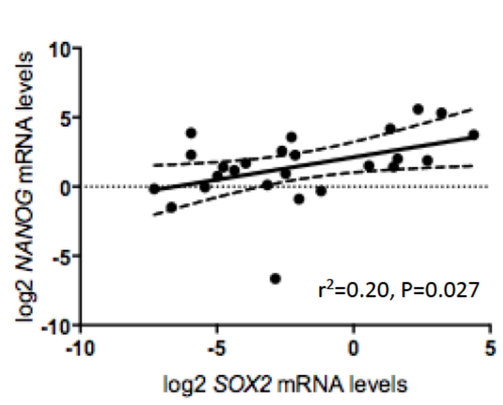


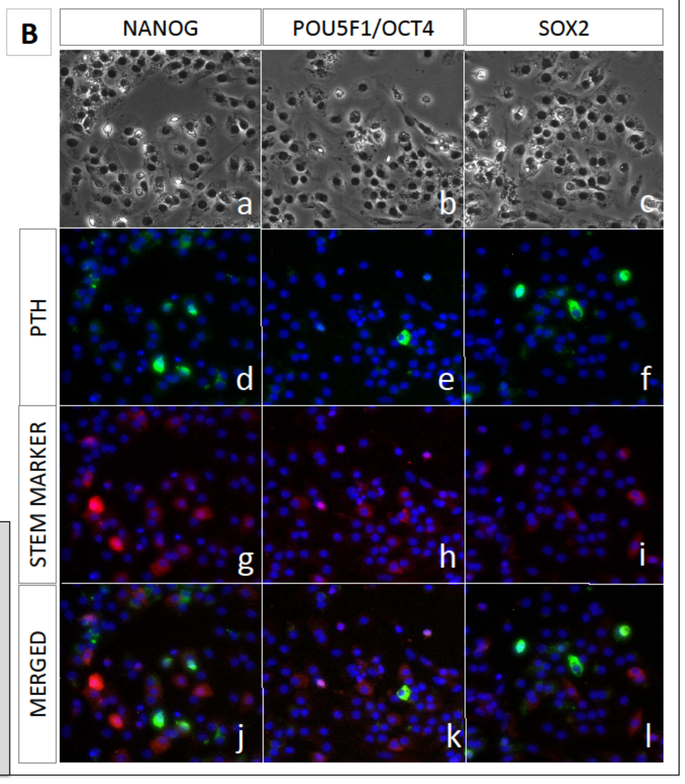


Immunofluorescence analysis of core stem genes in PAds-derived cells:



Immunofluorescence detected few cells coexpressing SOX2 and NANOG or OCT4 (**Panel A**), in agreement with the positive correlation between *NANOG* and *SOX2* mRNA levels in Pads.
Parathyroid tumor cells expressing PTH were negative for all the stem cell genes (**Panel B**).





Conclusions We firstly identified an embryonic pattern of gene expression in parathyroid tumors, where β-catenin signaling might be involved in regulating the expression of the core stem genes. SOX2, in particular, was associated with a more severe presentation of primary hyperparathyroidism.

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