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Upper Gastrointestinal Cancers (L Rajdev, Section Editor)

Targeting the mTOR Signaling Pathway in Neuroendocrine Tumors

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Opinion statement

Neuroendocrine tumors (NETs) are a heterogeneous group of malignancies characterized by variable but most often indolent biologic behavior. Well-differentiated NETs can be broadly classified as either carcinoid or pancreatic NET. Although they have similar characteristics on routine histologic evaluation, the 2 tumor subtypes have different biology and respond differently to treatment, with most therapeutic agents demonstrating higher response rates in pancreatic NETs compared with carcinoid. Until recently, systemic treatment options for patients with advanced NETs were limited. However, improvements in our understanding of signaling pathways involved in the pathogenesis, growth, and spread of NETs have translated into an expansion of treatment options. Aberrant signaling through the mechanistic pathway of rapamycin (mTOR) pathway has been implicated in neuroendocrine tumorigenesis. Additionally, altered expression of mTOR pathway components has been observed in NETs and has been associated with clinical outcomes. Targeting the mTOR pathway has emerged as an effective treatment strategy in the management of advanced NETs. In a randomized, placebo-controlled study of patients with advanced pancreatic NET, treatment with the mTOR inhibitor everolimus was associated with improved progression-free survival (PFS). Largely based upon these data, everolimus has been approved in the United States and Europe for the treatment of patients with advanced pancreatic NET. The activity of everolimus remains under investigation in patients with carcinoid tumors. In a randomized study of patients with advanced carcinoid tumors associated with carcinoid syndrome, the addition of everolimus to octreotide was associated with improved PFS compared with octreotide. However, the results did not meet the prespecified level of statistical significance based on central review of radiographic imaging. Results from a randomized study examining the efficacy of everolimus in patients with nonfunctional gastrointestinal and lung NETs are awaited. In addition, further investigation is needed to determine whether primary tumor site or other clinical and molecular factors can im-

pact response to mTOR inhibition. Although everolimus can slow tumor progression, significant tumor reduction is rarely obtained. Targeting multiple signaling pathways is a treatment strategy that may provide better tumor control and overcome resistance mechanisms involved with targeting a single pathway. Results of ongoing and future studies will provide important information regarding the added benefit of combining mTOR inhibitors with other targeted agents, such as VEGF pathway inhibitors, and cytotoxic chemotherapy in the treatment of advanced NETs.

Introduction

Neuroendocrine tumors (NET) are a rare and heterogeneous group of neoplasms that arise from neuroendocrine cells located throughout the body. These tumors are characterized by their ability to secrete peptides resulting in distinctive hormonal syndromes. NETs consist of a spectrum of disease ranging from well-differentiated, low-grade tumors to poorly differentiated, high-grade carcinomas [1•, 2]. In general, poorly differentiated, high-grade carcinomas represent aggressive cancers that have a different natural history and response to treatment compared with well-differentiated, low-grade NETs.

A number of different complex classification systems exist for grading NET pathology [1•]. In the 2010 World Health Organization (WHO) classification, neuroendocrine neoplasms of the digestive system are categorized as low-grade (G1), intermediate-grade (G2), and high-grade (G3) based upon mitotic count and proliferative index (Ki-67) [3]. High grade carcinomas are those with a mitotic count of >20 per 10 high powered fields (HPF) or a Ki-67 proliferation index of >20 %. High grade carcinomas have a more aggressive biology and are generally treated with platinum-based chemotherapy regimens used to treat small cell lung cancer. In contrast, well-differentiated, low- and intermediate-grade NETs have a more indolent biology and lower measures of cell proliferation.

Well-differentiated NETs can be broadly subclassified as either carcinoid or pancreatic NETs. Carcinoid tumors may arise from multiple different organs and historically have been classified according to site of embryonic origin, namely foregut (gastric, bronchial), midgut (small intestine, appendix, proximal large bowel), and hindgut (distal colon, rectum, genitourinary) [4]. While carcinoid and pancreatic NETs may have similar histologic characteristics, these 2 tumor subtypes have different biology and respond differently to therapy, with most agents demonstrating higher response rates in pancreatic NET patients compared with carcinoid.

When NETs are diagnosed at an early stage, surgical resection is often curative. Unfortunately, curative surgery is rarely an option for patients with metastatic disease. Recent studies have demonstrated that in addition to improving symptoms related to hormone hypersecretion, somatostatin analogs slow disease progression in patients with small bowel carcinoid tumors and gastrointestinal neuroendocrine tumors, including pancreatic NET [5, 6]. Treatment approaches with targeted therapy, including the use of agents inhibiting the vascular endothelial growth factor (VEGF) and mTOR signaling pathways and other pathways involved in neuroendocrine tumorigenesis, also provide new therapeutic options for patients with NET [7, 8••].

Notably, there are a subset of patients with NETs that appear histologically well- or moderately differentiated but are associated with Ki-67 proliferation indices >20 % that fall into the high-grade range. The most appropriate therapy for this subgroup of patients has not been well established. A recent series of patients with high-grade gastrointestinal neuroendocrine carcinomas demonstrated that response rates to platinum-based chemotherapy were lower in patients with a Ki-67 <55 % [9]. Because sensitivity to platinum-based chemotherapy appears to be associated with higher Ki-67 proliferation rates, other cytotoxic agents, such as temozolomide, or targeted agents, such as mTOR inhibitors or angiogenesis inhibitors, may play a role in the management of the management of these patients.

The aim of this review is to provide an overview of the role of the mTOR pathway in the pathogenesis of neuroendocrine tumors and to review the role of mTOR inhibitors in the treatment of this disease.

The mTOR pathway

The mechanistic target (originally referred to as "mammalian target") of rapamycin (mTOR) is an intracellular serine/threonine kinase that regulates key cell functions involved in cell survival, proliferation, and metabolism. mTOR interacts with several proteins to form 2 multiprotein complexes referred to as mTOR complex 1 (mTORC1) and 2 (mTORC2) [10]. By integrating signals from growth factors and nutrients, mTOR regulates various anabolic and catabolic cellular processes [11, 12•, 13].

mTORC1, which is the better characterized of the 2 complexes, is activated by extracellular growth factors and nutrients (Fig. 1). When active, mTORC1 phosphorylates the translational regulators eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1). These events lead to cell proliferation by promoting translation of specific mRNAs encoding proteins regulating cell-cycle progression, angiogenesis, energy metabolism, and metastasis [14]. mTORC1 also promotes lipid biosynthesis and suppresses autophagy through phosphorylation of other key cellular effectors [12•].

Compared with mTORC1, less is known about mTORC2. It also responds to growth factor signals, and when active, mTORC2 regulates cell survival, cytoskeletal remodeling, and cell migration [15, 16]. It also serves to regulate

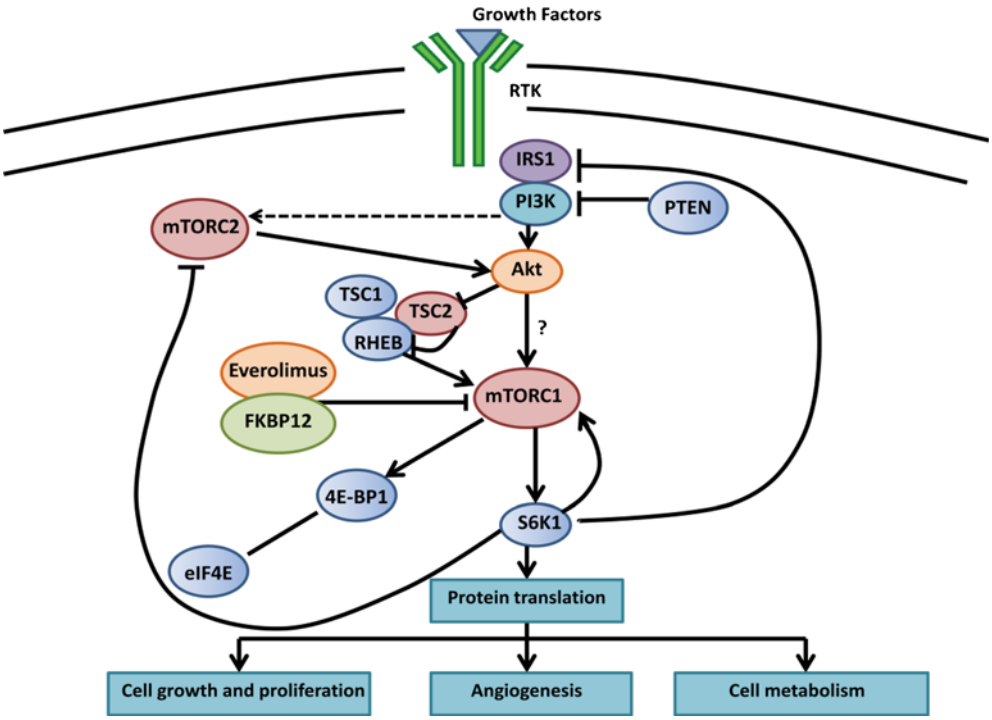

Fig. 1. The mTOR signaling pathway. Simplified representation of key components of the mTOR signaling network. The mTOR pathway plays an important role in mediating growth factor signals that stimulate cell growth and proliferation and regulate angiogenesis and cell metabolism. Arrows represent activation; bars represent inhibition. Adapted from Yao et al, 2013 [66].

the PI3K/AKT pathway via phosphorylation and activation of Akt [17]. Whereas mTORC1 is sensitive to inhibition by rapamycin, mTORC2 is considered insensitive to rapamycin [12•].

In addition to regulation by energy and nutrient status, the mTOR pathway responds to growth factors through signaling involving the phosphatidylinositol 3-kinase (PI3K) pathway (Fig. 1) [13]. Binding of insulin or insulin-like growth factors to their receptors leads to phosphorylation of insulin receptor substrate (IRS). PI3K is subsequently recruited to the cell membrane, leading to phosphorylation of phosphatidylinositiol-4,5-bisphospate (PIP2) to phosphatidylinositiol-3,4,5-bisphospate (PIP3), and ultimately activation of Akt. The phosphatase PTEN is an inhibitory regulator of the PI3K-Akt-mTOR pathway that antagonizes the action of PI3K by dephosphorylating PIP3 to PIP2, causing suppression of PI3K-dependent cell signaling.

mTOR is linked to the PI3K-Akt pathway by the tuberous sclerosis proteins TSC1 and TSC2, which act as a heterodimer that negatively regulates mTOR signaling. In response to insulin and other growth factors, TSC2 is phosphorylated and inactivated by Akt, which then leads to mTOR activation [18–20].

The mTOR pathway and pathogenesis of NET

Several observations support the importance of the mTOR pathway in the pathogenesis of NET. First, although most NETs arise sporadically, NETs can arise within the context of several familial cancer syndromes that are due to mutations in genes encoding proteins that lie upstream from mTOR. Neurofibromatosis type 1 (NF-1) and tuberous sclerosis (TS) are autosomal dominant tumor susceptibility syndromes caused by inactivating mutations in the tumor suppressor genes *NF1* and *TSC1* and *TSC2*, respectively [21]. *NF1* encodes the protein neurofibromin, which regulates *TSC1* and *TSC2* [22]. Loss of NF1 in neurofibromatosis leads to constitutive activation of mTOR and is associated with NETs involving the ampulla of Vater, duodenum, and mediastinum. Loss of function of TSC1 and TSC2 leads to mTOR activation in patients with tuberous sclerosis, which has been associated with pancreatic NETs [23].

Second, whole exome sequencing analysis of sporadic pancreatic NETs has identified somatic mutations in genes involved in the mTOR pathway, including PTEN, TS2, and PIK3CA, in 15 % of cases [24•]. Additionally, chromosomal changes, including loss of 16p, the region containing TSC2, and loss of 10q, which contains PTEN, have been reported in pancreatic NET [25, 26].

Altered expression of mTOR pathway components also has been observed in NETs and has been associated with clinical outcomes in several studies. In an analysis of gene expression profiles of 72 primary pancreatic NETs, TSC2 and PTEN were found to be downregulated in most of the primary tumors [27•]. In this study, 85 % of primary tumors showed altered protein levels of TSC2, PTEN, or both. Low levels of expression of TSC2 and PTEN were associated with shorter disease-free and overall survival. Moreover, 8/25 (32 %) patients with low levels of TSC2 and PTEN developed liver metas-

tases and progression of disease compared with none of 20 patients with normal levels of both TSC2 and PTEN. Studies have also demonstrated that expression of mTOR and its downstream targets are associated with clinical outcome [28, 29•]. In an analysis of tumor from 195 patients with NETs arising in various sites, primarily small intestine, expression of mTOR or its activated downstream target *p*-EIF4EBP1 was associated with a higher proliferative index. Furthermore, high expression of mTOR or its activated downstream products were associated with shorter survival [29•].

Interestingly, there appears to be differential expression of mTOR depending on the primary tumor site. Expression levels of mTOR and activation of its downstream targets have been found to be higher in foregut tumors compared with midgut tumors [28]. Additionally, although low expression of PTEN, TSC1, and TSC2 have been found in pancreatic NETs, TSC1 and TSC2 expression appear preserved in small intestinal NET [29•]. This suggests that there may be potential differences in the mechanisms of mTOR activation in different subgroups of NETs.

Treatment

Targeting the mTOR pathway

- The mTOR inhibitor rapamycin and its analogs bind FK506 binding protein, and this complex binds to mTORC1, inhibiting downstream signaling [30]. Everolimus and temsirolimus are rapamycin derivatives that have been evaluated in the treatment NET (Tables 1 and 2).

Everolimus

Pancreatic NET

- The activity of everolimus in pancreatic NET was explored in the RADIANT-1 trial, an international multicenter phase II trial of 160

Table 1. Clinical trials of mTOR inhibitors in carcinoid tumors

	Agent	No. patients	Tumor response rate (%)	Median TTP or PFS	Reference
Phase II studies					
	Everolimus [a]	30	17	63 wk	Yao et al. 2008 [37]
	Temsirolimus [a]	21	5	6.0 mo	Duran et al. 2006 [41]
Phase III studies					
RADIANT-2	Everolimus + octreotide LAR vs.	216	2	16.4 mo	Pavel et al. 2011[38••]
	Placebo + octreotide LAR	214	2	11.3 mo	
RADIANT-4	Everolimus vs. Placebo	Ongoing			

PFS progression-free survival, *TTP* time to progression
[a] Data from the subset of patients with carcinoid tumors in these phase II studies of unselected patients with NET are presented

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