Akt as a potential prognostic marker in neuroendocrine tumors: a possibility?

“...current evidence suggests that p-Akt may be of prognostic value as part of a panel of phospho-proteins indicating activity of the PI3K/Akt/mTOR pathway in neuroendocrine tumors.”

Angela Hague^{*1} & Helen L Robbins²

First draft submitted: 13 October 2016; Accepted for publication: 14 October 2016;
Published online: 15 November 2016

Neuroendocrine tumors (NET) include intestinal and pancreatic NET (P-NET), bronchiopulmonary NET (BP-NET), pheochromocytomas, paragangliomas, neuroblastoma, medullary thyroid carcinoma, pituitary tumors, small cell lung cancer and Merkel cell tumors. The diversity and relative rarity of these tumors create important challenges to our understanding of their molecular biology and also to identifying markers of prognosis or response to therapy. Although NET are rare, the incidence has increased in recent years, at least in part due to improved diagnosis. Prognosis depends on the tissue of origin, tumor grade and tumor stage. The proliferative index is considered in the grading since it contributes to poor prognosis. Often these tumors are slow growing and well differentiated with an indolent course, but some are aggressive and poorly differentiated.

Chemotherapeutic approaches have not shown consistent benefit for patients

and attention has turned to molecular targeted therapy. The PI3K/Akt/mTOR pathway holds great promise, at least for a proportion of NET. In brief, various G-protein-coupled receptors, tyrosine kinase receptors and mutant *RAS* stimulate PI3K/Akt signaling. Phosphatidylinositol 3-kinase (PI3K) phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP₂), forming phosphatidylinositol 3,4,5-triphosphate (PIP₃), which recruits Akt to the cell membrane. Akt is then activated by phosphorylation enabling it to phosphorylate a plethora of downstream targets including mTOR, and hence Akt drives key cell processes such as cell survival, cell cycle progression, angiogenesis and cell migration.

Defects in the PI3K/Akt/mTOR pathway have been highlighted through exome sequencing of small intestinal NET (33%) and P-NET (14%) [1]. Multiple tyrosine kinase receptors and G-protein-coupled receptors that stimulate this pathway are

KEYWORDS

- Akt • biomarker
- everolimus • mTOR
- neuroendocrine tumors • p-Akt

¹School of Oral & Dental Sciences, University of Bristol, Lower Maudlin Street, Bristol, BS1 2LY, UK

²Department of Medicine, University Hospitals Coventry & Warwickshire, Clifford Bridge Road, Coventry, CV2 2DX, UK

*Author for correspondence: a.hague@bristol.ac.uk

overexpressed in NET [2]. Critically, the mTOR inhibitor everolimus (RAD001) increased progression-free survival (PFS) over placebo in clinical trial, and has been approved for treatment of progressively advanced P-NET, nonfunctional (nonsecreting) gastrointestinal and lung NET. Further clinical trials of everolimus and other mTOR inhibitors (either as single agents or in combination therapy) are underway or recruiting [3]. Of note, mTOR is only one of a large number of Akt target proteins, and drugs acting upstream through inhibiting tyrosine kinase receptors, PI3K and Akt are being evaluated as potential therapeutic options.

It is relevant to question whether activation of Akt, detected as phospho-Akt (p-Akt), can be used as a prognostic marker for NET, or as a method or predicting response to treatment. Akt1 is considered to be ‘fully active’ when phosphorylated at both threonine 308 (by PDK1) and serine 473 (primarily by mTOR complex 2: mTORC2). Expression of p-Akt in NET has been demonstrated by immunohistochemical staining using antibodies to p-Akt(Ser473), for example, in combined sets of gastroenteropancreatic NET [4–7]; BP-NET [8,9], pheochromocytomas [10,11] and pituitary adenomas [12]. Interestingly, p-Akt may also be detected in tumor stroma and blood vessels supplying the tumors. Comparisons with corresponding normal cells are not made directly in all studies, but the general picture is indicative of p-Akt(Ser473) overexpression and/or overactivation in a large proportion of NET. These studies and others showing elevated p-mTOR or its downstream targets suggest frequent overactivation of this pathway in these tumors.

Few studies of p-Akt expression examined PFS or overall survival of NET patients, possibly in part due to the extended follow-up needed due to the indolent course of many of these tumors. Fernandez *et al.* [13] found that higher staining scores for p-Akt(Ser473) or phospho-S6 ribosomal protein (p-S6) (a downstream target of mTOR), predicted shorter median PFS in a series of 25 metastatic neuroendocrine carcinomas of mixed anatomical sites. The same study also reported an association between staining for p-Akt(Ser473) and p-S6. In medullary thyroid carcinomas, studies showed no relationship between p-Akt(Ser473) and prognosis [14,15], although there was an association between p-Akt(Ser473) staining and p-mTOR and p-S6K staining, suggesting that Akt is actively stimulating mTOR activity in these tumors [14]. In incompletely

resected nonfunctioning pituitary adenomas, p-Akt(Ser473) expression was associated with recurrence (n = 35) [16]. In a series of 210 BP-NET, tumorlets and low-to-intermediate grade carcinoids tended to have a higher percentage of p-Akt(Ser473) positive cells than high-grade tumors [8], and although p-Akt-positive staining correlated with lymph node status in a study of 110 BP-NET [9], there was no association with disease-free survival or overall survival. Qian *et al.* [7] used a series of 195 gastroenteropancreatic NET to examine p-Akt(Ser473) and, although they observed association with p-mTOR, there was no association with prognosis.

The regulation of the PI3K/Akt/mTOR pathway is complex, and is controlled at a number of levels. Akt is regulated by ubiquitination, sumoylation and glycosylation as well as phosphorylation, and therefore measurement of downstream substrate activation in conjunction with p-Akt measurement will give a clearer picture of its activation, and may provide further prognostic information. Assessment of inhibitors of this pathway is also potentially informative. PTEN and TSC2 are key suppressors of the PI3K/Akt/mTOR pathway, and reduced levels of TSC2, PTEN (or both) were found in 85% of P-NET tumors in tissue microarray analysis. Furthermore, low levels of each of these proteins were associated with shorter PFS [17]. While PTEN is classically downregulated by allelic loss, there is strong evidence that regulation via microRNAs plays a role, as microRNAs that target PTEN are upregulated in NET [2].

Treatment with everolimus results in increased p-Akt, believed to be because elimination of negative feedback mechanisms downstream of mTORC 1 results in stabilization of IRS-1, a protein which potentiates PI3K signaling. Meric-Bernstam *et al.* [18] used paired pretreatment and on-treatment fine needle aspirates from 17 neuroendocrine carcinoma patients on a Phase II trial of everolimus and octreotide (a somatostatin analog) to determine whether baseline p-Akt would be predictive of response and whether induction of p-Akt by everolimus would engender resistance. In this study, p-Akt(Thr308) was detected using a reverse phase protein array. Pretreatment of p-Akt(Thr308) was reported to be associated with PFS, but this was of marginal significance (p = 0.0533); however, patients who had a partial response to everolimus treatment were significantly more likely to show an increase

“...assessment of the dual phosphorylation status of Akt may prove to be a more robust marker of prognosis.”

in p-Akt(Thr308) than patients with stable or progressive disease. Moreover, on-treatment p-Akt(Thr308) showed an association with PFS, suggesting that this is a marker of everolimus response. What is interesting is that no such association was detected using archival tumor blocks from 23 patients on the same trial, probably because use of fine needle aspirates and reverse phase protein array is a more sensitive technique. Monitoring p-Akt(Thr308) or both p-Akt(Thr308) and p-Akt(Ser473) together improved the prognostic significance of Akt activation in a series of 116 primary neuroblastoma samples by tissue microarray analysis compared with p-Akt(Ser473) alone [19]. In this study p-Akt, but not S6 ribosomal protein, was indicative of poor prognosis. Relatively few studies of NET have included measurement of p-Akt(Thr308), yet assessment of the dual phosphorylation status of Akt may prove to be a more robust marker of prognosis.

As stated above, mTOR inhibition results in elevated p-Akt through disruption of negative feedback. Critically, this is potentially a mechanism of drug resistance and reduced efficacy of mTOR inhibitors. Dual PI3K/mTOR inhibitors should prevent this escape mechanism, and therefore could perhaps be a more effective therapeutic approach. One such inhibitor, BEZ235, was put into Phase I trial after promising preclinical results, however toxicity proved problematic. The RIP1-Tag2 mouse develops P-NET that shows elevated p-Akt similar to human P-NET and has proved useful in evaluating the effectiveness of everolimus [1]. Using this model, p110 α PI3K was highlighted as the isoform contributing to carcinogenesis. Genetic and pharmacological inhibition of the p110 α PI3K led to decreased

p-Akt, increased tumor cell death, and reduced angiogenesis and metastatic dissemination [20].

Current research is hampered by the rarity and diversity of these tumors, and further research would perhaps benefit from larger more homogeneous cohorts of patients. Further genomic, transcriptomic (including microRNAs) and proteomic analyses are needed to provide clearer molecular profiles to help define prognostic biomarkers. Using a single phospho-protein as a marker of pathway activation is perhaps over-simplistic: there is a move towards identifying a panel of phosphoproteins to provide a more rigorous assessment of PI3K/Akt/mTOR pathway activation, and to evaluate activity of compensatory pathways such as the MEK/ERK pathway. In addition, the bulk of current research utilizes p-Akt(Ser473) as a marker of pathway activation, but it will be worthwhile to include p-Akt(Thr308) as a potential marker.

In summary, current evidence suggests that p-Akt may be of prognostic value as part of a panel of phosphoproteins indicating activity of the PI3K/Akt/mTOR pathway in NET. There is a promising evidence to suggest that such a biomarker panel will be useful in predicting and monitoring tumor response to therapies that target the signaling nodes of this pathway.

“...a biomarker panel will be useful in predicting and monitoring tumour response to therapies...”

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Capdevila J, Meeker A, Garcia-Carbonero R *et al.* Molecular biology of neuroendocrine tumors: from pathways to biomarkers and targets. *Cancer Metastasis Rev.* 33(1), 345–351 (2014).
- Briest F, Grabowski P. PI3K–AKT–mTOR-signaling and beyond: the complex network in gastroenteropancreatic neuroendocrine neoplasms. *Theranostics* 4(4), 336–365 (2014).
- ClinicalTrials.gov. <https://clinicaltrials.gov/>
- Shah T, Hochhauser D, Frow R, Quaglia A, Dhillon AP, Caplin ME. Epidermal growth factor receptor expression and activation in neuroendocrines. *J. Neuroendocrinol.* 18(5), 355–360 (2006).
- Ghayouri M, Boulware D, Nasir A, Strosberg J, Kvols L, Coppola D. Activation of the serine/threonine protein kinase Akt in enteropancreatic neuroendocrine tumors. *Anticancer Res.* 30(12), 5063–5067 (2010).
- Shida T, Kishimoto T, Furuya M *et al.* Expression of an activated mammalian target of rapamycin (mTOR) in gastroenteropancreatic neuroendocrine tumors. *Cancer Chemother. Pharmacol.* 65(5), 889–893 (2010).
- Qian ZR, Ter-Minassian M, Chan JA *et al.* Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. *J. Clin. Oncol.* 31(27), 3418–3425 (2013).
- Ali G, Boldrini L, Capodanno A *et al.* Expression of p-AKT and p-mTOR in a large series of bronchopulmonary neuroendocrine tumors. *Exp. Ther. Med.* 2(5), 787–792 (2011).
- Bago-Horvath Z, Sieghart W, Grusch M *et al.* Synergistic effects of erlotinib and everolimus on bronchial carcinoids and large-cell neuroendocrine carcinomas with activated EGFR/AKT/mTOR pathway. *Neuroendocrinology* 96(3), 228–237 (2012).
- Fassnacht M, Weismann D, Ebert S *et al.* AKT is highly phosphorylated in

- pheochromocytomas but not in benign adrenocortical tumors. *J. Clin. Endocrinol. Metab.* 90(7), 4366–4370 (2005).
- 11 Chaux A, Brimo F, Gonzalez-Roibon N *et al.* Immunohistochemical evidence of dysregulation of the mammalian target of rapamycin pathway in primary and metastatic pheochromocytomas. *Urology* 80(3), 736 e737–e712 (2012).
- 12 Musat M, Korbonits M, Kola B *et al.* Enhanced protein kinase B/Akt signalling in pituitary tumors. *Endocr. Relat. Cancer* 12(2), 423–433 (2005).
- 13 Fernandes I, Pacheco TR, Costa A *et al.* Prognostic significance of AKT/mTOR signaling in advanced neuroendocrine tumors treated with somatostatin analogs. *Oncotargets Ther.* 5, 409–416 (2012).
- 14 Rapa I, Saggiorato E, Giachino D *et al.* Mammalian target of rapamycin pathway activation is associated to RET mutation status in medullary thyroid carcinoma. *J. Clin. Endocrinol. Metab.* 96(7), 2146–2153 (2011).
- 15 Erovc BM, Kim D, Cassol C *et al.* Prognostic and predictive markers in medullary thyroid carcinoma. *Endocr. Pathol.* 23(4), 232–242 (2012).
- 16 Noh TW, Jeong HJ, Lee MK, Kim TS, Kim SH, Lee EJ. Predicting recurrence of nonfunctioning pituitary adenomas. *J. Clin. Endocrinol. Metab.* 94(11), 4406–4413 (2009).
- 17 Missiaglia E, Dalai I, Barbi S *et al.* Pancreatic endocrine tumors: expression profiling evidences a role for AKT–mTOR pathway. *J. Clin. Oncol.* 28(2), 245–255 (2010).
- 18 Meric-Bernstam F, Akcakanat A, Chen H *et al.* PIK3CA/PTEN mutations and Akt activation as markers of sensitivity to allosteric mTOR inhibitors. *Clin. Cancer Res.* 18(6), 1777–1789 (2012).
- 19 Opel D, Poremba C, Simon T, Debatin KM, Fulda S. Activation of Akt predicts poor outcome in neuroblastoma. *Cancer Res.* 67(2), 735–745 (2007).
- 20 Soler A, Figueiredo AM, Castel P *et al.* Therapeutic benefit of selective inhibition of p110alpha PI3-kinase in pancreatic neuroendocrine tumors. *Clin. Cancer Res.* doi:10.1158/1078–0432.CCR-15–3051 (2016) (Epub ahead of print).