Oral Diseases (2009) 15, 388–399. doi:10.1111/j.1601-0825.2009.01563.x

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REVIEW ARTICLE

Oral squamous cell carcinoma: overview of current understanding of aetiopathogenesis and clinical implications

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MEDLINE contains well over 14 000 papers revealed by a search using keywords 'oral squamous cell cancer' or 'oral squamous cell carcinoma', over 27 000 using 'oral carcinoma' and over 48 000 using the keywords 'oral cancer'. It is difficult to see how clinicians could keep abreast of such a subject. This paper attempts to help by providing an overview of the aetiopathogenesis of oral squamous cell carcinoma (OSCC), discussing changes in epidemiology and increasing awareness of the wide range of risk factors, emphasising the genetic background to cancer susceptibility and the genetic changes associated with progression to OSCC and highlighting clinical implications.
Oral Diseases (2009) **15**, 388–399

Keywords: oral cancer; tobacco; betel; alcohol; diet; viruses; genetics

Introduction

Most oral cancer is oral squamous cell carcinoma (OSCC) – a disease found particularly in low income communities and mainly a problem of older men, 90% being in the over 45-year-age group who are exposed to the known risk factors of tobacco and/or alcohol (IARC 2004). Clinically, OSCC includes lip cancer, which accounts for the majority of OSCC and intra-oral cancer, which mainly affects the tongue (Table 1).

Oral squamous cell carcinoma is the eighth most common cancer world-wide but parts of Northern France and East Europe, particularly Hungary, and parts of South America and South East Asia have particularly high prevalences (Moore *et al*, 1999, 2000a,b).

Lip cancer is particularly a problem of older people and is especially a disease of males and particularly a lesion of the lower lip, which is largely related to the exposure to ultra violet irradiation from the sun. It is particularly seen in people who are exposed on a prolonged, or a repeated basis, to sunlight, and that includes fishermen, farmers, skiers and windsurfers. It is found mainly in Caucasians particularly in hot climates. There is a very good survival – up to 95% 5-year survival – probably related to the early diagnosis of a lesion, which is clinically very obvious (Scully and Moles, 2008).

Cancer within the mouth affects mainly the tongue, particularly the lateral border, especially posteriorly, in older people, in males, and often related to life-style habits, which are largely tobacco- or alcohol-related (Scully and Moles, 2008). It is a particular problem in some black and ethnic minority populations (Scully and Bedi, 2000). With around a 40% 5-year survival, it is quite different from lip cancer, with about half as good a prognosis.

There are changing patterns in both lip and intra-oral cancer. There has been a decrease in male incidence of lip cancer over about a 30-year period, but several studies show an increase in tongue cancer, particularly in younger patients, currently attributed to smoking and binge drinking amongst younger people. In males in East and Central Europe, there has been a rise in mortality rates since the 1980s along with an increase in tobacco use, and the rise is the largest for any of the common neoplasms (Bray *et al*, 2002; La Vecchia *et al*, 2004). In Western European males, there has also been a rise, but in some countries where lung cancer has decreased which suggests that it is not in those populations related so much to the classical risk factor of tobacco as to alcohol. In females, there has been a slight increase associated with more alcohol and tobacco use. OSCC remains a lethal disease for over 50% of cases diagnosed annually (Warnakulasuriya, 2008).

Aetiopathogenesis

The aetiopathogenesis of potentially malignant oral disorders and OSCC has been reviewed a number of times this millennium (e.g., Scully *et al*, 2000a,b,c; Patel *et al*, 2001; Reibel, 2003; Brinkman and Wong, 2006; Mithani *et al*, 2007; Haddad and Shin, 2008).

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Received 9 March 2009; accepted 10 March 2009

Table 1 International Classification of Diseases; oral cancer

	ICD-9	*ICD-10*
Lip	140	C00
Tongue	141	C01–02
Gum	143	C03
Floor of mouth	144	C04
Other and unspecified mouth	145	C05–06
Salivary gland	142	C07–08
Oro-, naso-, and hypo-pharynx	146–149	C09–14
Other and ill-defined sites of lip, oral cavity and pharynx		

The cell of origin of OSCC is the oral keratinocyte, in which DNA mutation can be spontaneous, but mutagens increase the mutation rate. Genetic variation in the xenobiotic metabolising enzymes (XME), which influence carcinogen (cancer-causing chemical) metabolism, DNA repair mechanisms and other protective mechanisms may well help explaining differing susceptibilities to the OSCC – causing effects of the risk factors such as tobacco and alcohol (see below, and Scully *et al*, 2000a,b,c).

Risk factors

The various risk factors for OSCC appear mainly to act by increasing the rate of mutations. Lifestyle factors, especially tobacco and alcohol, appear particularly important but, in some cases, betel, sunlight exposure, ionising radiation, human papillomavirus (HPV) or other infections or immuno-incompetence are relevant (Scully and Moles, 2008). Genetics such as single nucleotide polymorphisms (SNPs) may also influence the risks, as discussed below.

Tobacco

Tobacco is by far the main risk factor for OSCC (Vallecillo Capilla *et al*, 2007; Hirota *et al*, 2008), and this applies not only to smoked but also to smokeless tobacco (Johnson, 2001; Warnakulasuriya and Ralhan, 2007), although some have suggested a somewhat lesser risk from the latter (Vigneswaran *et al*, 1995; Boffetta *et al*, 2008). Tobacco use generates carcinogens such as the TSNAs, tobacco-specific nitrosamines [N′-nitrosonornicotine,4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] as well as free radicals, resulting in alterations in the antioxidant enzymes glutathione-*S*-transferase (GST), glutathione reductase, superoxide dismutase, catalase and glutathione peroxidase, as well as lipid peroxidation and total thiol, although tobacco products vary widely in their potential for producing carcinogens (Brunnemann *et al*, 1996; Hoffmann and Hoffmann, 1997).

There is about a 20-fold risk of OSCC with heavier tobacco smokers and a strong dose–response relationship, and the risk increases with the number of cigarettes smoked per day and the duration of smoking. Tobacco in other forms is also a risk factor: for example, data from 282 incident oral cancer cases and 1410 matched controls in Trivandrum, India analysed using multivariate conditional logistic regression models confirmed tobacco chewing to be the strongest risk factor associated with OSCC. Effects of chewing pan (betel) with or without tobacco on OSCC risk were elevated for both genders. Bidi smoking increased the risk of OSCC in men. Dose–response relations were observed for the frequency and duration of tobacco chewing and alcohol drinking, as well as in duration of bidi smoking (Muwonge *et al*, 2008).

Betel

There is also a relationship of OSCC with betel (areca nut) use, a habit of something like 20% of the world's population (Cogliano *et al*, 2004). The IARC long ago declared that betel was carcinogenic to humans, and that has been confirmed (Merchant *et al*, 2000; Jacob *et al*, 2004; Carpenter *et al*, 2005; Guh *et al*, 2007; Thomas *et al*, 2007, 2008; Reichart and Nguyen, 2008). Gene expression may be altered and arecoline, a main alkaloid in the areca nut (the main component of betel quid) may, by hypermethylation, block tumour suppressor genes (TSGs) p14, p15 and p16, and it inhibits the p53 TSG, represses DNA repair and triggers DNA damage responses in human epithelial cells (Chen *et al*, 2008; Park *et al*, 2008; Takeshima *et al*, 2008; Tsai *et al*, 2008; Cheong *et al*, 2009). Similar chewing habits – such as khat use – may also be implicated in OSCC in some communities (Fasanmade *et al*, 2007; Sawair *et al*, 2007). The carcinogenicity of products such as marijhuana is more controversial in the aetiopathogenesis of OSCC (Hashibe *et al*, 2005).

Alcohol

Alcohol (ethanol) is oxidised to acetaldehyde – a suspected carcinogen – (Boccia *et al*, 2009a) by alcohol dehydrogenases (ADHs), and acetaldehyde is then degraded to acetate (non-carcinogenic) by aldehyde dehydrogenases (ALDH). Alcohol produces about a fivefold risk for heavy drinkers, and there is a strong dose–response relationship between alcohol drinking and alcohol consumption (Pelucchi *et al*, 2008). Use of alcohol-containing mouthwashes is more controversial in the aetiopathogenesis of OSCC (McCullough and Farah, 2008; La Vecchia, 2009).

There is a greater than joint multiplicative risk for OSCC in people who are both alcohol drinkers and heavy tobacco smokers (Hashibe *et al*, 2009). Smoking increases the acetaldehyde burden following alcohol consumption, and alcohol-drinking enhances the activation of pro-carcinogens present in tobacco due to increased metabolic activation, by an induced cytochrome P450-2E1-dependent microsomal biotransformation system in the mucosa and the liver (Seitz and Cho, 2009).

Other factors

In one study of OSCC in young adults, tobacco accounted for 77%, alcohol for 52%, low vegetable consumption for 52% and all three combined for 85% of OSCC (Rodriguez *et al*, 2004). Worldwide, 25% oral cancers are attributable to tobacco usage (smoking

and/or chewing), 7–19% to alcohol drinking, 10–15% to micronutrient deficiency and more than 50% to betel quid chewing in areas of high chewing prevalence (Petti, 2008). These behaviours are widespread: 2 billion consume alcohol; 1 billion men and 250 million women smoke cigarettes; 600–1200 million people chew betel quid and an unbalanced diet is common amongst both developing and developed countries (Petti, 2008). However, a substantial proportion of OSCC cannot be attributed to tobacco or alcohol use, particularly among women and among young-onset cases (Llewellyn *et al*, 2001; Hashibe *et al*, 2009) and, in these, other factors, such as micro-organisms may thus be at play.

Viruses

There is a relationship with HPV and OSCC, particularly oropharyngeal carcinoma (D'Souza *et al*, 2007). A number of studies have shown DNA from HPV in OSCC, especially in oropharyngeal carcinoma. OSCC is also increased in people who have a high number of sexual partners and if they start intercourse at a young age. OSCC is increased in females who have cervical carcinoma which is related itself to HPV, and it is also increased in their partners. The possibility of sexual transmission has thus been raised (Scully, 2002, 2005). An interesting anecdotal report showed a couple-husband and wife diagnosed synchronously with OSCC: their tumours were positive for HPV16 by polymerase chain reaction (PCR) and both viral genomes were identical and closely related to the revised European prototype, HPV16R (Haddad *et al*, 2008). These tumours probably represented transmission between the couple (Haddad *et al*, 2008). Some tumours are associated with HPV and some with viruses of the herpes family, although the exact role of these viruses needs careful evaluation (Shillitoe, 2008).

Oral health

The dentition may also play a role in OSCC (Zheng *et al*, 1990). Head and neck and oesophageal dysplasia/cancer has been found associated with dental neglect (Dye *et al*, 2007; Guha *et al*, 2007; Abnet *et al*, 2008), and OSCC is less likely in people who have received dental care (Holmes *et al*, 2008). Periodontal disease or tooth loss has been implicated in OSCC (Meyer *et al*, 2008), each millimetre of alveolar bone loss being associated with a 5.23-fold increase in the risk of tongue cancer (Tezal *et al*, 2007). Possible mechanisms revolve around microbial interactions with dental bacterial plaque: polymicrobial supragingival plaque has a mutagenic interaction with saliva (Bloching *et al*, 2007), and both oral streptococci (Kurkivuori *et al*, 2007) and neisseria (Muto *et al*, 2000) may synthesise acetaldehyde from alcohol.

Host defences

Other factors implicated in OSCC may act by undermining host defences against carcinogens, or repair or defence mechanisms. These include genetic, immune and dietary defects, drugs and deprivation.

Single nucleotide polymorphisms in various genes have been implicated: these are discussed below.

Conditions associated with an increased risk of OSCC include organ transplantation, Fanconi anaemia, dyskeratosis congenita and more recently diabetes and scleroderma (Dikshit *et al*, 2006; Goutzanis *et al*, 2007). Factors that are more controversial include HIV/AIDS and various inherited cancer syndromes (e.g., Li-Fraumeni syndrome).

Diet

Diet may play a role in OSCC development as evidenced by multiple epidemiological studies worldwide (Pavia *et al*, 2006). Apart from the dietary risk factors for OSCC such as alcohol and other factors (Zain, 2001), the most consistent dietary findings across multiple cultural settings are a protective effect of high fruit consumption (Winn, 1995) and high vegetable consumption (Lucenteforte *et al*, 2008), especially yellow/orange vegetables (Sapkota *et al*, 2008), and diets varied in vegetables and fruit (Garavello *et al*, 2008). Dietary antioxidants from fruit and vegetables may be protective (Suzuki *et al*, 2006). Folate in particular may be protective (Pelucchi *et al*, 2003) whilst, in contrast, mild iron deficiency and low glutathione (GSH) levels which are associated with increased oxidative stress appear to increase the risk of OSCC (Richie *et al*, 2008). Although fruits and vegetables can be protective against OSCC (Pavia *et al*, 2006), probably via folate and dietary antioxidants (carotene, carotenoids, flavonoids, flavanones, vitamins A, C and E and phytosterols) (Rossi *et al*, 2007), preserved vegetables can be a risk factor (Sapkota *et al*, 2008). Even in the presence of high alcohol consumption or tobacco use, a high intake of fruit and vegetables might prevent the development of around one quarter of cases of SCC in the head and neck (Boccia *et al*, 2008) and possibly one half OSCC (Pavia *et al*, 2006). The risk of oral potentially malignant disorders is also significantly reduced with higher consumption of fruits, particularly citrus fruits and juices (Maserejian *et al*, 2006). Case–control studies indicate that vitamins C, E, A and carotenoids in food may decrease the risk of oral potentially malignant disorders and OSCC, but clinical trials of vitamin supplements have failed to find protective effects of beta-carotene and suggest indeed, that beta carotene and vitamin E may increase the risk (Maserejian *et al*, 2007). This is discussed further, elsewhere (Scully, 1995; Scheer *et al*, 2004; Brown and Kane, 2006).

Potentially malignant oral disorders is the term adopted at a recent WHO workshop to cover the most common ones (leukoplakia and erythroplakia) that may transform to OSCC, and others such as lichen planus, oral submucous fibrosis, actinic cheilitis (van der Waal, 2008).

Carcinogenesis

DNA mutations affect a number of genes, disrupting growth control. Multiple genetic and epigenetic events include the aberrant expression and function of

molecules regulating cell signalling, growth, survival, motility, angiogenesis and cell cycle control, and underlie the progressive acquisition of a malignant phenotype by the keratinocyte which progresses via a series of genetic change steps to a premalignant or a potentially malignant cell – characterised by an ability to proliferate in an uncontrolled fashion – that may result in cancer, characterised by invasion across the epithelial basement membrane and eventual metastasis. Changes in over one hundred genes have now been implicated (Roepman *et al*, 2005). The cell cycle is disturbed particularly by various oncogenes and their over-expression or over-activity (amplification), which will drive cell proliferation.

Working in the opposite more protective direction are the TSGs: for example, the more important TSGs are p16 which acts as a checkpoint in growth control and p53, which will either repair a potentially malignant cell or it will destroy it by apoptosis (see below). Protective mechanisms include TSGs and the liver – protective particularly because of its ability to metabolise carcinogens. The carcinogen metabolising enzymes vary from person to person on a genetic basis. There are also a whole series DNA repair enzymes, which can repair the mutations and, again, there are differences genetically between individuals.

The fundamental and simplified concept of the genetic basis behind cancer is the over-expression of oncogenes and/or the silencing of TSGs. Genetic analysis now involves a detailed high-resolution mapping of regions of chromosomal gain, loss and translocation using techniques such as comparative genomic hybridization and fluorescent *in-situ* hybridization. PCR-based techniques can identify if there is loss of genetic material, represented by complete deletion, or loss, of one allele (known as loss of heterozygosity or LOH), and it is evident that there is a relatively common pattern of DNA allelic loss as one progress from the premalignant to malignant stage. Presumably, if a TSG is in the area of allelic loss, this would make the host more susceptible to dysfunction of the gene, leading to the development of cancer. The common regions of chromosomal loss reported in OSCC prove to be at 1p, 3p, 4p, 5q, 8p, 10p, 11q, 13q and 18q, with gains at 1q, 3q, 5p, 7q, 8q, 9q, 11q, 12p, 14q and 15q. Microarray technology involves the miniaturisation of DNA sequence hybridization onto microscopic surfaces, which can then be read by laser, able to detect and interpret signals from minute fluorophores. Microarray technology has facilitated the ability to produce huge amounts of data from nearly the entire human genome, and such DNA and messenger RNA (mRNA) arrays are proving powerful in identifying key elements involved in OSCC. For example, one microarray-based gene-expression analysis found 601 genes to be significantly regulated in OSCC tissue compared to adjacent intra-individual mucosa controls, and 25 genes with differences in their regulation comparing samples from early-stage cancer with those from advanced disease (Fialka *et al*, 2008). Some of the mechanisms that might be implicated in carcinogenesis are summarised elsewhere (Haddad and Shin, 2008; Bagan and Scully, 2009).

Tumour suppressor genes

Tumour suppressor genes are genes that normally function in growth control – by regulating the cell cycle, apoptosis, cell adhesion and DNA repair. The normal cell cycle includes programmed cell death, or apoptosis, and cells that avoid this have the potential to become cancerous. Aberrations such as deletions or mutations in TSG genes, or silencing by hypermethylation, can thus lead to cancer.

There are several putative TSGs involved in OSCC, but the most important at present appear to be p53 (*TP53*) and p16 (Angiero *et al*, 2008). *TP53*, a gene involved in apoptosis and cell cycle regulation, is silenced (most often through point mutations), leading to premalignant disease and OSCC. Furthermore, the *MDM* class of oncogenes has been shown to inhibit *TP53* in the absence of *TP53* mutation. There is also a high incidence of LOH at chromosome 9p21, where the cyclin-dependent kinase *CDKN2a* locus codes for both *p16* and *p14* (*ARF*) – two putative TSGs that respectively regulate the cell cycle and stabilise *TP53* via *MDM2*. These TSGs are probably silenced by epigenetic control, such as promoter hypermethylation (Mithani *et al*, 2007).

There could be a myriad of other TSGs silenced in OSCC, but the pattern of silencing may differ between patients.

Oncogenes

The over-expression of oncogenes – genes that promote growth, survival and spread of cells – can lead to the development of cancer. However, the identification of oncogene over-expression is not easy. Most studied has been the epidermal growth factor receptor (*EGFR*) gene, which appears to be over-expressed in most OSCC. For example, EGFR is amplified in areca-associated OSCC compared with matched adjacent oral mucosa (Chiang *et al*, 2008).

The Δ*Np63*-α oncogene is also over-expressed and may inhibit *p73*, a TSG related to *TP53*, which is also involved in the *bcl-2* mediated apoptotic pathway, and Δ*Np63*-α may cause the accumulation of beta-catenin, a transcription factor that may play a role in cell adhesion and in the *Wnt* signalling pathway. Matrix metalloproteinases (*MMPs*) are a family of genes thought to be involved in cell adhesion, proliferation and migration, and MMPs may act as oncogenes related to infiltrative growth and lymph node involvement. Other oncogenes involved in OSCC may include 11q13, which probably influences the *Fas*-associated death domain. As with TSGs, the range of oncogenes being identified is quite broad, and the mechanisms by which they act appear complex (Glazer *et al*, 2008; Ha *et al*, 2008).

Mitochondrial mutations

Mitochondria play a crucial role in cellular respiration and ATP generation, and appear to have a role in carcinogenesis, possibly via the accumulation of succinate (part of the tricarboxylic acid cycle), which inhibits

the degradation of hypoxia-inducible factor (*HIF*)-1 alpha, an angiogenic factor activated by tumour hypoxia (Ha *et al*, 2008; Sun *et al*, 2009).

Stromal cell derived factor (SDF-1) and chemokine (C-X-C motif) receptor (CXCR4) may be involved in invasion and metastasis via angiogenic factors such as vascular endothelial growth factors (VEGFs) (Carmeliet and Jain, 2000). Some of the range of mechanisms that might be implicated in carcinogenesis and recently reported are summarised elsewhere (Bagan and Scully, 2009).

Single nucleotide polymorphisms

Single nucleotide polymorphisms are genome areas that have altered DNA sequences which may not lead to an amino acid alteration, or altered DNA sequences that do not seem to have any adverse effect in 'normal' individuals but may be markers for disease predisposition, or may be used to genetically identify patients, as they tend to cluster with ethnic background. SNPs in TSGs may play a role in cancer development (Drummond *et al*, 2002; Izzo *et al*, 2003): for example, SNPs in cell-cycle control pathway genes such as the CCND1 splice variant P241P may contribute to the risk of potentially malignant lesions (Ye *et al*, 2008).

Single nucleotide polymorphisms have also been detected in enzymes responsible for detoxifying environmental toxins and carcinogens (XMEs) and for DNA repair (DNA repair enzymes), and some may be markers for OSCC development (Duarte *et al*, 2008). However, this has not been confirmed in all studies (Losi-Guembarovski *et al*, 2008).

Xenometabolising enzymes

Xenometabolising enzymes have been fairly extensively studied. Alcohol (ethanol) is oxidised to acetaldehyde (the suspected carcinogenic agent in alcohol) by ADHs and cytochrome P-4502E1 (CYP2E1), both of which exhibit great inter-individual variability in activity. Individuals with a high production rate of acetaldehyde from ethanol have an increased cancer risk when they drink chronically. These include individuals with a genetically determined increased acetaldehyde production caused by ADH polymorphism (Yokoyama *et al*, 2007a, 2007b), as well as those with a decreased detoxification of acetaldehyde to acetate due to ALDH mutation.

Alcohol dehydrogenase-3 (now ADH1C) (ADH3*2 allele) may predispose to OSCC (Bouchardy *et al*, 2000; Schwartz *et al*, 2001; Zavras *et al*, 2002). ADH-2 (now ADH1B) homozygotes avoid alcohol because of adverse reactions (and are at low cancer risk), but heterozygotes (who can drink alcohol) are liable to OSCC (Seitz and Stickel, 2007). ADH-1B and ADH-7 are independently and strongly associated with protection against OSCC and aero-digestive cancers (Hashibe *et al*, 2008).

Aldehyde dehydrogenases genotypes may influence cancer susceptibility, presumably as acetaldehyde can accumulate. Some head and neck carcinomas appear to be ALDH2-associated, possibly by increases in acetaldehyde-derived DNA adducts (Yokoyama and Omori, 2003; Matsuda *et al*, 2006; Asakage *et al*, 2007; Yokoyama *et al*, 2008). The less-active homozygous ADH-1B (ADH1B*1/*1) and inactive heterozygous ALDH-2 (ALDH2*1/*2) increase the risk of upper aero-digestive tract cancer in Japanese alcoholics (Yokoyama *et al*, 2007a,b).

Alcohol dehydrogenase and methylenetetrahydrofolate reductase (MTHFR) appear to influence cancer risk as the ADH1C*2/*2/MTHFR 677TT genotype combination appears to be more susceptible to OSCC, with a 20-fold increase in risk in heavy drinkers and a 5.9- and 2.8-fold increase in risk, respectively, in moderate drinkers and light drinkers (Solomon *et al*, 2008). Others have suggested that MTHFR SNPs (C677T and A1298C) influence head and neck cancer (Boccia *et al*, 2009b), further implicating folate as a protective factor.

N-acetyl transferases (NATs) catalyse both the *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens. Epidemiological studies suggest that the isozymes *NAT1* and *NAT2* polymorphisms via acetylation modify the risk of developing head and neck, and some other, cancers. Fast NAT2 acetylation appears to be a risk factor for OSCC (Buch *et al*, 2008). Ethnic differences in *NAT1* and *NAT2* genotype frequencies may also be a factor in cancer susceptibility (Hein *et al*, 2000).

The enzyme, GST, catalyses conjugation of reduced glutathione via the sulphydryl group, activity which is useful in the detoxification of endogenous compounds such as peroxidised lipids. GSTM1 (glutathione *S*-transferase mu 1) null genotype significantly increases susceptibility to OSCC in Asians (Patel *et al*, 2008; Zhuo *et al*, 2009) but not in Caucasians (Zhuo *et al*, 2009). Studies on OSCC susceptibility of genetic polymorphisms at GSTM1, GSTT1 and GSTP1 gene loci have not generally supported the hypothesis of an increased risk of GSTP1 G/G, GSTM1 or GSTT1 null genotypes for developing OSCC: in contrast, the GSTM1 A/B genotype appears protective (Hatagima *et al*, 2008). The isolated or combined null genotype of GSTM1 and GSTT1 appear associated with oral leukoplakia development, and the null GSTT1 genotype shows an increased risk of p53 over-expression, in oral leukoplakia (Duarte *et al*, 2008). There may be joint effect for GSTM1 homozygous deletion and the CYP1A1 m1m2 variant (Varela-Lema *et al*, 2008). Polymorphisms at mitochondrial (mt) loci alone and in combination with the risk genotype at GSTP1 also increase the risk of OSCC (Datta *et al*, 2007).

DNA repair genes

Functional DNA repair genes are essential to protection against carcinogenesis, and SNPs may influence development of potentially malignant oral disorders and OSCC. Nucleotide excision repair enzymes *xeroderma pigmentosum complementation group A* (XPA [A23G]), XPC (Ala499Val) and XPD (Asp312Asn) appear to predict risk of developing potentially malignant oral disorders (Wang *et al*, 2007). Study of ERCC6 (excision

repair cross-complementing rodent repair deficiency complementation group 6) suggests that the heterozygous and homozygous A allele of the ERCC6 codon 399 may be associated with OSCC (Chiu *et al*, 2008a,b). However, gene–environment interactions with tobacco smoking and betel quid chewing, but not alcohol drinking, were significant (Chiu *et al*, 2008a,b).

Study of the DNA double strand break repair gene XRCC4 (X-ray repair complementing defective repair in Chinese hamster cells 4) showed that patients with heterozygous del/ins at XRCC4 intron three had an increased risk of OSCC compared to those with ins/ins, and there were significant gene–environment interactions with tobacco smoking and betel quid chewing, but not with alcoholism (Chiu *et al*, 2008a,b). Interactions of various of these SNPs may increase the risk of potentially malignant oral disorders and OSCC (Marques *et al*, 2006; Majumder *et al*, 2007).

Other genes

Polymorphisms affecting gene expression of cytokines such as interleukins IL-4, -6, -8, -10 as well as tumour necrosis factor-alpha (TNF-α) appear to be strongly associated with an increased risk for OSCC (Serefoglou *et al*, 2008). Smokeless tobacco has been shown to induce TNF-α, which, along with its receptors, is over-expressed in OSCC. SNPs in TNF-α and TNF receptor (TNFR) genes may affect their expression and may be a potential determinant of susceptibility to tobacco-related OSCC: TNF-α −308G/A may be related to susceptibility, whereas SNPs involving −609TT TNFR1 and 1690 C/T TNFR2 may be protective (Gupta *et al*, 2008).

The inducible cyclo-oxygenase (COX)-2 enzyme, which plays an important role in inflammation, but also in carcinogenesis, is over-expressed in oral potentially malignant disorders and OSCC, and SNPs in the COX-2 gene may modify the risk of developing potentially malignant disorders (Pu *et al*, 2009) and the risk of OSCC, where COX-2–765G > C polymorphisms: COX-2–765C allele *vs* −765G/G genotype may be protective (Lin *et al*, 2008).

Clinical implications

Clinical diagnostic aids

A variety of commercial diagnostic aids and adjunctive techniques are on the market to potentially assist in the screening of patients for evidence of otherwise occult cancerous change or to assess the potential of clinically abnormal lesions, but none has rigorously yet been confirmed to be superior to clinical examination (Lingen *et al*, 2008; Trullenque-Eriksson *et al*, 2009). Toluidine blue staining has been shown to be related to the genetic changes (allelic loss or LOH) associated with the progression of potentially malignant lesions to OSCC (Guo *et al*, 2001; Zhang *et al*, 2005). Furthermore, a longitudinal study showed that toluidine blue identified LOH-positive lesions that progressed to OSCC (Zhang *et al*, 2005).

There have been enumerable studies on markers that might be of diagnostic or prognostic value.

Genetic changes

Genetic characteristics such as described above can be found in OSCC tissue and offer the potential for use in diagnosis and prognostication, especially in the light of the limitations in histopathology discussed elsewhere (Abbey *et al*, 1995; Karabulut *et al*, 1995; Fischer *et al*, 2004, 2005).

Genetic changes are found in the oral mucosa in tobacco users and, in those with OSCC, are also found outwith the tumour (Boldrup *et al*, 2005; Gabriel *et al*, 2006; Proia *et al*, 2006; Sanz-Ortega *et al*, 2007; Sridhar *et al*, 2008). This is no surprise since, over half a century ago, Slaughter showed that 11% of patients with OSCC had another cancer elsewhere outside the primary OSCC, introducing the concept of field-cancerisation (Slaughter *et al*, 1953). Others have confirmed that histologically dysplastic epithelium may be found far removed from sites of OSCC (Thomson, 2002; Thomson and Hamadah, 2007). Furthermore, in patients who smoke tobacco, genetic changes not only affect the whole of the aero-digestive mucosa but also persist for a long period of time, even if the patient stops smoking. Second primary tumours are found in the upper aero-digestive tract in about 20% (Lippman and Hong, 1989).

Further, in many potentially malignant lesions, there can be a mixture of potentially malignant cells, malignant cells that have yet to invade, cells that have invaded and normal cells (Califano *et al*, 2000; Braakhuis *et al*, 2003, 2004, 2005a,b; Tabor *et al*, 2004). Thus, in a clinical potentially malignant lesion, there may be a range of cells present of different malignant potential, including some that traverse the epithelial basement membrane – defining the lesion as cancer. The histopathological interpretation of a biopsy from such a lesion could thus vary from benign to malignant. Thus, in patients with potentially malignant disorders, biopsy may not truly represent the worse components in a lesion. Indeed, as long as 25 or more years, an Italian study showed that leukoplakias, which were non-dysplastic upon incisional biopsy, on excision contained OSCC in around 10% (Chiesa *et al*, 1986). Similar findings were reported more recently (Holmstrup *et al*, 2007).

Genetic markers

Developments in genetic markers may have major implications in clinical practice, some of which are discussed elsewhere (van Houten *et al*, 2000a,b). Examination of tissue for TSG silencing might be of utility. p53 (chromosome 17) expression above basal layer in potentially malignant oral disorders is predictive of cancer (Cruz *et al*, 1998). p53 is mutated and inactivated, with LOH at 17p13, but p53 changes alone appear unsuitable for cancer prediction (Warnakulasuriya, 2000). P53 changes are seen at tumour surgical margins that appeared histologically clear of cancer (Brennan *et al*, 1995; Bilde *et al*, 2009), and this may help predict recurrence (Ball *et al*, 1997). There may also be p16 changes at surgical margins (Bilde *et al*, 2009), and there have been early reports on its utility and that of

RARbeta, E-cadherin, cyclin A1 and cytoglobin (Shaw *et al*, 2006, 2007). NF-kappaB and COX-2 have also been studied at tumour surgical margins (Santhi *et al*, 2006). In lymph node biopsies, p53 changes can detect unsuspected nodal metastases (Brennan *et al*, 1995; Tjebbes *et al*, 1999; Cortesina *et al*, 2000).

Telomerase (which is not expressed in normal epithelia) is expressed in potentially malignant oral disorders such as leukoplakia, and in epithelial dysplasia, OSCC and adjacent to carcinomas (Mutirangura *et al*, 1996; Kannan *et al*, 1997; Thongprasom *et al*, 1998; Sumida *et al*, 1999; Kim *et al*, 2001; Luzar *et al*, 2004, 2004; Yajima *et al*, 2004).

Other markers such as those of cell proliferation (Ki-67 antigen) and apoptosis (Bax, Bcl-2) may play a role in diagnosis: apoptotic Bcl-2 expression decreases significantly in dysplastic and early invasive lesions and then increases in consequent stages, while Ki-67 expression increases sharply in early OSCC, but significantly decreases later (Derka *et al*, 2006). A more aggressive tumour behaviour and worse prognosis may be signified by changes in a range of biomarkers – such as reduced E-cadherin expression (Hamidi *et al*, 2000; Diniz-Freitas *et al*, 2006), laminin (LN)γ2 chain expression (Kuratomi *et al*, 2006) and decreased tumour cell transmembrane proteoglycan syndecan-1 (Máthé *et al*, 2006).

However, the most predictive of the available tissue molecular markers reported thus far and assessed in OSCC development (Lee *et al*, 2000) include chromosomal polysomy, p53 protein expression and LOH in chromosomes 3p or 9p (probably due to changes in p16). It would seem probable that the use of these markers as an adjunct to routine histopathological examination may help prognostication and effective management of the lesions, but sadly, routine use still appears to be low, perhaps hampered by the test cost, complexity, lack of facilities in some laboratories and limited outcome studies to date (Scully *et al*, 2003).

Salivary markers

Cancer-specific variations have also been demonstrated in body fluids. Saliva may contain p53 mutations, microsatellite alterations, increased mitochondrial DNA content and TSG promoter hypermethylation, and the concept of a saliva test to diagnose OSCC is very appealing (Hu *et al*, 2006, 2007, 2008; Wong, 2006; Park *et al*, 2007; Viet *et al*, 2007; Zimmermann *et al*, 2007; Xie *et al*, 2008).

RNAs in saliva have been tested in over 300 saliva samples from OSCC patients and healthy people, and the signature was always present in higher levels in the saliva of OSCC patients than in saliva from healthy people, with an overall accuracy rate of about 85% (Wang *et al*, 2006). CD44, a multistructural and multifunctional cell surface transmembrane glycoprotein molecule is also detectable in saliva. CD44 is involved in cell proliferation, cell differentiation, cell migration, angiogenesis, presentation of cytokines, chemokines and growth factors to the corresponding receptors, and docking of proteases at the cell membrane, as well as in signalling for cell survival. CD44 isoforms containing the variant 3 (v3) exon include a growth factor binding site and may be involved in OSCC progression (Franzmann *et al*, 2001; Reategui *et al*, 2007). Salivary soluble CD44 (solCD44) levels were significantly raised in head and neck cancer patients compared with normal controls, but solCD44 levels did not vary significantly with tumour size, stage, recurrence, history of radiation treatment or tobacco and alcohol risk factors (Franzmann *et al*, 2005).

Free radicals (Bahar *et al*, 2007), ALDH (Giebutowicz *et al*, 2008), endothelin (Pickering *et al*, 2007), Cyfra 21-1, the soluble fragment of cytokeratin 19 (CK19) (Zhong *et al*, 2007), insulin-like growth factor (IGF), MMP-2 and MMP-9 (Shpitzer *et al*, 2007) and p53 (Boyle *et al*, 1994; Tavassoli *et al*, 1998) are among the other substances present in saliva that are being examined for potential diagnostic value.

Serum markers

Serum could possibly also be helpful in identifying common mutations, promoter hypermethylation and LOH (Glazer *et al*, 2008; Molinolo *et al*, 2008), but not all studies have given promising results. For example, p53 antibodies in serum show no correlation with prognosis (Friedrich *et al*, 1997; Maass *et al*, 1997; Tavassoli *et al*, 1998; Gottschlich *et al*, 1999). Multicentre studies in large populations at risk of OSCC and those at low risk are needed.

Summary

Genetic changes can be found in OSCC tissue and body fluids and offer the potential for use in diagnosis and prognostication, especially in the light of the limitations in conventional histopathology.

Author contributions

Drs Scully and Bagan contributed equally in writing this paper.

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