Molecular genetics of head and neck squamous cell carcinoma: a review

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Abstract

Objective: The objective of this review article is to summarise the plethora of advances in knowledge of the molecular and genetic profiles of HNSCC with regard to risk factors, pathogenesis, clinical behaviour and novel therapeutic strategies for HNSCC.
Method: A review of the English medical literature on head and neck cancer was undertaken using the PubMed database.
Result: Several genomic lesions accompany the transition from normal epithelium, via precursor lesions, to full blown head and neck squamous cell carcinoma (HNSCC), in line with the multistep theory of carcinogenesis. These include inactivation of tumour suppressor genes such as P53, RB, P16 and p14 and activation of proto-oncogenes such as cyclin, EGFR and STAT genes. Molecular players in the genesis of HNSCC include epithelial-stromal reactions, tumour metastasis and micro-RNAs. Chemoprevention, gene therapy and targeted therapy in the management of HNSCC are also reviewed.
Conclusion: Molecular genetics has improved our understanding of events in early stages of HNSCC and has several potential applications including evaluation of surgical resection margins, assessment of risk factors and development of novel therapies with greater potency and less toxicity. It is anticipated that these advances shall feature prominently in the development of chemopreventive and cancer treatment regimens in the near future.

Key words: Molecular genetics, squamous cell carcinoma, head and neck

Introduction

Head and neck cancer is the sixth commonest cancer worldwide with an estimated 600,000 incident cases annually, including 300,000 oral cancers, 160,000 laryngeal cancers and 60,000 oropharyngeal cancers; and overall annual death rate of 300,000[1]. Head and neck cancers arise from the mucosa lining the oral cavity, oropharynx, hypopharynx, larynx, sinonasal tract and nasopharynx, the commonest histological type being squamous cell carcinoma[2].
Modern radiotherapy techniques and more potent chemotherapy agents have resulted in improved overall quality of life for patients with HNSCC. However, the 5-year survival rates of HNSCC have remained unchanged at less than 50%. This development has led both to emphasis on preventive health campaigns and advocacy for research on novel molecular methods aimed at early cancer detection and improved treatment outcome of HNSCC[3-5]. Recognition of early and late molecular genetic lesions in HNSCC tumour progression has afforded clinicians the opportunity to detect and treat pre-neoplastic lesions that appear morphologically normal but have latent tumour potential. The usual late presentation of head and neck cancers is potentially preventable with molecular screening techniques at early stages, which will also give greater chance for cure and better survival rate[6,7].
This review documents recognised distinct molecular and genetic profiles of HNSCC with regard to risk factors, pathogenesis, clinical behaviour and novel applications of molecular characterisation to improved therapeutic strategies for HNSCC.

Overview of genetic alterations in HNSCC

A plethora of chromosomal aberrations can be found in HNSCC and, as for other cancers, the number of aberrations increase steadily during cancer progression. Oral leukoplakia has fewer chromosomal aberrations than oral cancer and lower tumour stage (T1) is associated with fewer aberrations than higher tumour stage (T2)[8]. Phenotypic progression from pre-malignant progenitors to invasive malignancy is due to clonal proliferation of cells with cumulative genetic alterations[9].
Early genetic aberrations have been linked to important target genes like TP53 (17p13), RB1 (3q14) and CDKN2A (9p21). Loss of heterozygosity (LOH) or homozygous deletions in 3p, 9p, 13q and 17p in early lesions have also been documented[10-12]. Chromosome 3 frequently hosts allelic imbalance in several regions, especially 3p25, 3p21 and 3p1314. The 3p14 region encompasses the fragile site FRA3B and the FHIT gene and is probably one of the most vulnerable areas of the genome in many cancer types[13,14].
Aberrations that are associated with advanced tumour stage or poor differentiation include allelic losses in 5q21-22, 22q13, 4q, 11q, 18q and 21q. Gains in 3q are also common in advanced oral cancer[14].

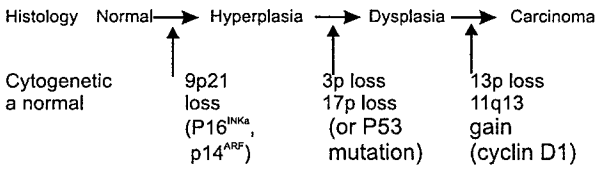

Figure 1. Genetic events in HNSCC development (tumour suppressor genes and oncogenes are in parenthesis)[9].

1. Loss of chromosome region 9p21-22 and P16/P14ARF: Loss of 9p21-22 (bearing the P16/P14ARF gene loci) is the commonest mutation in HNSCC occurring in 70% of cases. P16 (CDKN2/MTS1) and P14ARF produce 2 distinct transcripts at the same locus via alternative splicing and alternate reading frames. The p16 protein prohibits cell cycle entry by inhibiting cdk4 and cdk6, abrogating Rb phosphorylation and leading to G1 cell cycle arrest. P14ARF inhibits the association of P53 with its inhibitor MDM2, thereby also exerting an anti-proliferative effect in the wild-type form[6].
An alternative mechanism to P16/P14ARF induced inactivation of P53 is promoter hypermethylation[15]. Hypermethylation of CpG islands in promoter regions abrogates gene expression in the absence of sequence mutations in the genetic code.

2. Loss of chromosome regions 3p and 3q: Benign hyperplastic lesions and the earliest precancerous lesions exhibit loss at 3p[16]. RASSF1A is a tumour-suppressor gene on 3p21.3 that is inactivated via promoter hypermethylation in a large variety of head-and-neck cancers. FHIT is another putative tumour-suppressor gene that was cloned from 3p and shown to exhibit altered transcripts in aerodigestive tract cancers[17, 18]. The P40/p63 gene resides on 3q27-29 and a variant of p63, Np63, acts a positive regulator of the β-catenin signalling pathway, providing a basis for its oncogenic property[19].

3. Loss of chromosome region 17p13 and P53**:** A later event in head-and-neck cancer tumour progression is loss of 17p13, the locus of P53. P53 is one of the commonest mutated genes in all human malignancy and is implicated in up to 79% of HNSCC[20, 21]. P53 mutation frequency is directly proportional to histological grade. Its loss of function results in a transformation from a pre-invasive to an invasive phenotype as further genetic alterations are allowed to continue through the cell cycle unrepaired[21].

4. Amplification of 11q13 (cyclin D1): Amplification of 11q13 (the locus of cyclin D1, also known as PRAD1 and CCD1) occurs in one-third of HNSCC. The function of the proto-oncogene, is to activate Rb via phosphorylation, thereby facilitating progression from G1 (growth) to S (synthesis) phase[22]. Constitutive activation of this gene creates increased proliferation, which can contribute to tumourigenesis and has been associated with a worse prognosis[23,24].

Oncogenes in HNSCC

Epidermal Growth Factor Receptor (EGFR): EGFR is a tyrosine kinase receptor encoded on 7p. Increased activity of EGFR and its ligand Transforming growth factor alpha (TGF-α) is an early event in head and neck carcinogenesis. High-grade dysplastic oral pre-malignant lesions are associated with higher EGFR activity. Overexpression of EGFR occurs in 40 to 65% of HNSCC and higher levels of expression are associated with worse prognosis[25,26].

Phosphatidylinositol-3-kinase (PI3K): PI3K is located on chromosome 3q26.3 which is one of the commonest areas of gain in HNSCC. An important role of PI3K is the activation of Akt which is an anti-apoptotic serine/threonine kinase. PI3K mutation occurs in 10% of HNSCC[6,27].

Signal Transducers and Activators of Transcription (STATs): STATs are transcription factors that, upon becoming tyrosine-phosphorylated, dimerise and migrate to the nucleus where they mediate transcription of genes involved in cell proliferation, differentiation, and apoptosis. In HNSCC, STAT3 is activated downstream of TGF-a/EGFR as an early event in tumourigenesis. STAT activity may also result from tyrosine phosphorylation by Src kinase. High tumour levels of phosphorylated STAT3 have been associated with lower patient survival rates[27,28].

Src tyrosine kinase: Src encodes a non-receptor tyrosine kinase attached to the cytoplasmic aspect of the plasma membrane. Activated Src phosphorylates STAT molecules on tyrosine residues, inducing the formation of STAT homodimers and heterodimers. The dimers migrate to the nucleus to activate transcription of genes that mediate cell proliferation. Src-mediated disruption of the focal adhesion complexes, leading to increased cellular motility, is an early step in the process of cancer cell invasion. In studies with dasatinib, a small-molecule inhibitor of Src family kinases, inhibition of Src reduced the levels of phosphorylated FAK and paxillin. Synergism between Src and EGFR may contribute to a more aggressive phenotype in multiple human tumours, including HNSCC[29,30].

Tumour suppressor genes in head and neck cancers

P53**:** P53 is a nuclear phosphoprotein that is activated in response to DNA damage, in turn activating p21, with resulting cell cycle inhibition, permitting cells to repair the damaged DNA. P53 however triggers apoptosis when the DNA damage is severe[20].
In one study, the frequency of P53 mutation in HNSCC was 58% in those that both smoked and drank alcohol, 33% among smokers but non-drinkers and 17% among non-smokers and non-drinkers. For HNSCC patients without a history of tobacco or alcohol exposure, loss of p53 activity may result from human papilloma virus-16 (HPV-16) infection. As with other solid tumours, most (80%) mutations occur within exons 58. The importance of P53 in the biology of HNSCC is emphasised by a series demonstrating that, for patients with histological negative margins, the presence of P53 mutations at the surgical margin was associated with an increased risk of tumour recurrence. In a study of 44 patients with laryngeal cancer P53 mutation was associated with decreased survival.

Another review of HNSCC patients receiving induction chemotherapy, showed that P53 mutation were less likely to respond to chemotherapy[31].

Due to the central role of P53 in HNSCC biology, gene therapy strategies have been developed to exploit this target. For example, Onyx-015 is a replication competent adenovirus lacking the E1B gene, which inactivates cellular P53. Intratumoural injection of Onyx-015 leads to necrosis in P53 wild-type tumours. In phase 2 and 3 studies of Onyx-015, intratumoural injection of virus improved the efficacy of systemic chemotherapy in HNSCC, independent of tumour p53 status[21,32,33].

The CDKN2A Locus, p16 and p14: Loss of 9p21, containing the CDKN2A locus, occurs in 70% of HNSCCs. CDKN2A encodes for p16 and p14. The function of p16 is to inhibit the cyclin D1/cyclindependent kinase complex, thereby preventing inactivation of pRB via phosphorylation. Whereas active hypophosphorylated pRB blocks the onset of S phase by suppressing the programme of the E2F-1 transcription factor, inactive phosphorylated pRB is unable to block the G1 to S phase transition in the setting of p16 loss[34].

The normal function of p14 is to allow for induction of p53 in response to DNA damage. P53 activity is constrained by the negative regulator MDM2, but p14 inactivates MDM2. When the ability of p14 to constrain MDM2 is lost, the normal p53 damage response is also hindered. In a study of 148 surgically resected squamous cell carcinomas of the anterior tongue, 54% of patients were negative for p16 by immunohistochemistry, and lack of p16 staining was a significant predictor of shorter overall survival by multivariate analysis. In addition, there was p14 negativity in 20% and p14 negativity was associated with poor overall survival[15].

Phosphatase and Tensin Homologue: Phosphatase and tensin homologue (PTEN), encoded by a gene located at chromosome 10q23, functions as a tumour suppressor by counteracting the activity of PI3K. PTEN has many substrates, but its most important reaction is the dephosphorylation of PIP3 to PIP2. Absence of functional PTEN in cancer cells leads to constitutive activation of downstream components of the PI3K survival pathway, including Akt and mTOR kinases. Loss of PTEN activity by genetic deletion, somatic mutation, epigenetic alteration or altered transcription occurs in approximately 10% or fewer of HNSCC specimens[35].

Field cancerisation and surgical margins

Many techniques, including chromosome X inactivation, microsatellite analysis, and p53 mutational analysis have confirmed the presence of genetic alterations in the mucosa adjacent to primary HNSCCs. Most tumours arising in these abnormal fields are clonally related and originate from a common pre neoplastic progenitor. The transformed cells have a survival advantage and eventually displace or replace the surrounding mucosa through a process known as "clonal expansion"[36].

Califano et al[9] described a patient with multiple squamous cell carcinomas from the hypopharynx and distal oesophagus showing similar allelic loss separated by 40 cm[37].

A new classification of secondary HNSCCs was proposed arising after treatment of oral or oropharyngeal carcinoma based on the degree of clonal relation (**Figure 2**). They defined field cancerisation as mucosa with "the presence of one or more areas consisting of epithelial cells that have genetic alterations. A field lesion (or field) has a monoclonal origin, and does not show invasive growth or metastatic behaviour"[37,38].

These investigators proposed that tumours with similar genetic profile should be classified as "recurrence" or a metastasis depending on whether the second lesion occurs in the same or a distant anatomical site. The secondary lesion should be regarded as a "true" second primary tumour if the individual tumours show different genetic alterations. The third group consists of lesions with a common genetic origin but which diverge in later stages; therefore some show similar allelic imbalances and mutations but others show different genotypes. This latter group is classified as "second field tumours"[39].

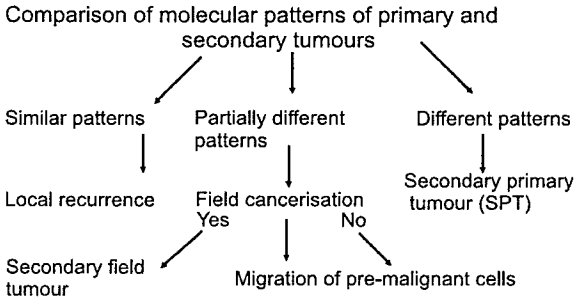

Figure 2. Proposed classification of Braakhuis et al second tumours after a primary HNSCC[37]

Molecular aspects of surgical margins: Several studies have investigated the presence and clinical implications of molecular alterations in seemingly tumour-free mucosa in the surgical margins of patients undergoing curative surgery for HNSCC. Detection of genotypically abnormal cells at the surgical margins of resection may be helpful in identifying individuals with a heightened risk of local recurrences after complete surgical resection. Apart from molecular studies, no other clinicopathological index was predictive of development of local or regional failure[4,5].

Molecular studies are also helpful in detecting small foci of abnormal mucosa overlooked by conventional histopathology assessment[40]. Data from several studies by Nathan et al[41] have suggested some prognostic value for overexpression of the proto-oncogene eIF4E in histologically negative margins of HNSCC; eIF4E is a eukaryotic protein synthesis initiation factor increased in almost all HNSCC cases. Using immunohistochemistry, it also showed statistically significant differences in local recurrence rates and disease-free intervals between eIF4E positive and eIF4E negative margins; patients with eIF4E-positive margins had a 6.5-fold risk of developing local recurrences[41]. Demonstration of eIF4E was reported to be a more sensitive prognostic indicator for local recurrences than p53 immunohistochemistry[41].

Viral Pathogenesis in HNSCC:

Human papillomavirus (HPV): The increased incidence of oropharyngeal squamous cell carcinoma despite the recent overall reduction in other squamous cell carcinomas of the head and neck has been attributed to the oncogenic

HPVs, especially HPV16 with reported prevalence in the oropharynx ranging from 19-72%. Patients with HPV positive HNSCCs are clinically distinct from their HPV negative counterparts being younger at age of presentation, less inclined to be heavy smokers and drinkers and highly curable with ionising radiation with or without chemotherapy. Furthermore there is a recent advocate for the use of HPV vaccination in the prevention of progression of HPV-related oropharyngeal cancers with probable vaccination of male as the high risk group in a similar fashion to female vaccination for cervical cancers[42, 43].

HPV-associated HNSCC arise mostly in palatine and lingual tonsils. HPV targets the highly specialised tonsillar crypt epithelium and once the virus DNA genome is integrated within the host cell nucleus, it deregulates the expression of oncoproteins E6 and E7. E6 induces deregulation of p53 through proteolysis with resulting loss of p53 activity; E7 binds and inactivates pRb causing the cell to enter S-phase leading to cell cycle disruption, proliferation and malignant transformation[38, 44].

Epstein Barr Virus (EBV): Nasopharynx carcinoma (NPC) is an EBV-associated malignancy. NPC is uncommon in the Western world, but is endemic in Southern China, other parts of Southeast Asia, and the Mediterranean basin. EBV is a double-stranded DNA herpes virus that infects B-lymphocytes and pharyngeal epithelium. Pre-invasive lesions of the nasopharynx harbour clonal EBV infections. In EBV-infected NPC, the viral gene expression patterns are consistent with latent infection. A prospective study of 99 patients with locoregional advanced NPC demonstrated that pre-treatment plasma EBV DNA levels increase with TNM stage, and higher pre-treatment EBV DNA levels are associated with poor prognosis[40, 45].

Tumour microenvironment in HNSCC:

Over 100 years ago, Paget hypothesised that the "soil" was as important to the development of tumours as the tumour "seed" itself. The stromal components involved in tumour genesis include macrophages, fibroblasts, and some of the molecules involved in the communication between the microenvironment and the cancer cells[46].

The stromal microenvironment of HNSCC consists of fibroblasts, adipocytes, macrophages, mast cells, vascular components, and inflammatory cells of the innate and acquired immune system, as well as the extracellular matrix (ECM). All of these components communicate with each other and with the neoplastic cells to contribute to the aberrant tumour organ including epithelial tumour. Although the epithelium in carcinomas certainly is mutated, the events that promote tumour progression involve the stroma also[47].

Although normal stroma may protect the epithelium from tumourigenesis, aberrant stroma can initiate tumourigenesis. In most cases the stroma is "activated," or tumour promoting, but genotypically normal; however, tumour-suppressor gene mutations within the stroma can be found[48, 49].

Tumour-associated macrophages (TAMs): Much evidence points to macrophages as being active co-conspirators in cancer progression through responses to micro environment changes that modify macrophage abilities and functions. A high density of TAMs correlates with poor prognosis and reduced survival in a number of different cancers (e.g., breast, prostate, endometrial, bladder, kidney, oesophageal, squamous cell carcinoma, malignant uveal melanoma and follicular lymphoma)[50].

Macrophages regulate tumour angiogenesis, in part by producing vascular endothelial growth factor (VEGF) and then mobilising it through the production of proteolytic enzymes. Also, macrophages and related myeloid cells may prepare a niche in the distant sites that facilitate the metastatic growth of the disseminated cells[51, 52].

TAMs are recruited to the tumours through cytokines and chemokines secreted by the cancer cells. Unlike macrophages in healthy tissue or wound-healing environment, TAMs are modified in the context of the tumour microenvironment and lose the ability to phagocytose cancer cells or present tumour antigens to T cells. Through the secretion of factors, including growth factors and proteases, macrophages promote cancer cell proliferation, survival, motility, and growth. Thus, the macrophages contribute to tumour progression at several stages, from the chronic inflammatory response and tumour initiation, matrix remodelling, angiogenesis, tumour invasion, and intravasation to metastasis[53].

Macrophages may actually create a mutagenic environment through the secretion of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS are known to cause lesions in DNA, RNA, proteins, and membranes through their free-radical intermediates, and these defects can drive carcinogenesis[54].

Carcinoma associated fibroblasts: The reactive fibroblasts that arise during neoplasia are referred to as carcinoma-associated fibroblasts (CAFs). CAFs differ from normal fibroblasts by having an abnormally high expression of smooth muscle actin and increased expression of proteolytic enzymes and ECM proteins, such as tenascin-C[55].

The effects of CAF on epithelial carcinogenesis are achieved through fibroblast secreted protein (FSP1, also called S100A4, metastasin or mts1), which is expressed in CAFs, carcinoma cells, and macrophages during tumour progression. FSP1 is a calcium-binding protein with intracellular and extracellular protein-binding partners. In the cell, it interacts with and possibly inactivates p53. FSP1 also interacts with non-muscle myosin heavy chain, actin filaments, and non-muscle tropomyosin, thereby potentially influencing the cytoskeleton and regulating cell motility. FSP1 is pro-angiogenic, and this may be mediated by plasminogen activation or up-regulation of matrix metalloproteinase MMP 13, which may facilitate endothelial cell invasion. FSP1 is also up-regulated in metastatic carcinoma cells, perhaps as a result of epithelial mesenchymal transformation (EMT), which gives the carcinoma cells a fibroblastic phenotype. EMT has been proposed to be the mechanism responsible for the metastatic phenotype induced by FSP1[56].

TGF-β is one of the key players involved in the communications between CAFs and carcinoma cells, but again TGF-β is expressed by multiple cell types, including the stromal fibroblasts, the inflammatory cells, and carcinoma cells. TGF-b acting on the fibroblasts normally protects the epithelium from developing into carcinomas, whereas TGF-b secreted by CAFs and acting on the epithelium promotes carcinogenesis[57, 58].

Epithelial mesenchymal transformation (EMT): This is the process by which epithelial cells undergo a profound change in their differentiation programme and acquire many of the phenotypes of mesenchymal cells, including motility and invasiveness. Transcription factors (such as Snail, Slug, Twist, Goosecoid, and SIP-1) acting during various stages of early embryogenesis are capable of

programming EMTs. Each of these factors is able to act pleiotropically to programme an EMT, and thereby is able to cause the repression of epithelial genes and the induction, in their stead, of mesenchymal genes[59].
Carcinoma cells exploit these early embryonic genes to execute most of the steps of the invasionmetastasis cascade. Expression of these embryonic genes seems to be induced by contextual signals that these carcinoma cells experience in the tumour microenvironment and that originate in the tumour-associated stroma. For example, TGF-β impinging on certain cancer cells is able to elicit the expression of several of the transcription factors that are capable, in turn, of programming an EMT.
Once carcinoma cells have disseminated and landed in distant tissue sites, they no longer encounter the mix of signals that were released by the activated stroma of the primary tumour and that led initially to their passing through an EMT. This new tissue microenvironment may therefore allow these cells to revert, via a mesenchymalepithelial transition (MET) to the epithelial phenotype of their progenitors in the primary tumour, thereby generating once again epithelial histomorphology[60].

Matrix metalloproteins (MMPs): Proteolytic enzymes of many classes have been implicated in tumour cell invasion, including serine proteinases plasmin, plasminogen activator, seprase, hepsin; several kallikreins; cathepsin-B; cathepsin-D; and metal dependent proteinases of the matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase (ADAM) families. Other matrix-degrading enzymes such as heparanase, which cleaves heparin sulphate proteoglycans, and hyaluronidase cleavage of its substrate hyaluronic acid have also been causally associated with tumour progression and invasion[61]. Because of the putative roles of MMP-2, MMP-9 and MMP-13 in HNSC invasiveness, using the expression of these genes as markers of tumour progression may be useful in disease prognostication and cancer treatment. Experimental treatments include chelators that bind zinc ions required for MMP function, MMP signalling pathway inhibitors and MMP antibodies; but these strategies are far from clinical application[62].

Tumour metastasis and metastasis suppressor genes in HNSCC

Metastasis is the dissemination of neoplastic cells to non-contiguous nearby or distant secondary sites where they proliferate to form a mass and is the most significant contributor to cancer-related morbidity and mortality[63]. In the past few decades, interest has grown in the field of metastasis suppressor genes (MSGs), which are functionally defined by their ability to suppress in vivo development of metastases without affecting the growth of the primary tumour[64,65].
NM23 (Non metastatic gene 23) was first identified in 1988 based on its reduced expression in highly metastatic as compared to poorly metastatic cells[64-66]. Metastasis suppression by NM23 involves three mechanisms: demethylation of specific CPG islands, reduced motility of tumour cells and downregulation of MMP2 to inhibit metastasis. Expression of NM23-H1 homologue is associated with decreased lymph node metastasis, while reduced NM23-H1 expression is associated with increased cisplatin resistance in HNSCC patients[67,68].

Role of microRNAs in HNSCC

MicroRNAs (miRNAs), are a group of small non-coding RNAs and genetic regulators that were identified in the late 1990s and 2000s. After transcription, primary miRNAs (pri-mirs) are processed by double-stranded ribonuclease (Drosha) to yield approximately 70-nucleotide hairpin-shaped precursor miRNAs (premirs) in the nucleus. Premirs are then exported to cytosol and further cleaved by ribonuclease (Dicer) into approximately 22-nucleotide mature miRNA duplexes[69].
The seed sequence of a miRNA binds to its target sequence in the 3'untranslated region (3'UTR) of the target gene via base pairing to form an RNA-induced silencing complex (RISC) that triggers an RNA interference mechanism; this can involve mRNA cleavage, translational repression or de-adenylation of the targeted mRNA. One miRNA is able to target several mRNAs and one mRNA can be regulated by several miRNAs. Various sets of miRNAs are expressed in different cell types. Thus, a miRNA may play several roles in the modulation of a range of biological or pathological processes[70]. Aberrant miRNAs may contribute to the multistep HNSCC carcinogenesis process, either as oncogenic miRNAs or tumour suppressor miRNAs[71].

Oncogenic microRNAs in HNSCC: miR-21 is the most frequently upregulated oncogenic miRNA in HNSCC and is a marker for poor prognosis in HNSCC and other human malignancies, including gastric cancer, colorectal cancer and lung cancer[71,72]. miR-21 plays critical roles in tumour cell survival, invasion, metastasis and drug resistance.
Several important tumour suppressor genes, including pTEN and TGFβ, are known targets of miR-21. In addition plasma miR-21 levels are upregulated in HNSCC patients and post-surgical reduction of plasma miR-21 level is an indicator of good prognosis[73].
miR-31 is another important oncogenic miRNA that is significantly upregulated in HNSCC. miR-31 targets the 3'UTR of factor-inhibiting hypoxia-inducible factor (FIH), a hypoxia-inducible factor (HIF) regulatory enzyme that inhibits the ability of HIF to act as a transcriptional regulator under normal oxygen conditions. By impeding FIH expression, miR-31 activates HIF and its downstream elements, thus contributing to the development of HNSCC. An increase in the level of miR-31 has been identified in the plasma and saliva of OSCC patients compared with control individuals, and this increase was significant among early-stage patients[74]. miR-31 levels in plasma and saliva have been found to be decreased after tumour resection, and therefore, secreted miR-31 is being considered a potential marker for HNSCC surveillance[75].
miR-210 is a well-known miRNA that is upregulated by hypoxia. miR-210 upregulation has been associated with locoregional recurrence and a reduced HNSCC survival[76].
Other potential oncogenic miRNAs that are located within frequently amplified chromosome regions have been reported. For example, miR-30b is found at chromosome 8q24. Genomic analysis has revealed that this region is frequently amplified in HNSCC tissues. In addition, it has been reported that gene amplification affects various other oncogenic miRNAs, including miR-29a and c, miR-30b and miR-1403p[77,78].

Tumour suppressor microRNAs in HNSCC: The let-7 miRNA family is an important miRNA cluster that includes Let-7a-j, miR-98 and other members. They are expressed abundantly and drive numerous processes, including the regulation of cell renewal, mobility, the EMT properties and miRNA processing[79].
Inhibition of DICER, high-mobility group (AT-hook 2) and

Ras expression by let-7 has been reported in a wide variety of malignancies including HNSCC. Twist1 is an important EMT driver; it represses let-7i, and then in turn upregulates neural precursor cell expressed, developmentally downregulated 9 (NEDD9) and dedicator of cytokinesis 3 (DOCK3), which leads to Rac activation and the initiation of HNSCC carcinogenesis. Co expression of Twist1, NEDD9 and DOCK3 leads to a worse disease-free survival among patients. A combined expression profile wherein there is upregulation of miR-205 and downregulation of let-7d has been found to define a poor prognosis for HNSCC[80].

The miR-99 family, including miR-99 and miR-100, are tumour suppressors. Activation of the mTOR signalling pathway is crucial for HNSCC progression, and miR-99 and miR-218 target mTOR and inhibit AKT phosphorylation. miR-133a is another important HNSCC suppressor, It downregulates caveolin-1 (CAV1) and glutathione s-transferase p1 (GSTP1), which enhances apoptosis and reduces the migration of HNSCC cells. miR-133a also suppresses actin-related protein 2/3 complex subunit 5 (ARPC5) and moesin (MSN), which are important regulators that contribute to cell migration and invasion[81-83].

The miRNA (miR-375) is a tumour suppressor that is significantly downregulated in HNSCC and has been shown to inhibit cell proliferation and to induce apoptosis by targeting the oncogene AEG-1/MTDH in SAS and Fadu HNSCC cell lines. miR-375 downregulation is associated with metastasis and poor prognosis in HNSCC; miR-375 also inhibits cell invasion in other HNSCC cell lines. The expression ratio of upregulated miR-221 and downregulated miR-375 has been shown to have a strong predictive power for HNSCC[84-86].

MicroRNAs associated with cancer stem cells (CSCs) and EMT in HNSCC: Both the CSCs and cancer cells exhibiting EMT characteristics cause tumour progression and resistance to therapy in HNSCC. Evidence of crosstalk between CSCs and EMT has been demonstrated in the tumour genesis. Twist1, Snail, BMI1 and ZEB are also important EMT regulators, and E-cadherin, vimentin and N-cadherin are known to be EMT markers[87].

MicroRNAs in head and neck premalignant disorders: Overexpression of miR-21, miR-345, miR-181b, miR-184, miR-520g, miR-649, miR-518b and miR-146a has been documented in progressive dysplasia and OSCC tissues. Expression of miR-21, miR-181b and miR-345 was found to positively correlated with increasing severity of dysplasia during progression to OSCC[88].

MicroRNAs as predictive HNSCC biomarkers: miRNAs may help with the development of a range of platforms, either custom-made platforms containing specific candidate miRNAs or commercialized platforms containing the global range of human miRNAs. The latter would allow a comprehensive annotation of an individual's miRNA profile that could then be used for general disease diagnosis.

Compared with mRNA, miRNA is much more stable during long-term storage. This advantage further increases the feasibility of high throughput miRNA analysis using tumour tissue. As a small molecule, the release of miRNA into the extracellular environment, which includes plasma, saliva and other body fluids, is relatively easy. miRNAs are often bound to RNA-binding proteins such as Argonaute2 (Ago2) or are entrapped in vesicles such as exosomes. Both of these events help to maintain the stability of miRNAs in the circulating system[89].

Changes in the level of tumour-associated miRNAs in the circulation have been identified in HNSCC patients. High throughput analysis performed on the serum or plasma from patients could easily be a non-invasive and convenient procedure for biomarker validation. Saliva samples from OSCC patients may yield even more useful information than plasma samples because of closer proximity of saliva to the head-and-neck lesion. In this context, changes in tumour-associated miRNA levels have been detected in saliva or oral rinse, both collected by non-invasive procedures[73,74].

Molecular targeted therapy in HNSCC

Molecular targeted therapy uses agent(s) that act with a high degree of specificity on a well-defined target or biological pathway that drives the cancer phenotype, with minimal harm to normal cells. Good molecular targets for cancer therapy are characterised by self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replication potential, sustained angiogenesis, and tissue invasion and metastasis[90]. The target should also show minimal redundancy, meaning that its inhibition gives a major change in the cancer phenotype being measured, without other pathways taking over[91].

Personalized medicine or individualized treatment is the model of the way medicine will evolve through the use of specific treatments and therapeutics best suited for an individual's genotype. Cancer therapy presents a unique example of personalized medicine where individual's genotype is important, because it determines the therapeutic and toxic response to a drug (the pharmaco-genotype) and the genotype of the cancer cell is also important, since it determines the response to therapy[92].

The protein products of oncogenes, tumour-suppressor genes, regulatory genes, repair genes, and stress signalling genes are all potential cancer drug targets. DNA microarray analysis of genome-wide gene expression patterns are widely used in drug target discovery and provide leads to potential clinical applications[93].

EGFR genes are upregulated in head-and-neck cancer and may provide a target for molecular treatment. Uno et al showed that the HER2/neu monoclonal antibody (rhumAbHER2) was effective at inhibiting growth of oral squamous cell carcinoma cell lines. Additionally, IMC-225, a humanmouse chimerised monoclonal antibody directed against EGFR, has undergone a phase 1b clinical trial in HNSC. In conjunction with cisplatin, IMC-225 demonstrated high levels of EGFR saturation; 67% of patients showed a partial response, while 22% demonstrated a complete response[94,95].

Gene therapy in HNSCC

Gene therapy is another molecular directed approach that attempts to replace a gene and its function. Such therapy centres on the frequently mutated tumour-suppressor gene P53. ONYX-015 is an adenovirus that lacks the E1B 55K gene. This gene encodes a protein that binds p53 and targets it for degradation. In the absence of E1B 55K, ONYX-015 was expected to replicate poorly in normal cells, in which functional p53 could abort lytic productive replication. In contrast, cancer cells are permissive for ONYX-015 since E1B 55K is unnecessary in cells that lack p53[96].

Replicating ONYX-015 virus was found in head and neck cancer following intratumoural injection. Meanwhile, a closely related adenovirus, H101, was approved for treatment of head and neck cancer in China after a clinical phase 3 study demonstrated a tumour response rate of 78.8% in patients who had received H101 in combination with cisplatin and 5-fluorouracil[97].

Chemoprevention of HNSCC

Chemoprevention was first proposed by early workers who looked at the protective effects of vitamin A and other retinoid derivatives on tumour genesis[98]. The mechanism of action is the binding of retinoic acid receptor molecules which then function as angiogenic and growth-regulating factors. One study showed that high-dose 13-cis-retinoic acid in patients with known HNSCC decreased the rate of development of second primary tumours and successfully treated oral leukoplakia. Furthermore, when therapy was discontinued, risk of disease development reverted to baseline after 2 years of non treatment[99,100].

Cox 1 and 2 inhibitors are also chemopreventive agents via their mediation of prostaglandins and inflammation. In head-and-neck cancer, prostaglandin levels are elevated, presumably due to upregulation of Cox1 and Cox2. One possible mechanism for the efficacy of non-steroidal anti-inflammatory drugs is their telomerase inhibiting properties as HNSC cell lines treated with indomethacin and ibuprofen caused dose-dependent reduction of cell numbers and telomerase activity[101].

Conclusion

Molecular genetic analysis allows for better understanding of events in early stages of HNSCC and the potential applications to evaluation of the risk of tumour recurrence following apparent adequate surgical resection. Molecular genetic differences also allow for recognition of subtypes of HPV-positive and negative HNSCC with differing risk factors and therapeutic options. Additionally, the increasingly refined knowledge of HNSCC molecular genetics is paving the way to development of novel therapies with greater potency and less toxicity. Knowledge of miRNA contribution to biological pathways involved in cancer such as cell cycle, oncogenesis, tumour suppressor pathways, apoptosis, epithelial-mesenchymal transition and metastasis is likely to be important in the management of patients with or at risk of HNSCC. Novel biological agents that target oncogenic signalling pathways are anticipated to feature prominently in the development of chemopreventive and cancer treatment regimens in the near future.

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