MOLECULAR ALTERATIONS AND TARGETED THERAPY IN OESOPHAGEAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Oesophageal cancer is one of the most fatal cancers. Oesophageal squamous cell carcinoma is the predominant histological subtype worldwide with a high incidence rate in Asia. Although significant advancement has been made in surgery and chemoradiotherapy, still it has a very dismal prognosis. These insights have led to the new-found interest into the molecular alterations that may occur in Oesophageal squamous cell carcinoma with the objectives to discover novel biomarkers for improving and guiding targeted therapy. Many molecular alterations may occur in oesophageal carcinogenesis. Basically they are of two types: oncogenes and tumour suppressor genes. Oncogenes related to ESCC are growth factors, growth factor receptors, signal transducers and nuclear factors. Among the tumour suppressor genes, the important ones are p53, Rb, p16 and p21. In addition, there are genes related to apoptosis and metastasis. This review summarizes all these genes and their deregulated proteins, which have proved to have strong prognostic and therapeutic implications in these patients.

KEY WORDS: Oesophageal squamous cell carcinoma; Molecular alterations; Oncogenes; Tumour suppressor genes; Targeted therapy

INTRODUCTION

Oesophageal cancer is one of the lethal cancers worldwide. The major histological subtypes of oesophageal cancers are squamous cell carcinoma and adenocarcinoma. Other malignant tumours like melanoma, leiomyosarcoma, carcinoids and lymphomas may develop in the oesophagus as well.¹

Incidence of oesophageal squamous cell carcinoma (ESCC) varies geographically, the highest risk areas include the so called “Asian oesophageal cancer belt” extending across Asia from the southern shore of the Caspian sea in Iran, through Soviet Central Asia and Mongolia to northern China.² In contrast, in the United States incidence of oesophageal adenocarcinoma is increasing and recent trends have shown that the incidence of early stage oesophageal adenocarcinoma has reached a plateau.³,⁴

Oesophageal carcinogenesis is a multifactorial process, which is influenced by environmental condition, lifestyle and genetic predisposition. Although substantial progress has been made in surgery and adjuvant chemoradiotherapy, the prognosis of the patients with ESCC still remains poor.⁵ These insights have lead to vigorous investigations into the molecular alterations that may occur in ESCC with the objectives to discover novel markers for enhancing accurate diagnosis, predicting prognosis and guiding targeted therapy.

MOLECULAR ALTERATIONS IN ESCC

Many molecular alterations occur in oesophageal carcinogenesis. They are basically two types: oncogenes and tumour suppressor genes. Oncogenes are often related to increased stimulatory function, whereas tumour suppressor genes are associated with loss of inhibitory function.⁶ In addition, there are genes for regulating apoptosis, stimulating angiogenesis and assisting metastasis.⁷

Oncogenes

Major mechanisms for the pathogenesis of ESCC involving oncogenes include mutation, amplification and/or overexpression. Oncogenes related to ESCC are (i) growth factors (ii) growth factor receptors (iii) signal transducers and (iv) nuclear factors.

Growth factors

The growth factors implicated in ESCC are epidermal growth factor (EGF) and transforming growth factor (TGF-α) which is structurally related to EGF and binds to epidermal growth factor receptor (EGFR).⁸ These growth factors have been implicated in many aspects of cell growth, angiogenesis and tumour development.

Growth factor receptors

The growth factor receptors implicated in ESCC are EGFR and HER-2/neu (erbB-2), which is a member of the EGFR family. Recent evidence indicates that EGFR overexpression is a poor prognostic marker in ESCC. EGFR family members include erbB-1, erbB-2, erbB-3 and erbB-4. Its intrinsic tyrosine kinase activity is stimulated when ligands, such as EGF and TGF-α, bind to its extracellular domain. In the process, downstream signalling molecules are produced and the most important being the protein kinase Akt.⁹ Akt is responsible for sending anti-apoptotic signals and also plays a role in the regulation of cell cycle progression.⁹

Proto-oncogene HER-2/neu (erbB-2), localized to chromosome 17q, encodes a transmembrane tyrosine kinase growth factor receptor similar to EGFR. HER-2/neu antigen expression has been found in invasive breast cancers and enhances the aggressiveness of the tumour.¹⁰ HER-2/neu overexpression has been found in gastrointestinal tumours. HER-2/neu overexpression in ESCC ranges from 0%–2% with a mean of 23%. In ESCC, HER-2/neu overexpression has been correlated with clinical aggressiveness in the form of extramural invasion and resistance to neoadjuvant chemotherapy.¹¹

Signal transducers

Vascular endothelial growth factor (VEGF) is an important signal transduction protein. VEGF alteration is found in ESCC and is involved in both vascuogenesis and angiogenesis. Overexpression of VEGF is a poor prognostic marker in ESCC.¹² It produces various biologic effects to induce tumour growth and spread, including endothelial cell mitogenesis and migration, induction of proteinases,
increased vascular permeability, and maintenance of survival for newly formed blood vessels.\textsuperscript{13}

**Nuclear factors**

Gene amplification and overexpression of cyclin D1 are frequently demonstrated in ESCC. Overexpression and amplification of cyclin D1 is found in 22-73% of ESCC.\textsuperscript{14} Cyclin D1 overexpression was shown to be associated with poor prognosis such as poor response to chemotherapy and decreased survival.\textsuperscript{15} The cyclin D1 gene, located on chromosome 11q13, encodes a protein that complexes with a cyclin dependent protein kinase (CDK) to phosphorylated Rb and promote a cell’s advancement from the G1 phase to the S phase. Overexpression of cyclin D1 is thought to override the G1 checkpoint, promoting proliferation of tumour cells. Thus in ESCC, cyclin D1 overexpression is considered as an early event.\textsuperscript{16}

**TUMOUR SUPPRESSOR GENES**

Inactivation of tumour suppressor genes either by mutation, methylation or chromosomal loss may result in cell transformation. Tumour suppressor genes closely related to ESCC involve (i) p53 (ii) Rb (iii) p16 and (iv) p21.

**p53**

The p53 gene is located at the short arm of chromosome 17. It has been reported that approximately 50% of all human tumours including ESCC carry mutations in the p53 gene.\textsuperscript{4} These mutations can stabilize the protein, so it can accumulate in nuclei resulting in overexpression of p53.\textsuperscript{17} ESCC showing p53 overexpression has a poor prognosis and is related to invasiveness and metastasizing capacity of the cancer cells.\textsuperscript{18} The p53 network is normally "off". It is activated only when there is cell damage. p53 protein maintains the cellular integrity by suppressing the oncogenic transformation. It interrupts the G1 phase of cell cycle to buy time for repairing the damaged DNA or apoptosis induction if the damage is irreparable. p53 protein therefore provides a critical brake on tumour development. DNA damage acts as the "on" switch explaining the fact that it is often mutated and thereby inactivated in cancers.\textsuperscript{19}

**Rb**

The retinoblastoma (Rb) gene encodes the phosphoprotein retinoblastoma protein (pRb). Reduced expression of pRb has been found in 30 to 50% of ESCC cases, which has been associated with poorer survival in patients. One of the important causes of this reduced expression of pRb in tumour cells is loss of heterozygosity of the Rb gene.\textsuperscript{20} Phosphorylation of Rb occurs in an undulating fashion during the cell cycle. It becomes hyperphosphorylated in late G1 phase, and remains hyperphosphorylated in S, G2, and M phases. Cells in G0/G1 express Rb in a hypophosphorylated form. This pattern of phosphorylation during the cell cycle suggests that the Rb protein itself may act as a cell cycle regulator, the inactivation of which may lead to uncontrolled cell growth.\textsuperscript{21}

**p16**

Cells harbouring oncogenic mutations in vivo often respond by activating expression of the INK4a/ARF/INK4b locus on chromosome 9p21, which encodes three related genes - ARF (also known as p19ARF and p14ARF), p15INK4b, and p16INK4a-that encode critical tumour suppressor proteins.\textsuperscript{22} p16 (CDKN2a/INK4a) is a CDK inhibitor that regulates cell cycle progression through the G1/S restriction point by binding to CDK 4 and 6, preventing phosphorylation of pRb. p16 alterations are one of the most common genetic/epigenetic alterations in cancer leading to its inactivation.\textsuperscript{23} About 15-20% of ESCC cases exhibit reduced expression of p16, which has been associated with shorter survival in these patients.\textsuperscript{23, 24}

**p21**

p21 (WAF1/CIP1) is a well-characterized CDK inhibitor. p21 can bind to proliferating cell nuclear antigen thereby blocking DNA synthesis and it is also a transcriptional target of p53. Thus p21 plays a crucial role in mediating growth arrest when cells are exposed to DNA damaging agents.\textsuperscript{25} Binding of wild type of p53 protein to DNA stimulates the expression of p21. This then interacts with a cell division-stimulating protein (CDK2) forming a complex and this complex acts as a stop signal for cell division. Mutation of p53 makes p21 protein unavailable for it to function as the stop signal for division of cell. As a result there is unbridled cell growth leading to cancer development.\textsuperscript{26} Expression of p21 has been linked to independent prognostic factor ESCC with a better survival in the high expression group compared to the low expression group.\textsuperscript{26}

**APOPTOSIS RELATED GENES**

The biological process by which cells undergo programmed death is known as apoptosis. There are multiple signalling pathways that lead to activation of the apoptosis. Role of apoptosis in carcinogenesis can be explained by the fact that it involves participation of many oncogene and tumour suppressor gene products in the process of apoptosis including p53. If DNA damage is detected, p53 along with other regulatory proteins induces the cell to arrest in the G1 phase of the cell cycle. By doing so it enables DNA repair to take place or, alternatively, it induces the cell to undergo apoptosis.\textsuperscript{27} An inner mitochondrial protein, bcl-2, has a key role in the regulation of apoptosis.\textsuperscript{27} In addition to bcl-2 itself, bcl-XL and bcl-w are responsible for suppression apoptosis, whereas pro-apoptotic group of bcl-2 members are bax, bad and bak.\textsuperscript{28} Thus alterations of cell cycle regulators and apoptotic machinery may have a critical impact in carcinogenesis of ESCC with prognostic implications.

**METASTASIS RELATED GENES**

Invasion and metastasis consist of sequential multi-stage, multi-step processes involving adhesion molecules like E-cadherin and β-catenin, which perform a pivotal role. E-cadherin, located on long arm of chromosome 16, is a classical cadherin. It forms the functional component of adherens junctions between epithelial cells. β-catenin is a multifunctional cytoplasmic protein which links E-cadherin to cytoskeleton.\textsuperscript{29} Thus downregulation of E-cadherin and β-catenin have been reported to be closely associated with lymph node metastases and venous invasion in ESCC.\textsuperscript{29, 30}

**TARGETED AGENTS BASED ON MOLECULAR ALTERATIONS IN ESCC**

Molecular alterations in ESCC are not only important biologic prognostic factors but are also potential targets for the development of new antitumour drugs. Over the last decade, there has been a tremendous development in the field of drug development with the identification of specific molecular targets as well as the ability to direct treatment at these targets.\textsuperscript{31} Potential tumour targets for ESCC are those related to oncogenes like growth factor receptors (EGFR, HER-2/neu and VEGF) and nuclear factors (cyclin D1), tumour suppressor genes (p53, p16, p21), genes regulating apoptosis (p53, bax and bel-2) and those relating to metastasis (tissue inhibitor of metalloproteinase, E-cadherin). Many targeted therapies for ESCC are in different phases of clinical trials. These include EGFR monoclonal antibodies (cetuximab),
tyrosine kinase inhibitors for EGFR (gefitinib and erlotinib), monoclonal antibodies to the HER-2/neu receptor (trastuzumab) and VEGF ligand (bevacizumab). 31, 32 Although these novel targeted agents are in early phases of development, encouraging results have been reported in ESCC. 31

Cetuximab
Cetuximab is an antibody of IgG1 subclass and is a chimera between mouse and human. It blocks EGFR and TGF-α by binding erbB-1 and thus it prevents phosphorylation and activation of EGFR tyrosine kinase.33 Cetuximab also acts by internalizing EGFR by removing the receptor from the cell surface and as a result avoiding interaction with the ligand. This leads to variety of effects like inhibition of cell growth, apoptosis induction and production of VEGF. Cetuximab has shown potential for oesophageal cancer with minimal side effects. 31, 33

Gefitinib and Erlotinib
Tyrosine kinase inhibitors like gefitinib and erlotinib are a class of oral, small molecules. They inhibit ATP binding within the tyrosine kinase domain, which effectively inhibits EGFR autophosphorylation and signal transduction. 34 These two tyrosine kinase inhibitors have shown modest response rates in ESCC with few common side effects. 31, 32

Trastuzumab
Trastuzumab is a humanized monoclonal antibody of IgG1 subclass. It targets the HER-2/Neu antigen and has multiple mechanisms of actions. In early phases of clinical trials trastuzumab has been found to be safe in oesophageal adenocarcinoma as a combination therapy with other chemotherapeutic agents like paclitaxel and cisplatin, and radiotherapy. 35 Similar results are expected in ESCC also.

Bevacizumab
Bevacizumab is a recombinant humanized monoclonal antibody. It has high affinity for all VEGF isoforms and blocks the binding of VEGF to its receptor. 36 VEGF blockade decreases tumour interstitial pressure and vascular permeability. Thus bevacizumab enhances radiation and delivery of chemotherapy to tumours. Combination therapy with bevacizumab for oesophageal cancers are on trials although most of the trials are for oesophageal adenocarcinoma. 37

**p53 based gene therapy**
Utility of other molecular markers, in particular p53, has been primarily prognostication of ESCC. p53 undergoes mutation resulting in its overexpression in ESCC but wild type of p53 has a protective role in ESCC. Trials are going on for gene therapy via adenovirus-mediated p53 gene transfer in patients with chemoradiation resistant advanced ESCC. It has been observed that this treatment results in local antitumor effects in these patients. 38

**CONCLUSION**
Recently there has been a tremendous development in the field of molecular biology of cancers. Understanding the molecular aspects may provide clues to oesophageal squamous cell carcinogenesis. ESCC has a very dismal prognosis. Thus elucidating the molecular alterations may herald newer preventive, diagnostic and therapeutic strategies with scope for improvement in its prognosis. With the plethora of insights pouring in from this field, future strategies in the treatment of ESCC are bound to be based on understanding of the molecular intricacies of the disease.

**REFERENCES**