Review article:

The molecular mechanisms of esophageal cancer

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ABSTRACT

Esophageal Cancer ranks among the 10 most frequent cancers in the world, and recent evidence shows that its incidence is increasing. Prognosis of this disease is poor, with an overall 5-year survival rate of less than 10%. Unraveling the mechanisms or developing animal models for esophageal carcinoma have thus far not been successful. Many genes have been found that are believed to play a role in the development of esophageal cancer but the underlying mechanism by which this disease develops is still not clear. It is believed that esophageal cancer has an intricate molecular mechanism of evading apoptosis by the down-regulation of Bax, up-regulation of Bcl-2, Bcl-xl and Survivin, mutation of p53 and alteration in Fas expression. A great deal of research has been performed in order to determine the key genes that initiate and promote the growth of esophageal cancer. This review focuses on apoptosis and candidate genes linked to the development of esophageal cancer, which it is hoped may provide diagnostic and therapeutic tools, and potential therapeutic strategies for the management of this carcinoma.

Keywords: Human esophageal cancer, molecular genetics, apoptosis signalling pathway, genomics, esophageal cancer therapeutics

INTRODUCTION

Cancer of the esophagus ranks among the 10 most frequent cancers in the world (over 300,000 new cases/year), recent evidence shows that its incidence is rising. Prognosis of this cancer is poor with an overall 5-year survival rate of less than 10%(Stathopoulos et al., 2003). There are two major types of esophageal cancer: squamous carcinoma and adenocarcinoma. Squamous cell carcinoma(SCC) is very prevalent in three regions: the Asian belt (starting in eastern Turkey and extending through the southern states of the former Soviet Union, Iran and Iraq into northern China), parts of southern and eastern Africa, and northwestern France. The trend of the incidence rate of esophageal cancer had been decreasing for the last 30 years in China (He et al., 2006). But the incidence of esophageal adenocarcinoma is increasing, and is most prevalent in the USA (Lepage et al., 2005).

Esophageal cancer occurs much more frequently among men, rates are typically two to four times higher among males than females. From 1963 to 2003, there were 2085 esophageal cancer cases in our department, with 1534 males and 551 females (Gong et al., 2005). However, in high-rate areas, the cancers appear almost as often in women as men reflecting the exposure to the same causative factors(Stathopoulos et al., 2003 and Manuel et al., 2001)]. Incidence of esophageal cancer increases with age, with the lowest occurring at age 30 and the highest at age 70. The highest mortality rates are found in China accounting for 26.5% in males and 19.7% in females. The rates for SCC among black males continued to decline into the 1990s. The incidence of SCC is more than five times among US Black men (16.8 per 100,000) than among
US White men (1.0 per 100,000). To date, high incidence areas (expressed as crude incidence per 100,000) include: China (21 per 100,000), South America (13 per 100,000), Western Europe (11 per 100,000), South Africa (10 per 100,000), Japan (9 per 100,000) and the former Soviet Union (8 per 100,000) (Pearson et al., 2002) (Pickens et al., 2003).

Esophageal cancer is a multifactorial disease; no single agent has been identified so far as the cause of esophageal cancer. In developed countries, the etiology of SCC has been mainly attributed to tobacco smoking and heavy alcohol drinking. However, dietary deficiencies seem to play a major role in the pathogenesis of this neoplasia among high-risk populations of developing countries, and there is strong epidemiological evidence that supports the importance of some dietary components on esophageal cancer in western countries as well (Franceschi et al., 2000 and Brown LM et al., 2001). Brown LM et al observed that low income, as a social class variable, contributed to the higher incidence among Blacks than among Whites in the United States and appeared to be independent of other risk factors. This variation may reflect a set of lifestyle and other environmental factors including poor housing, limited access to medical care, stress and poor nutrition (Brown et al., 2001). Smoked food consist of a high content of nitrosoamines and nitrites. Methyl-alkyl-nitrosamines appear to be specific inducers of carcinoma of the esophagus, regardless of its route of administration. Fungal toxins and spices are believed to have a positive correlation with esophageal cancer. Human papilloma viruses (HPV), especially serotypes 16 and 18 have been found to be associated with the development of the disease. Barret’s Esophagus (BE) has also been found to be a risk factor for adenocarcinoma of the esophagus. BE is a metaplastic change of the esophageal epithelium from squamous to columnar mucosa, which is associated with repeated episodes of chronic gastro-esophageal reflux (GORD). It has been shown that 86% of primary esophageal adenocarcinomas originate from BE (Van der Woude et al., 2002). Current epidemiologic evidence suggests that lifestyle modifications including a reduction in alcohol and tobacco use and improvements in living conditions and diet characterized by daily consumption of fruits and vegetables, would markedly lower the incidence of SCC of the esophagus. Adenocarcinoma of the esophagus is increasing very fast in western countries, however, the reasons for this epidemiological change remain unclear. Barrett’s esophagus represents the precancerous lesion for most of these tumors but the majority of persons with this condition remain unrecognized in the general population. Identification of high-risk groups for developing esophageal adenocarcinoma is an urgent task for the coming years. Recent data also suggest that adenocarcinoma of the esophagus and esophagogastric junction (EGJ) can be preventable through dietary interventions (Marsman et al., 2005)

**Molecular genetics of esophageal cancer**

As esophageal carcinogenesis is poorly understood, many research works are being carried out to discover the precise mechanisms causing the metaplasia–dysplasia sequence of esophageal carcinoma at a molecular level. It is known that tumor suppressor genes, oncogenes, and apoptotic genes are involved in the initiation and development of esophageal cancer, but to date no gene directly related to esophageal cancer has been identified (Kwong et al., 2005). The key tumor related genes and their specific role which played in the development of esophageal cancer are discussed in more detail in following chapters.

(1) HPV and squamous esophageal cancer

There are more than 70 different HPV types that have been identified and divided into two groups, high and low risk HPVs. The high risk HPVs are the cancer causing types (types 16 and 18), and the low risk types that gives rise to warts and benign lesions (examples include types 6, 11, and 33) (Sur et al., 1998). In China, HPV were determined by Ultrasensitive S-P immunohistochemistry and in situ hybridization in esophageal carcinoma tissues (82 cases) and the normal esophageal mucosa (40 cases). The result suggests that HPV infection is high in esophageal carcinoma of Henan emigrants, local residents and patients in Hubei Cancer Hospital. HPV is closely related with esophageal squamous cell carcinoma. HPV infection may play an important role in esophageal squamous
It has been shown that many HPV types including the low risk types have been found in esophageal cancer tissues (Matsha et al., 2002). Particular studies also show no HPV detection in esophageal cancer tissues, and the argument is that the detection methods utilized are not sensitive enough. HPV has been associated with esophageal cancer and its high frequency implicates the possibility of being an etiological factor in this disease, but definite evidence is still required (Sur et al., 1998).

(2) Apoptosis—genetic regulation of esophageal cancer

Apoptosis is generally defined as a programmed cell death that eliminates unwanted cells and is essential for the homeostatic maintenance of an organism. It has been found that elevated levels of apoptosis as well as low levels of apoptosis can have a detrimental effect on the organism. Recently, reports have shown that esophageal tumor cells abundantly express Fas ligand (FasL) in vivo. As the triggering agonist for Fas receptor (Fas or APO-1/CD95)-mediated apoptosis of lymphocytes, Fas ligand expressed by esophageal cell lines has been shown to induce apoptosis of cocultured Fas-sensitive lymphoid cells in vitro. These studies support a ‘Fas counterattack’ mechanism of immune escape in esophageal cancer. By expressing functional Fas ligand, esophageal cancer cells can deplete antitumor lymphocytes by inducing apoptosis. To express functional FasL, esophageal carcinomas also acquire molecular mechanisms to resist autocrine Fas-mediated apoptosis of tumor cells (O’Connell et al., 1999).

(3) Telomerase activity

Repetitive telomere sequences are located at the ends of eukaryotic chromosomes to protect the ends from damage and rearrangement. Progressive shortening of telomeric sequences is associated with cell division and DNA replication (Li et al., 2003). Telomerase activity levels have been shown to correlate with tumor progression in several malignancies. In a study carried out by Bergqvist M, ten human esophageal carcinoma cell lines were investigated using the telomerase activity assay, telomerase activity levels were detected in all cell lines with a broad range of activity levels. Using a high correlation coefficient, r > 0.90, the following genes were found to be positively correlated with telomerase activity levels: N-myristoyltransferase 2; ribosomal protein L3; retinoblastoma-like 2 (pRb2/p130); and cyclin G2. The microarray provide primary validation data indicating possible candidates for prognostic and prediction factors in esophageal cancer in relation to telomerase activity (Li et al., 2003). Li et al. find that increased telomerase activity was associated with the progression of squamous esophageal carcinoma. Grade I (metaplasia) stage showed a 60% telomerase activity and Grades II (dysplasia) and III (high grade dysplasia) showed 90% and 91% telomerase activity respectively. This result supported other reports of telomerase activity being correlated with esophageal squamous carcinoma differentiation and lymphatic metastasis. The poorer the differentiation, the higher the telomerase activity occurred, and also patients with lymphatic metastasis showed a higher telomerase activity than those without lymphatic metastasis (Bergqvist et al., 2006).

Tumor suppressor genes

Tumor suppressor genes are inactivated by genetic or epigenetic changes such as point mutations, deletions (LOH), promoter methylation, abnormal splicing, deregulation of imprinting and haploinsufficiency. LOH (loss of heterozygosity) causing inactivation of most candidate tumor suppressor genes have been found on the critical regions of chromosomes 1p, 3p, 4, 5q, 9, 11q, 13q, 17q, and 18q in esophageal cancer. Chromosome region 17q25.2–25.3 carries the autosomal dominant esophageal disorder, tylosis (Stathopoulos et al., 2003 and Lepage et al., 2005).
(cell suicide). Mutations in these checkpoint genes result in defective proteins and unsuccessful check-points occur. Cells then complete mitosis but aberrant daughter cells arise which leads to diseases where uncontrolled cell growth occur, such as cancer. Accumulation of p53 in the normal esophagus, suggested that the loss of suppressor function p53 might be an early event in carcinogenesis of the esophagus (Lepage et al., 2005). Mutations in codons 175, 248, and 273 of p53 are considered to have growth advantages to progress to invasive squamous cell carcinoma and occur most frequently (Schrum et al., 2005), whereas codon 158, though considered as being highly sensitive to mutagenesis, does not have the same carcinogenic transformation properties (Hainaut et al., 1997). Somatic alterations of p53 abolish its ability to activate p21, Bax, and PIG3 reporter systems, thus altering cell cycle control and apoptosis overall (Robert et al., 2000). It was determined that 85% of the p53 mutations in esophageal adenocarcinoma occurred as GCYAT transitions, with 69% at the CpG dinucleotides (Schrum et al., 2005). GYA mutation pattern result from DNA methylation induced by nitrosamine (Wang et al., 2002). p53 mutated esophageal cancer is one of the malignancies that have been recognized as a conventional chemotherapy-resistant disease(Kihara et al., 2000)). But in our department, The expression of p73 mRNA and mutation of p73 gene were detected by reverse transcriptase polymerase chain reaction (RT-PCR), by single strand conformation polymorphism(PCR-SSCP) and by heteroduplex mobility assay (HMA) in 37 cases of esophageal tumor tissues, which relates to clinical pathologic characteristic of esophageal cancer was to explored. Over expression of p73mRNA was found in 21/37(51.8%) esophageal tumor tissues. The positive expression rate of p73 mRNA in the tumor tissues was significantly higher than that in the paracancer tissues, regionallylymphonodi and matched esophageal normal tissues (P<0.05). No significant relationship was observed between positive expression of p73 and differenti ated grades, pathological classification or P-TNM stages of the esophageal cancer(P>0.05). There was not any type mutation of p73 gene in esophageal tumor tissues.So p73 gene may be not play an important role in suppression of tumor development in esophageal cancer(Feng et al., 2002).

(2) APC and MCC
APC is a tumor suppressor gene, and like MCC located at chromosome 5q21 region. APC is mutated in adenomatous polyposis (FAP) and like MCC again, it has been shown to play a role in the pathogenesis of colorectal cancer and also lung cancer(Raja et al., 2002). It has been shown via linkage analysis that LOH involving the APC and MCC genetic loci occurs in the majority of human esophageal cancers and is involved in the development and/or progression of the disease(Nair et al., 2006). In China, Linzhou is a high incidence area for esophageal squamous cell carcinoma (ESCC). Analyses of the entire spectrum of the disease from basal cell hyperplasia (BCH), dysplasia (DYS), carcinoma in situ (CIS) to invasive cancer showed loss of heterozygosity (LOH) in as early as the BCH stage. From DYS onwards, LOH was frequently detected at 3p21 (RASSF1A), 9p21 (p16, p15), 13q12 (BRCA2, RNF6) and 17p13 (p53). Additional LOH at 3p14 (FHIT) and 5q21 (APC) began to accumulate in CIS. Allelic loss at 6p21 (p21Waf1) was detected exclusively in cancerous samples. The results shed light on genetic alterations involved in esophageal carcinogenesis(Cheung et al., 2005).

(3) p16INK4a and p15INK4b
Two tumor suppressor genes are localized at 9p21, Which has been shown to undergo hemizygous or homozygous deletion in a variety of tumor types. These two genes encode two cyclin dependent kinase (CDK) inhibitors which negatively regulate the cell from G1-S phase in proliferating cells, contributing to active pRb maintenance (Morgan D, 1995). During the G1-S phase p16INK4a binds and inhibits CDK4/6 activity (Retnisdottir et al., 1997), and p15INK4b binds to cyclin D-dependent kinase and prevents p27 association(Kunisaki et al., 2004). p27 then binds to E-CDK2 complex, blocking the cell cycle at the G1-S boundary, risking cells to abnormally proliferate(Kunisaki et al., 2004). Aberrant methylation of p16INK4a has been found to be a key feature in human carcinogenesis, and although aberrant methylation of
p15INK4b also occurs it is found to occur less frequently in human esophageal cancer in Lixian county, China (Xing et al., 1999). A common feature of p15INK4b is homozygous deletion, which also takes place in p16INK4a.

(4) WWOX
The WWOX (WW domain containing oxireductase) gene was recently discovered as a candidate tumor suppressor gene at chromosomal region 16q23.3–24.1 (Paige et al., 2001). It has been demonstrated in a study carried out by Kuroki et al., that both alleles of the WWOX gene are inactivated in squamous carcinoma of the esophagus, as a combination of tumor-specific mutations and LOH of the WWOX gene locus, which is also referred to as a two-hit mechanism (Bednarek et al., 2001). The WWOX enzymatic domain is considered to be encoded mostly by the exon 6–8 regions of the gene and mutations in this region have been found to occur in breast cancer, as well as in esophageal squamous carcinoma (Kuroki et al., 2002). This data suggests that WWOX could act as a tumor suppressor in esophageal squamous carcinoma.

(5) DLC1
DLC1 (deleted in lung cancer 1) is a putative tumor suppressor gene and commonly deleted region at 3p21.3 as defined by LOH studies in lung cancer, and aberrant splicing of this potential gene was found in a third of esophageal, lung, and renal cancers. After introduced into several cancer cell lines, Normal DLC1 cDNA caused significant suppression of growth, indicating that aberrant DLC1 transcripts may play a critical role in the carcinogenesis of those tissues. The encoding protein (Mr 166) unfortunately has no significant homology to known proteins and so putative functional annotations could not be made. It has been found though that DLC1 protein has 54 phosphorylation sites, 27 of which are casein kinase (CSNK) II phosphorylation sites, and is localized in the cytoplasm (Daigo et al., 1992). A ubiquitous, messenger-independent serine/threonine kinase, CSNKII is localized in both the cytoplasm and nucleus and functions as a protease, and so it is supposed that DLC1 may act as a downstream gene in the serine/threonine kinase pathway.

(6) Retinoblastoma (Rb)
Rb is a nuclear phosphoprotein that plays a role in cell cycle regulation. Hypophosphorylated Rb in the cell prevents cell progression when the cell is being assessed, and upon phosphorylation the Rb protein releases the E2F transcription factor that allows for the expression of important cell-cycle control genes (Ikeguchi et al., 2001). LOH of the Rb gene was found correlated with the loss of pRb protein expression and associated with p53 alterations in human esophageal cancer (Shi et al., 1999). It is suggested that associated Rb and p53 inactivation may be the major event in the development and progression of esophageal cancer, due to the greater selective advantage of the affected cells. It is also believed that other genes in the Rb and p53 pathways contribute to the malignant transformation of the cells; in the majority of cases an alteration of p16, p15, or even both were shown to occur (Shi et al., 1999).

Oncogenes
The oncogenes most frequently activated in esophageal cancer are cyclin D1, c-erbB1 and 2, FRAT1, c-myc, c-ras, Int-2/hst-1, and EGFR. Frequent mechanisms activating these oncogenes include point mutations, amplification, rearrangement and over-expression, with amplification and overexpression being the most common (Schrump et al., 2005 and Jiang et al., 1993).

(1) FRAT1
FRAT1 and FRAT2 genes, clustered in human chromosome 10q24, are human homologues to mouse proto-oncogene Frat1, which promotes carcinogenesis through activation of the WNT-beta-catenin-TCF signaling pathway. It is known that overexpression of FRAT1 leads to the dissociation of GSK-3β from Axin to inhibit h-catenin phosphorylation. Unphosphorylated h-catenin is not recognized by ubiquitin ligase complex including hTRCP2, and is stabilized and translocated to the nucleus. h-Catenin–TCF complex activates transcription of WNT target genes, such as c-Myc, WISP 1, WISPf2, and cyclin D1. FRAT1 and FRAT2 mRNAs were up-regulated together in a gastric cancer cell line TMK1, and also in 2 out of 10 cases of
primary gastric cancer (Saitoh et al., 2002). In one study, Frat1 expression was found to be relatively high in human esophageal cancer cell lines. It is therefore proposed that the up-regulation of Frat1 mRNA, not only in esophageal cancer but in several other malignancies, might promote carcinogenesis through the activation of the WNT–h-catenin–TCF signalling pathway (Saitoh et al., 2002).

(2) Cyclin D1
By through binding and activating CDK4 and CDK6, Cyclin D1 can phosphorylate the tumor suppressor protein, retinoblastoma (pRb) (Jiang et al., 1993). It was shown that altered expression of the cyclin D1 and Rb genes play a role in human esophageal cancer. Xiao et al detected cyclin D1 expression in esophageal carcinomas from southern China, 61% and 35% cases showed increased expression of cyclin D1 in esophageal carcinomas and the adjacent epithelia comparing to normal esophageal tissue, respectively. Significant difference for cyclin D1 expression was found between esophageal carcinomas and the adjacent epithelia. Cyclin D1 was found overexpressed in early stages of esophageal carcinomas and showed significant alterations. The results suggested that cyclin D1 was involved in the earlier event and accumulated as the cancer evolved to a later stage in some esophageal carcinomas. (Xiao et al., 2006).

Other apoptotic genes
(1) Bcl-2 family
This family consists of at least 15 proteins with either antiapoptotic or pro-apoptotic function. Proteins that have been found to be aberrantly expressed within esophageal cancer include the anti-apoptotic proteins bcl-2 and bcl-xl (which are both up-regulated) and pro-apoptotic protein bax (down-regulated) (Shimoji et al., 2000).

(2) PCNA
Proliferating cell nuclear antigen (PCNA) expression increases gradually in cell nuclei with the progress of G1 phase and reach a peak when entering the S phase. In a study, foetal esophageal epithelia showed a much higher expression pattern compared to normal adult esophageal epithelia and basal cell hyperplasia (BCH), and a much higher expression pattern was observed in malignant adult esophageal tissue compared to foetal esophageal epithelia. It was found that PCNA acts as a good marker for cell proliferation (Kimos et al., 2004). Another study showed that p53 and PCNA are already overexpressed to different extents in normal epithelia and also precancerous lesions of the esophagus, but an increase in expression is observed with progressive cancer stages (Chen et al., 2003). It was suggested that since PCNA has been found to play a role in DNA damage repair, it could combine with hMSH6 and hMSH3, the subunits of hMutSulpha and hMutSbeta that act as cofactors in a DNA mismatch repair system. Malignant tissue is characterized by high frequencies of DNA mismatch, breakages, and mutations, and therefore the increase in PCNA expression is thought to occur as a repair response (Kimos et al., 2004).

(3) Survivin
Survivin is a unique inhibitor of apoptotic proteins (IAP), and is only expressed in fetal tissue and a variety of human cancers, but almost undetectable in most normal adult tissue (Li et al., 1998). Survivin inhibits apoptosis by binding to microtubules of the mitotic spindles (Ikeguchi et al., 2003) and ultimately inactivating caspase-3 and caspase-7 activity (Tamm et al., 1998). It has been shown that increased expression of survivin can have a cancerous effect on the cell as it surmounts the G2/M phase checkpoint proceeding into mitosis and it has been proposed that survivin is only present in the G2/M phase (Ikeguchi et al., 2003). Rodriguez et al also discovered that survivin encouraged cell proliferation by interacting with CDK4 and displacing p21. The nuclear survivin expression in esophageal squamous carcinoma tissue was examined and found to correlate with poor prognosis, but it seems that localization of survivin expression is critical for activity in tumor cells and its negative effect in dysregulating cell cycle definitely plays a role in tumor progression (Grabowski et al., 2003). It is clear that survivin expression could be used as a diagnostic tool, and possibly a therapeutic strategy, in the near future.

(4) Matrix metalloproteinase-7
Matrix metalloproteinase-7 has been implicated in tumor initiation, growth, invasion and metastasis. Matrilysin is a member of the MMP family and it has wide variety substrate specificity, and potency to start an activation cascade of MMPs (Wilson et al., 1996). The study found the MMP-7-181A/G polymorphism might be a candidate marker for predicting individuals who are at higher risk to certain tumors but might not be used to predict potential of lymphatic metastasis in esophageal squamous cell carcinoma, atric cardiac carcinoma and non-small cell lung carcinoma (Zhang et al., 2005). MMP-7 expression correlates with penetrating tumor progression in esophageal cancer. Nuclear translocation of beta-catenin, without mutations in beta-catenin exon 3, is associated with MMP-7 expression (Saeki et al., 2002).

(5) Metallothionein (MT)
Metallothionein is a thiol-rich protein that plays a major role in detoxification of toxic metals and in protection against oxidative damage(Aloia et al., 2001). Li et al. studied the relationship of MT and apoptosis in the progression from metaplasia to dysplasia and adenocarcinoma in subjects with BE. It was found that tissue with high activity of apoptosis had high expression of MT and vice versa. It is believed that MT may contribute to cytoprotection, thereby inhibiting apoptosis and increasing the likelihood of BE to progress toward adenocarcinomas. It is hypothesised that MT might act as a zinc donor in favour of tumor proliferation or it is induced by the rapid growth of the tumor (Li et al., 2003). The exact mechanism of MT in the metaplasia–dysplasia–adenocarcinoma sequence has to be clarified.

(6) E2F-1
Overexpression of E2F-1 has been shown to induce apoptosis in several cancer cell types. Yang et al. studied the effect of adenovirus-mediated E2F-1 overexpression on human esophageal cancer cell lines, Yes-4 and Yes-6. Overexpression of E2F-1 resulted in cell growth inhibition due to apoptosis induction in the Yes-4 cell lines, but the Yes-6 cells were more resistant to E2F-1 overexpression. The resistance of Yes-6 cells to E2F-1 is believed to be caused by differential expression of cell death inhibitory proteins of the Bcl-2 family. These proteins include Bcl-2, Mcl-1, and Bcl-xl, which decreased after 48 h in the Yes-4 cells, but remained unchanged in Yes-6 cells. Restinoblastoma gene product (pRB) also declined after 48 h in Yes-4 cells and remained constant in theYes-6 cells. It is suggested that pRb inhibits apoptosis, as it binds to E2F-1 and negatively regulates its transactivation function(Yang et al., 2000). The caspases involved in the E2F-1 mediated pathway of apoptosis in the Yes-4 cells were demonstrated by caspase-3 and caspase-6, which cleaved caspase3/ CPP32 and poly-ADP-ribose polymerase (PARP), as well as fragmentation of the caspase-6 substrate, lamin B. p53 does not seem to play a role in this E2F-1 apoptosis mediated pathway. These findings suggest E2F-1 mediated apoptosis may be related to differential expression of Bcl-2 family member proteins and that therapy may be a promising treatment strategy for the treatment of this disease (Itoshima et al., 2000).

(7) DcR3/M68
A secreted decoy receptor (DcR3), member of the tumor necrosis factor (TNF) receptor superfamily, was found to be a negative regulator of Fas-mediated apoptosis by binding to Fas ligand, Fasl. (Pitti et al., 1998). DcR3 shows overexpressed in a variety of cancers including gastrointestinal tumors, and it is believed the blockade of FasL-induced cell death allows tumor cell growth. It seems that DcR3 over-expression occurs without gene amplification, but it is suggested that DcR3 overexpression precedes gene amplification in tumors (Bai et al., 2000).

Other genes believed to play a role in the development of esophageal cancer

(1) Annexin 1
It has recently been discovered by Liu et al. that the protein Annexin 1 was translocated from the plasma membrane in normal cells to the nuclear membrane in malignant cells. It was found that Annexin 1 usually formed a consecutive typical trammel net on the plasma membrane of normal esophageal epithelia, but in esophageal cancer a great decrease was found on the cellular
membrane and was highly expressed on the nuclear membrane, which was never found on normal esophageal epithelia. This data suggests that Annexin 1 translocation may be correlated with the tumorigenesis of esophageal cancer (Liu et al., 2003).

(2) Cathepsin B (CTSB)
CTSB is a cysteine protease that also maps to chromosome 8p22 and has been found overexpressed or altered in certain tumors of the lung, breast, stomach, colon and prostate (Hughes et al., 1998). CTSB was found amplified and over expressed in esophageal adenocarcinoma, showing genomic alteration involving CTSB (Li et al., 2005). It is therefore believed that CTSB plays a critical role in tumor progression or malignant transformation of esophageal adenocarcinoma, and even other types of malignancies (Lin et al., 2000).

(3) GASC1
Gene amplified in squamous cell carcinoma 1 (GASC1) was found to be amplified and overexpressed in several esophageal squamous cancer cell lines. The GASC1 locus is found on chromosome 9p23–24 and contains one PX domain and two PHD fingers. PHD-finger motifs are found in nuclear proteins that participate in chromatin-mediated transcriptional regulation and are present in a number of proto-oncogenes. It is assumed that GASC1 may be involved in the carcinogenesis or progression of multiple tumors, even though its function is not clear (Yang et al., 2001).

(4) FEZ1
FEZ1 was identified via genomic analysis of chromosome 8p22 in esophageal cancer, due to the loss of this region in esophageal cancer as well as many other types of cancers (Nonaka et al., 2005). It was found that Fez1 encodes a leucine-zipper protein. Fez1 expression was found almost ubiquitously expressed in normal cells, but undetected in most of the esophageal cancer cells. Three point mutations were detected in Fez1 from esophageal as well as prostate cancer cell lines. E44 alteration from TTCYCCC at codon 29, results in the substitution of SerYPro, which is a predicted cAMP-dependent kinase phosphorylation site. Second mutation, an E50 alteration of AAG/LysYGAG/Glu at codon 119 was found, resulting in the allelic loss of the marker D8S261. The third mutation change of CAG/GlnYTAG/Stop at codon 501 in prostate cancer resulted in coding a putative 1 66-aa protein lacking the C terminus. This data suggests the major mechanism for Fez1 inactivation is a two-hit mechanism, allelic loss and point mutations, and possibly, allele loss plus failure in transcription (Ishii et al., 1999).

(5) ODC
Ornithine decarboxylase (ODC) has been found to play a critical role in the biosynthesis of polyamines, which are important in cell proliferation (Garewal et al., 1999). Mafune K et al and Takashima T et al. demonstrated ODC overexpression in esophageal carcinomas. It has been shown that overexpressed ODC cannot cause tumor progression but increases the formation of polyamines in premalignant cells. It is believed that the constant overexpression of ODC mRNA in esophageal tumors, especially in esophageal squamous cancer, may be evidence that ODC plays a critical role in the tumorigenesis of the esophagus (Mafune et al., 1999 and Takashima et al., 2001).

(6) FzE3
FzE3 is a frizzled gene that forms part of the Frizzled family of seven-transmembrane proteins that acts as receptors for Wnt signalling. The protein contains cystein-rich residues in its extracellular N terminal region to which the Wnt proteins bind (Tanaka et al., 1998). FzE3 was found specifically expressed in esophageal tumor tissue compared with normal mucosa and is believed to alter the function of the tumor suppressor gene, adenomatous polyposis coli (APC). It has been shown that in normal cells, wild-type adenomatous polyposis coli APC protein bound to the serine–threonine glycogen synthase kinase (GSK)-3 h binds to h-catenin within the cytoplasm, resulting in APC degradation (Cadigan et al., 1997). In colon cancer cells, mutated APC was found stabilizing h-catenin to form a complex with transcription factors, Lef (lymphoid enhancer binding factor) and Tcf (T cell specific transcription factor), which then translocates to the nucleus where up-regulation of cell proliferation genes occur. In esophageal tumor tissue wild-type APC was found, so it has been suggested that the presence
of FzE3 acts as a negative regulator of APC function, allowing h-catenin signal transmission to up-regulate cell proliferation associated genes (Tanaka et al., 1998).

(7) ECRG4
Esophageal cancer related gene 4 (ECRG4) is a novel esophageal cancer related gene and found to be down-regulated in esophageal squamous cancer compared to normal esophageal tissues. It is located on chromosome 2q14.1–14.3 and contains 4 exons. ECRG4 down-regulation is believed to play a role in the development of OeSc and the mechanism inactivating it has been demonstrated to be aberrant methylation of CpG islands in the core promoter of the ECRG4 gene (Yue et al., 2003).

**Invasion and metastasis**
Invasion and metastasis of esophageal cancer is poorly understood. The cell–cell adhesion molecules (CAMs) hold cells together, and believed to play an important role in metastasis of the cancer cell (Kleespies et al., 2004). h-Catenin has been found to play a role in squamous esophageal cancer cells, by its cell–cell adhesion function and interactions with the cytoskeleton and cadherin junctions of cells. h-Catenin has been implicated in the transcription of oncogenes such as c-myc, c-jun and cyclin D1, which are oncogenes frequently active in esophageal cancer cells. The APC gene product targets h-catenin for degradation and prevents h-catenin dependent degradation. Increased h-catenin dependent transcription due to h-catenin binding to Fz receptors, mutations in h-catenin, APC, and increased h-catenin expression due to Fz receptor mutations, have all been found in adenocarcinomas and squamous esophageal carcinomas. It is therefore believed that down-regulation of h-catenin expression by antisense technology could be an effective treatment for esophageal cancer (Kuwano et al., 2005).

**Angiogenesis**
Angiogenesis is the development of new blood vessels, which provide blood and nutrient supply to tumors to survive. Once the tumor is stable, it can then invade neighbouring cells leading to metastasis. In esophageal cancer cells the increased expression of vascular endothelial growth factors (VEGFs) stimulates endothelial proliferation and migration. Increased expression of VEGFs and VEGFRs (receptors) were detected in metaplastic tissues of the lower esophagus but not in normal esophageal epithelium, indicating sustained neovascular development early in Barrett’s carcinogenesis (Feagins et al., 2005).

**Gene therapeutic strategies in esophageal cancer**
The management of esophageal cancer management to date remains an unsolved health problem. All over the world diagnostic markers and therapeutic analyses are carried out to generate a solution to this problem. It is believed that the cure to cancer would be a two directional method, one including chemotherapy or radiation, and a key drug that targets a specific molecule present only in the cancer cells and has a low or no toxicity effect on the normal surrounding cells (Buskens et al., 2005). Apoptosis inducing nucleosides (AINs) from CD57+HLA-DRbright-natural suppressor (57.DR-NS) cell lines were used to induce apoptosis in human esophageal cancer cells. This study revealed that AINs induce apoptosis in esophageal cancer cells through DNA strand breaks and caspase-3 activation. Further research is currently carried out to develop an ideal anticancer agent, since apoptosis generated in malignant cells lacked toxicity in normal cells, suggesting a possible evasion from side effects in clinical trials (Mori et al., 2001). An antagonist to the anti-apoptotic gene, survivin is a promising therapeutic strategy not only for esophageal cancer but various other types of cancers where survivin is highly expressed. It is believed that antagonists to Survivin would increase the effectiveness of chemotherapy by removing the protective role of Survivin on the cancer cell (Kato et al., 2001). Other drugs of interest would be those targeting angiogenesis. Anti-angiogenesis drugs developed to inhibit blood and nutrient supply to the tumor cells is a potential therapeutic strategy as well. Researchers are currently working on the development of antagonists to two angiogenesis molecules angiostatin and endostatin, and there is great hope that these drugs in combination with radiation or chemotherapy could be the cure to cancer (Mori et al., 2001 and Tabernero...
Esophageal cancer is a disease that urgently needs a consistent diagnostic tool for early diagnosis, and also an effective therapeutic strategy that ensures non-recurrence, best quality of life and an increased lifespan. Many genes have been found that are believed to play a role in the development of esophageal cancer but the underlying mechanism by which this disease develops is still not clear. A few genes with significant correlation to this disease have been found, and are currently being analyzed as potential candidates for determining prognosis and therapeutic strategies. A human esophageal tumor model would be of interest, as it would help other researchers to focus on the genes of interest, and diagnostic and therapeutic tools could be developed at a much quicker rate.

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