Tyrosine Kinase Inhibitors in Thyroid Cancer: May Axl/Gas6 Pathway be a Hidden Target?

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ABSTRACT

Tyrosine kinase inhibitors are molecules that block various intracellular signaling pathways. Trials with some tyrosine kinase inhibitors showed promising results in thyroid cancer. They are thought to owe their antitumor property mainly to their effect on vascular endothelial growth factor receptor which is important in angiogenesis. Axl is a receptor tyrosine kinase shown to be involved in proliferation migration and survival of cells. Recent studies implied axl in thyroid cancer development. In this review, we aimed to discuss the mechanism of action of tyrosine kinase inhibitors in general and specifically focus on the axl/gas6 signaling pathway.

Keywords: Tyrosine kinase inhibitor, axl/gas6 signalling, Thyroid cancer

ÖZET

Tiroid Kanserinde Tirozin Kinaz İnhibitörleri: Axl/Gas6 Yolağı Gizli Bir Hedef Olabilir mi?

Tirozin kinaz inhibitörleri çeşitli intraselüler sinyal yolaklarını bloke eden moleküllerdir. Bazı tirozin kinaz inhibitörleriyle yapılan çalışmalar, tiroid kanserinde ümit verici olduklarını göstermiştir. Antitümör özelliklerini başlıca, anjiogenezde önemli olan vasküler endotelyal büyüme faötrü reseptörü üzerindeki etkilerine borçlu oldukları düşünülmektedir. Axl, hücrelerin çoğalması, göçü ve sağkalımında rolü olduğu gösterilmiş bir reseptör tirozin kinazdır. Yakın zamanda yapılan çalışmalar tiroid kanseri gelişiminde axl'yi işaret etmektedir. Bu derlemede, genel olarak tirozin kinaz inhibitörlerinin etkisini tartışmak ve özellikle axl/gas6 sinyal yolağı üzerinde durmak istedik.

Anahtar Kelimeler: Tirozin kinaz inhibitörü, axl/gas6 sinyali, Tiroid kanseri

INTRODUCTION

Kinases are enzymes that transfer a phosphate group from high-energy donor molecules to specific substrates. Protein kinases phosphorylate proteins, resulting in functional changes of target proteins. There are 90 tyrosine kinases (TK) encoded by human genome.

TK can be classified in families (eg. Vascular endothelial growth factor receptor (VEGFR) family and the fibroblast growth factor receptor (FGFR) family) or as receptor TK and non-receptor (cytoplasmic) TK. Receptor TKs are needed for transducing extracellular signals into the cell, while non-receptor TKs act in intracellular communication.[1]

TKs are involved in oncogenesis through various mechanisms. For example it is a TK linked to a partner protein which creates BCR ABL, the constitutively active fusion protein. CSF1R-like tyrosine kinase 3, stem cell factor receptor and KIT result from mutation or deletion of tyrosine kinase domain of the receptor. Other TK related mechanisms in oncogenesis are, increased or aberrant expression of TK receptors and decrease in factors regulating tyrosine kinase activity.[2]

Tyrosine kinase inhibitors (TKI) are hydrophobic molecules that are able to pass through the cell membrane.[3] After they enter the cell they interact with the intracellular domain of receptors and intracellular signaling molecules. So, small molecule kinase inhibitors are able to block the various downstream signaling pathways intracellularly.

Most small-molecule kinase inhibitors compete with ATP for ATP-binding site of a kinase. Type I kinase inhibitors recognize the active conformation of a kinase.[4,5] Type II kinase inhibitors recognize the inactive conformation of a kinase.[6] A third class; 'covalent' inhibitors have been developed to covalently bind at specific sites of the kinase.[7]

Many anti-angiogenic tyrosine kinase inhibitors are so called multi-targeted kinase inhibitors. These agents target a number of different kinases, which are involved in several signaling pathways. The multi-targeted kinase inhibitors are expected to have a broader efficacy than a single-targeted inhibitor. For example, a multi-targeted kinase inhibitor that blocks VEGFR signaling as well as PDGFR signaling –both important in angiogenesis- will be more effective with regard to antiangiogenic function.[8]

TKI have anticancer properties and lower side effects when compared to cytotoxic chemotherapy, thus they emerged as a new class of therapy. Their efficacy is proven in several types of carcinoma – like Philadelphia chromosome positive chronic myelogenous leukemia (Ph CML)[9], metastatic renal cell cancer (RCC)[10] and gastrointestinal stromal tumors (GIST)[11]- and are currently under investigation for new indications.

TKI and Thyroid

Recently TKI has achieved promising results in the management of thyroid cancer. A phase I study with XL184 (cabozantinib) revealed partial response in 15 of the 34 medullary thyroid cancers (MTC), irrespective of RET mutation.[12] Vandetanib tried in hereditary MTC showed similar efficacy.[13,15] The authors attributed this effect to the prominent antiangiogenic properties of the drug.[15] This was also true in the subgroup analysis of patients with MTC in a phase II trial with axitinib.[16]

VEGFR is supposed to be the main target of TKI on thyroid cancer cell. Thyroid follicular cells express VEGF and VEGFR.[17] Animal studies showed regression in normal capillaries in thyroid tissue when treated with VEGFR inhibitors.[18,19] Sunitinib has selectivity for all isoforms of VEGFR, PDGFR, cKIT and RET(57) In a phase II trial with sunitinib response rate of thyroid cancer to the drug was 31%.[20]. On the other hand, imatinib, aTKI with no effect on VEGFR showed no effect on thyroid cancer.[21]

Sorafenib is an oral TKI inhibiting several kinases including VEGFR 2 -3 PDGFR RET and B –Raf.[22] In a phase II trial in which 60% of the patients had differentiated thyroid cancer (DTC), 7 patients (23%) had a partial response and 16 patients (63%) had stable disease.[23] Another trial of sorafenib in metastatic thyroid cancer confirmed these results.[24]

Axitinib is another multikinase inhibitor inhibiting all isoforms of VEGFR. In a phase II trial in which half of the patients suffered DTC, 30% of the patients showed partial response. Axitinib however is less selective for PDGFR and KIT.[15] On the other hand, partial response rate of thyroid cancer to motesanib remained at 14% although it has selectivity for all isoforms of VEGFR.[25]

Is VEGFR the only explanation for TKI effect?

Several points related to the mechansim of action of TKI in thyroid cancer remains to be explained. As discussed above not all TKI are effective in thyroid cancer. TKI with similar efficacy on VEGFR isoforms show different efficacy on clinical grounds. Changing TKI may change the prognosis. For example; XL-184 (cabozantinib) has particular activity against hepatocyte growth factor receptor (tyrosine-protein kinase Met), vascular endothelial growth factor receptor 2 (VEGFR-2) and proto-oncogene tyrosine-protein kinase receptor Ret.[26] In the phase I study mentioned above, some of patients responding to XL184 were previously treated with other TKIs such as vandetanib, sorafenib and motesanib. All four agents are actually known to have VEGFR as a target.[27] If there is no cross resistance among XL-184 and others, then they may not be sharing a common mechanism of action.

Effects of Gas6/Axl Signaling

The receptor tyrosine kinase Axl was first identified from the DNA of chronic myelogenous leukemia patients.[28,29] Axl is stimulated by Gas6 (growth arrest specific 6) (30 ,31). Axl signaling in culture protects cells from starvation– or tumor necrosis factor-_-induced apoptosis.[32] The overexpression of Axl and/or its ligand, Gas6, has been reported in different solid human tumor types[33,34] and myeloid leukemias.[35]

Axl signaling modulates integrin function. A recent study showed that Gas6-dependent signaling results in phosphorylation of h3 integrin in platelets.[36] Similar crosstalk has been described between some angiogenic growth factor receptors (e.g., VEGFR2 and FGFR) and integrins (e.g., avh3 and avh5).[37] Integrins mediate substrate adhesion required for cell migration, proliferation, and survival of growth factor–stimulated cells. So blocking Axl signaling may alter the function of certain integrins necessary for tumor cells.

Axl is also important in angiogenesis. Axl knockdown is shown to impair endothelial tube formation in vitro and impair blood vessel formation in a mouse angiogenesis model. Endothelial cell Gas6 and Axl expression are required for proangiogenic processes. In a xenograft assay, inhibition of Axl expression is shown to reduce growth of MDA-MB-231 breast carcinoma cells.[38]

Gas6/Axl in Different Cancer Types

Axl overexpression is identified in Imatinib -resistant CML cell lines and patients. The reverse is also true; that is knockdown of Axl, sensitized TKI-resistant cells to imatinib.[39] The same applies to Nilotinib.[40] Axl overexpression and/or activation has been related to resistance to chemotherapy in some other cancer types ; gastrointestinal stromal tumor cell lines[41], rhabdomyosarcoma[42], HER-2 positive breast tumor cells[43], cutaneous squamous cell carcinoma (SCC)[44], Kaposi sarcoma[45] and ovarian cancer.[46]

Drugs targeting Axl are currently under investigation. For example, a small-molecule inhibitor of Axl kinase (R428) is shown to reduce tumor burden and extend survival in intracardiac and orthotopic mouse models of breast cancer metastasis.[47]

Gas6/Axl in Thyroid Cancer

A study in three thyroid carcinoma cell lines showed Axl mRNA and protein overexpression in two of the cell lines compared with that in normal tissue. Gas 6 demonstrated a modest mitogenic activity in thyroid carcinoma cells overexpressing Axl.[48] In another study, tissues obtained from 81 patients with thyroid carcinomas, 18 with adenomas, and 13 with adenomatous goiters are examined by immunohistochemistry and in situ hybridization. Axl was expressed faintly in adenomatous goiter and adenomas, but not in normal thyroid tissues. Of the 81 cases of thyroid carcinoma, 70 (86.4%) showed a positive staining for the Axl protein.[49]

A similar study in which tissues obtained from 17 patients with thyroid papillary carcinomas from Gomel, Belarus are examined, axl and Gas6 are found to be overexpressed in 76.5% and 70.6 % of cases, respectively. Eighty-five percent of Axl positive cases coexpressed Gas6. The authors proposed that, Axl and Gas6 expression might be involved in childhood thyroid tumorigenesis around Chernobyl.[50]

A gene expression profiling study of human papillary thyroid cancer cells revealed significant upre-

gulation of axl. Twenty-seven thyroid carcinoma samples (9 papillary, 10 follicular and 8 poorly differentiated/anaplastic thyroid cancers) were analyzed by immunohistochemical staining with an anti-human Axl antibody and 73% (19/27) scored positive for Axl expression. Normal thyroid was negative for Axl expression. Gas 6 staining applied in the same samples showed positive scoring for Gas 6 mainly in cytosol of carcinoma cells.[50]

Axl-Gas6 blockade inhibits thyroid cancer cell proliferation and survival. Axl silencing inhibits experimental tumor growth. The investigators evaluated the role of Axl in tumor growth by using xenografts of anaplastic thyroid cancer cells into (nu/nu) immunodeficient mice. Silencing Axl inhibited cancer cell viability, invasiveness, and growth of tumor xenografts in nude mice.[51]

CONCLUSION

To some extent TKI have an effect on thyroid cancer. The exact mechanism of action of these agents is not well known and today we do not exactly know why some TKI work efficiently on thyroid while some others fail to display an antitumor action.

Axl /Gas 6 signaling pathway is an important modulator of integrin function which is crucial for cell migration, proliferation, and survival of growth factor-stimulated cells. Like many other tumor cells thyroid cancer cells also express axl and blocking axl is reported to have antitumor effect in these cells. These findings suggest that Axl /Gas 6 signaling pathway may have a role in thyroid carcinogenesis. Further research on axl may enlighten the mechanism of action of TKI on thyroid cancer cells and may provide a basis for development of novel TKI, specifically targeting axl /gas 6 pathway.

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