

Molecular Biology Character of Esophageal Cancer

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1. Introduction

Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer death worldwide. Esophageal squamous cell carcinoma (ESCC) and adenocarcinoma (EAC) are two major histopathological type of esophageal cancer. The incidence of EC was increased in the past 3 decades. Five-year survival of advanced cancer is still very poor, even though improved surgical techniques and adjuvant chemoradiation therapy. It is very important to understand esophageal cancer biology.

2. Genetic changes in esophageal cancer

Genetic change is one of the major events in transforming normal esophageal epithelia to malignant cells. Mutations and genetic polymorphisms in coding gene sequences may cause functional alteration of genes. Functional mutation and single nucleotide polymorphism (SNP) (eg.p53, SULT1A1, CYP3A5, ALDH2, ADH1B1 and ECRG1) is related to susceptibility of esophageal cancer.

2.1 Effects of mutations and SNPs in esophageal cancer

P53 is involved in multiple cellular pathways including apoptosis, transcriptional regulation, and cell cycle control. Alterations in p53 have been reported to occur at an early stage of EC. P53 mutation was observed in exon 5 and accounted for about 77% of ESCC patients (Hu, Huang et al., 2001). Fanconi gene family is another interesting example. The risk of ESCC is associated with both heterozygous and homozygous mutations in several Fanconi anemia-predisposing genes, such as heterozygous insertion/deletion mutations in FANCD2 (p.Val1233-del), FANCE (p.Val311SerfsX2) and FANCL (p.Thr367AsnfsX13) (Akbari et al., 2011).

SNPs in p53 pathway also play important roles in EC tumorigenesis. SNP in p53 gene (Arg72Pro) decreased apoptosis and was associated with increased risk, earlier age of onset, reduced response to chemotherapy and early recurrence in esophageal cancers (Pietsch et al., 2006). T309G is located in the promoter region of MDM2, which is the regulator of p53 pathway. Transcription factor may easily bind to the G variant of MDM2, increase MDM2

expression and reduce apoptosis in response to DNA damage (Bond et al., 2004). MDM2 T309G G/G was associated with an increased risk of death in ESCC (Cescon et al., 2009).

SNPs in key genes are associated with EC, such as genes involved in nucleotide excision repair (NER) and base excision repair (BER) pathways. The increasing number of variant alleles in SNPs of NER showed a significant trend to EAC, including XPD Lys751Gln, ERCC1 8092 C/A and ERCC1 118C/T (Tse et al., 2008). Esophageal cancer related gene 1 (ECRG1) is reported as a novel tumor suppressor. ECRG1 is normally expressed in esophagus, but reduced in ESCC. ECRG1 (Arg290Gln) was identified as the susceptible SNP of ESCC (Li et al., 2006). It has been found that the increased risk of ESCC relates to combined SULT1A12*2 genotype and CYP3A5 heterozygous genotypes, especially in tobacco smokers (Dandara et al., 2006). SNP of ATP-binding cassette sub-family B (MDR/TAP) member 1 gene (ABCB1) was reported to be associated with lymph node and distant metastases in EC (Narumiya et al., 2011). SNP also impacted disease-free survival (DFS) of ECs. The MDM2 T/G and CDH1 GA/GA genotype confer risk of death in EAC patients (Boonstra et al., 2011). Vascular endothelial growth factor (VEGF) 936C/T is associated with an improved overall survival compared with wild type genotype in EC (Bradbury et al., 2009).

2.2 Effects of chromosomal abnormalities in esophageal cancer

Genomic alterations, such as amplification, deletion, translocation and loss of heterozygosity (LOH) play an important role in initiation and progression of cancer. Recently a panel of chromosome instability biomarkers, including LOH and DNA content, has been reported to identify patients at high and low risk of progression from Barrett's esophagus (BE) to EAC (Paulson et al., 2009).

Chromosomal aberrations have been discovered in BE and EAC, including frequent gain of chromosomes 6p (10–37%), 7q (17–37%), 7p (30–60%), 8q (50–80%), 10q (20–50%), 15q (10–40%), 17q (30–50%), and 20q (50–80%); and frequent loss of chromosomes 4q (20–50%), 5q (20–50%), 9p (20–50%), 14q (30–40%), 16q (36–40%), 17p (30%), 18q (20–60%) and Y (60–76%). The proto-oncogenes are often duplicated, such as MYC (8q), EGFR (7p) and ERBB2 (17q). But tumor suppressor genes are usually deleted in BE and EAC, including APC, CDKN2A, p53, and SMAD4 (Akagi et al., 2009). Genomic instability varied widely across chromosomal arms, with the highest frequency of LOH on 9p, CN (copy numbers) loss on 3p, and CN gain on 3q in ESCC (Hu et al., 2009).

ERBB2 and Topoisomerase (DNA) II alpha (TOP2A) genes are located in 17q12-q21.2 region which was reported to be amplified in EACs. Amplification of ERBB2 was found in 10% to 70% of EAC samples. Antagonist of ERBB2, Trastuzumab/Herceptin, inhibits growth of OE19 EAC cell line, which exhibits high expression of ERBB2. TOP2A gene is associated with cell proliferation, and amplified TOP2A has been reported in ESCC (Akagi et al., 2009). The epidermal growth factor receptor (EGFR) I, a tyrosine kinase (TK) involved in several tumor progression and may serve as an important therapeutic target (Erlotinib, Cetuximab). Homogeneous EGFR amplification defines a subset of aggressive Barrett's adenocarcinoma with poor prognosis (Marx et al., 2010). Numerous studies have been reported that chromosomal abnormalities (aneuploidy and tetraploidy) and loss of heterozygosity (LOH)

may be used as biomarkers to predict progression of Barrett's esophagus to EAC (Reid et al., 2000). It was demonstrated that a number of SNPs was highly correlated with chromosomal abnormalities in Barrett's esophagus and EAC (Li et al., 2008).

3. Epigenetic changes in esophageal cancer

The term epigenetics refers to the study of heritable changes in gene expression without changes in gene sequence. In addition to genetic alteration, epigenetic modifications are recognized as a common molecular alteration in human cancers. DNA methylation and histone modifications are important epigenetic changes during tumor initiation and progression (Sadikovic et al., 2008). Non-coding RNA (ncRNA) is another kind of epigenetic regulation factor, especially microRNA (miRNA) was recently regarded as the important gene expression regulator. Epigenetic regulation was involved in different pathways including cell cycle, apoptosis, DNA repair et al (W. Zhang et al., 2008; X. Zhang et al., 2010).

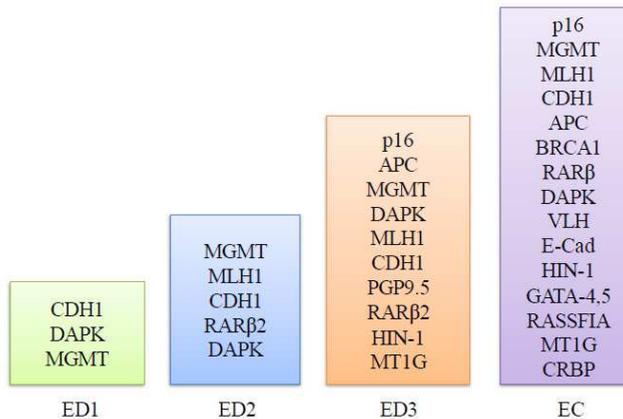
3.1 DNA methylation

DNA methylation leads to gene silencing either by directly block the transcriptional factors binding to DNA, or by MBP which recruits chromatin remodeling co-repressor complexes (Klose & Bird, 2006). Promoter region methylation was reported frequently in human esophageal cancer. DNMT1, DNMT3A and DNMT3B have been identified as DNA methyltransferases in eukaryotic cells. DNMT1 is involved in maintaining DNA methylation, DNMT3A and DNMT3B are responsible for *de novo* methylation. Overexpression of these DNMTs were reported to be involved in a variety of cancers including EC (Kassis et al., 2006). DNMT3L and DNMT2 were reported recently related to DNA methylation. DNMT3L is required for the methylation of imprinted genes in germ cells, and interacts with DNMT3a and 3b in *de novo* methyltransferase activity (Chen et al., 2005). And the function of DNMT2 remains unclear, its strong binding to DNA suggests that it may mark specific sequences in the genome.

Methylation profile is different in ESCC and EAC. Adenomatous polyposis coli (APC) is frequently methylated in EAC, but infrequently in ESCC (Zhang & Guo, 2010). CDKN2A/p16^{INK4a} methylation is a frequent and early event both in ESCC and EAC (Wang et al., 2009). Caudal type homeobox 2 (CDX2) is expressed in gut epithelia and plays an important role in establishing intestinal phenotype during development. CDX2 is frequently methylated in ESCC (49%), but rarely in EAC (5%) (Guo et al., 2007). Inactivation of CDX2 in EC associated with DNA methylation may be an important determinant of squamous or non-adenomatous phenotype. Multiple genes methylation increases during progression from esophageal mucosa to EC [Figure1] (Fang et al., 2007; Guo et al., 2006). No RAR β 2 methylation was observed in normal esophagus but increased methylation was found with the progression of esophageal carcinogenesis. Hypermethylation of p16 and APC is related to high-grade dysplasia or cancer in BE patients.

There is considerable epidemiological evidence suggesting that alcohol, tobacco, diets deficient in vitamins/protective antioxidants, carcinogens and thermal injuries are important in the pathogenesis of EC. Cigarette smoke is a key factor in esophageal carcinogenesis. It was reported that cigarette smoking is a cause of SSBP2 promoter

methylation and that SSBP2 harbors a tumor suppressive role in ESCC through inhibition of Wnt signaling pathway (Huang et al., 2011). A previous study demonstrated that duration of tobacco smoking is correlated significantly with DNA methylation of HOXA9, MT1M, NEFH, RSPO4, and UCHL1 in the background esophageal mucosa of EC patients (Oka et al., 2009).



ED: esophageal dysplasia; EC: esophageal cancer

Fig. 1. Accumulated methylation of genes in the progression of esophageal cancer.

3.2 Histone modification

Histone modification (acetylation, methylation, phosphorylation, ubiquitylation, et al.) has important functions in many biological processes including heterochromatin formation, X-chromosome inactivation and transcriptional regulation. In mammals histone arginine methylation is found on residues 2, 8, 17 and 26 of histone H3 and residue 3 of histone H4. Histone lysine methylation occurs on histones H3 and H4 and can be mono-, di- or trimethylated. Similar to histone lysine methylation, arginine methylation occurs in mono-methyl, symmetrical di-methyl or asymmetrical di-methyl state, and contributes to both active and repressive effects on chromatin function (Martin & Zhang, 2005). Methylation on the same site can lead to different outcomes depending on the number of methyl groups added. However the functional relevance of these modification states remains poorly understood. Although there is no evidence that lysine methylation directly affects chromatin dynamics, acetylation of lysine residues in histones is reported to antagonize folding of chromatin in vitro (Hansen, 2002). In mice, for example, it has been shown that pericentric heterochromatin is specifically enriched in trimethyl-H3-K9 and H4-K20, and the effect is silencing of transcription; while mono- and dimethyl-H3-K9 and H4-K20 are found in euchromatin, and play activating transcriptional function, even though there some argues (Peters et al., 2003; Schotta et al., 2004). The main sites of lysine methylation that have been associated with gene activity include K4, K36 and K79 of histone H3. Trimethylation of lysine 27 on histone H3 (H3K27me3) is an silencing epigenetic marker.

Acetylation neutralizes the positive charge of lysine, it has been suggested that this modification might operate through an electrostatic mechanism and histone acetylation is associated with active gene transcription. DNA methylation and histone modifications have recently been reported to cooperate in controlling gene expression (Johnson et al., 2002). Methylation of histone H3 lysine 9 was triggered by DNA methylation. DNA methyltransferases have been shown to interact with histone deacetylases (HDAC), histone methyltransferases, and methyl-cytosine-binding proteins in complex network (Fuks et al., 2000). Histone modifications and DNA methylation are epigenetic phenomena that play a critical role in neoplastic processes.

H3K18Ac and H3K27triMe was correlated with worse survival of ESCC, especially in early stages patients (Langer et al., 2009). Zester homolog 2 (EZH2) is reported to be overexpressed and correlates with poor prognosis in human cancers. The expression frequency and expression levels of H3K27me3 were significantly higher in ESCCs than in normal tissues by immunohistochemistry. Expression of H3K27me3 was significantly correlated with WHO grade, tumor size, T status, locoregional progression and EZH2 expression. High expression level of H3K27me3 was significantly associated with poor locoregional progression-free survival (LPFS) in ESCC (He et al., 2009). A study of 237 ESCC patients showed that histone modifications have significant effects on recurrence-free survival (RFS) after esophagectomy in ESCC, such as acetylation of histone H3 lysine9 (H3K9Ac), histone H3 lysine 18 (H3K18Ac), and histone H4 lysine 12 (H4K12Ac), and the dimethylation of histone H3 lysine 9 (H3K9diMe) and histone H4 arginine 3 (H4R3diMe). 1% increased global level of H3K18Ac in pathologic stage III worsened RFS at 1.009 times, after adjusting for age, sex, and operative method (I et al., 2010). Global levels of histone modifications in ESCC may be an independent prognostic factor of RFS.

3.3 Non-coding RNA

Non-coding RNAs (ncRNAs) are functional RNA molecules that do not code for proteins. Based on size, they are divided into different classes: long ncRNAs (lncRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), microRNAs (miRNAs), etc (Brosnan & Voinnet, 2009). NcRNAs were regarded as important factors of cancer. MiRNA is only well-studied ncRNAs in different disease, including esophageal cancer. MiRNAs are a class of single stranded, evolutionarily conserved non-coding RNAs, only 17-25 ribonucleotides long, involved in a wide spectrum of basic cellular activities through their negative regulation of gene expression.

MiRNAs play important roles in cellular activities such as proliferation, apoptosis and differentiation (Bartel, 2004). MiRNAs are involved in the development, progression and prognosis of esophageal cancers (Feber et al., 2011). As shown in Table 1, expression of miRNAs is different in EAC and ESCC. It was reported that miR-25, miR-151 and miR-424 were up-regulated, whereas miR-29c, miR-99a and miR-100 were reduced in EC. The pattern of these miRNAs may be used to distinguish malignant from normal esophagus. Low level of miR-103/107 expression showed a strong correlation with high overall and disease-free survival periods for EC patients, which may be used for the diagnosis of esophageal cancer. Higher level of miR-196a was observed in EAC, BE and dysplastic lesions compared with normal mucosa. MiR-145, miR-133a and miR-133b inhibited cell

proliferation and invasion in ESCC. MiR-200a has been linked to the etiology and prognosis of ESCC. Expression levels of mature miR-21 and mature miR-145 were significantly higher in ESCC than those in normal epithelium, and were significantly associated with lymph node positive, recurrence and metastasis in ESCC (Akagi et al., 2011; Guo et al., 2008; Kano et al., 2010; Maru et al., 2009).

Pathological type	Overexpression	Downregulation	Predicted targets of miRNAs	Reference
EAC	miR-215, miR-560, miR-615-3p, miR-192, miR-326, miR-147	miR-100, miR-23a, miR-605, miR-99a, miR-205, let-7c, miR-203	HMGA2 (let-7c), ZEB1 and ZEB2 (miR-205)	(Fassan et al., 2010)
ESCC	miR-145, miR-133a, miR-133b	Let-7	FSCN1(miR-145,miR-133a, miR-133b) HMGA2 (Let-7)	(Kano et al., 2010; Liu et al., 2011)
ESCC, EAC	miR-21	miR-375	PDCD4, NFIB, PTEN, TPM1 (miR-21); PDK1 (miR-375)	(Mathe et al., 2009; Matsushima et al., 2010)
ESCC	miR-93	miR203,miR205	FUS1, E2F1, TP53INP1 (miR-93); ΔNp63 (miR-203)	(Feber et al., 2008; Yuan et al., 2011)
ESCC	miR-373, miR-129	miR-10a	Rab11, APC, LATS2 (miR-373); LATS2 (miR-129) HOX family (miR-10a)	(Matsushima et al., 2010)

EAC: esophageal adenocarcinoma; ESCC: esophageal squamous cell carcinoma

Table 1. MiRNAs expression profile in EAC and ESCC.

In the progression from low-grade dysplasia (LGD) to high-grade dysplasia (HGD) of esophagus, miR-513, miR-125b, miR-101 and miR-197 were up-regulated; miR-23b, miR-20b, miR-181b, miR-203, miR-193b, and miR-636 were down-regulated. MiR-345, miR-494, miR-193a, let-7a, let-7b were down-regulated in progression from HGD to EAC (Yang et al., 2009). MiR-196a level is increased with the progression from normal mucosa to EAC (Maru et al., 2009).

In the past few years, increasing evidence has indicated that a substantial number of miRNAs were regulated by DNA methylation in cancers. Like protein-coding genes, hypermethylation in promoter region of miRNAs was recognized as the mechanism of miRNA regulation in cancers. For example, miR-375, miR-34a, miR-34b/c and miR-129-2 were down-regulated by hypermethylation in EC, and frequent methylation of miR-129-2 was regarded as early detection biomarker of ESCC (Chen et al., 2011; Li et al., 2011).

4. Biology of esophageal precancerous lesion

Most tumors are adenocarcinomas in western societies, squamous cell cancers constitute over 80% of EC in the world. The development of human esophageal cancer is a multistep, progressive process. An early indicator of this process is an increased proliferation of esophageal epithelial cells morphologically including basal cell hyperplasia, different grades

of dysplasia, carcinoma in situ (CIS) and advanced esophageal squamous cell carcinoma (ESCC) (Guo et al., 2008). The widely studied precancerous lesion is Barrett's esophagus.

Barrett's esophagus (BE) is an acquired condition in response to chronic gastro-esophageal reflux. EAC was developed through progression from normal epithelium to metaplasia, and different grade of dysplasia (Flejou, 2005). Barrett's esophagus is defined as replacement of normal squamous epithelium with intestinal column epithelium in distal portion of esophagus. The incidence rate of HGD or cancer per patient-year for non-dysplastic Barrett's esophagus was 0.49%. 13.4% of LGD will become HGD or cancer in one year. 10% or greater of high-grade dysplasia may develop to invasive cancer per patient-year (Curvers et al., 2010; Shaheen & Richter, 2009). Barrett's esophagus is thought to be a precancerous lesion with the following changes: augmentation of cell cycle and proliferation, increased angiogenesis and aneuploidy, decreased antiproliferative signaling and apoptosis. The molecular basis of the development of EAC, although extensively studied (Brabender et al., 2004; McManus et al., 2004), is still remains unclear. Better understanding of the molecular alterations during its development might improve prevention and treatment.

In the last three decades, the incidence of Barrett's esophagus-associated esophageal adenocarcinoma (BEAC) is increasing very fast in western world (Blot & McLaughlin, 1999). Despite improvements in treatments of EAC, the prognosis is still poor (Falk, 2002). Therapeutic advances in BEAC have lagged behind other cancers due to its paucity of reliable models in vitro and in vivo. Although Bic-1 and OE33 cells have been established as BEAC-derived cell lines, molecular character remains unclear. BEACs have been shown to undergo loss of heterozygosity at chromosome 18q, the location of smad2 and smad4, in up to 69% of patients, and in as many as 46% of patients with non-dysplastic BE (Barrett et al., 1996; Wu et al., 1998). For the treatment, 2-methoxyestradiol (2-ME(2)) is increasingly recognized as a novel chemotherapy drug to activate a wide array of anti-cancer targets with a relative sparing of normal tissues (Dahut et al., 2006; Sweeney et al., 2005). 2-ME(2) was reported to play an important role in chemoprevention and therapy of BEAC (Kambhampati et al., 2010).

Bile acids may play an important role in progression from BE to EAC. It is reported that bile acid reflux present in patients with BE may increase cell proliferation via activation of PI-PLC γ 2, ERK2 MAP kinase, and NADPH oxidase NOX5-S, thereby causing DNA damage and gene mutation, which contribute to the development of EAC (Hong et al., 2010). Trefoil factor 3 (TFF3) was identified as a promising biomarker to screen asymptomatic patients for Barrett's esophagus (Lao-Sirieix et al., 2009). Increased expression of cyclinD has been implicated in predisposition to transform from metaplastic epithelium to cancer (Trudgill et al., 2003).

5. Key protein and pathway involved in esophageal cancer

More than 500,000 patients are diagnosed as esophageal cancer annually. Molecular factors are including aberrant regulation of cyclooxygenase-2 (COX-2), TNF- α and several pathways such as Wnt signaling pathway, TGF- β signaling pathway, NF- κ B signaling pathway and so on.

5.1 Wnt signaling pathway

Wnt/ β -catenin signaling pathway plays crucial roles in regulation of cellular activity during embryonic development and human diseases including cancers (Logan & Nusse, 2004). Numerous Wnt signaling components, including WNT, secreted frizzled-related proteins (SFRPs), β -catenin, are also of pivotal importance in carcinogenesis of esophageal cancers (Clement et al., 2006).

Epigenetic regulation of key genes in Wnt signaling pathway was described above. Aberrant activation of Wnt signaling pathway has significant effect on the development of esophageal cancer from Barrett's esophagus. WNT2 is upregulated along the progression from LGD to EAC, its expression was higher in dysplasia and EAC than in BE, with 77% of EAC showing high expression of WNT2 (Clement et al., 2006). β -catenin has emerged as a key regulator of Wnt signaling pathway, which plays an important role in development and progression to cancers. Accumulation of nuclear β -catenin in esophagus squamous epithelium might be the crucial step for the carcinogenesis of ESCC (Veeramachaneni et al., 2004). Reduced membranous β -catenin expression has been associated with progression, invasion and poor prognosis in EC (Krishnadath et al., 1997). SRY-box containing gene 17 (SOX17) is reported to play critical roles in regulation of development and stem/precursor cell function through repression of Wnt pathway activity (Gubbay et al., 1990). Hypermethylation of SOX17 was found frequently in ESCC (Zhang et al., 2008). Several studies have reported nuclear accumulation of β -catenin is an indicator of activation of Wnt/ β -catenin signaling, and nuclear translocation of β -catenin was observed during progression of BE towards EAC (Osterheld et al., 2002).

5.2 TGF- β pathway

Transforming growth factor- β (TGF- β) was initially identified and named on the basis of its ability to stimulate fibroblast growth in soft agar, but it is now the best-studied growth inhibitory protein. TGF- β family has emerged as a major source of signals that control cell growth and differentiation (Massague, 2000). TGF- β signaling pathway is reported to be frequently involved in gastrointestinal carcinogenesis (Blaker et al., 2002).

TGF- β is regarded as both tumor suppressor and oncogene (Pardali & Moustakas, 2007). In human prostate cancer, overexpression of TGF- β 1 enhanced angiogenesis around the tumor, which increased metastasis of prostate cancer. On the other hand, gallbladder tumors secrete TGF- β , which inhibits angiogenesis and results in reduced tumor growth. Thrombospondin1 (THBS1), cysteine-rich protein 61 (Cyr61) and connective tissue growth factor (CTGF) are all involved in TGF- β signaling pathway, which plays an important role in tumorigenesis. In human breast cancer THBS1 reduces tumor growth, metastasis and angiogenesis (Sheibani & Frazier, 1995). TGF- β signaling pathway can be activated by THBS1 through its interaction with latent TGF- β binding proteins (LTBP), so that TGF- β is capable of binding to its receptors and stimulating Smad pathway (Crawford et al., 1998). Smad proteins bind to Cyr61 and CTGF promoters, which leads to transcription of Cyr61 and CTGF and activation of angiogenesis and tumor growth (Bartholin et al., 2007; Holmes et al., 2001). It has been reported that THBS1 expression in stroma of ESCC was correlated with lymph node metastasis and Cyr61 expression in Barrett's tissue of EAC was

significantly higher than that in Barrett's esophagus with no cancer (Di Martino et al., 2006; Oshiba et al., 1999). Recently, CTGF expression was found to be upregulated in ESCC and significantly related to survival of ESCC patients (Koliopoulos et al., 2002). Moreover, CTGF, CYR61 and THBS1 were overexpressed in ESCC, and Cyr61 and CTGF could serve as independent prognostic markers for ESCC (Zhou et al., 2009). Expression level of Smad4 was profoundly reduced at all stages of progression from Barrett's dysplasia to esophageal carcinoma. And 70% of EACs had hypermethylation of Smad4 gene. In Barrett's metaplasia-dysplasia-adenocarcinoma sequence, downregulation of Smad4 occurs due to several mechanisms, including methylation, deletion, and protein modification. And the resulting functional effects of impaired TGF- β signaling are profound throughout this carcinogenesis (Onwuegbusi et al., 2006).

TGF- β signaling has been shown to be paradoxical in tumorigenesis. In addition to inhibitors of TGF- β signaling, as tumor suppressor, many factors may activate TGF- β signaling, such as HDAC inhibitor, SAHA and synthetic terpenoid. It is a good strategy to block the initiation of tumorigenesis through the development of TGF- β mimics in order to achieve chemoprevention.

5.3 NF- κ B signaling pathway

NF- κ B signaling pathway plays important roles in regulation of cell growth and motility. The NF- κ B family is composed of p50, p52, RelA/p65, c-rel, and Rel B. The homodimers and heterodimers are sequestered in cytoplasm as an inactive form by the inhibitor of kappa B (I κ B). Upon stimulation, the I κ B kinase complex (IKK) phosphorylates κ B inhibitor, which releases NF- κ B and allows its phosphorylation, nuclear translocation, and subsequent activation of target genes involved in the regulation of cell proliferation, survival, angiogenesis and metastasis (Brown et al., 1995). Constitutively active NF- κ B is commonly detected in human cancer cell lines and tumor tissues including ESCC, but is rare in normal cells (Sethi et al., 2008). There is strong evidence of NF- κ B being involved in cancer progression, thus NF- κ B and its downstream signaling may serve as therapeutic targets (Basseres & Baldwin, 2006). However, the role of NF- κ B signaling pathway is not quite understood during esophageal carcinogenesis. It is reported that inhibition of NF- κ B can increase the chemosensitivity of EC cells in vitro (Li et al., 2006).

NF- κ B inhibitors (Bay11-7082 and sulfasalazine) were found to reduce proliferation, induce apoptosis, increase chemosensitivity (5-fluorouracil, and cisplatin), inhibit migration and invasion of ESCC cell lines. More importantly, Bay11-7082 had significant antitumor effects on ESCC xenografts in nude mice by promoting apoptosis, and inhibiting proliferation and angiogenesis, as well as reduced the metastasis of ESCC cells to lungs without significant toxic effects. NF- κ B inhibitors may be potential therapeutic agents for patients with esophageal cancer (Li et al., 2009).

5.4 Proteins involves in the other pathways

Except to signaling pathways mentioned above, there are other key proteins were also involved in esophageal carcinogenesis. Short survival and disappointing prognosis of EC is due to its resistance to many clinical therapies such as chemotherapy and radiotherapy

(Toshimitsu et al., 2004). Aurora-A kinase, a serine/threonine protein kinase, is a potential oncogene. Amplification and overexpression of Aurora-A have been found in ESCC. Overexpression of Aurora-A lead to resistance to cisplatin-induced apoptosis and promoted proliferation in esophageal cancer cell lines (Tanaka et al., 2005).

RAR β 2 is reported to be a putative tumor suppressor and is necessary for growth inhibition of retinoic acid (RA) (Chambon, 1996). Loss of RAR β expression was an early event associated with esophageal carcinogenesis and the status of squamous differentiation (Qiu et al., 1999). Frequent methylation and loss of RAR β 2 expression was found in ESCC. DNA methylation of RAR β 2 and tumor grade were correlated significantly in EC. And the correlation of methylation and loss of RAR β 2 expression was only found in G2 stage. RAR β 2 expression was restored and cell growth was inhibited by 5-aza-dc treatment (Liu et al., 2005).

Extensively study of key proteins and signaling pathways will help further understanding the mechanisms of esophageal carcinogenesis, and may improve traditional therapy.

6. Biomarker for esophageal cancer diagnosis and prognosis

Esophageal cancer is one of the most common malignancy worldwide. The overall 5-year survival rates are 10% to 15% due to late diagnosis, metastasis, and resistance to radiotherapy and chemotherapy. Novel early detection marker is urgently needed.

6.1 Potential markers for clinical application in esophageal cancer

Increasing number of studies are focused on EC early detection and promising results were obtained. CDC25B-Abs were reported to be a possible prognostic serological marker for poor survival in advanced ESCC. Expression of HIWI in ESCC is significantly associated with poorer prognosis. WDHD1 is a potential therapeutic target and a candidate biomarker for patients with EC. IGF2 LOI may be a clinically relevant molecular marker of risk for EAC and imprinting status is associated with post-operative outcome following esophageal resection. As shown in Table 2, methylation of HLA-I, CDH1, Integrin α 4, RUNX3 and Claudin-4 is associated with poor prognosis, whereas methylation of APC and FHIT is related to better prognosis in ESCC.

Frequent methylation of CDKN2A/p16^{INK4a}, O⁶-methylguanine-DNA methyltransferase (MGMT), E-cadherin (CDH1) and RAR β 2 was found in esophageal cancer. Accumulation of gene methylation was detected in the progression of esophageal cancer (Guo et al., 2006). HIN-1 (High in normal-1) is a tumor suppressor gene that is highly expressed in many normal tissues. Loss of HIN-1 expression and promoter region methylation was found in 13 (72%) of esophageal cancer cell lines. And methylation of HIN-1 was present in 0% of normal mucosa, 31% of grade I dysplasia, 33% of grade II dysplasia, 44% of grade III dysplasia, and 50% of esophageal cancer specimens (Guo et al., 2008). Methylation of HIN-1 is an early event in dysplastic transformation to esophageal cancer.

Cytokeratin (CK) is an essential cytoskeletal component involved in fixation of nucleus and maintenance of cell morphology. No expression of CK18 or CK8 was found in non-cancerous squamous epithelium. CK18 and CK8 were found of 42.9% and 40.5% positive

respectively in esophageal carcinoma. Prognosis is poorer in patients with CK18-positive than in negative ESCC. CK18 expression was reported to be an independent prognostic factors in ESCC. And CK18/CK8 correlated with progression of ESCC (Makino et al., 2009).

Gene	Histologic al type	Prognostic value	Follow-up period	Reference
HLA-I	ESCC	poor prognosis, lymph node metastasis	Shorter in 3 years	(Qifeng et al., 2011)
APC	ESCC	superior prognosis, decreased metastatic lymph nodes	35 months	(Kim et al., 2009)
FHIT	ESCC	superior prognosis	35 months	(Kim et al., 2009)
CDH1	ESCC	increased recurrence and poor RFS after surgery in stage I cancer	3.3 years	(Lee et al., 2008)
Integrin $\alpha 4$	ESCC	increased recurrence and poor RFS in stage II cancer	3.3 years	(Lee et al., 2008)
RUNX3	ESCC	poor prognosis	Shorter in 4 years	(Tonomoto et al., 2007)
Claudin-4	ESCC	poor prognosis	31.5 months (median)	(Sung et al., 2011)

EAC: esophageal adenocarcinoma; ESCC: esophageal squamous cell carcinoma; BE: Barrett's esophageal; ED: esophageal dysplasia; RFS: recurrence-free survival

Table 2. Prognostic value of gene methylation in esophageal cancer.

Increased β -catenin expression was noted in 18.2% ESCC samples. Reduced expression of Axin, β -TrCP and APC was observed in 46.0%, 24.4%, and 48.2% specimens, respectively. Axin is a negative regulator of Wnt signalling pathway, and genetic alterations of AXIN1 have been suggested to be an important factor in carcinogenesis. Reduced Axin expression was observed in 46% of ESCC. Expression of Axin was found to be correlated inversely with depth of invasion, lymph node metastasis, and lymphatic invasion in ESCC. Reduced Axin protein expression, lymph node involvement, and distant metastasis were significant negative predictors for overall survival and disease-free survival (Li et al., 2009; Nakajima et al., 2003).

MiRNA expression profiling could provide prognostic utility in staging esophageal cancer and treatment plan by endoscopic and neoadjuvant therapies. The alterations of specific miRNAs may further elucidate the metastatic mechanism and allow development of targeting therapy (Feber et al., 2011). Elevated levels of miR-21, miR-155, miR-146b, and miR-181b and reduced expression level of miR-223 were significantly associated with poor prognosis (Mathe et al., 2009).

7. Conclusion

The major goal of molecular biology study is curing of esophageal cancer. Although the molecular biological character was described above, the mechanism of esophageal

carcinogenesis remains unclear. Esophageal cancer is still one of the most lethal diseases even though the improved approaches of diagnosis, prevention and treatment. Therefore, greater effort is desired to comprehensively understand the molecular biology of esophageal carcinogenesis. The insight into cancer biology could be translated into practical approaches for the prevention, diagnosis and treatment of esophageal cancer. Due to the complexity of cancers, the early detection of esophageal cancer is more important at present time.

8. References

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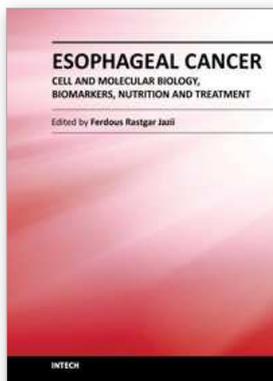
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Esophageal Cancer illustrates recent achievements and investigations in the esophageal tumorigenesis from different perspectives. Readers find mechanisms involved in esophageal tumorigenesis, cellular, molecular, genetic, epigenetics, and proteomics, their relevance as the novel biomarkers and application in esophageal cancer diagnosis and therapy. The book covers detailed effect of nutritional factors in addition to ethanol metabolic pathway in the inhibition of retinoic acid metabolism and supply. Diagnosis, classification, and treatment of esophageal cancer, application of both surgical and non surgical methods as well as follow up of the disease are described in detail. Moreover readers are endowed with especial features of esophageal cancer such as multiple early stage malignant melanoma and pulmonary edema induced by esophagectomy, the two features that received less attention elsewhere in literature.

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