Molecular Pathology of Neuroendocrine Tumor: The Era of Targeted Therapeutics

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Introduction

Neuroendocrine tumors are a heterogeneous neoplasm of diverse origin arising from a multitude of organs and tissues including the gastrointestinal tract, pancreas, bronchial tissues, thymus, parathyroid, adrenal, pituitary, and calcitonin-producing cells of the thyroid gland.

The prevalence is unfortunately increasing to a current rate of over 35/100,000 [1]. Complicating this is the fact that tumors in 80 % of cases present with advanced disease and metastasis with a 5-year survival rate of less than 40 %. Consequently, there is great need for effective systemic therapy [2].

Molecular Biology

Neuroendocrine neoplasms, of both endocrine and exocrine types, share the feature that they are generally under the control of the peptide hormone somatostatin [3].

Somatostatin primarily effects its action via interaction with somatostatin receptors (SSTRs). These membrane-associated receptors function via G-coupled signaling. Somatostatin also has effects on ion channels and tyrosine kinase receptors [4].

There are five main SSTRs that all bind somatostatin but have different cellular signaling effects [5].

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SSTR Biology

SSTR1 leads to MAPK pathway activation [6].

SSTR2 is most commonly expressed in GI-NETs and pancreatic NETS. SSTR2 activation results in activation of SHP1 downregulation of MAPK resulting in cell cycle suppression via Rb and p21. Its signaling is inactivated via recycling.

SSTR3 is connected with phosphotyrosine phosphatase (PTP)-dependent apoptosis, involving p53 and Bax activities. It may also have an effect on suppression of VEGF. It is downregulated via internalization and ubiquitination.

SSTR4 signaling results in upregulation of MAPK/ERK1/ERK2 pathway and subsequent cellular proliferations. SSTR4 is inactivated via internalization.

SSTR5 is connected with ion exchange channels K+/H+, voltage dependent Ca2+ and also with AMP and kainate glutamate signaling [7].

Somatostatin Analogues (SSAs)

SSTRs represent an attractive target for pharmacological targeting. Natural somatostatin has a very short half-life of only a few minutes due to rapid enzymatic digestion. Consequently more stable synthetic forms have been developed for therapeutic purposes; these include octreotide (SSTR2), lanreotide (SSTR5), pasireotide (SSTR1, SSTR2, SSTR3, SSTR5), and KE108 (SSTR1, SSTR2, SSTR3, SSTR4, SSTR5). These various SSAs have different affinities for SSTRs [8].

SSTRs Expression Patterns

SSTRs are also expressed broadly in non-endocrine cells including cells of blood vessels, stroma, and the immune system. Because of this broad distribution of SSTRs, SSAs have both direct and indirect effects on NET biology and growth [9].

Direct Effects of SSAs

The main direct effects of SSA on NETs are mediated through the action of the SSTR system and a variety of molecular signaling pathways within NET cells. These pathways and molecular mechanisms include the following:

1. Activation of p27kip1 via signaling through SHP1 and SHP2 and r-PTPeta
2. Downregulation of proliferation via suppression of PTP, cGMP, and RAS signaling

3. Triggering of apoptosis via p53 and BAX pathway activation via SSTR3 and dopamine receptor
4. Intracellular pH modification via NHE1 channel alterations
5. Induction of endogenous connexins (CX26 GJB2 and CX42) to form gap junctions

Indirect Effects of SSAs

Because SSTRs are expressed on non-NET tissues, SSAs also have indirect effects on NET growth. These indirect effects include the following molecular mechanisms:

1. Inhibition of trophic hormone release via calcium depletion. These pathways include GH, IGF1, EGF, insulin, gastrin, prolactin, VIP, serotonin, and others. This is probably mediated via transcription factor STAT5b through SSTR2 or SSTR3 activities.
2. Suppression of angiogenesis, via suppression of SSTR2 activity on neoangiogenetic endothelial cells. In addition, there may be suppression of VEGF and fibroblast growth factor via SSTR1, SSTR2, SSTR3, and SSTR5.
3. SSAs appear to possess immunomodulatory activity via triggering interferon release. In addition, there may be effects on lymphocyte proliferation and natural killer cell activities.

Interferon ALPHA

Interferon alpha appears to have a variety of inhibitory effects on NETs. These include direct effects on the neoplasms via induction of cell cycle arrest and apoptosis via a variety of signaling pathways and interferon receptors. In addition, interferon alpha has effects on angiogenesis via suppression of VEGF and the immune activity of the lymphocytes in the surrounding stroma. Unfortunately, INF-alpha has marked toxic side effects including induction of flu-like symptoms, fatigue, weight loss, and significant myelotoxicity [10, 11].

SSAs and INF-alpha have also been used in combination with synergistic results.

Carcinoid Syndrome

Carcinoid syndrome causes serious morbidity in some patients with NETs due to release of functional hormones from the neoplasm including serotonin, tachykinin, and substance P. The syndrome is characterized by flushing, diarrhea, tachycardia, abdominal pains, hypo- and hypertension, and cardiac abnormalities including heart valve fibrosis.

Carcinoid can be monitored biochemically by laboratory tests that measure chromogranin A or 5-hydroxyindoleacetic acid.

SSAs often have good effects on treating the symptoms of carcinoid syndrome by inducing stable disease or disease regression and reducing the levels of 5HIAA and chromogranin A [12].

Octreotide was approved in 1987 for the treatment of carcinoid, glucagonoma, and Verner-Morrison disease [13, 14]. Octreotide has a modified amino acid structure with a substitution of 3 amino acids resulting in an enhanced activity and stability of the molecule resulting in a 120-min half-life and an effective activity of over 12 h. The drug has low toxicity and has an effect against NET with low Ki67 proliferative indices.

Clinical Trials and SSAs

Trials such as PROMID which examined octreotide vs. placebo and CLARINET pitting lanreotide against placebo in phase II studies showed excellent signals with notable gains in progression-free survival [14–16].

Resistance to SSAs

Unfortunately, NETs often develop resistance to SSA treatment. This is via a variety of mechanisms including the following:

1. Tachyphylaxis, desensitization of the receptors
2. Development of mutated forms of the SSTR receptors (SSTR5/MD4 in pituitary adenoma)
3. Development of autoantibodies against the SSAs as SSAs are peptides and potentially immunogenic
4. Modification of other regulatory proteins such as amphiphysin IIb

Targeted Agents

Besides the SSTRs, NETs show a variety of potentially actionable molecular alterations. These include significant vascularity and overexpression of pro-angiogenic factors such as VEGF, VEGFR, and PDGF raising the possibility of using anti-angiogenic agents such as bevacizumab [17–19].

Further, several of the signaling pathways lead to mTOR activation, raising it as a potentially attractive target. This has led to the initiation of trials combining the anti-mTOR everolimus with octreotide (RADIANT 2, a phase III study) [20–22].

Sunitinib, an oral multitargeted RTK inhibitor of VEGFR, PDGFR, cKIT, RET FLT3, and others, has shown benefit in phase II and phase III trials with patients with NETs and has been approved for use by both European authorities and the FDA for treatment of metastatic unresectable PNET [23–25].

The Raf pathway has been investigated in NETs but with limited results. In melanomas and other tumors, BRAF is frequently activated via a mutational event (V600E) making BRAF an attractive and functional target. However, in NETs BRAF mutations are not observed. Some effects were noted with anti-RAF treatment using sorafenib, but the effects were most likely due to anti-angiogenic activities [26].

Following the report of EGFR expression and upregulation in some cases of NET [27], anti-EFGR therapies have also been tested in a limited number of patients.

Telotristat Etiprate (LX1606)

Telotristat etiprate is an inhibitor of tryptophan hydroxylase involved in serotonin biosynthesis and an attractive target to abrogate the effects of carcinoid syndrome [28, 29].

Chimeric Somatostatin

Evidence is emerging that there may be interactions between SSTR and D2 receptors leading to the development of a new class of chimeric molecules that target both, and these include BIM-23A758 and BIM-23A760 [30–32].

Radiotargeted Therapy

Trials are underway to evaluate the effectiveness of radiotargeted therapies using 90Y and 177Lu. Agents include 177Lu-DOTA-TYR3-octreotate [33].

Other Molecular Targets

Advances in genomic analysis technology and deep sequencing methods enable the uncovering of novel potentially actionable targetable mutations. Some targets that have emerged are of interest and include SMAD4 (targeting the TGF-beta pathway). DAXX and ATRX have also been shown to be altered [34, 35]. These genes are involved in chromatin remodeling.

Immunotherapy

Immunotherapy is rapidly emerging as a consideration in many tumor types, particularly after the spectacular results in several trials using immune checkpoint inhibitors for the treatment of advanced solid tumors including metastatic melanoma. These results are particularly encouraging given that in many instances, the checkpoint inhibitors display synergistic activity and extremely long durable responses unlike many targeted therapies. Current targets include PD-1, PD-L1, and CTLA4. These agents are currently under investigation in advanced NET [36–38].

Summary

NETs represent a diverse, heterogeneous group of neoplasms that frequently present with advanced stage and are often complicated by development of carcinoid syndrome. The mainstay of treatment is based on their proliferative activity. Tumors with high activity are subjected to cytotoxic therapy using combinations of platinum, temozolomide, and capecitabine. Biotherapy with SSAs is utilized in low- and mid-grade tumors. Interferon shows some effectiveness but has significant toxicity. Sunitinib (a multitarget TKI) and everolimus (against mTOR) have shown effect. Ongoing studies are investigating other targeted therapies and anti-angiogenic agents. Finally, immunotherapy using immune checkpoint inhibitors is under intense evaluation.

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