Relevance of neuroendocrine tumours models assessed by kinomic profiling

David Romano

Dochead : Klotz communication 2019 : New insights into the pathophysiology and treatment of Neuroendocrine Tumors

Relevance of neuroendocrine tumours models assessed by kinomic profiling

Pertinence des modèles de tumeurs neuroendocrines évalués sur le profil des kinases

David Romano

Marseille Medical Genetics, MMG, U1251 Inserm, Aix-Marseille Univ, Marseille, France

david.romano@univ-amu.fr

+33 4 91 69 89 21

Abstract

Although there is evidence of a significant rise of neuroendocrine tumours (NETs) incidence, current treatments are largely insufficient due to somewhat poor knowledge of these tumours. Despite many efforts achieved to expose driver oncogene mutations in NETs, the genetic landscape of NETs is characterized by relatively few mutations and chromosomal aberrations per tumour compared with other tumour types. In addition, NETs display few actionable mutations providing compelling rationale for targeted therapies. Recent works aiming at characterizing currently used NETs *in vitro* models at the genomic level raised concerns on their reliability as *bona fide* tools to study NETs biology. However, the lack of actionable mutation in NETs implies that sole use of genomic is not sufficient to describe these models and establish appropriate therapeutic strategies. Several kinases and kinase-involving signalling pathways have been demonstrated as abnormally regulated in NETs. Yet, kinases have only been investigated regardless of their involvement in large intracellular signalling networks. In order to assess the validity of in vitro NETs models to study NETs biology, "next-generation" high throughput functional technologies based on "kinome-wide activity" will demonstrate the similarities between signalling pathways in NETs models and patients' samples. These approaches will significantly assist in identifying actionable alterations in NETs signalling pathways and guide patient stratification into early-phase clinical trials based on kinase inhibition targeted therapies.

Keywords: neuroendocrine tumours cell lines, signalling pathways, kinases, proteomics

Résumé

Malgré une augmentation significative de l'incidence des tumeurs neuroendocrines (TNE), les traitements actuels sont largement insatisfaisants en raison d'une connaissance insuffisante de ces tumeurs. Malgré les nombreux efforts déployés pour identifier des mutations oncogéniques dans les TNE, le profil génétique des TNE est caractérisé par relativement peu de mutations et aberrations chromosomiques en comparaison avec d'autres types de tumeurs. En outre, les TNE possèdent peu de mutations conductrices justifiant de manière convaincante des stratégies de thérapies ciblées. Des travaux récents visant à caractériser les modèles *in vitro* de TNE actuellement utilisés au niveau génomique ont émis des doutes quant à leur fiabilité pour étudier la biologie des TNE. Cependant, l'absence de mutation conductrice dans les TNE signifie que la génomique à elle seule ne permet pas de caractériser les modèles d'étude de TNE ni d'établir des stratégies thérapeutiques appropriées. Plusieurs kinases et voies de signalisation ont été démontrées comme anormalement régulées dans les TNE. Cependant, ces travaux n'ont pas pris en compte l'implication de ces kinases dans de grands réseaux de signalisation intracellulaire. Afin d'évaluer la validité des modèles de TNE *in vitro* pour étudier la biologie des TNE, des technologies fonctionnelles à haut débit dites «de nouvelle génération», basées sur l'activité du « kinome », permettront de révéler les similitudes entre les voies de signalisation des modèles de TNE et les échantillons de patients. Ces approches aideront de manière significative à identifier des perturbations dans les voies de signalisation dans les TNE et guideront la stratification des patients pour développer des essais cliniques basés sur une pharmacologie ciblée des kinases.

Mots clés: lignées cellulaires de tumeurs neuroendocrine, voies de signalisation, kinases, protéomique

1. Introduction

High throughput genomics technologies, such as next-generation sequencing (NGS), allowed the identification of actionable mutations in tumours, to which targeted therapies can be developed with the potential to improve therapeutic index aiming at targeting the tumour versus normal tissues in contrast to conventional cytotoxic agents. Increasingly, NGS of patient tumour samples guides patient stratification into clinical trials, such that only the patients bearing specific molecular alterations will receive the corresponding targeted therapy. In parallel, extensive use of well-characterized *in vitro* preclinical models (e.g. cell lines) improved significantly our knowledge of tumour biology. Despite initial success and clinical deployment of this concept, several limitations have emerged [1, 2], such as the lack of driver mutations in a majority of tumours that are sequenced in the clinic and the differences between in vitro models and original tumours. These obstacles are particularly accurate in rare tumours or tumours with few, if any, actionable mutations, like neuroendocrine tumours, representing a significant unmet challenge in precision oncology. This highlights the need to bring forward cost-effective complementary approaches that will assist in identifying activated pathways that can be targeted therapeutically [2].

2. Genomic pitfalls and caveats of *in vitro* neuroendocrine tumours models

Neuroendocrine tumours (NETs) are neoplasms originating in hormone producing cells of the endocrine system. NETs can exhibit functional and non-functional symptoms and represent a heterogeneous group of neoplasm. Based on pathological analysis, NETs are generally classified into well-differentiated low-to-intermediate grade (grade 1 and 2) versus aggressive poorly differentiated tumours (grade 3, also named neuroendocrine carcinomas or NEC).

Alike more frequent type of tumours, the understanding of NETs biology depends essentially on advances in techniques such as tumour tissue and cell culture [3]. Patient tumour-derived

cell lines have been widely used to study the molecular mechanisms of tumours, their response to therapy and thus have considerably contributed to improve cancer research over recent decades. Cell lines, as disease models, present several outstanding advantages, such as their ease of manipulation and low cost, facilitating also their use for drug screening (e.g. concomitant study of multiple cell lines and multiple combinations of drugs). When a model system is used, it is essential to consider the similarities and differences between the model and reality, so that the model can be validly applied. There are also important limitations, such as the occurrence of changes/transformations in the original cell required for *in vitro* growth or differences from the original tumour microenvironment (deficit in vascularization and hypoxia). In addition, cell lines do not exactly reflect their tumour of origin and can differ substantially in terms of genomic alterations, protein expression and therapeutic sensitivity [4-8]. These biological differences between *in vivo/in vitro* tumour cell lines and human neoplasms must be considered when experimental systems are used as models for human cancer [9].

The establishment of NETs cell lines has proved to be difficult. This has been attributed, at least in part, to the low proliferation rate of NETs [10]. Despite these challenges, several cell lines of human and rodent origins have been established from small intestinal and pancreatic NETs [11]. The most commonly used human neuroendocrine cell lines origin include the pancreatic cell lines BON-1 [12] and QGP-1 [13], as well as the small intestinal cell lines GOT1 [14], P-STS, L-STS and H-STS [15], the neuroendocrine carcinoma cell lines NEC-DUE1, NEC-DUE2 [16] and N-TAK1 [17] and carcinoid lung cell lines, such as H727. As it stands, authentic NETs cell lines are rare, their genomic and mutational features poorly described and comprehensive information regarding their therapeutic sensitivity is incomplete. Recently, efforts have been made to characterize pancreatic NET-derived cell lines BON-1 and QGP-1 by exome sequencing and genome-wide copy number analysis.

These studies have raised questions regarding their relevance as models due to their deficiency in pancreatic NET-associated mutations [18, 19]. In addition, several NET-derived cell lines were recently comprehensively characterized, including evaluation of their neuroendocrine phenotype, genomic alterations and therapeutic sensitivity profiles [20]. This work found that neuroendocrine phenotype was preserved in only a subset of these NETs cell lines and that they harbour additional genomic alterations than those depicted for NETs. One major concern is the origin of NETs cell lines and whether they were derived from well-differentiated neuroendocrine tumours or poorly differentiated neuroendocrine carcinomas. It is straightforwardly questionable that available NET cell lines were established from more aggressive tumours than expected and thus should be classified as NEC. The occurrence of *TP53* mutations in several NET cell lines (P-STS, BON-1 and QGP-1) further strengthens this concern [19, 20]. The original publications on NETs cell lines do not contain sufficient data to determine the grade of the tumours from which cell lines were derived. As a consequence, these observations emphasises the need to be cautious when drawing conclusions from studies performed on NET cell lines.

In addition to traditionally obtained patient-derived cell lines, our group recently developed a new method for culturing primary pancreatic NETs cells using bovine extracellular matrix [21, 22]. This culturing method established on 30 pancreatic NETs allowed maintaining cells with neuroendocrine features and assessing their genomic mutations and drug responses [21, 22]. Primary cultures of pancreatic NET, as well as NEC models, have also been achieved by other laboratories [16, 23-25] and can be considered as appropriate models to use.

The common objective of cancer researchers and clinicians is to better match patients with therapies. So far, NGS-based matching has been the most advanced technology applied to this problem. Cancers accumulate genetic alterations, but we may be approaching a running time limit regarding detection these driver oncogenes [26]. Indeed, multiple studies revealed that

most malignancies lack actionable mutations or harbour mutations either in non-druggable oncogenes (e.g. RAS, MYC family proteins) or in genes of poorly characterized therapeutic value [27-30]. Moreover, even the best achievements demonstrate that identifying well-characterized mutations in an individual patient may generate transient, if any, benefit from single-agent targeted therapy. For instance, while mutation-directed therapy often achieves a remarkable initial response, this is almost inevitably followed by relapse and emergence of drug resistance [31, 32]. Finally, widespread analysis of hundreds of cell lines and compounds shows that, with some remarkable exceptions (e.g. BRAF, HER2/ERBB2, EGFR inhibitors), mutations are poor predictor of drug sensitivity [33, 34].

Therapeutic management of NETs faces multiple challenges due to tumour heterogeneity and relatively poor knowledge of their biology. The therapeutic route for NETs has recently rapidly evolved and the increasing number of treatment options for patients unveiled matters such as timing, sequencing and selection of therapies as core priorities [35]. Since traditional treatments usually induce tumour stabilization for limited length of time, there is a need to develop novel approaches to overcome treatment-related resistance in patients with advanced and progressive NETs. With this goal in mind, many efforts were recently put together in order to expose driver oncogene mutations in NETs [36, 37]. However, despite growing evidences that poorly differentiated NETs may harbour mutations commonly observed in pancreatic or colorectal adenocarcinomas [38-42], the genetic landscape of NETs is characterized by relatively few mutations and chromosomal aberrations per tumour compared with other tumour types [36]. In addition, whole-genome integrated analysis confirmed previous observations that NETs display few, if any, actionable mutations providing compelling rationale for targeted therapies [36]. As such, only a small proportion of mutations identified from whole-genome sequencing have functional data suggesting that they should be targeted with drugs [11, 36]. The overwhelming complexity of the cancer genome suggests

we are in the earliest phases of interpreting such results and translating that data into knowledge that is useful to clinicians. Thus, new transversal technologies are needed to hasten the era of precision medicine in cancer.

3. Kinome study to the rescue

In recent years, much attention has focused on identifying key cellular signal transduction pathways that are abnormally regulated in the cancer cell. Such pathways regulate cancer-relevant cellular processes, such as cell growth, cell division and cell survival. Typically, these pathways involve cascades of cytoplasmic kinases that ultimately impact on gene transcription. Recent advances in our understanding of the fundamental molecular mechanisms underlying cancer cell signalling have elucidated a crucial role for kinases in the carcinogenesis and metastases of various types of cancer [43, 44]. Since most protein kinases promote cell proliferation, survival and migration, when overexpressed or activated by unlicensed inputs (e.g. by constitutively active mutations or permanently activated by abnormally upstream signalling partners), they are also directly associated with tumorigenesis and/or tumour progression. Genome-wide studies of kinase mutations have revealed that genetically inherited variants of specific kinases are causally associated with cancer initiation, promotion, progression as well as recurrence [45-47]. Over the last decades, multiple human malignancies have been identified to be associated with modulation and dysfunction of protein and lipid kinases and deactivated phosphatases on account of chromosomal reshuffling and genetic mutations. Deregulation of kinases has also been demonstrated in many human disorders including immune, neurological and infectious diseases. However, there is probably no greater clinical niche for kinases as key targets for developing drugs than in cancer therapy. Kinome, the complete set of protein kinases encoded in its genome, has become an attractive target for the treatment of numerous types of cancer. Single and multiple

kinase inhibitors, both synthetic and natural molecules, are now targeted therapeutic strategies for treatment of human malignancies. As such, there is keen interest in elucidating the specific pathways altered in a given tumour in order to identify relevant targets and help predict treatment responses.

Several signalling pathways involving multiple kinases regulate NETs tumorigenesis and tumour progression (for extensive reviews, see [48] and [49]). To briefly summarize, pathways such as PI3K/Akt/mTor, Ras/MAPK and Notch have been shown as deregulated NETs. In particular, the PI3K/AKT/mTor pathway has been highlighted as a key player in the development of NETs, particularly from pancreas [50, 51]. In this context, the mTor inhibitor Everolimus has been largely studied in NETs and approved by both Food and Drug Agency (FDA) and European Medical Agency (EMA) for patients with advanced well/moderately differentiated pancreatic NETs. However, comprehensive clinical studies relying on Everolimus did not allow observing any improvement of overall survival of NETs patients despite a significant increase in progression free survival [52-55]. This relative lack of efficiency has been attributed to feedback mechanisms acting as compensation between Ras/MAPK and AKT/mTor pathways in order to maintain a prosurvival signal and cell homeostasis in the presence of these inhibitors [21, 56, 57]. Overall, studies focusing on specific candidate kinases sporadically identified abnormalities in expression and/or activation levels of isolated kinases in NETs [58-65] without considering their multiple interactions within signalling modules.

The human protein kinome comprises over 500 proteins controlling intracellular signalling networks. There is increasing appreciation among researchers and clinicians of the value of investigating biology and pathobiology at the level of cellular kinome activity. Kinome analysis provides valuable opportunity to gain insights into complex biology (including diseases), to identify biomarkers of critical phenotypes (for prognosis and therapeutic

efficacy) and targets for therapeutic intervention through kinase inhibitors [43]. Although genomic and transcriptomic approaches have identified many driver kinases in human cancer, the map portrayed by these approaches is incomplete. This is because protein kinases are regulated at multiple levels, including protein translation, stability and post-translational modification, presenting further mechanisms for deregulation in cancer that are not revealed by interrogation of samples at the DNA and mRNA levels. Consequently DNA- or mRNA-based studies can result in false negatives by not identifying protein kinases deregulated at one or more post-transcriptional levels, and false positives — for example, mutated kinases with low protein stability or kinases that are not overexpressed upon cognate gene amplification [43, 44]. A further confounding issue is that protein kinases usually function as components of larger pathways and networks, in which signal output can be subject to control mechanisms that act on separate pathway or network components. As such, kinases can also be deregulated as a result of molecular perturbations of interacting and/or regulatory partners. Consequently, pathway and network activity also needs to be taken into consideration when assessing the potential driver role of a given protein kinase. Importantly, these shortcomings can be addressed using proteomics.

Recently, considerable advances in methods to assess the activity of the kinome were achieved (for review see [66-68]). These technological innovations allow a comprehensive interrogation of kinome activity in different conditions, e.g. drug response. Two general approaches have emerged to assess the activity and architecture of the kinome in cells: one based on activation state-specific antibodies and another based on mass-spectroscopic analysis of phosphorylated substrates or of kinases captured in their activated state on inhibitor-coated beads. While there are obviously conceptual linkages in these approaches, they are based on distinct experimental techniques and priorities. It is important to emphasize that kinome analysis serves to define the activities of kinases that are responsible for

mediating many phosphorylation events to regulate distinct, but probably complementary, biological responses. In contrast, mass-spectrometric based phosphoproteome analysis seeks to provide information about the phosphorylation status of every kinase substrates (byidentification of phosphor-acceptor sites) within the proteome. The conceptual difference between the two approaches is perhaps best demonstrated by the consideration that complete coverage of the kinome could be achieved with around 500 data points, whereas comprehensive coverage of the phosphoproteome would require in the order of 100 000 data points.

In order to better depict NETs models, speed up acute data acquisition and thus establish a precise mapping of key signalling modules important for NETs biology, we have recently been using a novel micro-array based method to study kinome activity developed by PamGene (Hertogenbosch, The Netherlands). This highly sensitive method not only channels the study of kinomics from NETs cell lines, but also from tumour samples and then be an asset for the design of therapeutic strategies based on kinomics (unpublished data). It also provides a unique opportunity to compare kinome profiles from *in vitro* models and patients' samples in order to assess the relevance of preclinical models based on a functional assay despite their genetic discrepancies with NETs. This study will then fulfil the ID card of NETs *in vitro* models and will enable a better judgement towards their validity as good models to study NETs biology. Additional complexities arise from the ability of microenvironmental factors to influence phosphorylation-dependent signalling and from the tendency for some signalling processes to occur heterogeneously among tumour cells. However, kinomics profiling offers the prospect to select a therapeutic option not only based on proliferation rate or mutational background, as set by current standard strategies for NETs, but also on selective activated kinases identified by global kinome activity analysis.

4. Concluding remarks

Precision medicine is about matching the right drugs to the right patients and its application into clinical practice has considerably impacted the management of cancer over the last decade. Although this approach is technology agnostic, there is a clear inclination to make precision medicine synonymous with genomics in cancer. However, lack of detection of actionable mutations by sole use of genomic techniques is a frequent problem in the clinic. A recent cancer genomics study has shown that half of driver mutations in tumours occur outside of well-characterized cancer genes [69]. Such mutations will not be identified in tumour sequencing studies. Therefore, clinical implementation of unbiased signalling pathway analysis technologies to large patient cohorts or rare tumour cases, such as NETs, could improve (i) validation of preclinical models used for investigation, (ii) selection of patients to early-phase clinical trials for kinase-inhibition-based targeted therapies. Such need to broaden the spectrum of techniques to detect genetic and non-genetic targetable cancer vulnerabilities is currently increasingly raised in precision oncology.

References

[1] Tannock IF, Hickman JA. Limits to Personalized Cancer Medicine. N Engl J Med. 2016 Sep 29;375(13):1289-94.

[2] Friedman AA, Letai A, Fisher DE, Flaherty KT. Precision medicine for cancer with next-generation functional diagnostics. Nat Rev Cancer. 2015 Dec;15(12):747-56.

[3] Witkowski JA. Experimental pathology and the origins of tissue culture: Leo Loeb's contribution. Med Hist. 1983 Jul;27(3):269-88.

[4] Stein WD, Litman T, Fojo T, Bates SE. A Serial Analysis of Gene Expression (SAGE) database analysis of chemosensitivity: comparing solid tumors with cell lines and comparing solid tumors from different tissue origins. Cancer Res. 2004 Apr 15;64(8):2805-16

[5] Sandberg R, Ernberg I. Assessment of tumor characteristic gene expression in cell lines using a tissue similarity index (TSI). Proc Natl Acad Sci U S A. 2005 Feb 8;102(6):2052-7.

[6] Ertel A, Verghese A, Byers SW, Ochs M, Tozeren A. Pathway-specific differences between tumor cell lines and normal and tumor tissue cells. Mol Cancer. 2006 Nov 2;5(1):55.

[7] Gillet JP, Calcagno AM, Varma S, Marino M, Green LJ, Vora MI, et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. Proc Natl Acad Sci U S A. 2011 Nov 15;108(46):18708-13.

[8] Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun. 2013;4:2126.

[9] Rockwell S. In vivo-in vitro tumour cell lines: characteristics and limitations as models for human cancer. Br J Cancer Suppl. 1980 Apr;4:118-22.

[10] Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. J Natl Cancer Inst. 2008 Sep 17;100(18):1282-9.

[11] Kawasaki K, Fujii M, Sato T. Gastroenteropancreatic neuroendocrine neoplasms: genes, therapies and models. Dis Model Mech. 2018 Feb 26;11(2).

[12] Evers BM, Townsend CM Jr, Upp JR, Allen E, Hurlbut SC, Kim SW, et al. Establishment and characterization of a human carcinoid in nude mice and effect of various agents on tumor growth. Gastroenterology. 1991 Aug;101(2):303-11.

[13] Kaku M, Nishiyama T, Yagawa K, Abe M. Establishment of a carcinoembryonic antigen-producing cell line from human pancreatic carcinoma. Gan. 1980 Oct;71(5):596-601.

[14] Kölby L, Bernhardt P, Ahlman H, Wängberg B, Johanson V, Wigander A, et al. A transplantable human carcinoid as model for somatostatin receptor-mediated and amine transporter-mediated radionuclide uptake. Am J Pathol. 2001 Feb;158(2):745-55.

[15] Pfragner R, Behmel A, Höger H, Beham A, Ingolic E, Stelzer I, et al. Establishment and characterization of three novel cell lines - P-STS, L-STS, H-STS - derived from a human metastatic midgut carcinoid. Anticancer Res. 2009 Jun;29(6):1951-61.

[16] Krieg A, Mersch S, Boeck I, Dizdar L, Weihe E, Hilal Z, et al. New model for gastroenteropancreatic large-cell neuroendocrine carcinoma: establishment of two clinically relevant cell lines. PLoS One. 2014 Feb 14;9(2):e88713.

[17] Yamada T, Onda M, Tanaka N. Establishment and characterization of a human rectal neuroendocrine cell carcinoma in vitro. J Exp Clin Cancer Res. 2001 Dec;20(4):561-7

[18] Boora GK, Kanwar R, Kulkarni AA, Pleticha J, Ames M, Schroth G, et al. Exome-level comparison of primary well-differentiated neuroendocrine tumors and their cell lines. Cancer Genet. 2015 Jul-Aug;208(7-8):374-81

[19] Vandamme T, Beyens M, Peeters M, Van Camp G, de Beeck KO. Next generation exome sequencing of pancreatic neuroendocrine tumor cell lines BON-1 and QGP-1 reveals different lineages. Cancer Genet. 2015 Oct;208(10):523.

[20] Hofving T, Arvidsson Y, Almobarak B, Inge L, Pfragner R, Persson M, et al. The neuroendocrine phenotype, genomic profile and therapeutic sensitivity of GEPNET cell lines. Endocr Relat Cancer. 2018 Mar;25(3):367-380.

[21] Mohamed A, Romano D, Saveanu A, Roche C, Albertelli M, Barbieri F, et al. Anti-proliferative and anti-secretory effects of everolimus on human pancreatic neuroendocrine tumors primary cultures: is there any benefit from combination with somatostatin analogs? Oncotarget. 2017 Jun 20;8(25):41044-41063.

[22] Mohamed A, Blanchard MP, Albertelli M, Barbieri F, Brue T, Niccoli P, et al. Pasireotide and octreotide antiproliferative effects and sst2 trafficking in human pancreatic neuroendocrine tumor cultures. Endocr Relat Cancer. 2014 Oct;21(5):691-704.

[23] Falletta S, Partelli S, Rubini C, Nann D, Doria A, Marinoni I, et al. mTOR inhibitors response and mTOR pathway in pancreatic neuroendocrine tumors. Endocr Relat Cancer. 2016 Nov;23(11):883-891.

[24] Ohmoto A, Suzuki M, Takai E, Rokutan H, Fujiwara Y, Morizane C, et al. Establishment of preclinical chemotherapy models for gastroenteropancreatic neuroendocrine carcinoma. Oncotarget. 2018 Apr 20;9(30):21086-21099.

[25] Yachida S, Zhong Y, Patrascu R, Davis MB, Morsberger LA, Griffin CA, et al. Establishment and characterization of a new cell line, A99, from a primary small cell carcinoma of the pancreas. Pancreas. 2011 Aug;40(6):905-10.

[26] Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. 2014 Jan 23;505(7484):495-501.

[27] Chia S, Low JL, Zhang X, Kwang XL, Chong FT, Sharma A, et al. Phenotype-driven precision oncology as a guide for clinical decisions one patient at a time. Nat Commun. 2017 Sep 5;8(1):435.

[28] Garofalo A, Sholl L, Reardon B, Taylor-Weiner A, Amin-Mansour A, Miao D, et al. The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. Genome Med. 2016 Jul 26;8(1):79.

[29] Commo F, Ferté C, Soria JC, Friend SH, André F, Guinney J. Impact of centralization on aCGH-based genomic profiles for precision medicine in oncology. Ann Oncol. 2015 Mar;26(3):582-8.

[30] MacConaill LE, Garcia E, Shivdasani P, Ducar M, Adusumilli R, Breneiser M, et al. Prospective enterprise-level molecular genotyping of a cohort of cancer patients. J Mol Diagn. 2014 Nov;16(6):660-72.

[31] Cree IA, Charlton P. Molecular chess? Hallmarks of anti-cancer drug resistance. BMC Cancer. 2017 Jan 5;17(1):10.

[32] Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer. 2013 Oct;13(10):714-26.

[33] Goodspeed A, Heiser LM, Gray JW, Costello JC. Tumor-Derived Cell Lines as Molecular Models of Cancer Pharmacogenomics. Mol Cancer Res. 2016 Jan;14(1):3-13.

[34] Basu A, Bodycombe NE, Cheah JH, Price EV, Liu K, Schaefer GI, et al. An interactive resource to identify cancer genetic and lineage dependencies targeted by small molecules. Cell. 2013 Aug 29;154(5):1151-1161.

[35] Uri I, Grozinsky-Glasberg S. Current treatment strategies for patients with advanced gastroenteropancreatic neuroendocrine tumors (GEP-NETs). Clin Diabetes Endocrinol. 2018 Jul 11;4:16.

[36] Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature. 2017 Mar 2;543(7643):65-71.

[37] Jiao Y, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. Science. 2011 Mar 4;331(6021):1199-203.

[38] Konukiewitz B, Jesinghaus M, Steiger K, Schlitter AM, Kasajima A, Sipos B, et al. Pancreatic neuroendocrine carcinomas reveal a closer relationship to ductal adenocarcinomas than to neuroendocrine tumors G3. Hum Pathol. 2018 Jul;77:70-79.

[39] Simbolo M, Vicentini C, Mafficini A, Fassan M, Pedron S, Corbo V, et al. Mutational and copy number asset of primary sporadic neuroendocrine tumors of the small intestine. Virchows Arch. 2018 Dec;473(6):709-717.

[40] Shamir ER, Devine WP, Pekmezci M, Umetsu SE, Krings G, Federman S, et al. Identification of high-risk human papillomavirus and Rb/E2F pathway genomic alterations in mutually exclusive subsets of colorectal neuroendocrine carcinoma. Mod Pathol. 2019 Feb;32(2):290-305.

[41] Girardi DM, Silva ACB, Rêgo JFM, Coudry RA, Riechelmann RP. Unraveling molecular pathways of poorly differentiated neuroendocrine carcinomas of the gastroenteropancreatic system: A systematic review. Cancer Treat Rev. 2017 May;56:28-35.

[42] Klempner SJ, Gershenhorn B, Tran P, Lee TK, Erlander MG, Gowen K, et al. BRAFV600E Mutations in High-Grade Colorectal Neuroendocrine Tumors May Predict Responsiveness to BRAF-MEK Combination Therapy. Cancer Discov. 2016 Jun;6(6):594-600.

[43] Fleuren ED, Zhang L, Wu J, Daly RJ. The kinome 'at large' in cancer. Nat Rev Cancer. 2016 Feb;16(2):83-98.

[44] Wilson LJ, Linley A, Hammond DE, Hood FE, Coulson JM, MacEwan DJ, et al. New Perspectives, Opportunities, and Challenges in Exploring the Human Protein Kinome. Cancer Res. 2018 Jan 1;78(1):15-29.

[45] Maurer G, Tarkowski B, Baccarini M. Raf kinases in cancer-roles and therapeutic opportunities. Oncogene. 2011 Aug 11;30(32):3477-88.

[46] Kittler H, Tschandl P. Driver mutations in the mitogen-activated protein kinase pathway: the seeds of good and evil. Br J Dermatol. 2018 Jan;178(1):26-27.

[47] Bardelli A, Parsons DW, Silliman N, Ptak J, Szabo S, Saha S, et al. Mutational analysis of the tyrosine kinome in colorectal cancers. Science. 2003 May 9;300(5621):949.

[48] Patel P, Galoian K. Molecular challenges of neuroendocrine tumors. Oncol Lett. 2018 Mar;15(3):2715-2725.

[49] Aristizabal Prada ET, Auernhammer CJ. Targeted therapy of gastroenteropancreatic neuroendocrine tumours: preclinical strategies and future targets. Endocr Connect. 2018 Jan;7(1):R1-R25.

[50] Qian ZR, Ter-Minassian M, Chan JA, Imamura Y, Hooshmand SM, Kuchiba A, et al. Prognostic significance of MTOR pathway component expression in neuroendocrine tumors. J Clin Oncol. 2013 Sep 20;31(27):3418-25.

[51] Missiaglia E, Dalai I, Barbi S, Beghelli S, Falconi M, della Peruta M, et al. Pancreatic endocrine tumors: expression profiling evidences a role for AKT-mTOR pathway. J Clin Oncol. 2010 Jan 10;28(2):245-55.

[52] Yao JC, Fazio N, Singh S, Buzzoni R, Carnaghi C, Wolin E, et al. Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): a randomised, placebo-controlled, phase 3 study. Lancet. 2016 Mar 5;387(10022):968-977.

[53] Lee L, Ito T, Jensen RT. Everolimus in the treatment of neuroendocrine tumors: efficacy, side-effects, resistance, and factors affecting its place in the treatment sequence. Expert Opin Pharmacother. 2018 Jun;19(8):909-928.

[54] Lamberti G, Brighi N, Maggio I, Manuzzi L, Peterle C, Ambrosini V, et al. The Role of mTOR in Neuroendocrine Tumors: Future Cornerstone of a Winning Strategy? Int J Mol Sci. 2018 Mar 6;19(3).

[55] Fazio N, Buzzoni R, Delle Fave G, Tesselaar ME, Wolin E, Van Cutsem E, et al. Everolimus in advanced, progressive, well-differentiated, non-functional neuroendocrine tumors: RADIANT-4 lung subgroup analysis. Cancer Sci. 2018 Jan;109(1):174-181.

[56] Passacantilli I, Capurso G, Archibugi L, Calabretta S, Caldarola S, Loreni F, Delle Fave G, Sette C. Combined therapy with RAD001 e BEZ235 overcomes resistance of PET immortalized cell lines to mTOR inhibition. Oncotarget. 2014 Jul 30;5(14):5381-91.

[57] Zitzmann K, Rüden Jv, Brand S, Göke B, Lichtl J, Spöttl G, Auernhammer CJ. Compensatory activation of Akt in response to mTOR and Raf inhibitors - a rationale for dual-targeted therapy approaches in neuroendocrine tumor disease. Cancer Lett. 2010 Sep 1;295(1):100-9.

[58] Corbo V, Beghelli S, Bersani S, Antonello D, Talamini G, Brunelli M, et al. Pancreatic endocrine tumours: mutational and immunohistochemical survey of protein kinases reveals alterations in targetable kinases in cancer cell lines and rare primaries. Ann Oncol. 2012 Jan;23(1):127-34.

[59] Di Florio A, Adesso L, Pedrotti S, Capurso G, Pilozzi E, Corbo V, et al. Src kinase activity coordinates cell adhesion and spreading with activation of mammalian target of rapamycin in pancreatic endocrine tumour cells. Endocr Relat Cancer. 2011 Aug 30;18(5):541-54.

[60] Di Florio A, Capurso G, Milione M, Panzuto F, Geremia R, Delle Fave G, Sette C. Src family kinase activity regulates adhesion, spreading and migration of pancreatic endocrine tumour cells. Endocr Relat Cancer. 2007 Mar;14(1):111-24.

[61] Capurso G, Lattimore S, Crnogorac-Jurcevic T, Panzuto F, Milione M, Bhakta V, et al. Gene expression profiles of progressive pancreatic endocrine tumours and their liver metastases reveal potential novel markers and therapeutic targets. Endocr Relat Cancer. 2006 Jun;13(2):541-58.

[62] Banck MS, Kanwar R, Kulkarni AA, Boora GK, Metge F, Kipp BR, et al. The genomic landscape of small intestine neuroendocrine tumors. J Clin Invest. 2013 Jun;123(6):2502-8.

[63] Kidd M, Schimmack S, Lawrence B, Alaimo D, Modlin IM. EGFR/TGFα and TGFβ/CTGF Signaling in Neuroendocrine Neoplasia: Theoretical Therapeutic Targets. Neuroendocrinology. 2013;97(1):35-44.

[64] Shah T, Hochhauser D, Frow R, Quaglia A, Dhillon AP, Caplin ME. Epidermal growth factor receptor expression and activation in neuroendocrine tumours. J Neuroendocrinol. 2006 May;18(5):355-60

[65] Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009 Mar 3;15(3):220-31.

[66] Baharani A, Trost B, Kusalik A, Napper S. Technological advances for interrogating the human kinome. Biochem Soc Trans. 2017 Feb 8;45(1):65-77.

[67] Cann ML, McDonald IM, East MP, Johnson GL, Graves LM. Measuring Kinase Activity-A Global Challenge. J Cell Biochem. 2017 Nov;118(11):3595-3606.

[68] Radu M, Chernoff J. Recent advances in methods to assess the activity of the kinome. F1000Res. 2017 Jun 26;6:1004

[69] Martincorena I, Raine KM, Gerstung M, Dawson KJ, Haase K, Van Loo P, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. Cell. 2017 Nov 16;171(5):1029-1041.

Disclosure of interests

The author declares that he as no competing interest.