Tetraspanin CD9: A friend or foe of head and neck cancer (Review)

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Abstract. Head and neck cancers are diverse and complex diseases characterised by unregulated growth of tumour cells in various parts of the head and neck region, such as in the buccal mucosa, floor of the mouth, tongue, oropharynx, hypopharynx, oesophagus, nasopharynx and salivary glands. Partial or total glossectomy, radiation or chemotherapy greatly affect patient quality of life. However, even following treatment, patients may relapse. Nicotine-derived nitrosamines and alcohol are the major etiological factors underlying this deadly disease. These compounds induce DNA damage that may lead to mutation in crucial genes, such as p53 and p21, which are important to regulate cell proliferation, thus leading to cancer. CD9 is a tetraspanin, which are a group of transmembrane proteins that have a role in cell motility and adhesion. The present review aimed to explore the role of CD9 in head and neck cancer. Epidermal growth factor receptor activity and cell proliferation are regulated by the CD9-integrin/CD9-transforming growth factor interaction. Hence, CD9 can play a dual role in various types of cancer.

Contents

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1. Introduction

Head and neck cancer is common in several regions of the world such as India, Hong Kong and Sri Lanka (1). Head and neck squamous cell carcinomas (HNSCCs) are a type of epithelial cancer arising in the mucosa of the upper aerodigestive tract (1). The oral cavity, hypopharynx, oropharynx and larynx are sites that have the potential to be affected by this cancer (1). A tetraspanin member, CD9 is found on the epithelial cells. Hence, it may have a role in the carcinogenesis of head and neck cancer. HNSCCs are aggressive, genetically complex and difficult to treat. HNSCCs can develop from dysplastic or premalignant lesions in the oropharyngeal mucosa that have occurred due to chronic exposure of the upper aerodigestive tract to carcinogenic agents (2).

HNSCCs are associated with different types of epidemiologies, aetiologies and therapies (2). Treatment has to be undertaken by multidisciplinary teams with training in supportive care that considers swallowing, nutrition, dental and voice impairment due to the effects of clinical intervention. In total, 6-90% of patients at early stages of this cancer show positive responses to local therapy. Early diagnosis and appropriate treatment results in cure and survival. The majority of patients with HNSCC who present with stages III and IV locally advanced head and neck cancer require multimodality treatment (3).

HNSCCs begin in the flat squamous cells that make up the thin layer of tissue on the surface of the epithelium in the head and neck. Directly beneath the epithelium, some areas of the head and neck have a layer of moist tissue, called the mucosa. A cancer that is only found in the squamous layer of cells is called carcinoma *in situ*. Cancer that has grown beyond the mucosa and has moved into the deeper tissue is called invasive squamous cell carcinoma (4). Head and neck cancer, the sixth most common malignancy, accounts for >650,000 cases and 330,000 deaths annually world-

wide (1-3). Women are less likely to be affected than men, with ratios of 1:2 to 4:1 worldwide thus far. In the Indian subcontinent, mouth and tongue cancer are more common, whereas nasopharyngeal cancer is more common in Hong Kong, and pharyngeal and laryngeal cancers are more common in other populations (5).

Oral cancer accounts for 1-3% of all cancer cases worldwide (6-8). The most adverse factors leading to the death of patients with tongue squamous cell carcinoma are lymph node metastasis and distant metastasis (9,10). The capacity to invade locally and metastasize to regional lymph nodes is the main clinical characteristic of squamous cell carcinoma (11).

2. Risk factors associated with HNSCC

The use of tobacco and alcohol are associated with HNSCC. Consumption of alcohol and long-term use of tobacco are the main oncogenic drivers and primary risk factors associated with head and neck cancer (5). Using alcohol and tobacco together increases this risk even more (12). Heavy metals, Fanconi anaemia (FA), the plasminogen activator (PA) system, matrix metalloprotease (MMP), human papilloma virus (HPV) and Epstein-Barr virus (EBV) are also etiological factors that are associated with head and neck cancer.

Tobacco. A variety of chemicals, including nicotine and other carcinogens, are present in tobacco. The type of tobacco products used and the duration of exposure are two factors that have a major impact on human health. The main constituent of tobacco products and smoke is nicotine. As such, nicotine is non-carcinogenic and addictive, but it has the capacity to activate tumour progression related to various signalling pathways (13,14).

Nicotine-derived nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonornicotine, can cause cancer in humans through the formation of DNA adducts and mutations, and they can promote tumour progression by altering receptor-mediated pathways (7,15-30).

Activation of nicotinic acetylcholine and β-adrenergic receptors by nicotine and nitrosamines in turn activates the downstream signal transduction pathways that aid tumour progression (21).

NNK in tobacco smoke naturally occurs in an inert form as a procarcinogen, which is converted to DNA reactive forms by several cytochromes, leading to methylation, pyridyloxobutylation and pyridylhydroxybutylation of nucleobases in DNA (22). The other carcinogens present in tobacco are polycyclic aromatic hydrocarbons, aromatic amines, aldehydes, phenols, volatile hydrocarbons and nitrocompounds (15,23) (Fig. 1).

Alcohol. The combination of alcohol consumption with cigarette smoking increases the risk of head and neck cancer (24). Alcohol dehydrogenase converts ethanol into acetaldehyde, which is considered a carcinogen of the human upper respiratory tract (24). Cytochrome P450 2E1 (CYP2EI) also has the ability to convert ethanol into acetaldehyde when the amount of alcohol consumed is high. This leads to the formation of reactive oxygen species (ROS) (25). Exocyclic DNA adducts are formed when malonaldehyde and 4-hydroxynonenal, which are the by-products of lipid peroxidation, accumulate by the action of ROS produced by CYP2EI (26). The upregulation of vascular endothelial growth factor and monocyte chemotactic protein-1, which play an important role in tumour angiogenesis and growth, is caused by the accumulation of ROS (27). An increase in the expression of MMPs, such as MMP2 and MMP9, leads to the degradation of the extracellular matrix (ECM), resulting in cell motility, invasion and metastases (28) (Fig. 1).

Heavy metals. According to the International Agency for Research on Cancer (IARC), arsenic (As), cadmium (Cd), chromium (Cr) and nickel (Ni) are category I heavy metals that disrupt tumour suppressor gene expression (29). These heavy metals damage the DNA repair process and metabolism-related enzyme activities (30,31). As is present in organic and inorganic forms, but the organic form of As is less toxic when compared with the inorganic form. Inorganic As compounds are pentavalent and soluble in water and produce salts, such as arsenate (32). Oxidative stress is the major mechanism of As-related damage (33,34). DNA repair processes are inhibited and ROS are the metabolic products in the spleen and liver of the methylated forms of As (35,36). ROS accumulation results in abnormal gene expression and lesions of cellular components that induce cell death (37). Residues of As bind to the DNA-binding proteins and increase the risk of carcinogenesis (38). Cd is an environmental pollutant that is released from industry and agricultural waste (39). B cell lymphoma 2 protein-associated X protein and mitogen-activated protein kinase 1 are associated with Cd (40), which exists in different forms. The trivalent and hexavalent compounds of Cd are biologically toxic as they can induce oxidative stress, DNA damage and apoptosis (41-43).

The levels of As, Cd, Cr and Ni have been found to be significantly high in patients with head and neck cancer compared with those in healthy individuals (44). This may be due to altered cellular metabolism during cancer. Occupational or environmental factors might be the reason for this difference in the concentration of heavy metals between patients with cancer and healthy individuals (44).

FA. FA is a genetic disease that is characterised by alteration in one of the 23 genes of the FS pathway or in the 23rd FA gene, DNA repair protein RAD51 homolog 1 (45). Genome stability induced by interstrand DNA crosslink repair in the FA pathway has the potential to induce tumorigenesis (45). Patients with FA are more prone to HNSCC and are more sensitive to severe radiation-induced side effects. Patients with FA who are at higher risk for HNSCC must abstain from other risk factors, such as tobacco, alcohol and HIV infections (45). The main characteristics of this rare autosomal recessive disorder are congenital malformations, such as abnormal thumbs and arms, skeletal abnormalities of the hips, ribs or spine, small reproductive organs in male patients, low body weight at birth, mental retardation, hyperpigmentation, progressive bone marrow failure, and the development of solid tumours (46-48).

PA system. An extracellular proteolytic enzyme system, the PA system, comprises various components, such as urokinase-type

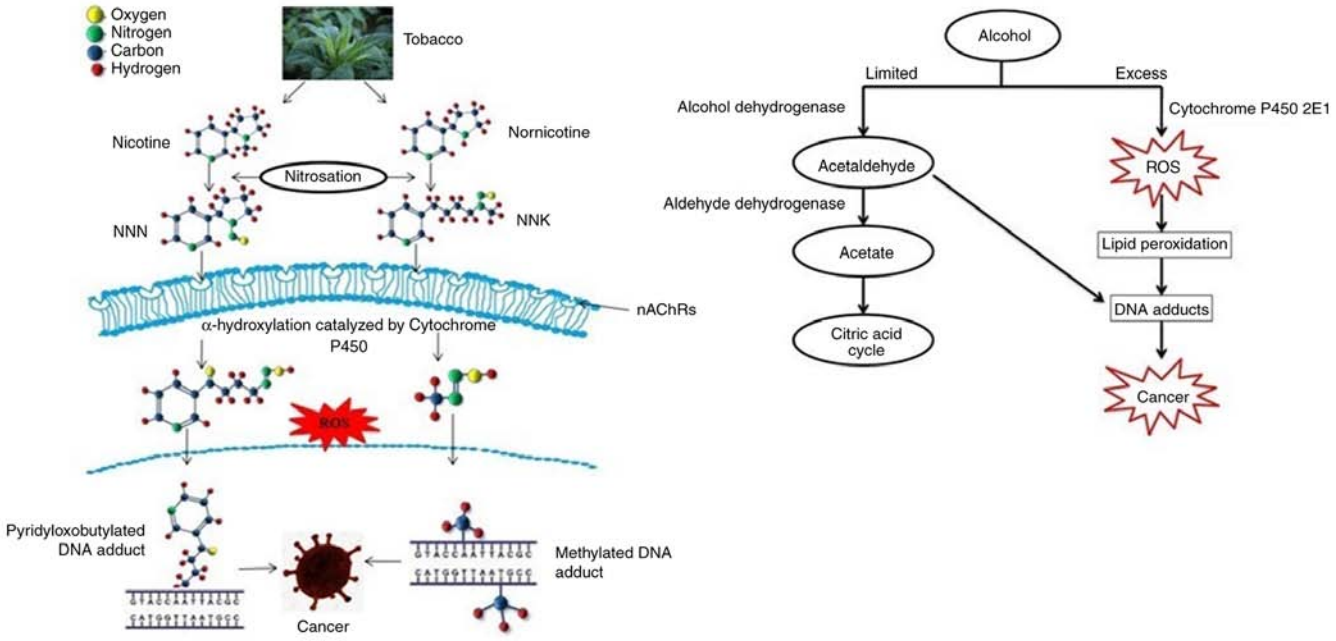

Figure 1. Role of nicotine-derived compounds and alcohol in head and neck squamous cell carcinoma. NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-nitrosonornicotine; ROS, reactive oxygen species; nAChR, nicotinic acetylcholine receptor.

PA (uPA), its receptor (uPAR), and PA inhibitor-1 and -2. They have a major role in cancer progression and metastasis (49). The activation of plasminogen to plasmin by binding of uPA to uPAR initiates a proteolytic cascade that degrades ECM components, thus facilitating cancer cell migration from the site of origin to distant organs (50). uPA/uPAR overexpression increases tumour cell migration and invasion, playing a key role in metastasis and conferring poor prognosis of patients with head and neck cancer (51). It is associated with focal adhesion kinase 1 and ERK1/2 signalling activation and an increase in HNSCC tumour growth (51,52). Activation of plasmin, ECM degradation and indirect activation of signalling pathways, such as the PI3K-Akt pathway, may be the reasons for this effect (50).

MMP. MMPs are enzymes that degrade the ECM, connective tissue and the basement membrane collagen, which are crucial in cancer cell invasion and progression. They require zinc for their catalytic activity. Type VI collagenase, MMP2 and MMP9 are members of the MMP family of enzymes (53-59). In HNSCC, immunohistochemical staining of MMP9 demonstrated that it has prognostic values that are not dependent on tumour stage. Patients with extensive positive MMP9 staining had relatively higher risk of mortality. No correlation has been found between MMP9 and the stage or grade of the tumour (60).

HPV and EBV infection. Inactivation of cellular tumour antigen p53 and cyclin-dependent kinase inhibitor 2A by cell cycle dysregulation leads to cell proliferation and inhibition of apoptosis in head and neck cancer (61). In oropharyngeal squamous cell carcinoma caused by HPV, the virus integrates into the host DNA genome, leading to the deregulation of oncoproteins (E6 and E7), which leads to the p53 and retinoblastoma tumour suppressor gene product pRb. P16 upregulation is the result of negative feedback of pRb inactivation. In nasopharyngeal squamous cell carcinoma caused by EBV, the cell cycle is the most deregulated pathway. Progression of the G1/S phase is promoted by the inhibition of p16 expression and pRb upregulation (61,62).

Wood and leather dust are the two types of occupational dusts that are classified as type 1 carcinogens by IARC (63). Dusts are small solid particles present in the air with a size ranging from 1 to 100 μm (64). They are a heterogenous group of exposures that can be either organic or inorganic. The carcinogenic effect of dust is exerted through the induction of chronic inflammation, their intrinsic chemical properties or they act as carriers of other carcinogenic compounds (63). Occupational sawdust exposure has been found to increase the risk of laryngeal carcinoma (OR, 1.2; 95% CI, 1.0-1.3) and metal dust (OR, 1.2; 95% CI, 1.0-1.4). Exposure to occupational leather dust can increase the risk of head and neck cancer (OR, 1.5; 95% CI, 1.2-1.9) (65).

1,1-thiobis, also known as sulphur mustard, causes blisters on contact with the skin and mucous membrane (66). A reactive intermediate, a cyclic sulfonium ion, is produced as sulphur mustard eliminates a chloride ion by intramolecular nucleophilic substitution. This intermediate causes alkylation of guanine nucleotide of DNA that prevents cell division, which may lead to malignant transformation (67,68).

Radiation is used widely to treat cancers. Radiation-induced sarcomas are seen in long-term survivors of head and neck cancer with a risk of up to 0.3% (69). Treatment of head and neck cancer include surgical eradication, chemotherapy and radiotherapy, which reduce quality of life (including loss of taste and excessive hair loss), and are ineffective. Genetic heterogeneity that results in the loss of function of genes, such as p53 and p16, and the activation of oncogenes, such as epidermal growth factor receptor (EGFR) and PIK3CA, plays an important role in HNSCC (70-72).

3. Biomarkers in head and neck cancer

A biomarker is an objective feature that can be precisely assessed to determine a specific biological, pathological or therapeutic development of the host (73). There are several biomarkers for head and neck cancer. MMPs are enzymes that degrade the ECM and induce cell migration. Serum levels of MMP2, 3 and 9 are elevated in patients with HNSCC (74). Inflammatory markers, such as IL-8 and IL-6, are increased in saliva and serum, respectively (75,76). Cytokeratin 17 is a cytoskeletal intermediate filament that is upregulated in oral squamous cell carcinoma (OSCC) when compared with normal cells, and it has been identified as a immunohistochemical marker for squamous cell carcinoma of the larynx (77,78).

MircoRNAs (miRNAs/miRs) are small non-coding sequences that regulate gene expression after transcription. Levels of miRNAs, such as miR-125a and miR-200a, are significantly lower in subjects with OSCC compared with those in normal subjects (79).

Interferon-γ (IFN-γ) released from activated $CD8^+$ T cells in the tumour microenvironment triggers the transmembrane protein, programmed death ligand 1 (PD-L1). T cell energy and programmed cell death can be induced by PD-L1 upregulation when it interacts with programmed death receptor-1 (PD-1), a checkpoint present on the immune cell surface. PD-L1 plays a prognostic role by regulating the relationship between tumour-infiltrating lymphocytes and tumour cells (80,81). HNSCC is a highly immunosuppressive cancer. Blocking the PD-1/PD-L1 pathway has been found to improve the survival of patients with head and neck cancer and reduce tumour growth (82). Progression-free survival was improved in PD-L1-positive patients with head and neck cancer (P=0.01). PD-L1 expression was increased in patients who had HPV-positive HNSCC (P<0.001). Poorer overall survival was observed in patients with positive PD-L1 who had low levels of $CD8^+$ tumour-infiltrating T cells (P=0.03) (83) (Fig. 2).

Fluorodeoxyglucose-positron emission tomography is a powerful imaging tool that can be used to identify cervical node metastasis and is a standard of care for patients with III and IV stage HNSCC (84). Patients with lower $\Delta SUV_{max10/20}$ showed lower overall survival compared with those with higher $\Delta SUV_{max10/20}$ (P=0.02). The decrease in the SUV_{max} before and after chemoradiotherapy acts as a potential prognostic marker in patients with head and neck cancer (85).

CD62, also known as L-selectin, is a lectin receptor expressed on leucocytes that regulate the entry of naïve and central memory T cells into lymph nodes (86). The spread of tumour cells to lymph nodes is a multistep process that includes invasion of the tumour cells into the lymphovascular compartment and lodging and growth of the tumour cell in the new environment. The lymph node is the most common region of metastasis for head and neck cancer. Head and neck cancer cells express unrecognized L-selectin that mediates the binding to lymphocytes and thus aids tumour node metastasis (87).

Likewise, tetraspanins are one of the markers for HNSCC. Tetraspanins play a major role in a wide array of cellular processes, including cell adhesion, motility, intracellular signalling, cell matrix adhesion and proliferation (88). Of the 33 tetraspanin proteins, CD9 is being extensively studied (89-91).

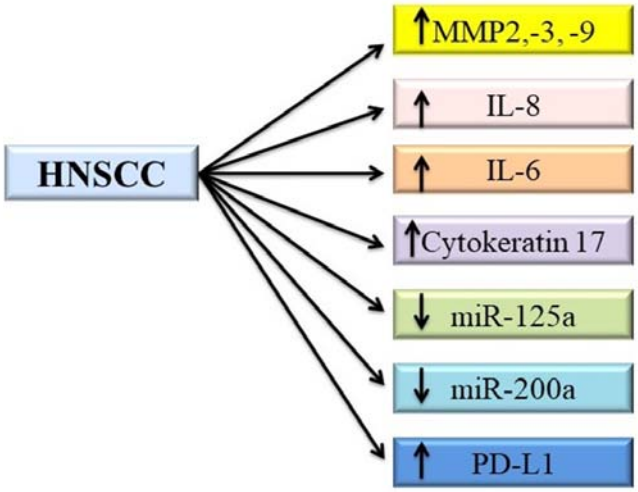

Figure 2. Biomarkers of HNSCC. HNSCC, head and neck squamous cell carcinoma; MMP, matrix metalloproteinase; miR, microRNA; PD-L1, programmed death ligand 1.

4. Tetraspanin CD9

Tetraspanin is a glycoprotein family containing four transmembrane domains. These proteins form multimeric complexes with each other and other cell surface proteins, including integrins, leukocyte antigens and signalling molecules, at specialized tetraspanin-enriched microdomains (92). They also contain distinct palmitoylation sites and most members are glycosylated (93).

The large extracellular loop has highly conserved motifs that aid in the recognition of tetraspanins (94). Cys-Cys-Gly, Phe-X-Ser-Cys and Glu-Gly-Cys are the conserved motifs of CD9 protein (95-97). 'Tetraspanin webs' are formed by the heteromultimerization of tetraspanins, which are stabilized by the transmembrane domains (97-99). There are two subdomains in the EC2 domain, a highly conserved subdomain with residue differences and a subdomain that has variability in size, amino acid sequence and protein folding for the disulphide bridge (90). The interaction between tetraspanins and other transmembrane proteins, such as integrins and other signalling molecules, is regulated by the EC2 domain of the tetraspanin (90,98-101) (Fig. 3). Tetraspanins recruit cell surface proteins, which stabilize the functional signalling complexes and act as molecular facilitators (102).

Kersey *et al* (103) identified CD9 using a monoclonal antibody (binds to acute lymphoblastic leukaemia cells) as the human lymphohematopoietic progenitor cell surface antigen p24. In the systematic nomenclature, Tspan 29 belongs to the tetraspanin family with a molecular weight of 21-24 kDa. CD9 is made up of four transmembrane domains with a small and large extracellular loop (SEL or EC1 and LEL or EC2, respectively) and short intracellular N- and C-terminal tails (104).

Among the tetraspanins, CD9 is unusual as it has only one N-glycosylation site located in its SEL domain, whereas other tetraspanins have a number of glycosylation sites (105). Critical physiological and pathological processes, such as sperm-egg

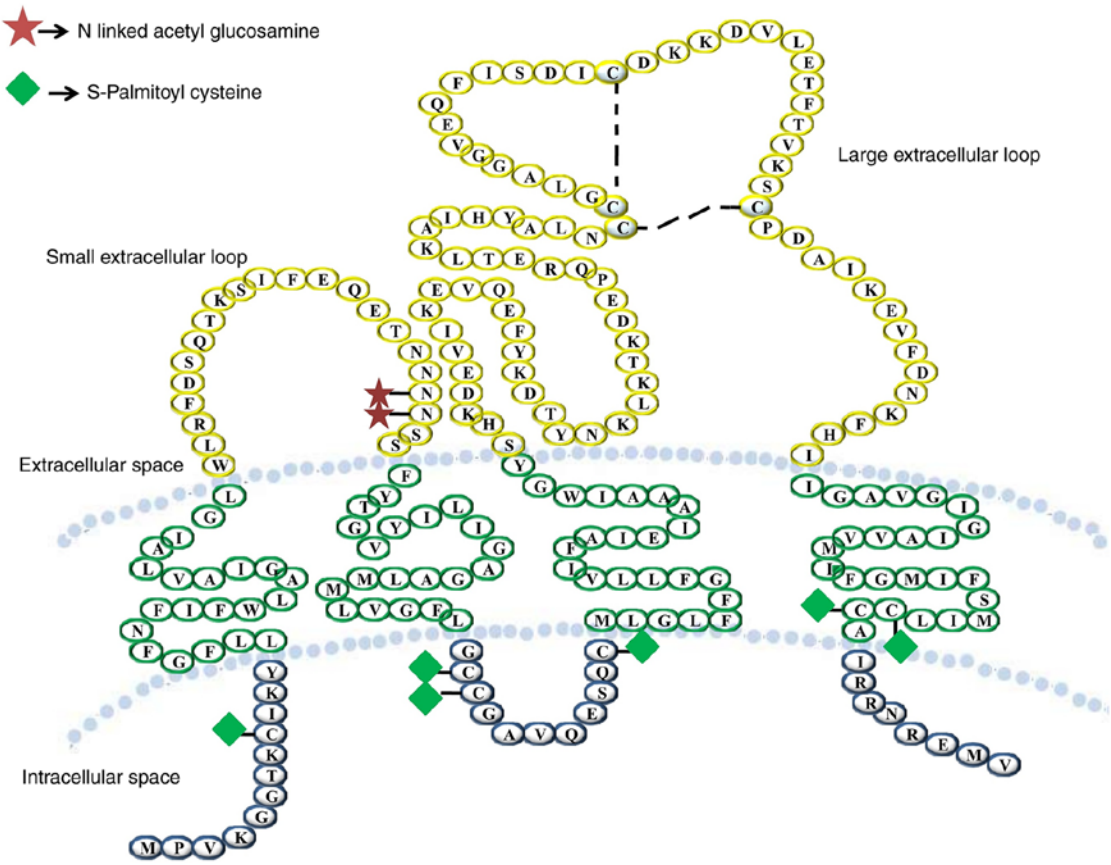

Figure 3. Structure of tetraspanin CD9. It has a small and large extracellular loop and four transmembrane domains that span the plasma membrane.

fusion, neurite outgrowth, myotube formation, tumorigenicity and metastasis, are regulated by CD9 (106-108).

5. Mechanism of action of CD9

The molecule that interacts with CD9 decides the role of this tetraspanin in cancer cell motility. The adhesion of tumour cells to the ECM increases when integrin expression is upregulated in combination with CD9. Transcription of MMP2 can be inhibited by CD9 complexes with fibronectin-bound integrins (109). Increased invasiveness of tumour cells can be the result of the activation of intracellular signalling molecules, such as PI4K and Src homology 2, by the transcription of MMP2 induced by CD9 crosslinking (110). Growth factors of the transforming growth factor (TGF) family activate the EGFR. Ectodomain shedding is a process where TGFα is proteolytically cleaved to release an EGF-core containing ligand. Ectodomain shedding and the release of TGFα is affected when it interacts with CD9, as it regulates the cleavage TGFα, which may lead to constant activation of EGFR, resulting in cell proliferation (110,111) (Fig. 4).

In CD9-overexpressed cells, the NF-κB signalling pathway has been found to be activated and dependent on CD9 expression. CD9 also induced tumour necrosis factor α (TNFα) gene expression, which resulted in the increase of IL-6 and IL-8 levels. NF-κB subunits, upon activation by TNFα, activate the transcription of genes involved in cell proliferation and differentiation by translocating into the nucleus. CD9 activates the caspase-3 inhibitor, which reduces the activity of caspase-3. Blockage of CD9 expression with small interfering RNA increases the level of caspase-3 activity. This shows that CD9 has anti-apoptotic activity (112) (Fig. 5).

6. CD9 as a friend of HNSCC

Favourable clinical outcomes have been observed in HNSCC with elevated CD9 expression. Tetraspanins or α3β1 integrins show an association with CD9 on the cell-to-cell junctions of human umbilical vein endothelial cells (109-113). Migration of endothelial cells during wound repair has been reported to be inhibited by anti-CD9 antibodies (101,114-117), which indicates the stabilizing effect of CD9 antigen on the integrity of the vascular membranes. During tumour angiogenesis, downregulation of CD9 proteins may be linked to vascular supply reorganization (89). CD9 acts by setting up the junctions between the cell surface and the intercellular matrix via the formation of a functional signalling complex with other cell surface proteins (98,118-121). Motility-related protein 1 (MRP-1)/CD9 expression was the only predictive parameter that seemed to be significant with respect to overall survival (P>0.049), whereas CD9 expression (P>0.006) and lymph node status (P>0.007) were significant for prolonged disease-free survival. Tumour patients with lower CD9 expression survived shorter periods of time than patients with high CD9 levels in the overall survival curves estimated by Kaplan-Meier analysis (P>0.04) (89). The potential effects of CD9 were confirmed when its expression was observed in the tumour vessels, indicating the involve-

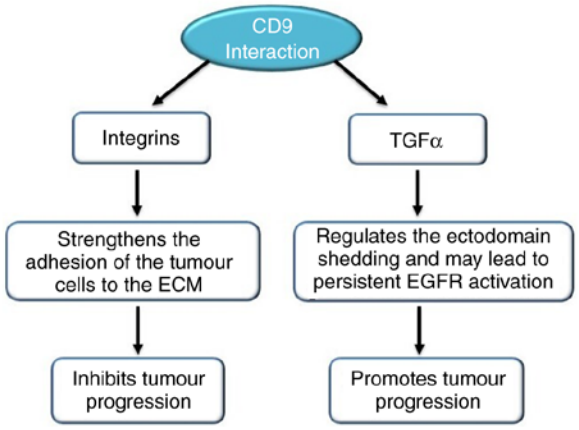

Figure 4. Interaction of CD9 with integrins and TGFα. TGFα, transforming growth factor α; ECM, extracellular matrix; EGFR, epidermal growth factor receptor.

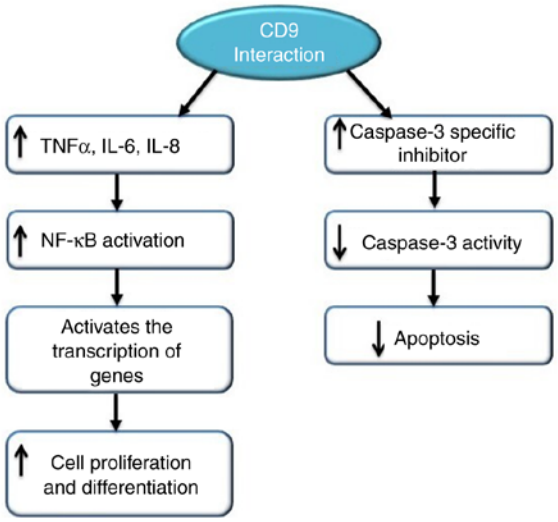

Figure 5. Interaction of CD9 with NF-κB, TNFα and caspase 3. TNFα, tumour necrosis factor α.

ment of this protein in tumour angiogenesis and endothelial cell migration (89).

Patients with positive CD9 tumours show shorter disease-free survival and overall survival than patients with negative CD9 expression in OSCC (100). Metastatic lesions have been reported in patients with lack of expression of these proteins, and they tended to have poorer prognosis and lower rates of survival (122-126). The incidence of cervical lymph node metastasis and survival has been found to be significantly associated with the abnormal expression of the CD9 protein (90).

One of the most common cancers in the head and neck region is laryngeal squamous cell carcinoma (LSCC) (91). The tumour grows in the glottic, supraglottic and subglottic areas. Death and the patient's quality of life are influenced by infiltration and metastasis, which have become the primary factors leading to an increase in the incidence of LSCC (91). Patients with negative CD9 protein expression have shorter median survival times compared with patients with positive CD9 protein expression ($P<0.01$) (91). LSCC may develop due to the combined participation of CD9 and another tetraspanin protein, CD82 (91). Infiltration, prognosis of LSCC and metastasis can be determined by using CD9 as a marker. Patients with TNM stage I-II, which is well-differentiated and non-metastatic LSCC, show higher CD9 positive expression than patients with TNM stage III-IV, which is well-differentiated and metastatic LSCC (91). These results show that as the expression of CD9 decreases, the invasiveness and the metastatic potential of the cancer cells increase (91).

Overexpression of CD9 by transfection leads to the suppression of cell motility (127,128). In oesophageal squamous cell carcinoma, lymph node metastasis may be facilitated by a decrease in CD9 expression (129). Patient prognosis can be predicted by the expression status of CD9 (129). A previous study reported that the cell membranes of normal oesophageal epithelial cells show positive CD9 expression, whereas CD9 expression is reduced on the membranes of cancer cells. As the tumours grew deeper, the levels of reduced CD9 expression significantly increased. As the stage of cancer advanced, the expression of MRP-1/CD9 was reduced. Lymph node metastasis and CD9 expression showed a significant inverse correlation, but there was no correlation between CD9 expression and distant metastasis. A correlation was found between lymph node metastases and lymphatic invasion. The 5-year survival rates of patients with CD9 positive expression were significantly improved compared with those patients with low or negative CD9 expression (129). The closest sites to the primary lesions may be affected by the loss of CD9, leading to local lymph node metastasis. Hence, there might be an inverse correlation between CD9 expression and lymphatic invasion (129). The adhesion effects of the interaction between CD9 and heparin-binding EGF-like growth factor associated with α3β1 integrin may play an important role in the initiation of the metastatic cascade (130,131). CD9 antibody activates platelets and their aggregation, thereby releasing the growth factors that facilitate tumour activation or growth (127,132).

In total, ~50% of gingival squamous cell carcinoma (GSCCs) cases show high oral malignant neoplasms and present with cervical lymph node metastasis (133). The jawbone and its surrounding tissues, such as nerves, muscles, the nasal cavity and skin, are invaded by GSCC. Logistic regression analysis with cervical lymph node metastasis as a target variable has shown that CD9/ACTB ($P=0.013$) and CD9/CD82 ($P=0.013$) have significant association (133). CD9 is related to the invasiveness of cancer cells by controlling the function of integrin receptors (133). Lymph node metastasis has been shown to be related to an increased level of the integrin a3 gene and a reduced level of CD9, as indicated in OSCC gene expression analysis (134,135). A previous *in vitro* study demonstrated that the main regulator of cell motility, the microvilli-like protrusions arising from the cancer cells, had clusters of tetraspanin-a3 integrin complexes on them. Upon treating the cells with tetraspanin and integrin antibodies, the cancer cells had increased invasive potential due to the stimulation of MMP2 production and elevated long invasive protrusion formation (136). Cancer cell motility is negatively influenced by CD9 via actin cytoskeleton reorganization. There

is a negative correlation between CD9/ACTB gene expression and lymph node metastasis. Cytoskeleton reconstruction related to elevated ACTB expression may be associated with a decrease in CD9 expression (137).

In papillary thyroid microcarcinoma, the patients' age, multifocality and extrathyroidal extension are known factors that can be used for prognosis (138). CD9 immunostaining intensity has been found to be higher in patients with lymph node metastasis than inpatients without metastasis (P=0.002) (138). CD9 intensity is also correlated with lymph node metastasis, suggesting that CD9 can be considered a prognostic marker for lymph node metastasis in papillary thyroid microcarcinoma (138).

7. CD9 as a foe

Through its association with other partner proteins, CD9 has various functions and has been identified as a tumour suppressor (139). CD9 is involved in and modifies the steps of tumour formation, such as proliferation, apoptosis, migration, adhesion and angiogenesis, and the communication with the environment, dissemination and metastasis (139). Thus, CD9 has a major role in cancer development and progression. Venous vessel invasion, metastasis and poor prognosis are related to tetraspanin CD9 (139). Upon treating patients with gastric cancer with CD9 antibody, tumour progression was found to be inhibited by antiproliferative, pro-apoptotic and anti-angiogenic effects. This indicates that CD9 may be target in patients with gastric cancer (139).

The EGFR has shown association with CD9. EGFR amplification is a characteristic of glioblastoma histology, affecting the signal transduction pathway. CD9 has the ability to attenuate the ligand-induced activation of the receptor via the destabilization of the surface expression of EGFR (140). Phosphorylation of EGFR at specific sites has been shown to be decreased by CD9 (141). Additionally, cell growth and proliferation pathways, such as EGFR signalling of PI3K/Akt and MAPK/Erk, can be attenuated by CD9. By contrast, activation of EGFR signal transduction pathways, including PI3K/Akt and MAPK/Erk, can be enhanced by the reduction in CD9 expression via small hairpin RNA-mediated knockdown of CD9. Inhibition of the activity of PI3K/Akt and MAPK/Erk signalling pathways and phosphorylation of EGFR maybe the mechanism underlying the CD9-induced suppression of cell proliferation (141). CD9, along with other transmembrane proteins, has the ability to regulate cell migration (142,143).

CD9 has been identified as a glioma stem cell-enriched protein. In a context-dependent manner, CD9 is associated with the progression of malignant tumours and plays a role in pro-tumorigenesis to promote cancer invasion and tumour growth in glioblastomas (144). Predicting patient survival using CD9 expression is a potential prognostic tool (145). According to previous reports, cell proliferation and tumour formation are facilitated by CD9 (129,133,144,146).

The progression of solid tumours is associated with CD9 downregulation. Patients with advanced stages lack these molecules, and reduced expression is observed less in primary site tumours than in metastatic tumours. CD9 may contribute to the highly invasive and metastatic phenotype of small cell lung carcinoma. Thus, CD9 is an indicator of poor survival (147).

CD9 expression is an independent prognostic factor of post-operation recurrence-free survival (RFS) for gastrointestinal stromal tumours (GIST), as shown by the Cox proportion hazards regression (HR, 0.104; 95% CI, 0.021-0.528; P=0.006). The RFS of patients with CD9-negative expression was significantly worse than that of the CD9-positive expression group (148). CD9 plays a role in the inhibition of proliferation and metastasis by inhibiting the activation, degradation and secretion of the Wnt signalling pathway, TGFα and metalloproteinase (143,149,150). Downregulation of CD9 is correlated with tumour invasion and metastasis and is a poor prognostic marker in various cancers, such as like breast, colon, small cell lung cancer. Malignant behaviour and tumour progression can be a result of reduced CD9 expression (148). The post-operative three-year RFS rate of the CD9-negative group was found to be lower than that of the CD9-positive group (33.3 vs. 78.4%; P<0.001), as shown in the universal analysis of comparison between the CD-negative and CD9-positive group (148). RFS can be predicted independently using CD9 expression via multivariate analysis (148). This result showed that CD9 is important in the invasion and metastasis of GIST, and the risk of metastasis and recurrence increases as the expression of CD9 decreases. Hence, the aggressive and progressive behaviour of GIST can be predicted using CD9 expression (148).

The survival rate of patients with colon cancer with CD9-positive tumours was reported to be significantly higher than that of patients with CD9-negative tumours (151). Cell motility inhibition and induction of apoptosis promoted by concurrent GM3 synthesis and N-glycosylation may be related to the suppression of malignancy by CD9 (152). The transmembrane 4 superfamily protein CD9 regulates cell motility by acting as a link between extracellular integrins and intracellular signalling molecules, such as phosphatidylinositol 4-kinase (153-155).

Increased invasiveness of breast cancer tumour cells may be the result of activation of intracellular signalling molecules, such as PI4K and Src homology, by CD9 crosslink-induced MMP2 transcription (110). In epithelial cells, cleavage of TGFα is protected by the interaction with CD9, which leads to the persistent activation of EGFR (105,106). Patients without CD9 expression had improved overall survival (P=0.051) and disease-free survival (P=0.014) compared with patients with CD9 expression (109). The survival of patients with breast cancer decreased due to altered cellular proliferation induced by activated EGFR signalling (108).

8. Conclusions

In several human cancers (Table I), CD9 has different effects on different types of cells. In epithelial cells, the expression of CD9 on the tumour cell has shown association with favourable clinical outcomes. Hence, CD9 can be regarded as a tumour prognostic biomarker. It is useful for making decisions regarding postoperative treatment. CD9 in tumour inhibition or tumour progression depends on the molecule that interacts with CD9 (Fig. 6). In conclusion, CD9 interacts with several molecules that result in altered behaviour of cancer cells. This behaviour is different for each cancer. Thus, determination

Table I. CD9 expression in different types of cancer.

Serial no.	Cancer	Observations	(Refs.)
1	Head and neck cancer	Lower survival rates of patients with lower CD9 expression compared with those with higher CD9 expression	(86)
2	Oral squamous cell carcinoma	A correlation was observed between positive CD9 expression and overall survival	(119)
3	Laryngeal squamous cell carcinoma	Patients with positive CD9 expression showed higher median survival. Non-metastatic tumours had higher CD9 expression	(88)
4	Oesophageal squamous cell carcinoma	CD9 expression and metastasis showed an inverse correlation. The survival of patients with CD9-positive expression was higher compared with those with CD9-negative expression	(126)
5	Gingival squamous cell carcinoma	CD9 showed a strong correlation with cervical lymph node metastasis	(130)
6	Papillary thyroid microcarcinoma	CD9 expression was higher in patients with lymph node metastasis	(135)
7	Glioblastoma	CD9 expression was associated with the progression of a malignant tumour	(141)
8	Small cell lung carcinoma	CD9 expression was higher in the primary tumour and reduced in advanced stages of cancer	(144)
9	Gastro-intestinal stromal tumours	As CD9 expression decreased, the risk of metastasis and invasion increased	(145)
10	Colon cancer	The survival rate of CD9-positive tumours was higher than that for CD9-negative tumours	(143)
11	Breast cancer	Patients with negative CD9 expression had improved overall survival compared with patients with positive CD9 expression	(102)

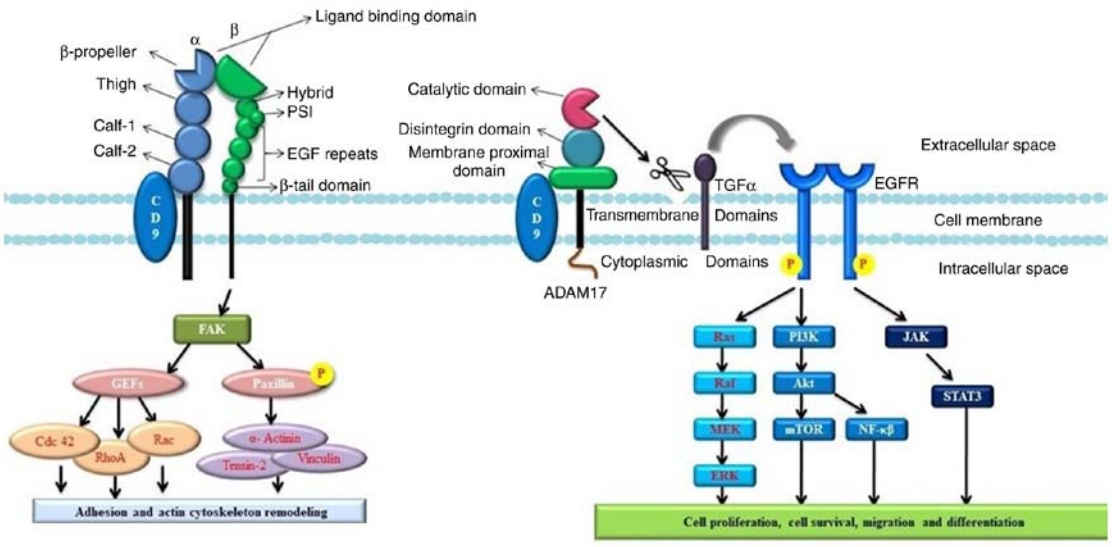

Figure 6. Interaction of CD9 with integrins, TGFα and EGFR. Interaction with these proteins may promote or inhibit cancer progression, migration and invasion. TGFα, transforming growth factor α; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; GEFs, guanidine exchanging factors; ADAM17, disintegrin and metalloprotease 17; PSI, plexin-semaphorin-integrin domain; P-, phosphorylated.

of the function and interaction of CD9 in various types of cancer that result in reduced cell motility may be of clinical importance.

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Authors' contributions

SPC wrote the manuscript, and was responsible for the original draft preparation, research and editing. SSS supervised, and wrote, reviewed and edited the manuscript. SKN supervised, validated the research, and wrote, reviewed and edited the manuscript. PKS and PP made revisions to the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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