mTOR inhibition, a potential novel approach for bronchial carcinoids

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Abstract

Although targeted therapy, including inhibitors of mammalian target of rapamycin (mTOR) and vascular endothelial growth factor, being developed for carcinoids arising from the gastrointestinal tract, treatment for locally advanced or metastatic bronchial carcinoids (BCs) remains lacking. Traditional cytotoxic chemotherapy offers essentially minimal benefit to this largely under-characterized tumor. In the September issue of *Endocrine-Related Cancer*, Zatelli *et al.* reported an anti-proliferative effect of mTOR inhibitor, everolimus, in cultured primary BC tumor cells by attenuation of IGF signaling pathway. This effect is more significant in aggressive tumors that carry higher levels of mTOR, and is consistent with the therapeutic benefit of everolimus for patients with BC observed in our phase II and III clinical trials. Although adding somatostatin analog to mTOR inhibitor did not provide a synergistic anti-tumor effect, development of rational combinations is highly warranted to further improve the outcome for patients with neuroendocrine tumors.

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Bronchial carcinoids

Bronchial carcinoid (BC), the second most common carcinoid tumor after gastrointestinal carcinoids, accounts for 20–30 percent of all cases of well-differentiated neuroendocrine tumors. With an incidence rate of 1.35 per 1 000 000 populations per year, it accounts for ~1–2% of all lung malignancies in adults (Yao *et al.* 2008a). Although BC can arise as a part of multiple endocrine neoplasia type 1 (MEN 1), vast majority is not associated with genetic cancer syndromes. Similar to other sporadic carcinoids, environmental risk factors have not been conclusively established (Hassan *et al.* 2008).

Although the genetic basis for sporadic BC remains to be fully elucidated, studies suggest the possible involvement of *MENIN*, the gene responsible for MEN1. For example, loss of heterozygosity (LOH) of chromosome 11 has been documented to be the most frequent genetic abnormality in BC (Jakobovitz *et al.* 1996, Debelenko *et al.* 1997, Walch *et al.* 1998). A study also reported that many sporadic BC may have inactivation of both copies of *MENIN* through a combination of deletion and mutation (Debelenko *et al.* 1997). *MENIN* has been shown to be a

component of a histone methyltransferase complex that regulates gene transcription (Hughes *et al.* 2004, Scacheri *et al.* 2006). Recently, *MENIN* has also been shown to have an important role in the modification of endocrine mass during pregnancy through regulation of p27 (Karnik *et al.* 2007).

Histologically, BCs can be classified into three groups with significantly different clinical behavior and prognosis. Typical BC is low grade, well differentiated, and often follows an indolent course with a 5-year survival rate of 87–100%; atypical BC is of intermediate grade with a higher tendency of local recurrence (3–23%) and metastasis (16–23%) and a lower 5-year survival rate of 30–95% (Travis *et al.* 1998, Kaplan *et al.* 2003), and high-grade tumors, more properly classified as small cell or large cell neuroendocrine tumors of the lung, are poorly differentiated with rapid tumor growth, early metastasis, and 5-year survival rate of 1–10% (Tai *et al.* 2003). Although the more aggressive high-grade neuroendocrine tumors respond very well to radiotherapy and platinum-based chemotherapy, typical and atypical BCs are generally resistant to both conventional radiation and cytotoxic chemotherapy.

Therefore, its cure largely relies on early detection and surgical resection (Cardillo *et al.* 2004, Asamura *et al.* 2006, Garcia-Yuste *et al.* 2007). For patients with unresectable, locally advanced, or metastatic disease, effective therapeutic options are lacking.

Preclinical study of everolimus using primary culture of BC

In the September issue of *Endocrine-Related Cancer*, Zatelli *et al.* (2010) reported an anti-proliferative effect of everolimus in human BC tumor cells in primary culture. In addition, they demonstrated that everolimus abolished the growth driven by insulin-like growth factor (IGF). Everolimus treatment was associated with decreased chromogranin A and vascular endothelial growth factor (VEGF) production. Tumors with aggressive clinical features, for example, atypical BC, and with higher expression levels of mammalian target of rapamycin (mTOR) exhibit a greater sensitivity to this effect (responders). Furthermore, pasireotide, a pan-somatostatin receptor ligand, did not show a synergistic effect with everolimus, but it does decrease cell survival on its own. It is yet to be determined whether this effect is also present in everolimus non-responders. In addition, it is also worthwhile to characterize the underlying mechanisms leading to the differential responses to everolimus observed in typical BC.

This study provides important confirmation for the role of mTOR signaling pathway in neuroendocrine tumors. A prior study using a single pancreatic neuroendocrine cell line demonstrated co-expression of IGF1 and IGF receptor (IGFR) (von Wichert *et al.* 2000). Similar to this study, exogenous IGF activate mTOR and increase cellular proliferation. mTOR inhibition in neuroendocrine cell lines has been shown to inhibit tumor growth (von Wichert *et al.* 2000, Moreno *et al.* 2008). However, the limited availability of representative cell lines has hindered the ability to replicate these findings in neuroendocrine tumors of other primary sites. The challenges in developing additional neuroendocrine cell lines are related to the indolent nature of tumor growth. In fact, it can be argued that a neuroendocrine cell line maintaining the growth rate of a well-differentiated tumor in human would have such a long doubling time that it would not be useful in the laboratory. Conversely, the available cell lines used now grow aggressively and may not fully represent the biology of well-differentiated tumors in humans.

The study of novel agent using primary culture of neuroendocrine tumors cells provides an important tool

to characterize its effect in the clinical setting. However, this has its own limitations. For example, in clinical practice, the vast majority of resections are for more slow-growing tumors that may exhibit a more indolent course and have a lower metastatic potential. Furthermore, growth of these cells in tissue culture limits the ability to evaluate the effect of growth factors and stroma tumor interaction. For example, somatostatin analogs such as pasireotide have been known to decrease VEGF expression and circulating IGF1 (Ruan *et al.* 2006, Zatelli *et al.* 2007). Potential additive or synergistic effect of somatostatin analogs and mTOR inhibitors cannot be fully addressed using cell cultures. The exogenous addition of growth factors does not fully solve this problem. Nonetheless, studies using primary culture add a valuable tool for discovery in neuroendocrine tumors.

Clinical studies of everolimus in patients with neuroendocrine tumors

The importance of the mTOR signaling pathway in human neuroendocrine tumors (NETs) is supported by this study as well as the recognition that genetic cancer syndromes in the mTOR pathway are linked in the development of neuroendocrine tumors. In a phase II study conducted at the University of Texas M. D. Anderson Cancer Center, including 30 patients (four patients with BC) with carcinoids and 30 patients with pancreatic neuroendocrine tumors, we have observed a promising intent-to-treat response rate of 20%, and a promising median progression-free survival of 60 weeks (Yao *et al.* 2008b). This was followed by a multi-national phase II study of everolimus among patients with pancreatic neuroendocrine tumors having progression during or after chemotherapy, and two randomized phase III studies (Öberg *et al.* 2010, Yao *et al.* 2010, 2011). RADIANT-3 is a randomized phase III study that enrolled 410 patients with progressive advanced pancreatic NETs and demonstrated a significant improvement in progression free survival (PFS) associated with everolimus compared with placebo (11 months versus 4.6 months; $P < 0.001$; Yao *et al.* 2011). RADIANT-2 examined the role of everolimus in carcinoid tumors. The study randomized 429 patients between everolimus plus octreotide LAR and placebo plus octreotide LAR. Although the study narrowly missed the significance boundary by central radiology review, everolimus therapy was associated with a clinically important 5.1 (11.3 vs 16.4; $P = 0.026$) months improvement in median progression-free survival (Öberg *et al.* 2010). Significant benefits by investigator review ($P = 0.018$) and in pre-specified

inverse probability of censoring weighting (IPCW) analysis ($P=0.0014$) adjusting for informative censoring and covariates confirms the benefits of everolimus. Although the number of patients with BC was modest ($n=44$), subgroup analysis showed benefit consistent with general study population with median PFS improved from 5.4 to 13.6 months. In addition, clinical trial, RAMSETE, examining the efficacy and safety of daily everolimus for the treatment of advanced patients with nonfunctioning NETs, including BC, will help us to gain further insight into this regard.

Whether the addition of somatostatin analogs such as octreotide, lanreotide, and pasireotide further improve the outcome among patients receiving everolimus cannot be answered using currently available data. In the RADIANT-1 study, the cohort of patients receiving the combination of everolimus and octreotide LAR had longer median PFS (16.7 vs 9.7 months). This, however, was not a randomized study and thus cannot definitively answer whether the difference was due to the addition of octreotide LAR or difference in the biology of functional versus non-functional tumor.

The development of target therapies, including inhibitors of mTOR and VEGF pathways in the recent years has brought new hope to carcinoid treatment. Pivotal phase III studies of everolimus have shown that everolimus slows tumor growth and improves progression-free survival. Development of rational combinations, such as VEGF inhibitor with cytotoxic chemotherapy, or with mTOR inhibitor, may further improve the outcome.

Declaration of interest

The authors declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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