Stem cell markers in Non-Functioning Pituitary Neuroendocrine tumours

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Background: The pituitary gland has a complex development and maturation during different life cycles. Pituitary Neuroendocrine tumours (PitNETs) are relatively common, and non-functioning PitNETs (NF-PitNETs) among the most abundant of these. Nevertheless, their origin and pathogenesis are still mostly unknown. Cells with stem cell features have previously been found both in normal and tumourous pituitary tissue (1,2). SOX2 and SOX9 are pituitary stem cell markers, while PROP1, a transcription factor present in pituitary progenitor cells, is involved in anterior pituitary cell lineage development (3). We aimed to investigate the presence of these markers in the NF-PitNETs.

	Gonadotroph	TPIT	PIT1	Null-cell	P-value
Female/male	39/73	7/9	6/3	1/3	
Age med (IQR)	60 (51-72)	57 (52-71)	38 (26-58)*	67 (60-73)	0.01
Follow-up (months)	128 (99-160)	110 (95-171)	125 (117-168)	116 (100-145)	0.6
Tumour volume (mm^3)	6480 (4055-10795)	6351 (2104-17697)	2670 (1962-4950)*	3440 (2104-)	0.04
Available IHC (N)	101	16	8	4	
IHC SOX2 med (IQR)	0 (0-1)	0(0-1)	0(0-0)	0(0-0)	0.14
ΔCT SOX2 med (R) (N=67)[ab]	0.00 (0.00-0.17)				
IHC SOX9med (IQR)[c]	1(0-2)	1(0-2)	0.5(0-1)	0(0-0)	0.04
ΔCT SOX9 med (R) (N=71)[ab]	0.00 (0.00-0.11)				
IHC PROP1 med (IQR)	0 (0-0)	0(0-0)	0(0-0)	0(0-0)	0.28
ΔCT PROP1 med (R) (N=70)[ab]	0.00 (0.00-2.48)				
Total	112	16	9	4	141

Table 1: Immunohistochemical staining (IHC) of the stem cell markers SOX2 and SOX9, and the transcription factor PROP1 in clinically non-functioning pituitary neuroendocrine tumours (NF-PitNETs). Median (med) and inter quartile range are given for continuous data.
**There was a significant difference in age at primary surgery between the PIT1 and TPIT group, PIT1 and null-cell group and PIT1 and SF1 group, and also in tumour volume between the SF1 and PIT1 group. However there were only 9 patients in the PIT1 group and preoperative MRI was only available for 5 patients.*
[a]Range is given rather than IQR for the gene expression data because of the low relative expression.
[b]Due to low number of tumours tissue available in the TPIT, PIT1 and Null-cell NF-PitNET groups (N=3, N=3 and N=2, respectively) , median and range are not given for these tumours.
[C]There was a significant difference in the SOX9 scoring between SF1 and null-cell group.

Methods: We investigated the distribution of SOX2, SOX9 and PROP1 in a previously established tissue micro array (N=101) and in frozen tumour tissue by RT-qPCR (N=71) from a retrospective cohort of NF-PitNETs (4, 5). Immunohistochemical (IHC) staining scores were compared to clinical data, and to previously investigated regulators of the gonadotroph axis in the same cohort. The markers were scored based on the percentage of positive staining cells, ranging from 0 (no positive staining cells) to 6 (>50% positive staining cells). RT-qPCR were performed as previously described (6).

	SOX2 neg	SOX2 pos	P-value	SOX9 neg	SOX9 pos	P-value	PROP1 neg	PROP1 pos	P-value
Female (N)	28 (35%)	6 (29%)	0.58	26 (36%)	8 (28%)	0.41	28 (34%	6 (32%)	0.83
Age (years)	61 (51-72)	55 (47-73)	0.26	62 (52-72)	55 (47-72)	0.13	61 (52-72)	55 (42-67)	0.11
Reintervention	28 (35%)	5 (24%)	0.33	26 (36%)	7 (24%)	0.25	26 (32%)	7 (37%)	0.67
Early reintervention	3 (4%)	1 (5%)	0.5	3 (4%)	1 (3%)	0.5	4 (5%)	0 (0%)	0.82
Invasiveness	14 (40%)	4 (36%)	0.83	13 (43%)	5(31%)	0.42	16 (42%)	2 (25%)	0.45
Tumor volume mm^3	6196 (3765–9999)	6693 (5127-5486)	0.13	6405 (3740-10137)	6520 (5065–14610)	0.37	6653 (3855-10581)	6340 (5065–14610)	0.62
Total	80	21		72	29		82	19	

Table 2 : Distribution of SOX2, SOX9 and PROP1 in gonadotroph NF-PitNETs (N=101). A staining score of ≤1 was considered as a negative staining score. Invasiveness is defined as Knosp≥3. MRI data was available for 46 tumours, 35, 30 and 38 of these presented a staining score ≤1 for SOX2, SOX9 and PROP1 respectively.

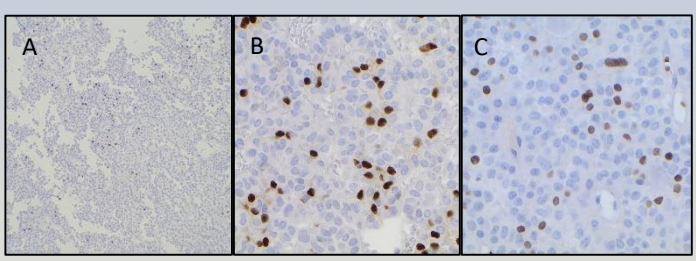

Figure 1: Examples of immunohistochemical staining for SOX2 (A), SOX9 (B) and PROP1 (C) in PitNETs.

		SOX9	PROP1	ΔCT SOX2	ΔCT SOX9	ΔCT PROP1
SOX2	R	0.696	0.726	**0.538**	0.534	0.486
	P-value	<0.001	<0.001	**<0.001**	<0.001	<0.001
	N	101	101	**60**	63	63
SOX9	R		0.617**	0.377	**0.601**	0.347
	P-value		<0.001	0.003	**<0.001**	0.005
	N		101	60	**63**	63
PROP1	R			0.546	0.535	**0.434**
	P-value			<0.001	<0.001	**<0.001**
	N			60	63	**63**
ΔCT SOX2	R				0.703	0.818
	P-value				<0.001	<0.001
	N				64	62
ΔCT SOX9	R					0.694
	P-value					<0.001
	N					64

Table 3: Spearman's correlation coefficient (R) between immunohistochemical protein staining and its relative gene expression for each marker (in bold characters) and between the different markers.

Conclusion: The stem cell markers SOX2 and SOX9 and the transcription factor PROP1 are present at low levels in gonadotroph NF-PitNETs. They are strongly correlated with each other, and might be associated with the regulation of gonadotropins.

Results:

- Most of the NF-PitNETs showed no or scattered cells with positive staining for SOX2, SOX9 and PROP1 (Table 1).
- There was no association between the presence of SOX2, SOX9 and PROP1 and gender, age at primary pituitary surgery and the rate of reintervention (Table 2).
- The IHC staining of SOX2, SOX9 and PROP1 correlated to the relative gene expression counterpart for all markers (Table 3).
- SOX2, SOX9 and PROP1 staining and gene expression correlated positively to each other (Table 3).
- The staining for SOX2 and SOX9 correlated to the immunoreactive score of ERα (ie SOX2 and ERα: N=97, ρ =0.315, p=0.002), the staining for FSH (ie SOX2 and FSH: N=99, ρ=0.359, p<0.001) and to the gene expression of GnRHR (ie SOX2 and GNRHR: N=57, ρ=0.445, p<0.001), the latter two also correlated positively with PROP1. The association remained significant when dividing the tumours in negative and positive staining (as exemplified by SOX2 in Figure 1).

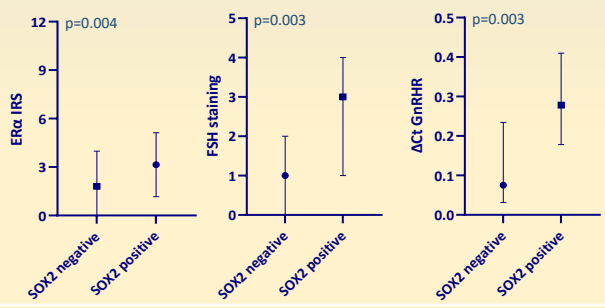

Figure 2: Immunohistochemical staining for ERα and FSH and gene expression of GnRHR according to the presence of SOX2 staining. SOX2 staining grade 0-1 was defined as negative, while staining grade 2-5 were defined as positive. Median and interquartile range are given for all variables.

References: 1. Chen J et al; Pituitary progenitor cells tracked down by side population dissection. Stem cells 2009; 27:1182-1195. 2. Nys C et al; Pituitary disease and recovery: How are stem cells involved? Molecular and Cellular Endocrinology; 525 (2021) 111176. 3.Laporte E et al; Pituitary Remodelling Througout Life: Are Resident Stem Cells Involved? Frontiers in Endocrinology January 2021; Volume 11: Article 604519. 4. Casar-Borota O et al;KIT protein expression and mutational status of KIT gene in pituitary adenomas. Virchows Archiv: an international journal of pathology. 2012;460(2):171-81. 5. Øystese KA et al; Estrogen Receptor α, a Sex-Dependent Predictor of Aggressiveness in Nonfunctioning Pituitary Adenomas: SSTR and Sex hormone Receptor Distribution in NFPA. J Clin Endocrinol Metab, September 2017, 102(9):3581-3590. 6. Normann et al; Selection and Validation of reliable reference genes for RT-qPCR analysis in a large cohort of pituitary adenomas. Mol Cell Endocrinol. 2016;437:183-189.