Expression and regulation of the early embryonic stem cell genes in parathyroid tumours

GP-118

Verdelli C[1], Forno I[2], Guarnieri V[3], Ferrero S[2], Scillitani A[3], Cetani F[4], Vicentini L[5], Balza G[6], Beretta E[7], Bosari S[2], Vaira V[2], Corbetta S[8].

[1]Laboratory of Experimental Endocrinology, IRCCS Istituto Ortopedico Galeazzi, Milan; [2]Pathology Unit, University of Milan, IRCCS Fondazione Cà Granda, Milan; [3]Genetics and Endocrinology Units, IRCCS Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG); [4]Dept.Endocrinology and Metabolism, University of Pise, Pise; [5]Endocrine Surgery, IRCCS Fondazione Cà Granda, Milan; [6]Medicine Unit, A.O. Manzoni, Lecco; [7]Endocrine Surgery, IRCCS Ospedale San Raffaele, Milan; [8] Endocrinology Unit, Department of Biomedical Sciences for Health, University of Milan, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy.

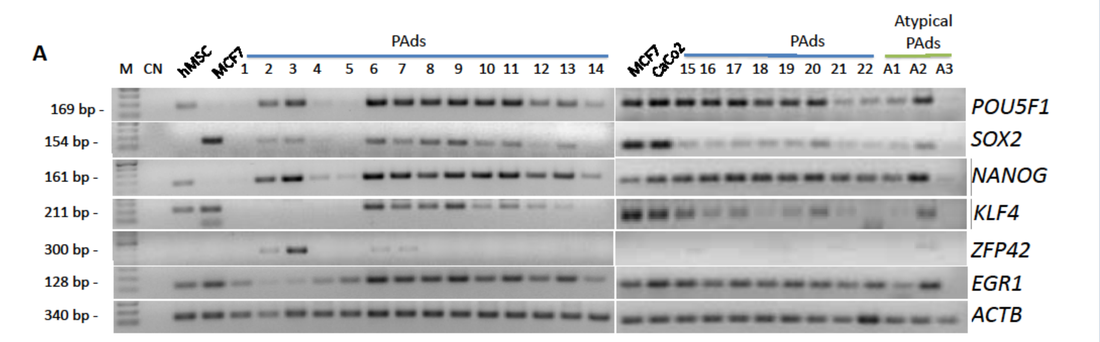
Introduction

Evidence suggested an embryonic epigenetic signature in parathyroid tumours, with deregulated microRNAs 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.

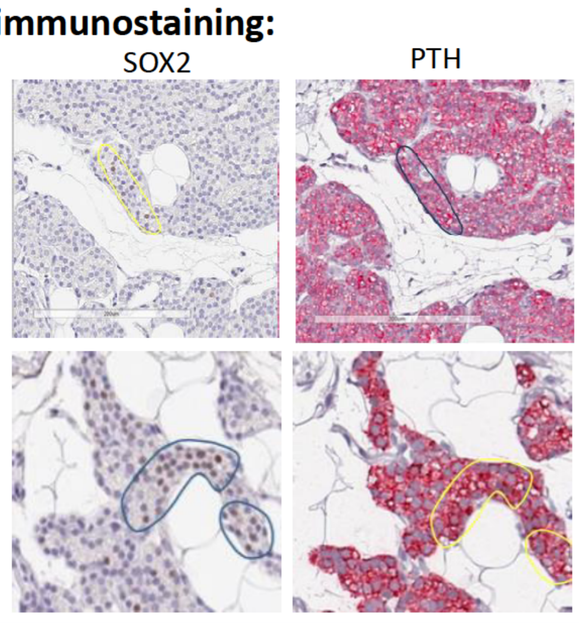
Aim of the study To investigate the expression of early embryonic stem cells (ESC) genes in adult human parathyroid tumours.

Results

Core stem genes analysis in parathyroid tumours: *POU5F1/OCT4*, *SOX2* and *NANOG* transcripts were detected in almost all parathyroid adenomas (PAds; n=22) and atypical PAds (n=3), besides the variable expression of ESC genes *KLF4*, *EGR1*, and *REX1/ZFP42* [**A**]. OCT4, SOX2 and NANOG proteins expression were analysed by immunohistochemistry in archival series of tumours and normal parathyroid glands [**B**]. Parathyroid carcinomas (n=8) had more NANOG-expressing cells (mean positive cells 40%) compared to PAds (n=11; mean positive cells 10%), while PAds (n=22) showed a higher proportion of SOX2-expressing cells, though SOX2-expressing cells occurred in half of tumours [**C**]. *NANOG* and *SOX2* mRNA levels showed a positive correlation [**D**].

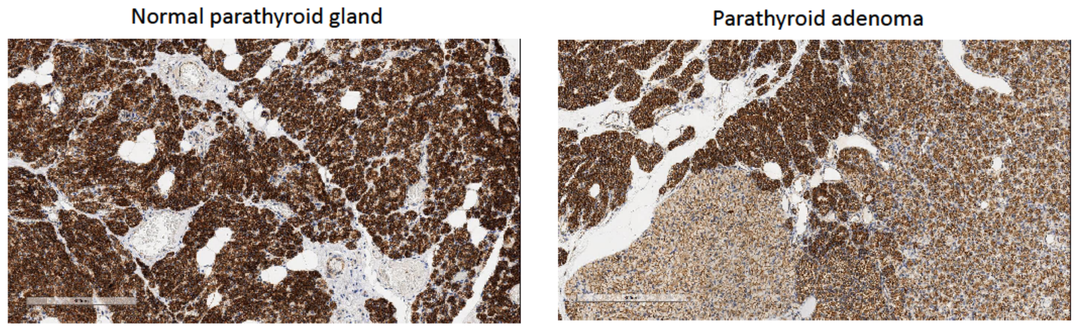


Some PAds-derived cells expressing SOX2 were positive for PTH immunostaining:

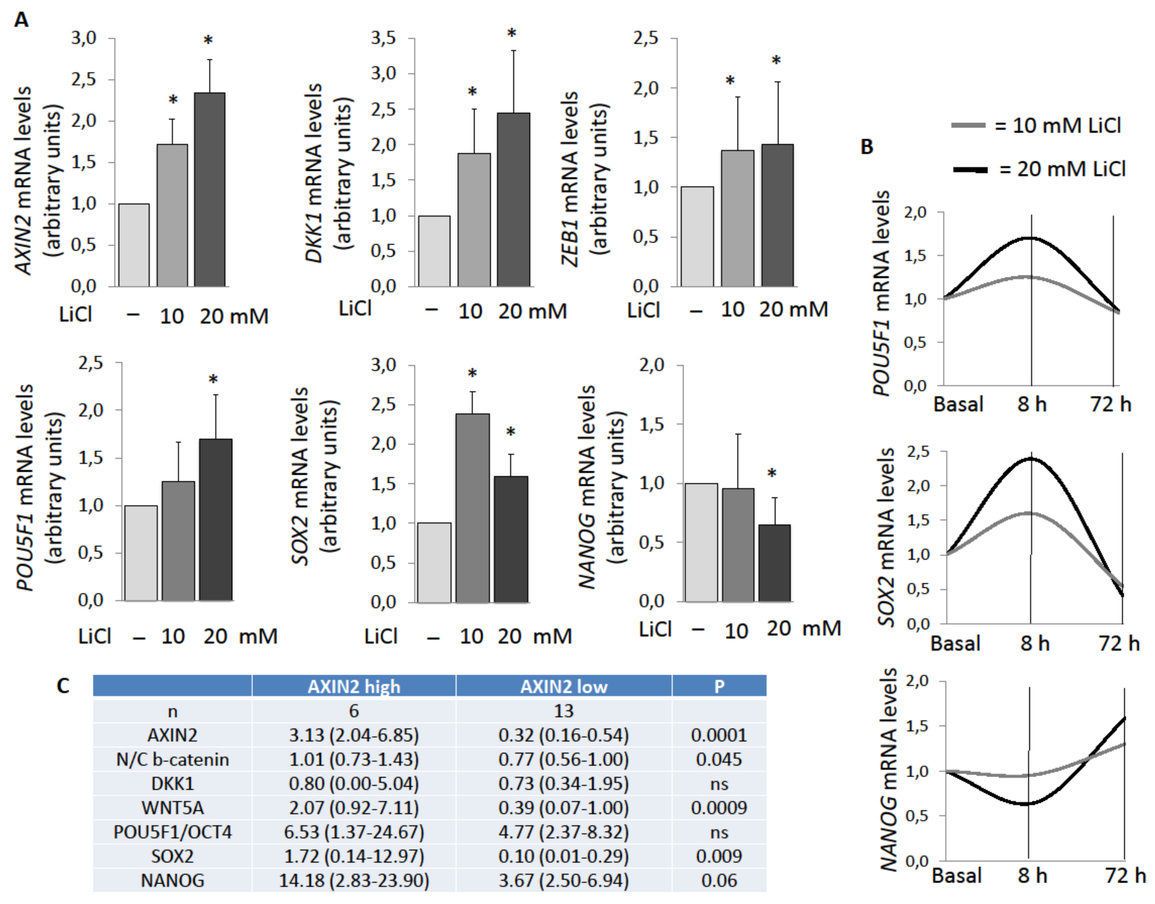


Staining of contiguous sections showed that clustered SOX2 expressing cells also coexpressed PTH.

Expression of β-catenin in parathyroid tissues: β-catenin highly accumulated at membrane and cytoplasm levels in normal glands (n=4) and in PAds (n=16), though PAds were heterogeneous showing parenchymal zones where cells had very low active β-catenin levels confined at membrane.



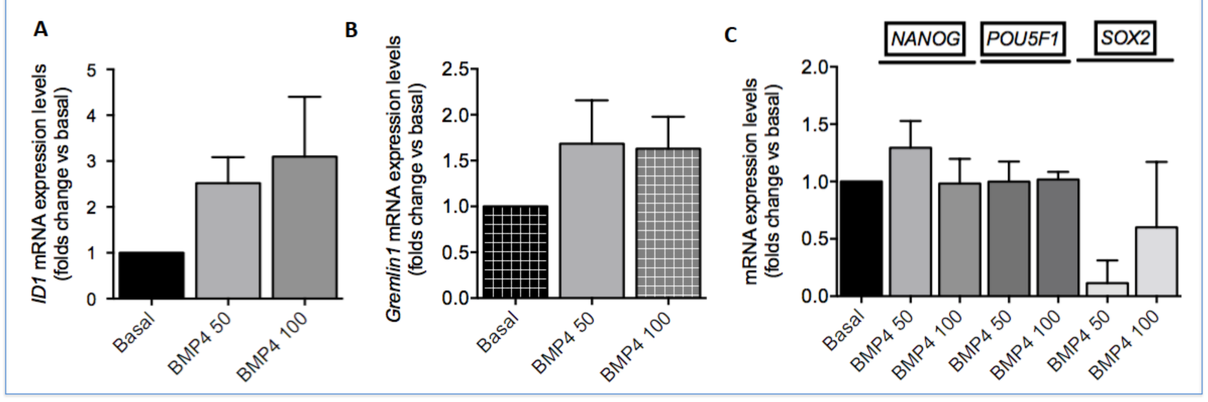
The ESC pluripotency is regulated by the Wnt/β-catenin and β-catenin is transcriptionally active in parathyroid neoplasia: treatment of PAds-derived cells (n=6) with 10-20 mM Lithium Chloride increased the Wnt gene targets *AXIN2, DKK1, ZEB1,* and modulated the expression of *POU5F1/OCT4*, *SOX2* and *NANOG* mRNA levels depending on the time course of β-catenin activation [**A-B**]. Investigating samples from 25 PAds, we observed that PAds expressing *AXIN2* (n=6) had abundant *NANOG*, *SOX2* and *WNT5A* transcripts [**C**].



C

	AXIN2 high	AXIN2 low	P
n	6	13	
AXIN2	3.13 (2.04-6.85)	0.32 (0.16-0.54)	0.0001
N/C b-catenin	1.01 (0.73-1.43)	0.77 (0.56-1.00)	0.045
DKK1	0.80 (0.00-5.04)	0.73 (0.34-1.95)	ns
WNT5A	2.07 (0.92-7.11)	0.39 (0.07-1.00)	0.0009
POU5F1/OCT4	6.53 (1.37-24.67)	4.77 (2.37-8.32)	ns
SOX2	1.72 (0.14-12.97)	0.10 (0.01-0.29)	0.009
NANOG	14.18 (2.83-23.90)	3.67 (2.50-6.94)	0.06

The ESC pluripotency is regulated by the bone morphogenetic proteins (BMP) signalling: stimulation of PAds-derived cells for 24 hours with 50 and 100 nM BMP4 induced significant increases in *ID1/inhibitor of DNA binding 1* (about 3 fold the basal levels) and *Gremlin* transcripts suggesting that PAds-derived cells are responsive to BMP signalling [**A-B**] . Any effect could be detected on ESC genes transcripts levels by a short term BMP pathway activation [**C**].



Conclusions We firstly identified an embryonic pattern of gene expression in parathyroid tumours, 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.