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Growth Factors, Signal Transduction Pathways, and Tumor Suppressor Genes in Esophageal Cancer

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1. Introduction

Esophageal cancer is the eighth common cancers in the world and the sixth most common cause of cancer-related death throughout the world [1, 2]. Histologically, esophageal cancer can be divided into adenocarcinoma and squamous cell carcinoma (SCCE). Esophageal cancer is among the most malignant type of cancers which rapidly invade into the surrounding tissues, metastases to the surrounding lymph nodes, and distant organs. Since clinical symptoms of esophagus cancer appear in the advanced stages of carcinogenesis, the majority of patients are diagnosed and receive medical attention only when the tumor has already gained substantial volume, spread into surrounding tissues, and cause obstruction when food is swallowed. Despite large improvements in the detection of cancers, surgical procedures and treatments, the prognosis of esophageal cancer remains poor and the 5-year survival rate is still low [3]. Therefore, early detection, seeking new strategies for treatment, comprehensive understanding of the molecular and genetic alterations of esophageal carcinogenesis are essential.

In addition to molecular alterations environmental and nutritional factors, as well as cultural habits are thought to be contributing factors in the development of esophageal cancer. The two major habitual risk factors are tobacco smoking and alcohol consumption. Chronic irritation and inflammation of the esophageal mucosa, which might be caused by substantial alcohol intake, achalasia, and frequent consumption of extremely hot beverages, increases the incidence rate of squamous cell carcinoma of the esophagus. In addition, a clear link between squamous cell carcinoma of esophagus and low socioeconomic status has also been established.

While the major risk factors for esophageal adenocarcinoma are the two altered physiological conditions: gastroesophageal reflux disease (GERD) and Barrett's esophagus [3], such association have not been proposed for squamous cell carcinoma of esophagus. In turn, a large number of molecular events were found to be involved in the development and progression of squamous cell carcinoma of esophagus. These events include genetic and epigenetic alterations in oncogenes, tumor suppressor genes, cell adhesion molecules, DNA repair genes, cell cycle regulatory genes, genetic instability as well as telomerase activation, and aberrant regulation of growth factors and their receptors. Recent studies have indicated that activation of cyclin D1, *erbB-2*, and *c-myc* oncogenes and inactivation of *p53*, *Rb*, *APC*, and *p16* tumor suppressor genes are frequently involved in esophageal cancers [3-6].

2. Growth factors

The significant role of growth factors and growth factor-mediated signaling pathways in the tumorigenesis of esophagus has been well established and similar to many other types of cancers as a preferred target for esophageal cancer therapy [4].

Growth factors regulate growth and development of cells. They might be supplied by distant glands and tissues, neighboring cells, or *in situ* by tumor cells themselves. Thus growth factors might be provided by endocrine, paracrine or autocrine mechanisms among which autocrine mechanism is thought to play a significant role in the growth of cancer cells [7, 8]. Most growth factors are polypeptides that regulate numerous cellular responses, notably cell proliferation. They exert their effects by binding to specific receptor on the cell surface; which most often is associated with an intrinsic tyrosine kinase activity, or by forming a complex with an intracellular tyrosine kinase [9]. Following to binding of growth factors to their corresponding receptors the tyrosine kinase activity is induced and phosphorylation of specific residue(s) in the intracellular domain of receptors occurs. Such phosphorylated cytoplasmic domains serve as docking sites for downstream signal transduction molecules and trigger signaling pathways that induce expression of cyclin D1, promoting cellular proliferation and survival (Fig 1, Fig 2) [10]. Aberrant regulation of growth factors and their corresponding receptors in addition to structural alterations in receptors play important role in tumorigenesis of esophageal cancer [4].

2.1 Epidermal Growth Factor Receptor (EGFR)

The epidermal growth factor receptor (EGFR) family and their ligands including epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) are implicated in the development of esophageal cancer [7, 11-17]. The EGFR family composes of four members: EGFR (HER-1, erbB-1), HER2 (erbB-2, Neu), HER3 (erbB-3) and HER4 (erbB-4) [4, 18], all of which are tyrosine kinase receptors that are activated by ligand-induced homo or hetero dimerization. Overexpression of EGFRs is common in esophageal cancer and has been reported in several cell lines of SCCE, 29-92% of tumor samples of SCCE [19, 20] and 80% of patients with adeno and squamous cell carcinoma [21, 22]. EGFR upregulation correlates with poor prognosis, low survival rate and minimal response to chemotherapy [20, 23-25]. Amplification of EGFR gene has been found approximately in 8-30% of esophageal adenocarcinomas [21, 26]. Additionally, expression of EGF or TGF-α ligands along with overexpression of EGFR is correlated with esophageal cancer [7, 11, 12, 14-17]. EGF overexpression has also been detected in Barrett's-associated adenocarcinomas [13, 27, 28].

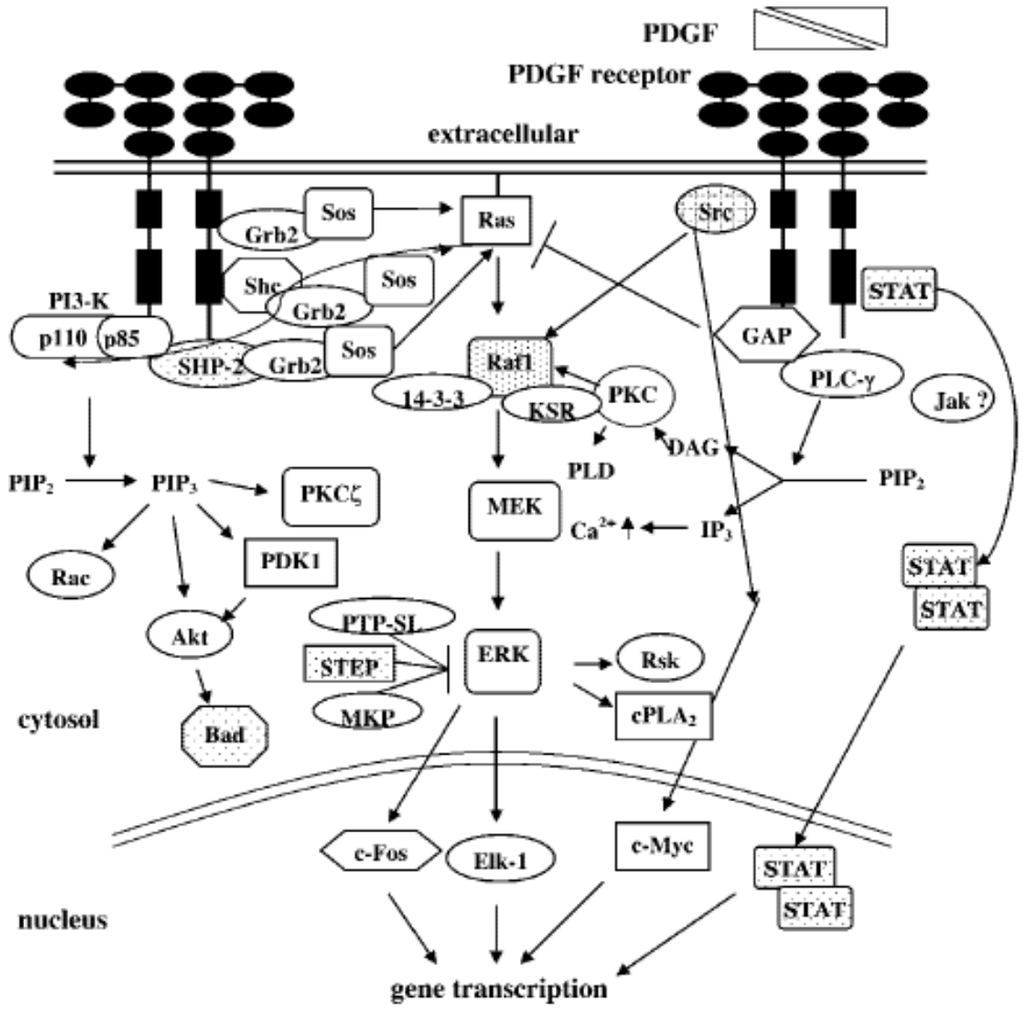

Fig. 1. Schematic presentation of growth factor–mediated signaling pathways. The picture illustrates signaling pathways downstream of PDGF receptors, activated by ligand-induced dimerization. Other tyrosine kinase receptors induce similar pathways. Arrows indicate activation; inhibitory interactions are indicated by blunted lines [4].

EGF may also serve growth inhibitory effect that shown to be mediated by STAT-1 (signal transducer and activator of transcription1) pathway and its mediation in the upregulation of p21 cyclin dependent kinase inhibitor [4, 29, 30].

EGFR activation is associated with metastasis as it modulates cell adhesion, angiogenesis, invasion and migration. Since EGFR activation increases expression of matrix metaloproteases (MMPs) it causes degradation of extracellular matrix and promotes invasion and metastasis in esophageal cancer [31, 32]. In addition, it has also been shown that EGF is implicated in relocalization of E-cadherin from the lateral adherent sides to cell surface, resulting in cell morphology change and increased invasiveness [33].

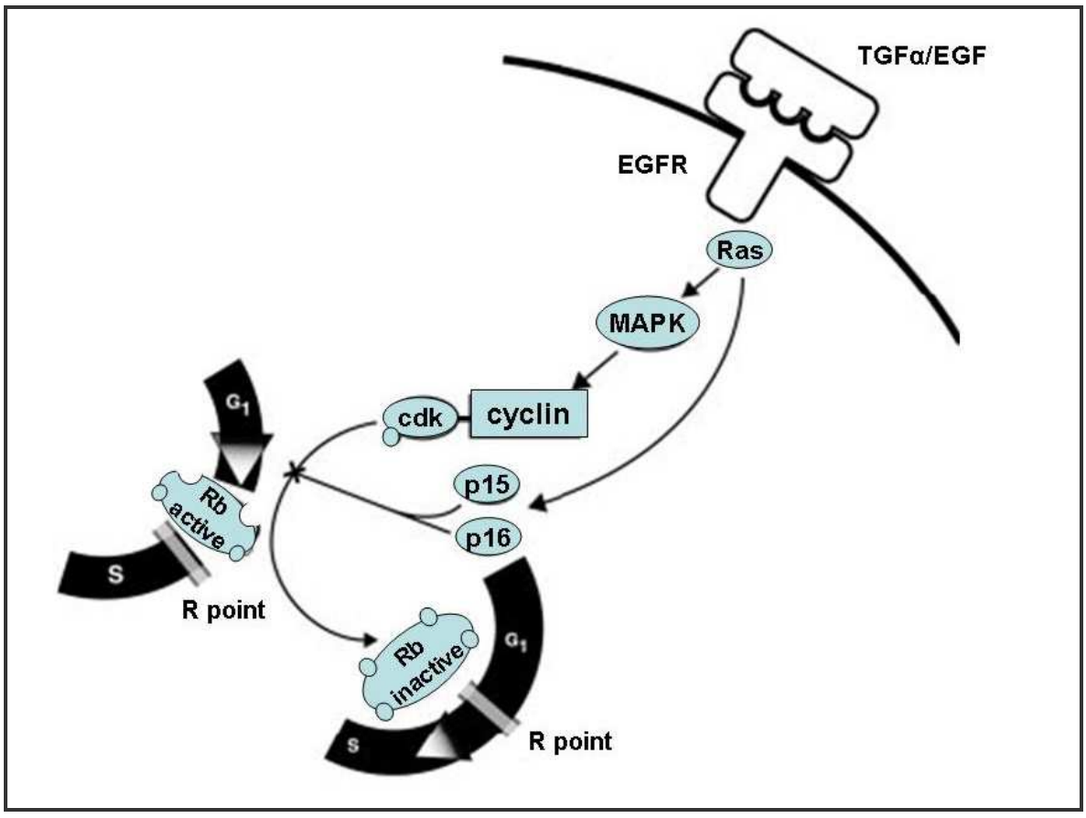

Fig. 2. Growth factors, Ras signaling and cell cycle regulation. Binding of growth factors TGF-α and EGF with EGFR (a tyrosine kinase receptor) promotes cell cycle progression by activating Ras and MAPK. MAPK signaling induces the expression of cyclins which bind CDKs and inactivate Rb. Activation of Ras also induces growth inhibitory effectors including p16 and p15. Similar to p53, p16 and p15 induce G1 arrest by inhibiting the function of cyclins and CDKs, thereby preventing cell cycle progression through the R-point [27]. By definition restriction point (R-point) is a point or event in G1 of a cell cycle at which cell becomes committed to progress cell cycle without requirement to extracellular proliferation stimulant.

2.2 Human Epidermal Growth Factor Receptor 2 (HER2)

Activation of certain tyrosine kinase receptors, such as EGF receptors and HER2 (erbB-2) results in phosphorylation of catenins and prevention of their binding to cadherins [34-36]. Regarding to this notion, overexpression of EGFR and HER2 (erbB-2) in esophageal cancer could possibly lead to sequestration of β-catenin, which result in the altered cell adhesion and increased tumor aggressiveness [27].

The role of HER-2 overexpression in esophageal cancer has been reported in 9%-60% of cases; depending on the stage of disease, tumor histology, or the applied methodology [4, 8, 11, 37-41]. There is no known ligand for HER-2, as it does its function by forming heterodimer with other tyrosine kinase receptors. In fact, *HER-2* (*erbB-2*) is an oncogenic form of the normal receptor tyrosine kinase and overexpression of erbB-2 by tumor cells is associated with hyperproliferation [4, 42]. Moreover, HER-2 expression may change during

tumor progression [4, 43-45]. Although some studies demonstrate that HER-2 overexpression correlates with invasion, lymph node metastasis, and chemoresistance in esophageal cancer [46-48], others have shown that its expression is associated with favorable response to chemo or radiotherapy in esophagus cancer [49].

2.3 Insulin-like Growth Factor-1 (IGF-1) and IGF-1 receptor (IGF-1R)

Insulin-like growth factor-1 (IGF-1) and its tyrosine kinase receptor: IGF-1R, contribute to esophageal cancer. Tumor growth upon overexpression of IGF-1R, prevention of apoptosis via IGF-1 autocrine loop, and mitogenic effects of IGF-1 and IGF-2 has been reported in esophageal cancer as well as Barrett's-associated neoplasia [7, 50-52]. In addition, IGF binding protein-3 (IGFBP3); the major regulator of IGF-1 or IGF-2, is frequently overexpressed in SCCE in parallel with EGFR overexpression [53]. IGFBP3 has been shown to promote transforming growth factor β1-mediated epithelial to mesenchymal transition and motility in esophageal cancer [54]. Furthermore, the level of serum IGF-1 and IGFBP3 significantly increases in esophageal cancer patients, which correlates with tumor invasion, poor prognosis, and low survival rate of patients [55] .

Platelet-derived growth factor (PDGF) comprise a family of dimeric isoforms including the related A, B, C, and D polypeptides chains which bind to α- and β-tyrosine kinase receptors [56]. The significance of PDGF and its receptors in esophageal cancer is unclear. In physiological condition, normally, there is no expression of PDGF receptors in epithelial cells, while a number of studies have indicated the expression of different PDGF isoforms in esophageal cancer. It was found that PDGF-BB isoform promotes the growth of human esophageal carcinoma cell line and prevents apoptosis of cancer cells [57]. Additionally, overexpression of PDGFR-β receptor has been shown in tumor tissues of esophageal cancer [23, 58].

2.4 Vascular Endothelial Growth Factor (VEGF) and other angiogenesis factors

Vascular endothelial growth factor (VEGF) is composed of a family of closely related members, including VEGF-A, VEGF-B, VEGF-C, and VEGF-D as well as placental growth factor, among which VEGF-A is usually known as VEGF which is the main growth factor of endothelial cells. VEGF contributes to the vascular permeability, proliferation, as well as prevention of endothelial cell apoptosis. VEGFs utilize tyrosine kinase receptors of VEGFR family, including VEGFR-1 and VEGFR-2, and VEGFR-3, in which VEGFR-1 and VEGFR-2 transmit growth signals for blood vascular endothelial cells while, VEGFR-3 is involved in the regulation of lymphatic endothelial cells [59-61].

Overexpression of VEGF has been found in 30-60% of esophagus cancer cases. It is significantly correlated with advanced stage of disease, extent of microvessel density, distant metastasis, and poor survival rate in patients [62-64]. Upregulation of VEGF along with fibroblast growth factor has been shown in Barrett's esophagus and adenocarcinomas of esophagus as well as gastroesophageal junction tumor [65]. Since VEGF overexpression is associated with malignant potential of esophageal carcinoma and a higher level of which could be observed in serum (S-VEGF) of esophageal cancer patients; it could be considered as a significant and independent prognostic factor and a useful clinical biomarker for evaluation of patient's prognosis [66].

The major role of VEGF is in the process of angiogenesis where it plays an essential role in growth and metastasis of esophageal carcinoma. Among VEGF family, VEGF-C has shown to be correlated with the process of lymphangiogenesis leading to lymph node micrometastasis (LMN). It is considered as one of the most important prognostic factors of esophagus squamous cell carcinoma [67-69]. The association between VEGF-C expression with angiolymphatic invasion, lymph node metastasis and lower survival rate has been shown in esophageal adenocarcinoma as well [70]. Moreover, bone marrow micrometastases in esophageal cancer correlates with an increased level of plasma VEGF [71]. It has been shown that VEGF-C, and VEGF-D are involved in the early stages of esophageal carcinogenesis since they are also expressed in dysplastic lesions of both types of esophageal carcinomas [72, 73].

In addition to VEGF, other factors such as heparin-binding growth factor (midkine), fibroblast growth factor, thymidine phosphorylase, and hepatocyte growth factor contribute to tumor angiogenesis as well [66].

Overexpression and release of fibroblast growth factor (FGF-2) by stromal fibroblasts correlates with tumor recurrence and short survival in esophageal cancer patients [66, 74]. Stromal fibroblasts are also involved in tumor progression through degradation of extracellular matrix, secretion of growth factors, and regulation of epithelial cell behavior. It has also been shown that FGF receptor 2-positive fibroblasts provide a suitable microenvironment for tumor development and progression through stimulation of cancer cell proliferation, induction of angiogenesis, cell mobility, inhibition of cell adhesion, and promotion of epithelial-mesenchymal transition [75].

While many studies indicate an anti angiogenesis role for TGF-β, one study by using 3D in vitro model, has shown the role of fibroblasts and TGF-β in VEGF-induced angiogenesis in esophageal cancer, in which the paracrine TGF-β secretion by SCCE cells leads to the activation of stromal fibroblasts, which undergo a myofibroblastic transdifferentiation and expression of VEGF. Secretion of VEGF from activated fibroblasts, known as carcinoma-associated fibroblasts (CAFs), subsequently stimulate endothelial cells migration and vascular network formation (Fig 3) [76].

Midkine (MK) is a heparin-binding growth factor which overexpresses in esophageal carcinoma and plays a role in tumor angiogenesis and invasion [77]. Serum MK (S-MK) is an independent prognostic factor and may be a useful tumor marker for esophageal carcinoma, since the level of S-MK is increased in patients with esophageal carcinoma. It is also associated with tumor size, immunoreactivity, and poor survival of patients [66, 78, 79].

Hepatocyte growth factor (HGF), also known as scatter factor, is another factor that derives from specialized cancer-associated fibroblasts (CAFs) in the extracellular matrix that acts in a paracrine way to promote SCCE invasion via activation of VEGF and IL8 expression [80, 81]. It has been found that HGF and its tyrosine kinase receptor, c-Met, play significant role in esophageal carcinogenesis. Increased levels of HGF in serum, correlates positively with VEGF of serum, and significantly associates with the advanced stage of metastasis and low survival; provide an independent prognostic factor as well [66, 80]. Moreover, increased expression of c-Met tyrosine kinase receptor is significantly correlated with the reduced survival rate, distant metastasis, and local recurrence of cancer in esophageal cancer patients [80, 82, 83]. Grugan *et al*. have shown activation of HGF/Met signaling in human SCCE

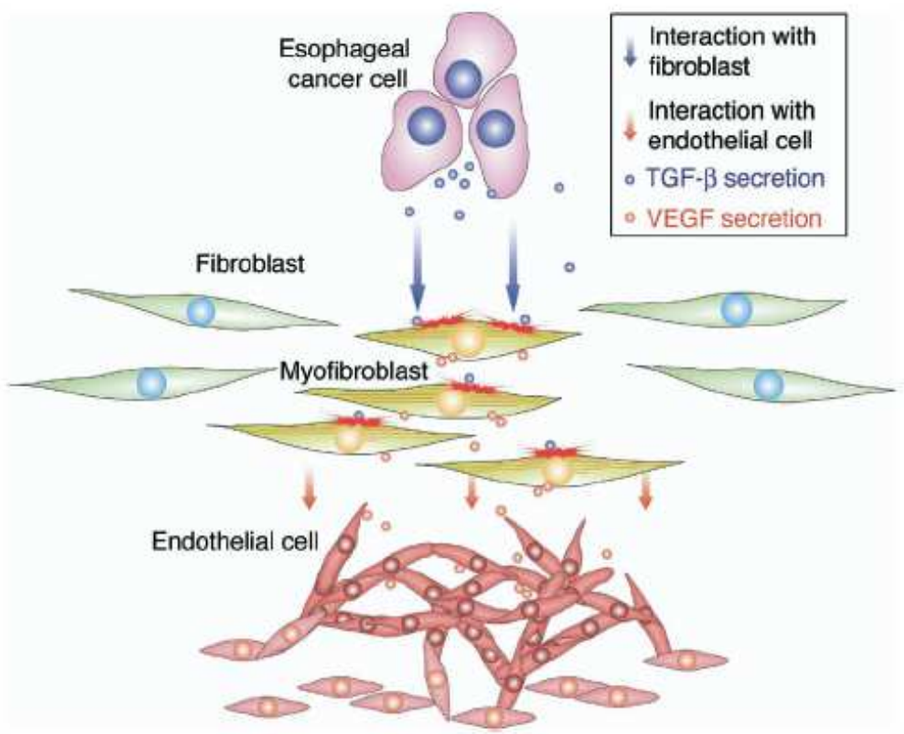

Fig. 3. Schematic illustration of SCCE cells and fibroblasts involvement in the vascular network formation. Esophageal cancer cells produce TGF-β to activate stromal normal fibroblasts. Tumor stromal fibroblasts become transdifferentiated into myofibroblasts that secrete VEGF, which in turn induce endothelial cell migration and the formation of a microcapillary network [76].

tissues and SCCE cell lines upon EGFR and p53 overexpression. Secretion of HGF by stromal fibroblasts induces the transformed esophageal epithelial cells to invade extracellular matrix; however, other unidentified factors may also cooperate with HGF in this process, which further highlight the significance of this pathway in esophageal carcinoma invasion and progression [84].

Recently another growth factor: connective tissue growth factor (CTGF, CCN2), has been introduced by Li and colleagues to be involved in the development and progression of SCCE in addition to poor survival of SCCE patients. It is suggested to be an independent factor for SCCE patients' prognosis as well as diagnosis of the precancerous lesions; as a result early detection of SCCE [85].

3. Signal transduction pathways

3.1 Ras signaling

Activation of the Ras pathway takes place upon a wide range of stimuli that could initiate its signaling. Ras activation begins with a vast array of upstream activated receptors including receptor tyrosine kinases, serpentine receptors, heterotrimeric G-proteins, integrins and cytokine receptors [86]. Among these activators, the best described mean of Ras stimulation

is via receptor tyrosine kinases such as EGF receptor (Fig 4). Binding of growth factors to their cognate receptors promote cellular proliferation through signal transduction cascades, initiated by the activation of membrane associated Ras proteins. Ras/Raf/mitogen activated protein kinase (MAPK) is one of the key Ras dependent growth-stimulating signaling cascade activated when growth factors bind their tyrosine kinase receptors, which ultimately leads to the induction of cyclin D1 [87, 88].

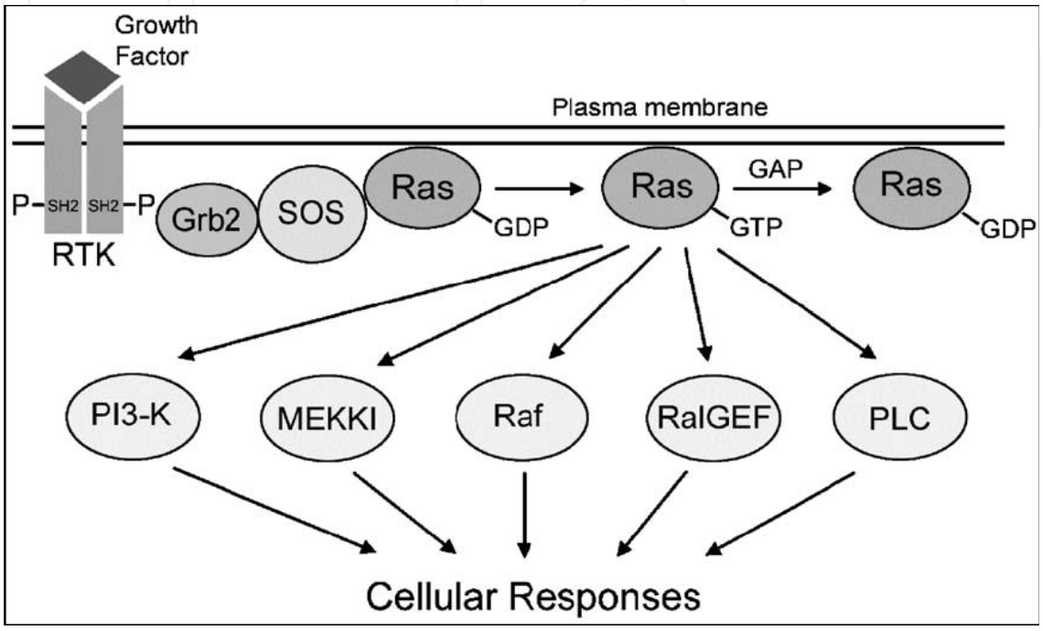

Fig. 4. A simplified overview of Ras activation and signaling cascade. Activation of a receptor tyrosine kinase (RTK) by an appropriate growth factor stimulates autophosphorylation of SH2 domains that recruit Grb2. Guanine nucleotide exchange factors (GEF) such as SOS are localized to the membrane by Grb2, which then stimulate Ras to exchange GDP for GTP. The activated Ras interacts with multiple signaling pathways, including phosphoinositide 3Vkinase (PI3-K), MEKKI, Raf kinase, RalGEFs and phospholipase C (PLC) to induce cellular responses. Ras signaling is terminated when GTPase activating proteins such as p120 and NF-1 stimulate Ras to hydrolyze GTP to GDP [86].

Ras pathway can also inhibit proliferation by inducing expression of p16 and p15 that are members of the INK4 family (Fig 2). These proteins block the Rb phosphorylation induced by cyclin D1/CDK complex, which results in cell cycle arrest in G1 [88]. Several lines of evidence have addressed the importance of Ras effectory pathways in the carcinogenesis of esophagus cancer; most notably through contribution of growth factors and their tyrosine kinase receptors, and in particular EGF and EGFR which are commonly deregulated and/or found at high level of expression in esophageal cancer [4, 5, 21, and 89].

In addition, mitogen activated protein kinase (MAPK) signal transduction pathway including Ras-Raf-MEK-ERK, PI3K/Akt, and JNK were found to be hyperactivated in esophageal cancer. This pathway modulates cell proliferation, invasion and metastasis, and resistance to chemotherapeutic agents, and ionizing radiation in esophageal cancer cells [24,

91-93]; in which, inhibition of MAPK signaling could enhance sensitivity of esophageal cancer cells to chemotherapeutic agents [94-96].

Lawler *et al*. [97] have also shown that mobility and invasiveness of metastatic esophageal cancer cells are potentiated by shear stress through the Rho kinase (ROCK) and Ras-signaling pathways, suggesting a novel physiological role for Rock and Ras in metastatic behavior of cancer cells [97].

The contribution of Ras/ERK signaling has been demonstrated by Senmaru *et al*. They have shown a dominant negative H-*ras* mutant (N116Y) inhibits EGF-stimulated activation of Erk2 in esophageal cancer cells. Furthermore, using adenoviral vectors and increased expression of this mutant significantly reduces the growth of human squamous cell carcinoma of esophagus cells *in vitro* and *in vivo* [98].

Furthermore, the significance of Ras signaling and its downstream pathways has also been recently indicated in tumorigenesis of esophageal cancer. Since, it was found that activation of MEK/ERK and PI3K/Akt effectory pathways play important role in downregulation of tropomyosin-1, which is a member of tropomyosin family and actin cytoskeleton-related proteins, in squamous cell carcinoma of esophagus [99].

Due to the importance of Ras dependent signaling drugs blocking these pathways were used in chemotherapy. Among such drugs are statins; a type of popular cholesterol-lowering agents including drugs such as Lipitor, were shown to inhibit tumor growth and proliferation of cancer cells as well as stimulation of apoptosis in esophageal cancer cell lines. These effects are achieved by inhibiting the Ras, ERK and protein kinase B (Akt) signaling pathways [90].

3.2 Wnt signaling

Wnt/β-catenin pathway initiates a signaling cascade critical for the normal development. The aberrant activity of this signaling pathway is associated with several forms of human carcinomas [100, 101]. Wnt ligands begin intracellular signaling pathways by binding to the G-protein -coupled receptors frizzleds (Fzs) [102]. In the absence of Wnt signals, GSK-3 phosphorylates cytosolic β-catenin within a destruction complex comprised of adenomatous polyposis coli (APC), Axin-1, casein kinase-1 (CK-1), and other proteins, the end result of which is targeting β-catenin for ubiquitin-mediated degradation (Fig 5a).

Upon Wnt binding to the Frizzled receptor and low-density lipoprotein receptor-related protein (LRP) co-receptors, the cytoplasmic Dishevelled (Dsh) protein becomes activated which in turn antagonizes the effects of GSK-3 through prevention of destruction complex formation and thus β-catenin phosphorylation. This in turn leads to the stabilization and accumulation of cytoplasmic β-catenin. GSK-3 could also be inactivated through phosphorylation by PI3-K (Fig 5c). As a result, accumulated cytoplasmic β-catenin enters into the nucleus where it binds to the T cell factor/TCF/LEF (T-cell factor/lymphoid enhancer-binding factor 1) transcription factor family and stimulates transcription of target genes including *c-myc*, *cyclin D1*, *c-jun,* and *fra-1* (Fig 5b), which plays critical roles in cell growth, proliferation, and differentiation [101-104]. Aberrant expression and function of Wnt signaling components result in aberrant nuclear accumulation of β-catenin which in turn contributes to the tumorigenesis of esophageal cancer through increased expression of cyclin D1 [105].

In addition to its role in Wnt signaling, β-catenin is also involved in cell adhesion, providing a link between actin cytoskeleton and cadherin(s) cell adhesion molecules [106, 107]. E-cadherin and β-catenin are primarily found in the cell membrane of the normal squamous mucosa of esophagus and the nondysplastic, specialized intestinal metaplasia of Barrett's esophagus [108, 109]. Immunohistochemical studies of dysplastic Barrett's esophagus has shown that membrane E-cadherin and β-catenin are decreased, while their level is increased in cytoplasm and nucleus [110].

Loss of APC tumor suppressor gene plays important role in the development of esophageal cancer, since loss of heterozygosity (LOH) of 5q21, the APC locus, occurs commonly in adenocarcinomas of esophagus [111-113]. Mutations in β-catenin and/or APC gene also alter degradation of β-catenin, as a result, its aberrant accumulation leads to the increased transcription of target genes. However, it has been shown that mutations of APC and β-catenin genes, unlike in colorectal carcinoma, involve in only a small subset of esophageal and esophagogastric junction carcinomas [114], or somehow are rare in esophageal cancer [115]. However some studies have confirmed that mutations of APC gene occur in human esophageal cancer [116, 117].

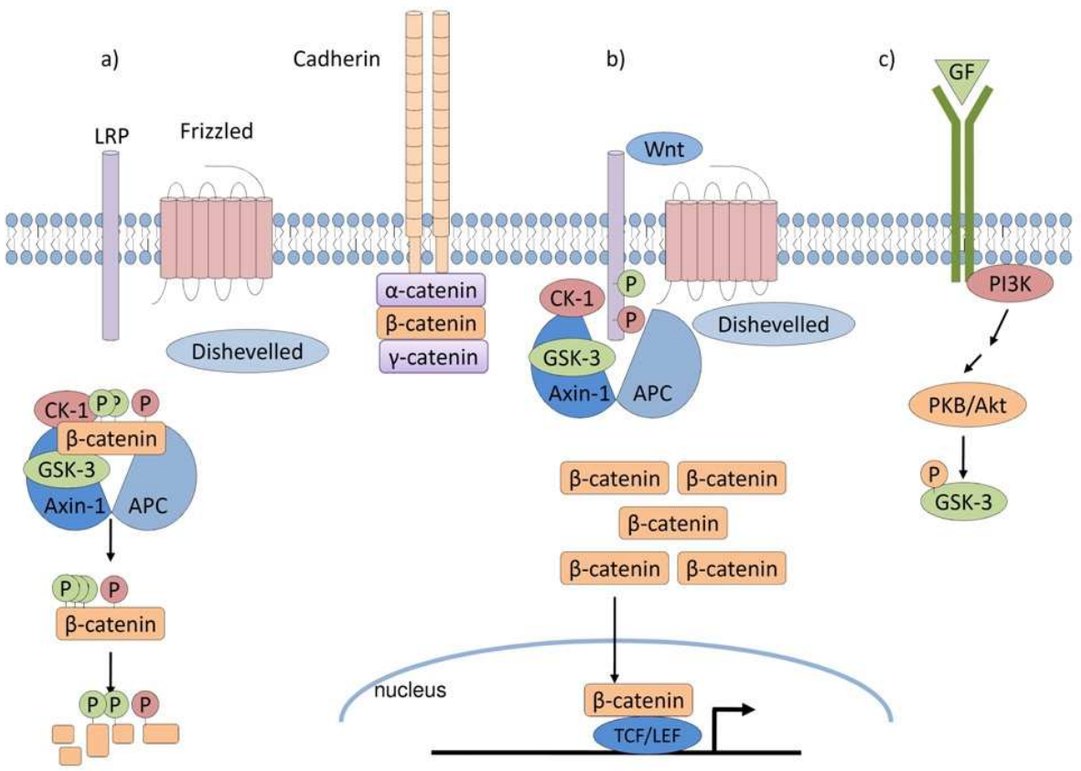

Fig. 5. The role of Wnt signaling in the activation of gene expression through mediation of β-catenin induced transcription. The non-phosphorylated form of β-catenin is active while the phosphorylated form of which is doomed to the inactivation and proteasomal degradation (a). Dishevelled antigonises β-catenin phosphorylation by GSK-3 (b) and PI3K does the same function by phosphorylating GSK-3 and thus inhibition GSK-3 mediated phosphorylation of β-catenin (c) [118].

Although mutations in *APC* or *β-catenin* are rare in esophageal cancer, alterations of upstream components, such as overexpression of Wnt2 ligand and Frizzled receptors or downregulation of Wnt antagonists and inactivation of secreted frizzeled-related protein (SFRP) genes by promoter methylation have been reported to play a dominant role in the activation of the Wnt pathway during esophageal carcinogenesis [119, 120].

Nonetheless, inactivation of APC by promoter methylation is involved in esophageal cancer, where it occurs in 83-92% of Barrett's high grade dysplasia and esophageal adenocarcinoma as well as 40-50% of Barrett's metaplasia without dysplasia [121, 122]. Higher level of promoter methylation of *APC* was also found by Clement, *et al*. in 100% of barrett's esophagus samples and in 95% of esophageal adenocarcinomas as well [119]. Several lines of evidence have also shown the role of *APC* promoter hypermethylation in squamous cell carcinoma of esophagus, as it was observed in about 50% of cases, to contribute in the progression of dysplasia to carcinoma in SCCE carcinogenesis along with low survival rate of patients [122-124]. Moreover, methylated *APC* DNA has been detected in the plasma of patients with esophageal adenocarcinoma and squamous cell carcinoma. Methylated APC promoter has shown to be associated with a significantly low patient survival [122], suggesting the capability of hypermethylated *APC* tumor suppressor gene to be a potential biomarker in esophageal cancer.

Wang *et al*. have recently shown a prominent role of Wnt signaling in SCCE carcinogenesis. They identified that Wnt2/β-catenin signaling pathway is activated in SCCE cells, as sodium nitroprusside (SNP) and siRNA against β-catenin not only inhibit expression of β-catenin and its major downstream effectors including c-myc and cyclin D1, but also induce cell cycle arrest and apoptosis, suggesting that Wnt2/β-catenin pathway may be a potential molecular target for SCCE therapy [125]. Inactivation of GSK3β, observed by higher phosphorylation of Ser9 GSK3β, has been found in most cancers with epithelial origin, including esophagus cancer [126, 127].

3.3 Dopamine and cyclic-AMP-regulated phosphoprotein

Recently, the role of t-DARPP (Dopamine and cyclic-AMP-regulated phosphoprotein) in the regulation of β-catenin has been investigated in esophageal cancer. DARPP-32 is a major regulator of dopaminergic neurotransmission in brain. It is the key factor for the functioning of the dopaminoceptive neurons [128]. DARPP-32 and t-DARPP, a truncated isoform of DARPP-32, are suggested as novel cancer-related genes [129].

Overexpression of t-DARPP has been reported in gastrointestinal malignancies as well as esophageal adenocarcinomas [130, 131], which leads to activation of Wnt signaling and increased cell proliferation through phosphorylation of GSK-3β, nuclear accumulation of β-catenin, and upregulation of *cyclin D1* and *c-myc* target genes. It has also been shown that t-DARPP mediated GSK-3β phosphorylation is AKT-dependent [132].

3.4 PI3K signaling

The involvement of phosphatidylinositol 3-kinase (PI3K)/AKT pathway in esophageal tumorigenesis was also subject of investigations. Phosphatidylinositol 3-kinases (PI3Ks) are a ubiquitous family of lipid kinases that catalyse the phosphorylation of phosphatidylinositol

(PI), PI(4)P and PI(4,5)P2 that leads to formation of PI(3)P, PI(3,4)P2 and PI(3,4,5)P3, respectively [133]. These phosphorylated lipid products are then able to activate a variety of downstream targets, such as protein kinase B (PKB/AKT), that regulate a wide range of important cellular processes, including cell proliferation, survival, migration, apoptosis, oncogenic transformation and intracellular trafficking of proteins [134].

Constitutive activation of PI3K and AKT is common in cancers including esophageal cancer [135-137]. Amplification of the *PIK3CA*, the gene coding for the $p110_{\alpha}$ catalytic subunit of PI3K, has been reported in SCCE [136] and in a low percentage of adenocarcinoma [138]. In addition, mutation of *PIK3CA* has been found to be an important event in the etiology of esophageal cancer [139].

Recent studies have indicated that inhibition of PI3K reduces proliferation and enhances radiosensitivity of esophageal cancer cells [92, 140]. In addition, the level of p-AKT expression in SCCE increases during chemotherapy, and a high expression of p-AKT correlates with poor prognosis [141].

3.5 Hedgehog signaling

The Hh (Hedgehog) signaling is critical for embryonic development which initiates following to binding of Hh to patched (Ptch) receptor, which releases smoothened (Smo), a potential G protein-coupled receptor (GPCR), from Ptch mediated repression. Smo signal transduction eventually leads to increased expression and activation of Gli, a transcription factor that regulates corresponding target genes [142-144]. Deregulation of this pathway leads to abnormal proliferation and transformation of cells in different tumors, such as small cell lung cancer, pancreatic cancer, prostate cancer and digestive tract cancer, including esophageal cancer [145-149] Hyperactive Hh signaling has been reported to be implicated in esophageal cancer [146, 147]. It has also been shown that PI3K/AKT pathway plays a critical role in epidermal growth factor (EGF), Gβγ and Shh-induced Hh signaling. Conversely, PI3K/AKT and MAPK signaling cooperate with the Shh pathway to promote esophageal cancer cell survival and proliferation [150].

3.6 Role of Id-1

Overexpression of Id-1, the inhibitor of differentiation or DNA binding, has also been shown in esophageal squamous cell carcinoma. Id-1 promotes proliferation [151], tumorigenicity and metastasis of human esophageal cancer cells through activation of PI3K/AKT signaling pathway [152-154]. Id-1 is a helix-loop-helix protein, which heterodimerizes with the basic helix-loop-helix transcription factors and inhibits them from DNA binding, therefore regulating gene transcription [155].

4. Tumor suppressor genes

4.1 P53

Tumor suppressor genes' inactivation occur by the genetic or epigenetic events such as mutations, allele deletions (LOH), promoter hypermethylation, abnormal splicing, and posttrancriptional silencing by microRNAs [156] in cancers. Alterations in multiple tumor

suppressor genes including *Rb*, *p53*, *APC*, *p16*, and *MCC* implicated in carcinogenesis of esophageal cancer. The majority of tumor suppressor genes are involved in the regulation of cell cycle. Cell cycle is controlled precisely through two major regulatory mechanisms, the p53 (p14–MDM2–p53–p21) and pRb (p16–cyclinD1–pRb). Deregulation of both mechanisms play critical role in the development of most human cancers including esophageal cancer.

The *p53* tumor suppressor gene regulates cell cycle progression, apoptosis and DNA repair. It also inhibits vascular endothelial growth factor. *p53* is the most frequent mutated gene in all human malignancies. P53 is normally expressed at low level but accumulates in the nucleus of the damaged cell and transactivates target genes including genes involved in G1 cell-cycle arrest (*p21/WAF1*) and apoptosis (*BAX*) [157-161]. In fact, it plays a role as a genomic policeman to prevent replication of damaged DNA; either by arresting cells in G1 and facilitating DNA repair or by apoptotic elimination of damaged cells [162]. *p53* mutations are common in esophageal cancer which occur in approximately 50-80% of esophageal cancers. More than 92% of these mutations occur in the four conserved domains of the *p53* gene; exon 5 to exon 8, with hot spots at Arg175, Cys176, Arg248, Arg273, and Arg282, 80% of which are point mutations including 46% transition and 36% transversion [5]. Several studies have indicated that alteration of *p53* gene in esophageal cancer occurs in the early stage of carcinogenesis and is associated with tumor progression; suggesting that loss of p53 function is critical for the development of this type of cancer [5, 156].

Although the presence of mutant *p53* have shown to be correlated with the poor prognosis [163, 164], other studies have not found such correlation [165, 166]. It has also been shown that measurement of circulating anti p53 antibody in serum of patients with SCCE is useful for detection of *p53* mutations, and as a tumor marker or prognostic marker [167-169].

4.2 p21/WAF1

The *p21/WAF1* gene is located on chromosome 6p21.2 and encodes a cyclin-dependent kinase inhibitor. Induced by wild-type p53, it mediates G1 arrest following to DNA damage [170]. Mutations and deletions of the *p21/WAF1* gene are less common in human cancers [171]. However, polymorphisms in exon 2 of the *p21/WAF1* gene has been documented to play important role in esophageal tumorigenesis [172]. Moreover, induction of *p21/WAF1* may occur via *p73* [173], a transcription factor that also regulates *p21/WAF1* expression [174] in esophageal cancer. The role of *p21/waf1/CIP1* expression in SCCE prognosis seems to be controversial. Several studies have indicated reduced expression of p21 as an indicator of poor esophageal cancer prognosis [175, 176], while others have found no significant correlation [173, 177]. Conversly others have claimed that p21 overexpression is correlated with a poorer prognosis [165, 178].

4.3 p16/INK4a and p15/INK4b

p16/INK4a, which is located on chromosome 9p21 is another member of cyclin dependent kinase inhibitors and is involved in *p53* independent G1 arrest through inhibition of D type cyclin dependent kinases (CDK4/CDK6) in the Rb phosphorylation [179, 180] and inacivation. Inactivation of *p16/INK4a* is a common event in esophageal cancer and occurs through homozygous deletion, point mutation and/or hypermethylation [181-184]. In

SCCE, homozygous deletion and promoter methylation are the major causes of *p16/INK4a* gene silencing, while somatic mutation is a rare event [185]. *p16/INK4a* methylation or loss of heterozygosity (LOH) occurs in the early stages of SCCE tumor progression [186, 187], while homozygous deletion of its locus is a late event [188]. The two common mechanisms of *p16/INK4a* inactivation in esophageal adenocarcinoma were found to be promoter hypermethylation, which occurs in the early stages of carcinogenesis and loss of heterozygosity [189, 190]; while homozygous deletion have not been documented to play significant role. In addition, loss of *p16/INK4a* expression together with overexpression of cyclin D1 may also be correlated with poor prognosis [191]. Detection of hypermethylated *p16/INK4a* could provide an appropriate biomarker for esophageal cancer screening as it could be observed in the early stages of carcinogenesis [192-194].

p15/INK4b, a homolog of INK4 family whose locus is close to INK4a locus could be subject of stimulation by TGF-β and activate G1 arrest [195]. Changes in *p15/INK4b* has been studied less often in esophageal cancer; however it has been found that inactivation of *p15/INK4b* occurs through homozygous deletion or abnormal methylation at the same time as *p16/INK4a*, which leads to the loss of Rb-regulated restriction point and plays an essential role in esophageal carcinogenesis [188].

4.4 Retinoblastoma (Rb)

The retinoblastoma protein (pRb or commonly known as Rb) is a nuclear phosphoprotein that plays essential role in the regulation of cell cycle. It negatively regulates transcription by forming complex with E2F transcription factor. Phosphorylation of Rb by the cyclin/CDK complex, results in E2Fs release, expression of target genes and cell division [196-198]. While deletions or mutations of *Rb* or inactivation by HPV infection are rare in SCCE, however several studies have indicated that alteration in *p16* and *p53* lead to the blockage of Rb function [199-201]. However, loss of heterozygosity of the retinoblastoma locus plays essential role in the inactivation of *Rb* gene and is associated with p53 alterations in esophageal cancer. It is suggested that association of *Rb* with *p53* inactivation may be the major event in the development and progression of esophageal cancer [199, 202].

5. Other novel tumor suppressor genes

In addition to the well established role of well-known tumor suppressor genes such as *p53*, *Rb*, *APC*, *p21*, and *p16* in the carcinogenesis of esophagus cancer; there are other novel tumor suppressor genes which also play role in the development of esophageal cancer.

ECRG4 (esophageal cancer related gene 4) is a novel candidate tumor suppressor gene for SCCE, which is downregulated through promoter hypermethylation. *ECRG4* is significantly associated with lymph node metastasis, tumor size, and tumor stage in SCCE, providing a candidate prognostic marker for SCCE [203].

ING (Inhibitor of growth gene) family, *ING1* to *ING5*, are new class of candidate tumor suppressor genes that are implicated in the cell cycle control, senescence, apoptosis, DNA repair, and chromatin remodeling. Downregulation of *ING1* was observed to be implicated in esophagus cancer [204].

The tetraspanin cell surface receptor uroplakin 1A (*UPK1A*) has been identified as another candidate tumor suppressor gene, which is downregulated by promoter hypermethylation in SCCE cells. *UPK1A* downregulation correlates with lymph node metastasis, tumor stage, and overall survival of patients, as well [205].

Expression of MAL (T-lymphocyte maturation associated protein), which is a component of protein machinery for apical transport in epithelial polarized cells, remarkably reduces in esophageal cancer. In addition, DNA methylation has shown to be associated with its downregulation [206].

*DLC*1 (deleted in lung cancer 1) is a putative tumor suppressor gene located on chromosome 3 (3p21.3) is supposed to act as a downstream gene in the serine/threonine kinase pathway. Its aberrant splicing was found in one third of esophageal, lung and renal cancers which plays a critical role in the carcinogenesis of these tissues [207].

The *WWOX* (WW domain containing oxireductase) gene located on chromosome 16 (16q23.3–24.1) is a candidate tumor suppressor gene for esophageal carcinoma. Both of *WWOX* alleles were seen to become inactivated in squamous carcinoma of the esophagus through combination of events among which mutations and LOH [208].

Annexin1, a member of annexin family which are calcium and phospholipid-binding proteins have also been shown to be lost or downregulated in esophageal cancer [209]. Additionally, its translocation from plasma membrane in normal cells to the nuclear membrane in malignant cells has proposed to be correlated with the tumorigenesis of esophageal cancer [210].

APC and *MCC* are the two tumor suppressor genes located on chromosome 5 (5q21) which are involved in the development of esophageal cancers through LOH of their corresponding genetic loci [111-113]. It has also been shown that mutations in *APC* and *MCC* genes take place in esophageal cancer as well as promoter hypermethylation [116, 122, and 124].

DCC (deleted in colorectal cancer) is a putative tumor suppressor gene whose loss has been implicated in colorectal tumorigenesis. Decreased or loss of *DCC* expression through promoter hypermethylation [211] as well as point mutations and LOH which are correlated with the degree of lymph node metastasis and differentiation [212], have been shown in esophageal cancer.

The involvement of tropomyosins (TMs) which are a family of cytoskeletal proteins and stabilizers of the actin microfilaments have been indicated in carcinogenesis of esophageal cancer; in which downregulation of β-TM (TM1), was described as a novel tumor suppressor gene [213]. The same role were also observed for TM2 and TM3 in esophageal squamous cell carcinoma [99, 214].

E-cadherin (*CDH1*), one of the most important molecules in cell to cell adhesion in epithelial tissues, localized on the surfaces of epithelial cells was reported to be lost in several cancers including esophageal tumors. Promoter hypermethylation was suggested to be involved in its inactivation as well. Loss of E-cadherin expression correlates with the high grade and advanced stages of disease along with poor prognosis [215, 216].

Downregulation of periplakin (a cell adhesion protein) [217] as well as loss of clusterin (a secreted glycoprotein) both in serum and tissue of esophageal squamous cell carcinoma [218] are among other events that have been correlated to the esophageal tumorigenesis.

6. References

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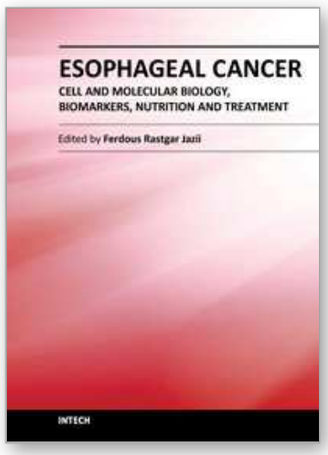

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Esophageal Cancer illustrates recent achievements and investigations in the esophageal tumorigenesis from different perspectives. Readers find mechanisms involved in esophageal tumorigenesis, cellular, molecular, genetic, epigenetics, and proteomics, 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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