RPS13 and cell cycle signaling pathways in pituitary tumorigenesis

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Introduction

• *CDKN1B* (p27) underexpression and Ribosomal proteins (RP) have been related to the pathogenesis of pituitary adenomas (1, 2).

• In gastric cancer, *RPS13* down-regulates p27 and promotes cell cycle progression (3); these mechanisms have not yet been explored in pituitary adenomas.

Objective

• To evaluate the relationship between *RPS13* and *CDKN1B, CDK2*, *CCNE1, MYC* gene expression in pituitary tumorigenesis and its association to clinical findings.

Methods

We studied four groups: corticotrophinomas (n=12), somatotrophinomas (n=18), non-functioning pituitary adenomas (NFPA, n=21), and normal pituitaries (NP, n=07). Clinical and pathological data of tumors are shown in table 1, 2 and 3. RNA was isolated by TRIzol method. Gene expression was assessed by qRT-PCR. Kruskal-Wallis test was used for continuous variables between groups and Fisher Exact test for categorical data.

TABLE 1: Clinical Findings of patients with ACTH-secreting pituitary tumors.

Patient	Age (years)	Gender	Tumor size (Resonance)	Immunohistochemistry	Remission
ACTH 1	31	M	without visible tumor	ACTH + ACTH +, GH +, TSH +, LH +, FSH	No
ACTH 2	30	F	0.5 cm	+	No
ACTH 3	45	F	0.9 x 1.0 x 0.8cm	ACTH+	Yes
ACTH 4	15	F	1.5 cm	ACTH +	No
ACTH 5	26	F	0.8 X 0.8 cm	ACTH +	No
ACTH 6	38	F	without visible tumor	Negative	Yes
ACTH 7	26	F	without visible tumor	ACTH +	No
ACTH 8	39	F	1.6 x 1.0 cm	Inconclusive	Yes
ACTH 9	11	F	0.4 cm	ACTH +	No
ACTH 10	36	F	1.0 cm	ACTH +	Yes
ACTH 11	26	F	0.8 x 0.8 cm	ACTH +	No
ACTH 12	17	F	3.5 x 1.6 cm	ACTH +	No

M- Male F-Female

TABLE 2: Clinical Findings of patients with GH-secreting pituitary tumors.

Patient	Age (Years)	Gender	Tumor Size (Resonance)	Immunohistochemistry	Post-surgery Remission	Visual Field	Basal GH (µg/L)	post-oGTT GH (µg/L)	IGF (%ULNR)	Disease Control
GH 1	28	F	2.4 x 1.5 cm	GH+, PRL+, FSH/LH (focal)	No	Normal	14.4	10.4	480	Yes
GH 2	29	M	3.3 x 2.0 cm	GH+, PRL+	No	Abnormal	92	52	210	No
GH 3	43	F	1.0 x 0.8 cm	GH+, PRL+, LH+	Yes	Abnormal	15.5	13.5	205	Yes
GH 4	42	M	1.5 x 1.3 cm	GH+, PRL+, LH+, FSH+, TSH+	Yes	Normal	23	22	921	No
GH 5	55	F	1.0 x 0.9 cm	GH+, PRL+	Yes	Normal	1.7	1.7	680	Yes
GH 6	50	F	1.2 x 1.0 cm	GH+	Yes	Normal	39.2	22	ND	Yes
GH 7	30	F	1.3 x 2.0 cm	GH+	No	Abnormal	13.7	7.1	410	Yes
GH 8	36	M	1.5 X 1.0 cm	GH+	No	Abnormal	70.1	32.4	404	No
GH 9	39	M	2.5 x 2.7 cm	GH+, PRL+	No	Abnormal	56.6	33.2	360	NA
GH 10	52	F	3.3 x 3.4 cm	GH+, PRL+, LH+	No	Normal	23	19.5	376	Yes
GH 11	54	F	2.3 x 1.7 cm	GH+, PRL+	No	NA	33	32.5	442	Yes
GH 12	33	M	2.0 x 1.7cm	GH+, TSH+, PRL+, LH+	No	Abnormal	10.8	8.5	485	No
GH 13	44	F	1.9 x 1.7 cm	GH+, PRL+	No	Abnormal	2.8	1.6	835	No
GH 14	57	F	1.8 x 1.3 cm	GH+	No	Normal	1.8	1.3	492	Yes
GH 15	54	F	2.5 x 1.8 cm	GH+	No	Normal	20.9	19.2	540	Yes
GH 16	42	M	2.3 x 1.5 cm	GH+	Yes	Normal	110	104	ND	NA
GH 17	31	F	2.1 X 2.0 cm	GH+, PRL+, TSH+	No	Normal	119	117	337	No
GH 18	26	M	5.8 x 2.0 x 3.0 cm	GH+, TSH+, PRL+,FSH+	No	Abnormal	392.5	338	ND	NA

M- Male F-Female oGTT- oral glucose tolerance test NA- not available

TABLE 3: Clinical Findings of patients with non-functioning pituitary adenomas

Patient	Age (years)	Gender	Tumor Size (cm) (Resonance)	Immuno histochemistry	Post-Surgery Remission	Visual Field	Hypopituitarism
NFPA1	65	F	2.7 x 2.5 x 2.1	LH+	Yes	Normal	GH/LH/FSH/TSH
NFPA2	71	M	3.1 x 1.9 x 1.9	TSH+, LH+, FSH+	Yes	Abnormal	No
NFPA3	64	M	3.0 x 2.6 x 2.4	LH+	No	Abnormal	LH/FSH
NFPA4	49	M	2.1 x 2.4 x 1.6	TSH+	Yes	Abnormal	GH/ACTH/LH/FSH
NFPA5	61	M	1.9 x 2.0 x 1.6	Negative	Yes	Normal	LH/FSH
NFPA6	45	M	2.4 x 1.6 x 1.9	PRL+, LH+, FSH+	No	Normal	GH/ACTH/LH/FSH/TSH
NFPA7	37	M	7.7 x4.0 x 4.4	LH+, FSH+	No	Abnormal	ACTH/LHFSH/TSH
NFPA8	37	F	3.8 x 3.4 x 2.8	Negative	No	Abnormal	ACTH/TSH
NFPA9	42	F	3.2 x 3.0 x 2.2	LH+	Yes	Abnormal	LH/FSH
NFPA10	43	M	4.3 x 3.7 x 2.9	Negative	Yes	Abnormal	ACTH/LH/FSH/TSH
NFPA11	42	F	3.5 x 2.8 x 2.5	Negative	Yes	Abnormal	GH/ACTH/LH/FSH/TSH
NFPA12	70	M	4.1 x 3.1 x 3.2	Negative	No	Abnormal	ACTH/LH/FSH/TSH
NFPA13	50	M	3.0 x 2.8 x 4.0	LH+	Yes	Abnormal	GH/ACTH/LH/FSH/TSH
NFPA14	58	M	4.1 x 2.4 x 2.5	Negative	Yes	Abnormal	ACTH/LH/FSH/TSH
NFPA15	62	M	3.0 x 1.8 x 1.7	Negative	Yes	Abnormal	LH/FSH/TSH
NFPA16	47	F	2.0 x 1.8 x 1.6	Negative	Yes	Abnormal	No
NFPA17	40	F	2.5 x 1.9 x 1.4	Negative	Yes	Abnormal	No
NFPA18	27	F	5.1 x 4.0 x 4.4	Negative	No	Abnormal	ACTH/LH/FSH
NFPA19	27	M	2.3 x 2.0 x 1.9	Negative	Yes	Normal	No
NFPA20	52	F	3.4 x 2.5 x 2.7	ACTH+,GH+	No	Abnormal	NA
NFPA21	50	M	2.1 x 4.2 x 2.5	PRL+, LH+, TSH+	No	Abnormal	LH/FSH

Results

We observed *CDKN1B* underexpression (fold=-2.0) in somatotrophinomas compared to NP (p=0.03), *CCNE1* overexpression (fold=2.0) in NFPA versus NP (p=0.02) and *MYC* underexpression (fold=-10.0) in NFPA compared to corticotrophinomas (p=0.002). No differential gene expression among the groups were observed in *RPS13* (p=0.1) and *CDK2* (p=0.07) (table 4; figure 1).

In corticotrophinomas: no association between gene expression and tumor size, remission or immunohistochemistry (IHC).

In somatotrophinomas: no relationship between gene expression and tumor size, visual field, IGF-1 levels, basal and post-oGTT GH levels, IHC, post-surgery remission and disease control. Tumors with higher *CDKN1B* expression tended to achieve control with somatostatin agonist (p=0.08).

In NFPA: higher *CDK2* expression was associated to *null cell* subtype (p=0.03) with a tendency to correlate with tumor size (p=0.08). Higher *CCNE1* expression was associated with remission (p=0.02).

TABLE 4: Relative Gene Expression among groups expressed as mean and standard deviation and median and interquartile range.

Gene		Normal pituitaries (NP)	ACTH-secreting tumors	GH-secreting tumors	Non-functioning Pituitary Adenomas (NPFA)	Kruskall Wallis (p)
cMYC	mean ± SD	0.976 ± 0.548	2.132 ± 1.894	0.994 ± 1.115	0.455 ± 0.627	**0.002**
	median [Q1-Q3]	1.0 [0.581-1.519]	1.741 [0.68-2.926]	0.564 [0.165-1.588]	0.174 [0.116-0.616]	
CDK2		1.095 ± 0.361	2.4 ± 1.344	1.261 ± 0.936	2.198 ± 2.444	0.07
		1.0 [0.749-1.539]	1.917 [1.495-3.574]	1.017 [0.417 -1.927]	0.990[0.733-2.436]	
CCNE1		0.966 ± 0.265	2.239 ± 2.052	3.17 ± 3.121	4.319 ± 2.848	**0.02**
		1.0 [0.850-1.169]	1.502 [0.801-3.406]	2.026 [0.662-4.687]	4.387 [1.620-6.102]	
CDKN1B		1.249 ± 0.596	0.922 ± 0.892	0.677 ± 0.806	1.373 ± 1.111	**0.03**
		1.0 [0.864-1.654]	0.697 [0.085-1.580]	0.491 [0.131-0.849]	0.964 [0.596-2.073]	
RPS13		0.92 ± 0.311	1.636 ± 0.896	1.101 ± 0.586	2.087 ± 3.875	0.19
		1.0 [0.581-1.122]	1.326 [1.076-1.762]	1.028 [0.606-1.482]	1.03 [0.620-1.765]	

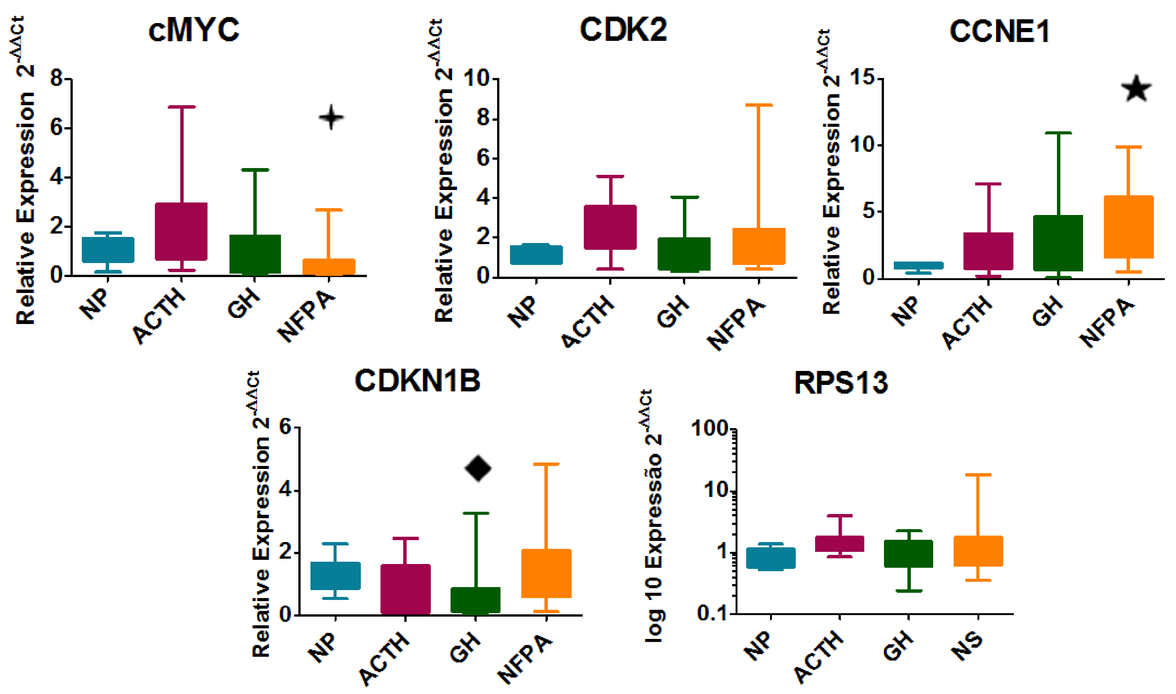

FIGURE 1: Relative Gene Expression among groups NP- normal pituitaries, ACTH- Corticotrophinomas, GH- somatotrophinomas, NFPA- non functioning pituitary adenomas.
✦ p=0.002 NFPA *vs* ACTH ◆ p=0.03 GH versus NP ★ p=0.02 NFPA versus NP

Conclusion

The p27-CDK2-CCNE1 pathway seems dysregulated in pituitary adenomas and may interact with other aberrant pathways, leading to an environment that may have putative role in pituitary tumorigenesis. Overexpression of *RPS13*, however, does not seem to be the underlying mechanism.

References

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