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Ann N Y Acad Sci. 2016 October ; 1381(1): 66–73. doi:10.1111/nyas.13144.**Personalized and targeted therapy of esophageal squamous cell carcinoma: an update****Yongjing Liu^{1,2,3}, Zhaohui Xiong³, Andrea Beasley³, Thomas D'Amico⁴, and Xiaoxin Chen^{3,4}**¹Department of Cardiothoracic Surgery, 105th Hospital of PLA, Hefei, Anhui Province, China.²Division of Thoracic Surgery, Duke University Medical Center, Durham, North Carolina.³Cancer Research Program, JLC-BBRI, North Carolina Central University, Durham, North Carolina.⁴Center for Esophageal Disease and Swallowing, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.**Abstract**

Esophageal squamous cell carcinoma (ESCC) is a deadly disease that requires extensive research. In this review, we update recent progress in the research area of targeted therapy for ESCC. Sox2 and its associated proteins (e.g., Np63 α), which regulate lineage survival of ESCC cells, are proposed as therapeutic targets. It is believed that targeting the lineage-survival mechanism may be more effective than targeting other mechanisms. With the advent of a new era of personalized targeted therapy, there is a need to move from the tumor-centric model into an organismic model.

Keywords

esophageal squamous cell carcinoma; targeted therapy; Sox2; lineage survival

Esophageal cancer is the eighth most prevalent cancer in the world. Each year, there are more than 480,000 incident cases and 400,000 deaths, with more than 80% occurring in developing countries.¹ Esophageal squamous cell carcinoma (ESCC) is the predominant histologic type worldwide. In China alone, more than 280,000 new cases and 200,000 deaths were estimated in 2010.² Despite many advances in diagnosis and treatment in the past decades, the 5-year survival rate for patients with esophageal cancer ranges from 15% to 20%.³ This is mainly due to late diagnosis, aggressiveness of this cancer, and a lack of effective treatment strategies.⁴

Surgery remains the mainstay of treatment for ESCC, although surgery alone achieves poor locoregional control and poor long-term outcome. The 5-year survival rate for non-

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Conflicts of interest

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metastatic ESCC is 10–40% if treated with surgery alone.⁵ Unfortunately, esophagectomy itself is a complex procedure with significant morbidity and mortality. Two percent to 25% of patients die within 30 days after surgery.⁵ Since 40–50% of surgical cases have stage III disease,^{4,6,7} most patients are given neoadjuvant chemotherapy with cisplatin/fluorouracil and carboplatin/paclitaxel. A recent meta-analysis showed a significant improvement in overall survival after neoadjuvant chemotherapy, with a 13% reduction of relative mortality risk and a 5.1% increase in 2-year survival. However, the difference was not statistically significant.⁷ More than 50% of patients with ESCC present with unresectable or metastatic disease at the time of diagnosis.⁴ Chemoradiotherapy is preferred as a nonsurgical approach if there are no contraindications. This combination approach yields a superior palliative outcome compared to radiotherapy alone and improves long-term progression-free survival,⁸ although its efficacy in locoregional control is inferior to surgery.⁹ For chemotherapy, fluorouracil and cisplatin with or without a third drug (such as epirubicin or taxane) is known as the most efficacious combination.¹⁰ Approximately 40% of patients for whom first-line treatment fails will be potential candidates for second-line therapy.¹¹ Unfortunately, salvage choices of second-line therapy are sparse, and there is no consensus on the optimum.¹⁰ Survival of these patients is poor, with a median survival of 5–10 months.^{12–22}

ESCC genomics: an update

Recently, tremendous progress has been made in cancer genomics and epigenomics with the advent of high-throughput techniques like next-generation sequencing. Four groups have reported the genetic landscape of human ESCC with whole-genome and exome sequencing.^{23–26} On the basis of these studies, we have proposed a strategy of personalized targeted therapy for ESCC.²⁷

Several reports on genomic alterations in human ESCC have been published this year using samples from China and Japan.^{28–35} In addition to single-nucleotide variants, copy-number alterations, and alterations of multiple signaling pathways in human ESCC, these studies further demonstrated (1) genomic alterations in a precancerous lesion, atypical hyperplasia; (2) mutual exclusivity of *NOTCH1* and *PIK3CA* mutations; (3) structural variations, including deletions and translocations through non-homologous end joining or alternative end-joining mechanisms and local chromosomal misarrangements through the mechanisms of chromothripsis, kataegis, and breakage–fusion bridge. Contributions of individual genes or pathways to carcinogenesis and prognosis were further analyzed *in vitro* and *in vivo*. Diagnostic, preventive, and therapeutic applications are proposed as future research directions. Targeted sequencing of a cancer gene panel was also applied to human ESCC, to improve cost-effectiveness.³⁶

On the other hand, the outcome of targeted therapy guided by the genomic alterations remains disappointing. Using EGFR as an example, clinical trials of targeted therapy have only shown limited success in improving the overall survival of patients with ESCC.^{37,38} Resistance shows up in cancer cells during the treatment, through multiple mechanisms. Combinations of multiple targeting agents may offer some small advantages.^{39,40} Off-label use of targeting agents based on tumor molecular profiling has not been shown to improve progression-free survival.⁴¹

The lineage-survival mechanism as a target

Among the single-nucleotide variants, copy-number alterations, and alterations of multiple signaling pathways, *SOX2* amplification is clearly a cancer driver leading to ESCC.²⁶ In fact, before these studies, *SOX2* was found to be an amplified oncogene at chromosome 3q26 in human ESCC by two independent groups.^{42,43} As a member of the Sox family of transcription factors, SOX2 plays a critical role in maintaining embryonic stem cells, as well as adult stem cells, in multiple tissues. During fetal development, SOX2 plays major roles in ectodermal, endodermal, and mesodermal development. SOX2 deficiency causes multiple human diseases, including anophthalmia–esophageal–genital syndrome. After birth, SOX2 expression remains in some adult tissues and cells. It continues to play critical roles in adult tissue homeostasis, regeneration, reprogramming, and pathogenesis.⁴⁴ The functional role of SOX2 in carcinogenesis has been extensively reviewed by multiple groups.^{45–47}

In human ESCC, *SOX2* is amplified in ~15% of cases and overexpressed in ~70% of cases, suggesting that multiple mechanisms other than amplification can lead to SOX2 overexpression. SOX2 overexpression is significantly associated with higher histological grade and poorer clinical survival of ESCC patients.^{48,49} Functionally, transgenic SOX2 overexpression drives the complete process of squamous cell carcinogenesis in the mouse forestomach.⁵⁰ On the contrary, mice with hypomorphic SOX2 exhibit an esophagus lined by a columnar epithelium instead of a keratinized stratified squamous epithelium, suggesting an essential role of SOX2 in esophageal epithelial development.⁵¹ *SOX2* is indeed an amplified lineage-survival oncogene in ESCC,⁴² as evidenced by its crucial role in esophageal squamous epithelial cell proliferation and survival during development, its overexpression in squamous epithelial cells of ESCC, its amplification in a subset of ESCC, its essential role for ESCC survival, and its function as a transcription factor.⁵² This discovery provides a possibility for developing targeted therapy of ESCC, even though targeting a transcription factor is a technical challenge. However, recent technical advances have improved the druggability of transcription factors.⁵³

As a transcription factor, SOX2 has a DNA-binding transcription domain and functional domains that interact with other cofactors. Eighty-two SOX2-associated nuclear proteins have been identified in two human ESCC cell lines (KYSE70 and TT) using tandem affinity purification followed by liquid chromatography–tandem mass spectrometry.⁵⁴ This list includes CBX3, DNMT1, HDAC2, KDM1A, KLF5, PARP1, TP63, and many other proteins. Among these, the most interesting is TP63, which is encoded by the p63 gene located ~7 Mb from *Sox2*. These two genes are often co-amplified in multiple cancers (Fig. 1A & 1B). It is known that SOX2 directly regulates transcription of p63 in lung cancer and lung basal cells.^{42,55} About 20% of ESCC cases harbor p63 gene amplification and ~60% overexpression.^{56–58}

The predominant isoform of TP63 in the basal cells of the esophageal epithelium is known as Np63 α . Similar to SOX2, Np63 α drives carcinogenesis via multiple mechanisms.^{59,60} It may antagonize the transcriptional activity of TAp73 and the p73-dependent proapoptotic transcriptional program^{61,62} and modulate expression of its target genes through physical interactions with HDAC1/2 and the histone H2A.Z.^{63–65} Silencing of Np63 α in ESCC

cells inhibited cell proliferation and colony formation via downregulation of Akt signaling.⁶⁶ Overexpression of Np63 α in squamous epithelial cells in transgenic mice leads to increased suprabasal cRel, Ki-67, and cytokine expression, together with epidermal hyperplasia and diffuse inflammation.⁶⁷ On the contrary, *TP63* knockout mice exhibit an esophageal phenotype similar to that seen in SOX2 hypomorphs.⁶⁸ These data suggest that Np63 α is also an amplified lineage-survival oncoprotein for ESCC.

In contrast to proteins directly associated with lineage survival (e.g., Np63 α), other SOX2-associated proteins in ESCC appear to regulate gene expression and may also be associated with lineage survival. CBX3 regulates RNA processing genome-wide, and loss of CBX3 leads to dramatic accumulation of unspliced nascent transcripts and alterations in target gene expression.⁶⁹ DNMT1 contains a DNA methyltransferase domain at its C-terminus for maintenance of global CpG methylation patterns and a large N-terminal domain for protein-protein interactions.⁷⁰ KLF5 regulates proliferation, apoptosis, and invasion in ESCC cells. Loss of KLF5 in the context of *TP53* deletion drives invasive progression of ESCC, and restoration of KLF5 leads to apoptosis and suppresses cell survival.^{71,72} PARP1 interacts with and poly(ADP-ribosyl)ates SOX2 directly for degradation of SOX2 protein. As a result, *PARP1* knockout enhances SOX2 expression and modifies cell differentiation.⁷³⁻⁷⁵ HDAC2 regulates expression of SOX2, and HDAC1/2 deficiency leads to loss of SOX2 expression and blocks proximal airway development.⁷⁶ HDAC1/2 also controls the transcriptional activity of Np63 α .⁷⁷

Genes and signaling pathways upstream or downstream of SOX2 have been extensively studied in the literature.^{45,47} However, cancer therapy targeting signaling pathways is known to be associated with a high rate of resistance, and resistance is predicted to appear in ESCC patients as well.²⁷ It may be less desirable to target SOX2 upstream or downstream signaling, even though targeting the downstream Akt/mTOR or IL6/STAT3 pathways seems to be effective.^{43,50} The high rate of resistance to targeted therapy has prompted the cancer research community to consider combination therapy (i.e., targeting multiple targets or pathways).^{39,40} Alternatively, transcription factors or regulators are potentially better therapeutic targets, simply because these transcription factors define the cancer phenotype.⁷⁸ Here, we argue that targeting the lineage-survival mechanism in ESCC cells is potentially more potent and less likely to produce resistance than targeting other signaling pathways. Four approaches are potentially applicable for ESCC: (1) using small interfering RNA (siRNA) to target SOX2 and/or Np63 α , although this approach is not feasible at this time; (2) enhancing the immune reaction against cancer cells with overexpressed SOX2 and Np63 α participate; and (4) targeting epigenetic modifications in which SOX2 and Np63 α are involved.

SOX2 itself has been targeted by vaccines^{79,80} and zinc finger-based artificial proteins.^{81,82} SOX2-associated proteins have been targeted as well. For example, specific inhibitors of KDM1A/LSD1, a SOX2-associated protein, selectively impair the growth of SOX2⁺ lung squamous cell carcinoma, but not that of SOX2⁻ cells. Inactivation of KDM1A reduces SOX2 expression, promotes G₁ cell cycle arrest, and induces genes for differentiation by selectively modulating the methylation states of H3K4 and H3K9.⁸³ It would be very

intriguing to target protein–protein interactions in which SOX2 and NP63 α are involved, with lineage survival as the readout.

There are two major concerns with regard to targeting SOX2 and/or NP63 α in ESCC. First, these genes are normally present in both normal tissue and cancer. Targeting the SOX2– NP63 α interaction may potentially alleviate the concern of side effects, because not many adult tissues coexpress these two proteins (only the larynx, bronchiole, tongue, esophagus, anus, tonsil, and exocervix).^{84,85} Second, there is a possibility of inducing phenotype switching of cancer cells (i.e., transdifferentiation of ESCC cells into adenosquamous cells or even adenocarcinoma cells), as loss of SOX2 and p63 is an early event in intestinal metaplasia of the esophageal squamous epithelium.⁸⁶ This approach may also potentially select poorly differentiated, lineage-independent cancer cells.⁵²

Targeting the lineage-survival mechanism requires a deep understanding of SOX2– NP63 α biochemistry and its interaction with other factors. The SOX2– NP63 α interactome in ESCC needs to be further clarified with better techniques, for example, stable isotope labeling using amino acids in cell culture. Biological replicates with forward and reverse experiments are needed to enhance confidence in protein interactions.⁸⁷ The functional roles of SOX2/ NP63 α -associated proteins in lineage survival need to be well understood. High-throughput screening has been used to screen inhibitors of protein–protein interactions. Although a few successes have been reported in the literature, classical target-based drug discovery using small molecules is challenging, in particular when the interactions involve multiple proteins.^{88,89} Recently, technical advances have been made in this area.⁹⁰ For example, *in silico* modeling of protein–protein interactions has been successfully used to identify raloxifene and bazedoxifene as novel inhibitors of the IL6/GP130 interaction.⁹¹ With the well-characterized ESCC cell lines (KYSE series and TE series) and a SOX2-transgenic mouse model of ESCC,^{43,50} it is believed that potent agents will be developed for treatment of SOX2-overexpressing ESCC.

The organismic model in ESCC treatment and its implications

With the advent of personalized targeted therapy, there is a need to move from the tumor-centric model into an organismic model.⁹² According to this model, clinicians need to consider not only the tumor itself but also its microenvironment and the whole body (Fig. 2). The idea that “there is an order in cancer” suggests that homeostatic mechanisms in the tumor have to be taken into consideration in targeted therapy.⁹³ The tumor microenvironment has been investigated for years for its capability to both promote and restrain cancer.⁹⁴ However, its potential use in ESCC treatment has not been well studied (see a recent review in Ref. 95). Antiangiogenesis and immune therapy targeting the microenvironment are currently under clinical investigation. At the organismic level, it is far more complex. Intriguing questions may be asked: how the body reacts to the tumor, how the tumor reacts to the body, and how to manipulate the body against the tumor and its microenvironment.

Nevertheless, this organismal model has multiple implications. Our therapeutic goal may turn into “keeping clinical cancer under control and living with microscopic cancer.” The

mindset of “5-year survival” may give away to “life-time treatment and monitoring.” Surgical resection, which is currently the mainstay for ESCC treatment, needs to be more precise and less invasive, and may need to be repeated over the course of treatment. Monitoring disease progression will be of critical significance. Emerging techniques, such as circulating tumor cells and DNA, will be essential in monitoring therapeutic response and disease status.^{96,97} There will be a great need to mitigate the various side effects of targeted therapy in order to improve the quality of life. The variety of issues (e.g., financial, social, psychological, behavioral) will make decision making in ESCC treatment sophisticated.

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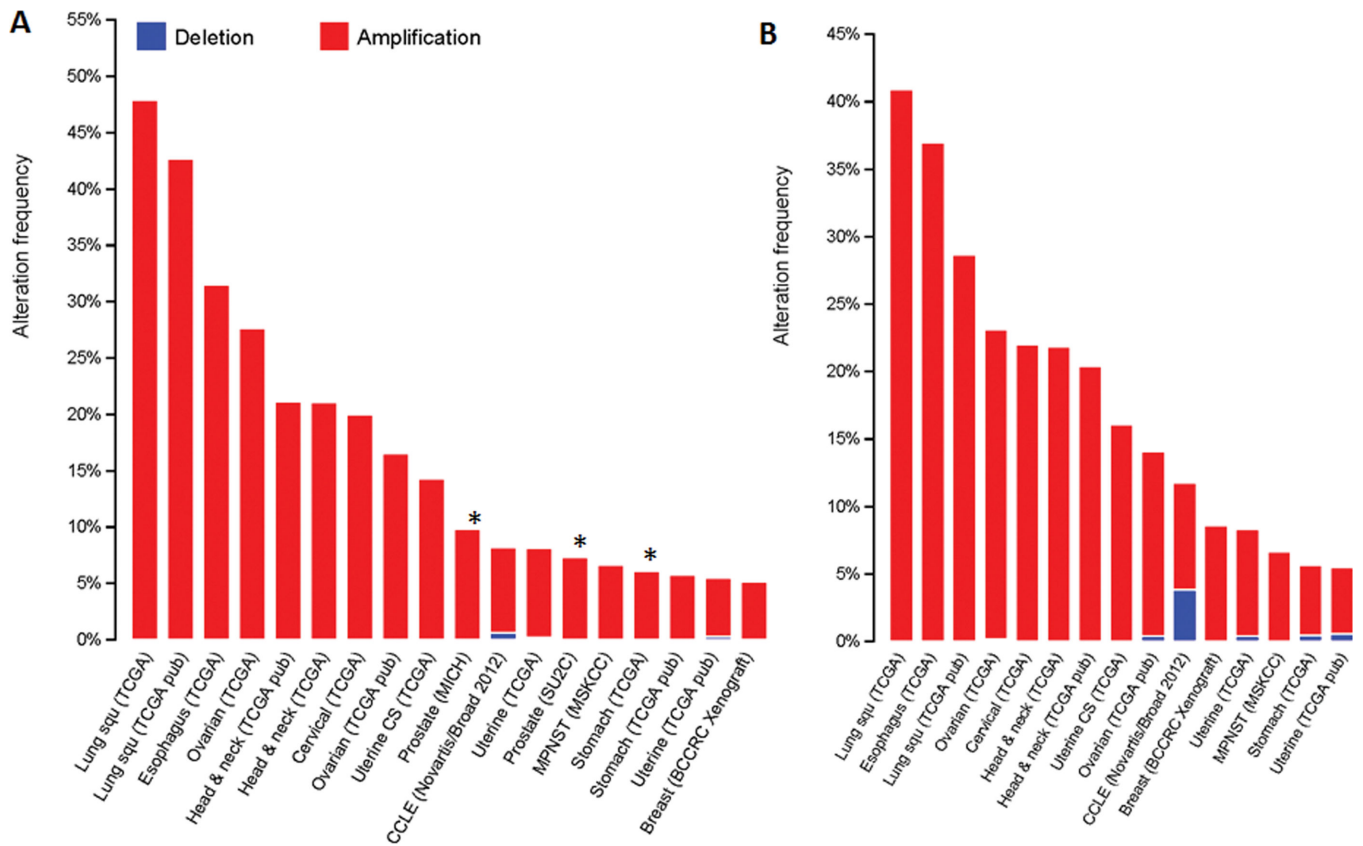


Figure 1. (A) *SOX2* and (B) *TP63* amplification in human cancers. Data are obtained from the TCGA database (www.cbioportal.org). Only cancers with a high incidence of gene amplification (> 5%) are included. There is an obvious overlap between *SOX2* and *TP63* amplification in these cancers.

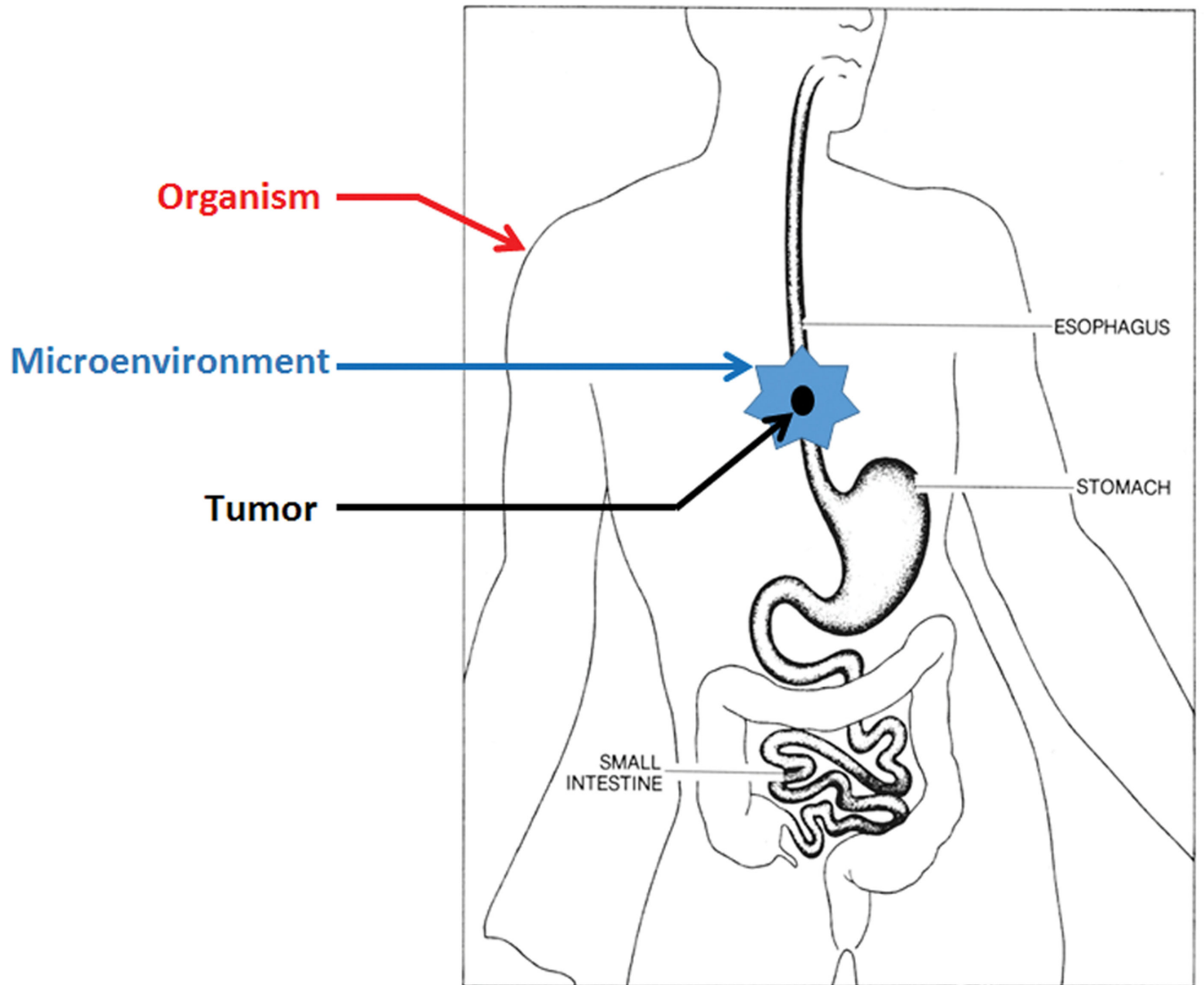


Figure 2. An organismic model of ESCC, as opposed to the tumor-centric model, calls for attention to the organism and the tumor microenvironment.