Endocrine (2012) 42:285–291
DOI 10.1007/s12020-012-9682-3

MINI REVIEW

PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways perturbations in non-functioning pituitary adenomas

Hadara Rubinfeld · Ilan Shimon

Received: 20 December 2011 / Accepted: 17 April 2012 / Published online: 3 May 2012

Abstract Non-functioning pituitary adenomas (NFPAs) comprise a heterogeneous group, which are considered the most common pituitary tumor. As no clinically hormone hypersecretion is apparent, non-functioning pituitary adenomas are often diagnosed only when they are large enough to cause tumor mass effects, such as hypopituitarism, visual field defects or headaches. Efficient medical therapy for NFPAs is currently unavailable and surgical treatment of these tumors is not always satisfactory. Characterization of signaling regulatory events in the context of NFPAs may enable the development of new attractive novel strategies. Although data regarding gene expression profiling of signaling pathways in NFPAs have accumulated, studies aimed at fine-classification of NFPAs-specific signaling regulatory mechanisms and feedback loops are scarce.

Keywords Non-functioning pituitary adenomas · Somatostatin · PI3K · mTOR · ERK

Introduction

Clinically non-functioning human pituitary adenomas constitute about 35 % of pituitary tumors. The majority of clinically NFPAs are of gonadotroph origin. Pituitary adenomas have the potential to become large and invasive macroadenomas and surgical treatment of these tumors is not always satisfactory. Unlike GH-, TSH-, and PRL-secreting adenomas, no effective medical therapy is available for these common adenomas. In a recently published meta-analysis of studies addressing the recurrence of pituitary adenomas after transsphenoidal surgery, the remission percentage found was significantly lowest in non-functioning pituitary adenomas (NFPAs) compared with other tumor types, owing probably to the large tumor size and extension at time of diagnosis [1]. The clinically available somatostatin (sst) analogs and dopamine agonists are poorly effective for the non-functioning adenomas [2, 3] and the more recently developed drug, SOM230, has promising potential in treating GH- and ACTH-secreting adenomas [4, 5] but no data regarding NFPAs treatments are available. Thus, new directions in the medical treatment of NFPAs are under research, based on the current knowledge of pituitary tumorigenesis mechanisms and the signaling pathways potentially involved. This will be summarized in this minireview.

H. Rubinfeld · I. Shimon (✉)
Institute of Endocrinology and Metabolism and Felsenstein Medical Research Center, Rabin Medical Center, Beilinson Campus and Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
e-mail: ilanshi@clalit.org.il

Differential expression of signaling components

Alterations in the mRNA and protein expression levels of signaling components have been studied throughout the years in pituitary adenomas using Northern, Reverse transcription real-time quantitative PCR, immunohistochemistry, immunoblotting and more recently, microarray, and proteomic analysis. NFPAs' unique expression patterns were found in some of the proteins investigated. For example, cyclin D1 [6–10], Cdk inhibitor, p15 [11] and p15-inducer, and clusterin [11] were discovered to be upregulated specifically in the non-functioning pituitary tumors compared with other tumor types or normal pituitary. HMGA2, another protein involved in cell cycle progression and other cellular processes, was shown to be overexpressed in most NFPAs studied, similar to that

Table 1 Differential expression of signaling components in NFPAs

PI3K pathway			ERK pathway			Tyrosine kinase receptors		
Gene	Expression[a]		Gene	Expression[a]		Gene	Expression[a]	
	mRNA	Protein		mRNA	Protein		mRNA	Protein
PIK3CA	NA	NA	Eps8 [13, 21]	↑[c]	↑	IGF-1R [61]	=	NA
Akt [20]	↑[b]	=	p-Eps8		NA	EGFR [32–36]	↑↓	↑↓
p-Akt [20, 21]		↑[c]	Raf [42]	↑[b]	↑[b]			
mTOR [22]	NA	=	MEK [22]	=	=			
p-mTOR [22]		=	p-MEK [22]		↑[b]			
TSC2 [22]	NA	=	ERK [22]	=	=			
p-TSC2 [22]		=	p-ERK [21, 22]		↑[c]			
p70S6K [22]	NA	=						
p-p70S6K [22]		=						
GSK3	NA	NA						
Zac1 [23]	↓[b]	↓[b]						
PTEN [20]	=	↓nuclear						

NA not available

↑↓ Contradictory data

[a] Compared to normal pituitary

[b] Highest/lowest in NFPAs

[c] In all tumor subtypes

= Similar to normal pituitary

observed in prolactinomas, although, HMGA2 up-regulation was not associated with chromosomal rearrangements in NFPAs [12]. Loss of expression of maternally expressed gene 3 was found selectively in NFPAs of gonadotroph origin [13–15]. Further information on epigenetic processes involved in pituitary tumorigenesis can be found in a review by Dudley et al. [16].

In this minireview, we have chosen to focus on PI3K/Akt/mTOR and Raf/MEK/ERK prominent signaling pathways known to trigger cell growth, proliferation, and survival. Components and regulators of these pathways are thus candidates for therapeutic targeting in NFPAs. For some of the proteins detailed below and summarized in Table 1, accumulating supporting data exist, whereas for others, limited reports are available for NFPAs.

PI3K/Akt/mTOR/Zac1

Overexpression of the components of PI3K/Akt/mTOR signaling pathway are found in numerous human cancers [17, 18]. To this end, a limited number of studies describe the expression levels of components and regulators of this pathway in pituitary adenomas.

PIK3CA

PIK3CA is the gene encoding the p110 subunit of PI3K. Similar frequencies of PIK3CA amplifications and mutations were found in the various types of pituitary adenomas [19] and significant association of p110 protein expression with PIK3CA copy gain was found [19]. However, to our knowledge, no report is yet available regarding the expression level of PI3K subunits in the different types of pituitary adenomas compared to the normal pituitary.

Akt/Zac1

In all pituitary tumors, and particularly in NFPAs, Akt was found to be over-expressed as well as overactivated [20, 21]. Interestingly, no overactivation was found in any pituitary tumor type for downstream components of this pathway, namely, mTOR, TSC2, and p70S6K [22]. Overactivation of Akt may, however, be reflected by the transcription factor zinc-finger protein Zac1 which is negatively regulated by PI3K pathway through GSK3 and p53. Indeed, the mRNA and protein levels of Zac1 were found to be significantly reduced or absent in NFPAs compared to normal anterior pituitary [23] and significant correlation was shown between low Zac1 expression and recurrence of NFPAs [24]. Additional information about Zac1 and pituitary tumorigenesis can be found in a recently published review [25]. In this context, it is worth mentioning that to the best of our knowledge, no report is currently available regarding the expression levels of total and phosphorylated GSK3 in pituitary adenomas.

PTEN

A negative regulator of PI3K pathway, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), also known as mutated in multiple advanced cancers is often lost in human tumors, especially those of the brain, prostate and endometrium, and was found, in human cancer, to be the second most frequently mutated gene after p53 [26]. However, in a study by Musat et al. [20] which analyzed the PTEN gene in pituitary adenomas, no mutation was found and PTEN transcript levels were not different than that in the normal pituitary. Interestingly, similar to other primary normal cells such as thyroid follicular cells and normal melanocytes, PTEN immunohistochemical expression in the normal pituitary is mainly nuclear [20]. NFPAs as well as secreting adenomas, and other tumors such as endocrine pancreatic tumor [27], express less nuclear PTEN than the normal tissue [20, 28], suggesting a post-translational regulation of PTEN in pituitary adenomas by a mechanism yet to be defined. It is of note that in follicular thyroid tumors, while nuclear PTEN diminishes during the progression from normal tissue to adenoma and carcinoma, the amount of phosphorylated Akt within the nucleus increases [29]. Thus, although a reduction in nuclear PTEN expression cannot address the cause for hyperactivation of Akt at the plasma membrane of pituitary adenomas, it may affect nuclear Akt. Accumulating data suggest that Akt may also play a role in tumorigenesis in the nucleus compartment (reviewed in [30]). In particular, a mouse model for epithelial cancers triggered by promyelocytic leukemia gene (*Pml*) loss highlights nuclear AKT function and identifies a PTEN–Pml–PP2a tumor-suppressive network for the inactivation of nuclear phospho-Akt [31]. Further studies, e.g., correlation studies of PTEN and phosphorylated Akt expression levels within the nucleus, are needed to establish the role of nuclear PI3K/Akt signaling pathway and the significance of nuclear PTEN as its negative regulator in pituitary adenomas.

Currently, few publications even partially address the expression and activation levels of the signaling components of PI3K/Akt/mTOR/Zac1 pathway in NFPAs (summarized in Table 1). The cause and the outcome of Akt overactivation requires further investigation.

EGFR/Eps8/Raf/MEK/ERK

Another signaling pathway which has been implicated in NFPAs tumorigenesis is the ERK pathway.

EGFR

Data regarding the expression level of epidermal growth factor receptor (EGFR) in NFPAs is controversial. Whereas in one study EGFR overexpression has been demonstrated by immunohistochemistry in most NFPAs [32]; in another study, EGFR mRNA and protein expression levels were extremely variable in some functional and non-functional adenomas [33]. Moreover, in later studies, only the minority of NFPAs displayed positive EGFR immunoreactivity and/or EGF binding sites [34–36]. An extensive review about the human EGFR family members (EGFR, ErbB, HER) and ligands in the pituitary has recently been published [37].

Eps8

Epidermal growth factor (EGF) receptor pathway substrate 8 (Eps8), initially identified as a downstream component of the EGF signaling pathway, is a docking protein that may be phosphorylated by various receptor tyrosine kinases including EGFR, fibroblast growth factor receptor, and platelet-derived growth factor receptor [38–40]. In a study aimed to further elucidate the molecular changes that contribute to the development of non-functioning tumors, one of the genes identified by microarray analysis with significant changes between NFPAs and normal pituitary tissues was Eps8 [13]. Semiquantitative RT-PCR in gonadotroph pituitary tumors, which comprise 40 % of NFPAs [41], confirmed Eps8 up-regulation [21]. The detection of phosphorylated Eps8 awaits the availability of a phospho-specific antibody.

Raf/MEK/ERK

Interestingly, a significant upregulation especially in NFPAs was also observed for Raf, ras effector, both at the mRNA and protein levels [42]. Overactivation of MEK was examined in hormone-secreting adenomas and NFPAs by the ratio of phospho-Ser217/221 MEK1/2/total MEK1/2 and was found highest in NFPAs compared to normal pituitary [22]. Overactivation of ERK1/2 was found higher in NFPAs and hormone-secreting adenomas compared to normal pituitary [21, 22].

In summary, overexpression of Eps8 and overactivation of Raf, MEK, and ERK may promote proliferation and cell survival of NFPAs, and may sensitize cells to growth factor activation independent of receptor up-regulation.

Targeting PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways in NFPAs

Somatostatin analogs

Sst analogs are not satisfactory in treatment of NFPAs patients [2, 43, 44], although they are successful in the

treatment of 60 % of GH-secreting pituitary tumors and reduce cell viability in cultured NFPAs in vitro [45–48]. An examination of their molecular mechanism of action further highlights this enigma: sst analogs were shown to deactivate both ERK (octreotide, SOM230) [49] and PI3K/Akt (octreotide) [50] fundamental signaling pathways, in cultured human pituitary adenoma cells (including NFPAs) and in the GH3 rat pituitary cell line, respectively. The ERK pathway was also shown to be involved in the significant cytostatic and cytotoxic effects of the dopamine–somatostatin chimeric compound BIM-23A760 in cultured cells from human NFPAs [51, 52]. Altogether, these data emphasize the specificity of the model used, namely, human versus rat, in vitro versus in vivo and the specific types of adenomas and sst analogs. Thus, the unique expression pattern profile of signaling components in NFPAs may account for the lack of somatostatin efficacy in these tumors; for example, the differential expression of the neuroprotective factor seladin-1 [53] and of Zac1 [23] in NFPAs compared to normal pituitary and secreting adenomas, as has already been discussed [46, 54]. Therefore, it seems that further studies specifically in the NFPAs model using various sst analogs are required to identify the molecular entity responsible for the lack of somatostatin efficacy in NFPAs treatment. An example of the impact of differential expression on sst efficacy was shown by Akt overexpression which eliminated the antiproliferative action of octreotide in the AtT-20 pituitary tumor cells [55]. These data raised the interesting possibility that sst2-expressing tumors with overactivated Akt, such as NFPAs, may be more resistant to the antiproliferative action of sst analogs that target mainly this receptor, such as octreotide.

mTOR inhibitors

NFPAs, however, may benefit by the overactivation of Akt regarding other compounds, such as the mTOR inhibitors. A screen of 13 human cancer cell lines treated with the rapamycin analog, everolimus, revealed the correlation of the antiproliferative response to everolimus with basal Akt S473 phosphorylation [56]. Indeed, rapamycin and everolimus inhibited pituitary cell proliferation in dispersed primary cultures in vitro [57, 58], although resistance of fraction of NFPAs was also shown [55]. This was attributed to the up-regulation of Ser473 Akt phosphorylation resulting from the inhibition of the negative feedback loop of P70S6K on IRS-1 [59]. Indeed, octreotide potentiated the antiproliferative effects of rapamycin in AtT-20 cells or human NFPA cells, sensitizing tumor cells even to low rapamycin concentrations [55]. Moreover, dual PI3K/mTOR inhibition by NVP-BEZ235, a synthetic small molecule that inhibits both PI3K and mTOR kinase activity, was more effective than everolimus in reducing cell viability of NFPA cells in a rat model of multiple endocrine neoplasia-like syndrome [60]. Interestingly, p27 expression positively correlated with the sensitivity of cells to NVP-BEZ235, suggesting that the expression of p27 in tumor cells may be a predictor of response to the dual PI3K/mTOR inhibition [60]. Notably, in a genetically defined mTORC1 deregulation model, the efficacy of NVP-BEZ235 did not correlate with the cellular response or Akt S473 phosphorylation induced by mTORC1 inhibition alone [56]. This suggests that the mechanism which underlies the potent efficacy of dual PI3K/mTOR inhibition may involve other parameters as well, a hypothesis that should be examined specifically in the NFPAs model.

IGF-1R inhibitors

Both PI3K/Akt and ERK signaling pathways diverge from the tyrosine kinase receptors IGF-1R and EGFR. mRNA expression of IGF-1R in NFPAs is similar to the normal pituitary [61] though IGF-1R protein expression level in NFPAs has not yet been reported. We have studied the effects of a selective IGF-1R inhibitor, NVP-AEW541, on human non-functioning pituitary tumor cells and non-secreting immortalized pituitary tumor cell line, MtT/E. Our results indicate the abrogation of IGF-1-induced cell proliferation and signaling by NVP-AEW541 [62].

EGFR inhibitors

As already mentioned above, in some studies the EGFR expression levels in NFPAs are low. However, EGFR blockade may still be effective as it does not necessarily correlate with EGFR expression [63]. Tyrosine kinase inhibitors (TKIs) of EGFR, such as lapatinib, a dual TKI of EGFR and HER2, and gefitinib, a TKI of EGFR, have been investigated in rat and human prolactinoma cells and were found to suppress cell growth [64, 65]. However, to the best of our knowledge, no study has yet been reported about the effects of EGFR inhibitors in NFPA cells. Moreover, recent findings indicate a possible cross-talk between somatostatin receptors and growth factor receptors such as EGFR in breast and glioma cancer cells [66], suggesting the high potential efficacy of sst analogs and EGFR inhibitors combinations. The relevance to NFPAs may be of great importance and requires further study.

Conclusions

Although data regarding the differential expression and function of signaling components in NFPAs are accumulating, they are insufficient. For example, the differential expression of microRNAs and their potential involvement in

pituitary tumorigenesis, are intriguing [67] and await further examination of NFPAs. There are additional specific questions that also require future research. In particular, the lack of efficacy of sst analogs in the treatment of patients with NFPA is in contrast to their in vitro efficacy and cannot be explained by the sst receptor expression profile. Another challenging question remaining to be answered is the cause for Akt hyperactivation: once revealed, it may offer new treatment opportunities. In addition, data are sometimes contradictory. For example, the gene expression profiling and the proteomic study of NFPAs by Moreno et al. [13] did not discover significant changes in any of the signaling molecules discussed here except for the Eps8. However, recent computational analysis of a series of mainly NFPAs proteomic expression data, demonstrated that IGF-1 and ERK cascades involved differentially expressed proteins [68]. Another example of contradictions found in the literature is with regard to EGFR expression levels in NFPAs [32–36]. Moreover, in each study, a significant variation in the rates of EGFR expression levels was reported [37], complicating the estimation of the potential efficacy of EGFR inhibitors in the treatment of patients with NFPA. Indeed, as NFPAs include different subtypes (gonadotroph adenomas expressing different hormonal profiles, null cell adenomas, silent corticotroph adenomas, etc.), it is not certain that the different signaling mechanisms involved in tumorigenesis are similarly active in the different adenoma subtypes.

Investigating the molecular mechanisms that confer sensitivity/resistance to available drugs such as pasireotide, mTOR, IGF-1R, and EGFR inhibitors, specifically in the NFPAs model, may enable the development of attractive novel strategies for treating these common tumors, for which no effective medical treatment is currently available.

Disclosure The authors have nothing to disclose.

References

1. F. Roelfsema, N.R. Biermasz, A.M. Pereira, Clinical factors involved in the recurrence of pituitary adenomas after surgical remission: a structured review and meta-analysis. Pituitary **15**, 71–83 (2011)
2. A. Colao, C. Di Somma, R. Pivonello, A. Faggiano, G. Lombardi, S. Savastano, Medical therapy for clinically non-functioning pituitary adenomas. Endocr. Relat. Cancer **15**(4), 905–915 (2008)
3. M.A. Tichomirowa, A.F. Daly, A. Beckers, Treatment of pituitary tumors: somatostatin. Endocrine **28**(1), 93–100 (2005)
4. A. Colao, S. Petersenn, J. Newell-Price, J.W. Findling, F. Gu, M. Maldonado, U. Schoenherr, B. Dipl, D. Mills, L.R. Salgado, B.M. Biller, A 12-month phase 3 study of pasireotide in Cushing's disease. N. Engl. J. Med. **366**(10), 914–924 (2012)
5. M. Boscaro, W.H. Ludlam, B. Atkinson, J.E. Glusman, S. Petersenn, M. Reincke, P. Snyder, A. Tabarin, B.M. Biller, J. Findling, S. Melmed, C.H. Darby, K. Hu, Y. Wang, P.U. Freda, A.B. Grossman, L.A. Frohman, J. Bertherat, Treatment of pituitary-dependent Cushing's disease with the multireceptor ligand somatostatin analog pasireotide (SOM230): a multicenter, phase II trial. J. Clin. Endocrinol. Metab. **94**(1), 115–122 (2009)
6. M.S. Elston, A.J. Gill, J.V. Conaglen, A. Clarkson, J.M. Shaw, A.J. Law, R.J. Cook, N.S. Little, R.J. Clifton-Bligh, B.G. Robinson, K.L. McDonald, Wnt pathway inhibitors are strongly down-regulated in pituitary tumors. Endocrinology **149**(3), 1235–1242 (2008)
7. N.A. Hibberts, D.J. Simpson, J.E. Bicknell, J.C. Broome, P.R. Hoban, R.N. Clayton, W.E. Farrell, Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin. Cancer Res. **5**(8), 2133–2139 (1999)
8. S. Jordan, K. Lidhar, M. Korbonits, D.G. Lowe, A.B. Grossman, Cyclin D and cyclin E expression in normal and adenomatous pituitary. Eur. J. Endocrinol. **143**(1), R1–R6 (2000)
9. D.J. Simpson, S.J. Frost, J.E. Bicknell, J.C. Broome, A.M. McNicol, R.N. Clayton, W.E. Farrell, Aberrant expression of G(1)/S regulators is a frequent event in sporadic pituitary adenomas. Carcinogenesis **22**(8), 1149–1154 (2001)
10. H.E. Turner, Z. Nagy, N. Sullivan, M.M. Esiri, J.A. Wass, Expression analysis of cyclins in pituitary adenomas and the normal pituitary gland. Clin. Endocrinol. (Oxf) **53**(3), 337–344 (2000)
11. V. Chesnokova, S. Zonis, C. Zhou, A. Ben-Shlomo, K. Wawrowsky, Y. Toledano, Y. Tong, K. Kovacs, B. Scheithauer, S. Melmed, Lineage-specific restraint of pituitary gonadotroph cell adenoma growth. PLoS One **6**(3), e17924 (2011)
12. G.M. Pierantoni, P. Finelli, E. Valtorta, D. Giardino, O. Rodeschini, F. Esposito, M. Losa, A. Fusco, L. Larizza, High-mobility group A2 gene expression is frequently induced in non-functioning pituitary adenomas (NFPAs), even in the absence of chromosome 12 polysomy. Endocr. Relat. Cancer **12**(4), 867–874 (2005)
13. C.S. Moreno, C.O. Evans, X. Zhan, M. Okor, D.M. Desiderio, N.M. Oyesiku, Novel molecular signaling and classification of human clinically nonfunctional pituitary adenomas identified by gene expression profiling and proteomic analyses. Cancer Res. **65**(22), 10214–10222 (2005)
14. X. Zhang, Y. Zhou, K.R. Mehta, D.C. Danila, S. Scolavino, S.R. Johnson, A. Klibanski, A pituitary-derived MEG3 isoform functions as a growth suppressor in tumor cells. J. Clin. Endocrinol. Metab. **88**(11), 5119–5126 (2003)
15. J. Zhao, D. Dahle, Y. Zhou, X. Zhang, A. Klibanski, Hypermethylation of the promoter region is associated with the loss of MEG3 gene expression in human pituitary tumors. J. Clin. Endocrinol. Metab. **90**(4), 2179–2186 (2005)
16. K.J. Dudley, K. Revill, R.N. Clayton, W.E. Farrell, Pituitary tumours: all silent on the epigenetics front. J. Mol. Endocrinol. **42**(6), 461–468 (2009)
17. D.A. Altomare, J.R. Testa, Perturbations of the AKT signaling pathway in human cancer. Oncogene **24**(50), 7455–7464 (2005)
18. I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat. Rev. Cancer **2**(7), 489–501 (2002)
19. Y. Lin, X. Jiang, Y. Shen, M. Li, H. Ma, M. Xing, Y. Lu, Frequent mutations and amplifications of the PIK3CA gene in pituitary tumors. Endocr. Relat. Cancer **16**(1), 301–310 (2009)
20. M. Musat, M. Korbonits, B. Kola, N. Borboli, M.R. Hanson, A.M. Nanzer, J. Grigson, S. Jordan, D.G. Morris, M. Gueorguiev, M. Coculescu, S. Basu, A.B. Grossman, Enhanced protein kinase B/Akt signalling in pituitary tumours. Endocr. Relat. Cancer **12**(2), 423–433 (2005)
21. M. Xu, L. Shorts-Cary, A.J. Knox, B. Kleinsmidt-DeMasters, K. Lillehei, M.E. Wierman, Epidermal growth factor receptor pathway substrate 8 is overexpressed in human pituitary tumors:

role in proliferation and survival. Endocrinology **150**(5), 2064–2071 (2009)
22. D. Dworakowska, E. Wlodek, C.A. Leontiou, S. Igreja, M. Cakir, M. Teng, N. Prodromou, M.I. Goth, S. Grozinsky-Glasberg, M. Gueorguiev, B. Kola, M. Korbonits, A.B. Grossman, Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. Endocr. Relat. Cancer **16**(4), 1329–1338 (2009)
23. U. Pagotto, T. Arzberger, M. Theodoropoulou, Y. Grubler, C. Pantaloni, W. Saeger, M. Losa, L. Journot, G.K. Stalla, D. Spengler, The expression of the antiproliferative gene ZAC is lost or highly reduced in nonfunctioning pituitary adenomas. Cancer Res. **60**(24), 6794–6799 (2000)
24. T.W. Noh, H.J. Jeong, M.K. Lee, T.S. Kim, S.H. Kim, E.J. Lee, Predicting recurrence of nonfunctioning pituitary adenomas. J. Clin. Endocrinol. Metab. **94**(11), 4406–4413 (2009)
25. M. Theodoropoulou, G.K. Stalla, D. Spengler, ZAC1 target genes and pituitary tumorigenesis. Mol. Cell. Endocrinol. **326**(1–2), 60–65 (2010)
26. Y. Yin, W.H. Shen, PTEN: a new guardian of the genome. Oncogene **27**(41), 5443–5453 (2008)
27. A. Perren, P. Komminoth, P. Saremaslani, C. Matter, S. Feurer, J.A. Lees, P.U. Heitz, C. Eng, Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. Am. J. Pathol. **157**(4), 1097–1103 (2000)
28. M.L. Tena-Suck, A. Ortiz-Plata, H.A. de la Vega, Phosphatase and tensin homologue and pituitary tumor-transforming gene in pituitary adenomas. Clinical-pathologic and immunohistochemical analysis. Ann. Diagn. Pathol. **12**(4), 275–282 (2008)
29. V. Vasko, M. Saji, E. Hardy, M. Kruhlak, A. Larin, V. Savchenko, M. Miyakawa, O. Isozaki, H. Murakami, T. Tsushima, K.D. Burman, C. De Micco, M.D. Ringel, Akt activation and localisation correlate with tumour invasion and oncogene expression in thyroid cancer. J. Med. Genet. **41**(3), 161–170 (2004)
30. A.M. Martelli, I. Faenza, A.M. Billi, L. Manzoli, C. Evangelisti, F. Fala, L. Cocco, Intranuclear 3′-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? Cell. Signal. **18**(8), 1101–1107 (2006)
31. L.C. Trotman, A. Alimonti, P.P. Scaglioni, J.A. Koutcher, C. Cordon-Cardo, P.P. Pandolfi, Identification of a tumour suppressor network opposing nuclear Akt function. Nature **441**(7092), 523–527 (2006)
32. S.S. Chaidarun, M.C. Eggo, M.C. Sheppard, P.M. Stewart, Expression of epidermal growth factor (EGF), its receptor, and related oncoprotein (erbB-2) in human pituitary tumors and response to EGF in vitro. Endocrinology **135**(5), 2012–2021 (1994)
33. V.K. LeRiche, S.L. Asa, S. Ezzat, Epidermal growth factor and its receptor (EGF-R) in human pituitary adenomas: EGF-R correlates with tumor aggressiveness. J. Clin. Endocrinol. Metab. **81**(2), 656–662 (1996)
34. M.L. Jaffrain-Rea, E. Petrangeli, C. Lubrano, G. Minniti, D. Di Stefano, F. Sciarra, L. Frati, G. Tamburrano, G. Cantore, A. Gulino, Epidermal growth factor binding sites in human pituitary macroadenomas. J. Endocrinol. **158**(3), 425–433 (1998)
35. A. Rishi, M.C. Sharma, C. Sarkar, D. Jain, M. Singh, A.K. Mahapatra, V.S. Mehta, T.K. Das, A clinicopathological and immunohistochemical study of clinically non-functioning pituitary adenomas: a single institutional experience. Neurol. India **58**(3), 418–423 (2010)
36. M. Theodoropoulou, T. Arzberger, Y. Gruebler, M.L. Jaffrain-Rea, J. Schlegel, L. Schaaf, E. Petrangeli, M. Losa, G.K. Stalla, U. Pagotto, Expression of epidermal growth factor receptor in neoplastic pituitary cells: evidence for a role in corticotropinoma cells. J. Endocrinol. **183**(2), 385–394 (2004)
37. O. Cooper, G. Vlotides, H. Fukuoka, M.I. Greene, S. Melmed, Expression and function of ErbB receptors and ligands in the pituitary. Endocr. Relat. Cancer **18**(6), R197–R211 (2011)
38. P.P. Di Fiore, G. Scita, Eps8 in the midst of GTPases. Int. J. Biochem. Cell Biol. **34**(10), 1178–1183 (2002)
39. F. Fazioli, L. Minichiello, V. Matoska, P. Castagnino, T. Miki, W.T. Wong, P.P. Di Fiore, Eps8, a substrate for the epidermal growth factor receptor kinase, enhances EGF-dependent mitogenic signals. EMBO J. **12**(10), 3799–3808 (1993)
40. M. Innocenti, E. Frittoli, I. Ponzanelli, J.R. Falck, S.M. Brachmann, P.P. Di Fiore, G. Scita, Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. J. Cell Biol. **160**(1), 17–23 (2003)
41. Y. Greenman, S. Melmed, Diagnosis and management of nonfunctioning pituitary tumors. Annu. Rev. Med. **47**, 95–106 (1996)
42. I. Ewing, S. Pedder-Smith, G. Franchi, M. Ruscica, M. Emery, V. Vax, E. Garcia, S. Czirjak, Z. Hanzely, B. Kola, M. Korbonits, A.B. Grossman, A mutation and expression analysis of the oncogene BRAF in pituitary adenomas. Clin. Endocrinol (Oxf) **66**(3), 348–352 (2007)
43. T.W. de Bruin, D.J. Kwekkeboom, J.W. Van't Verlaat, J.C. Reubi, E.P. Krenning, S.W. Lamberts, R.J. Croughs, Clinically nonfunctioning pituitary adenoma and octreotide response to long term high dose treatment, and studies in vitro. J. Clin. Endocrinol. Metab. **75**(5), 1310–1317 (1992)
44. M. Gasperi, L. Petrini, R. Pilosu, M. Nardi, A. Marcello, F. Mastio, L. Bartalena, E. Martino, Octreotide treatment does not affect the size of most non-functioning pituitary adenomas. J. Endocrinol. Invest. **16**(7), 541–543 (1993)
45. T. Florio, S. Thellung, S. Arena, A. Corsaro, R. Spaziante, G. Gussoni, G. Acuto, M. Giusti, G. Giordano, G. Schettini, Somatostatin and its analog lanreotide inhibit the proliferation of dispersed human non-functioning pituitary adenoma cells in vitro. Eur. J. Endocrinol. **141**(4), 396–408 (1999)
46. H. Padova, H. Rubinfeld, M. Hadani, Z.R. Cohen, D. Nass, J.E. Taylor, M.D. Culler, I. Shimon, Effects of selective somatostatin analogs and cortistatin on cell viability in cultured human nonfunctioning pituitary adenomas. Mol. Cell. Endocrinol. **286**(1–2), 214–218 (2008)
47. U. Renner, J. Mojto, M. Lange, O.A. Muller, K. von Werder, G.K. Stalla, Effect of bromocriptine and SMS 201-995 on growth of human somatotrophic and non-functioning pituitary adenoma cells in vitro. Eur. J. Endocrinol. **130**(1), 80–91 (1994)
48. M.C. Zatelli, D. Piccin, C. Vignali, F. Tagliati, M.R. Ambrosio, M. Bondanelli, V. Cimino, A. Bianchi, H.A. Schmid, M. Scanarini, A. Pontecorvi, L. De Marinis, G. Maira, E.C. degli Uberti, Pasireotide, a multiple somatostatin receptor subtypes ligand, reduces cell viability in non-functioning pituitary adenomas by inhibiting vascular endothelial growth factor secretion. Endocr. Relat. Cancer **14**(1), 91–102 (2007)
49. E. Hubina, A.M. Nanzer, M.R. Hanson, E. Ciccarelli, M. Losa, D. Gaia, M. Papotti, M.R. Terreni, S. Khalaf, S. Jordan, S. Czirjak, Z. Hanzely, G.M. Nagy, M.I. Goth, A.B. Grossman, M. Korbonits, Somatostatin analogues stimulate p27 expression and inhibit the MAP kinase pathway in pituitary tumours. Eur. J. Endocrinol. **155**(2), 371–379 (2006)
50. M. Theodoropoulou, J. Zhang, S. Laupheimer, M. Paez-Pereda, C. Erneux, T. Florio, U. Pagotto, G.K. Stalla, Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. Cancer Res. **66**(3), 1576–1582 (2006)
51. T. Florio, F. Barbieri, R. Spaziante, G. Zona, L.J. Hofland, P.M. van Koetsveld, R.A. Feelders, G.K. Stalla, M. Theodoropoulou, M.D. Culler, J. Dong, J.E. Taylor, J.P. Moreau, A. Saveanu, G. Gunz, H. Dufour, P. Jaquet, Efficacy of a dopamine-

somatostatin chimeric molecule, BIM-23A760, in the control of cell growth from primary cultures of human non-functioning pituitary adenomas: a multi-center study. Endocr. Relat. Cancer **15**(2), 583–596 (2008)
52. E. Peverelli, L. Olgiati, M. Locatelli, P. Magni, M.F. Fustini, G. Frank, G. Mantovani, P. Beck-Peccoz, A. Spada, A. Lania, The dopamine-somatostatin chimeric compound BIM-23A760 exerts antiproliferative and cytotoxic effects in human non-functioning pituitary tumors by activating ERK1/2 and p38 pathways. Cancer Lett. **288**(2), 170–176 (2010)
53. P. Luciani, S. Gelmini, E. Ferrante, A. Lania, S. Benvenuti, S. Baglioni, G. Mantovani, I. Cellai, F. Ammannati, A. Spada, M. Serio, A. Peri, Expression of the antiapoptotic gene seladin-1 and octreotide-induced apoptosis in growth hormone-secreting and nonfunctioning pituitary adenomas. J. Clin. Endocrinol. Metab. **90**(11), 6156–6161 (2005)
54. M. Korbonits, E. Carlsen, Recent clinical and pathophysiological advances in non-functioning pituitary adenomas. Horm. Res. **71**(Suppl 2), 123–130 (2009)
55. V. Cerovac, J. Monteserin-Garcia, H. Rubinfeld, M. Buchfelder, M. Losa, T. Florio, M. Paez-Pereda, G.K. Stalla, M. Theodoropoulou, The somatostatin analogue octreotide confers sensitivity to rapamycin treatment on pituitary tumor cells. Cancer Res. **70**(2), 666–674 (2010)
56. M. Breuleux, M. Klopfenstein, C. Stephan, C.A. Doughty, L. Barys, S.M. Maira, D. Kwiatkowski, H.A. Lane, Increased AKT S473 phosphorylation after mTORC1 inhibition is rictor dependent and does not predict tumor cell response to PI3K/mTOR inhibition. Mol. Cancer Ther. **8**(4), 742–753 (2009)
57. A. Gorshtein, H. Rubinfeld, E. Kendler, M. Theodoropoulou, V. Cerovac, G.K. Stalla, Z.R. Cohen, M. Hadani, I. Shimon, Mammalian target of rapamycin inhibitors rapamycin and RAD001 (everolimus) induce anti-proliferative effects in GH-secreting pituitary tumor cells in vitro. Endocr. Relat. Cancer **16**(3), 1017–1027 (2009)
58. M.C. Zatelli, M. Minoia, C. Filieri, F. Tagliati, M. Buratto, M.R. Ambrosio, M. Lapparelli, M. Scanarini, E.C. Degli Uberti, Effect of everolimus on cell viability in nonfunctioning pituitary adenomas. J. Clin. Endocrinol. Metab. **95**(2), 968–976 (2010)
59. K.E. O'Reilly, F. Rojo, Q.B. She, D. Solit, G.B. Mills, D. Smith, H. Lane, F. Hofmann, D.J. Hicklin, D.L. Ludwig, J. Baselga, N. Rosen, mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res. **66**(3), 1500–1508 (2006)
60. M. Lee, M. Theodoropoulou, J. Graw, F. Roncaroli, M.C. Zatelli, N.S. Pellegata, Levels of p27 sensitize to dual PI3K/mTOR inhibition. Mol. Cancer Ther. **10**(8), 1450–1459 (2011)
61. B. Kola, M. Korbonits, S. Diaz-Cano, G. Kaltsas, D.G. Morris, S. Jordan, L. Metherell, M. Powell, S. Czirjak, G. Arnaldi, S. Bustin, M. Boscaro, F. Mantero, A.B. Grossman, Reduced expression of the growth hormone and type 1 insulin-like growth factor receptors in human somatotroph tumours and an analysis of possible mutations of the growth hormone receptor. Clin. Endocrinol (Oxf) **59**(3), 328–338 (2003)
62. H. Rubinfeld, A. Gorshtein, E. Kendler, A. Kamar, O. Cohen, M. Hadani, I. Shimon IGF-1 induces tumorigenesis in human pituitary tumors—functional blockade of IGF-1 receptor as a novel therapeutic approach in non-functioning tumors, in *2nd ENEA Workshop, Aggressive Pituitary Tumors,* Munich, 2011, p. 71
63. F.M. Sirotnak, M.F. Zakowski, V.A. Miller, H.I. Scher, M.G. Kris, Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. Clin. Cancer Res. **6**(12), 4885–4892 (2000)
64. H. Fukuoka, O. Cooper, J. Mizutani, Y. Tong, S.G. Ren, S. Bannykh, S. Melmed, HER2/ErbB2 receptor signaling in rat and human prolactinoma cells: strategy for targeted prolactinoma therapy. Mol. Endocrinol. **25**(1), 92–103 (2011)
65. G. Vlotides, E. Siegel, I. Donangelo, S. Gutman, S.G. Ren, S. Melmed, Rat prolactinoma cell growth regulation by epidermal growth factor receptor ligands. Cancer Res. **68**(15), 6377–6386 (2008)
66. U. Kumar, Cross-talk and modulation of signaling between somatostatin and growth factor receptors. Endocrine **40**(2), 168–180 (2011)
67. X. Shi, B. Tao, H. He, Q. Sun, C. Fan, L. Bian, W. Zhao, Y.C. Lu, MicroRNAs-based network: a novel therapeutic agent in pituitary adenoma. Med. Hypotheses **78**(3), 380–384 (2012)
68. X. Zhan, D.M. Desiderio, Signaling pathway networks mined from human pituitary adenoma proteomics data. BMC Med. Genomics **3**, 13 (2010)