Journal of Biological Research-Thessaloniki **16**: 313–336, 2011
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

— REVIEW ARTICLE —

Reviewing the oral carcinogenic process: key genetic events, growth factors and molecular signaling pathways

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Received: 9 September 2010 Accepted after revision: 4 March 2011

The development of Oral Squamous Cell Carcinoma (OSCC) is characterized by a multistep row of events governed by the early detectable, gradual accumulation of genetic changes in genes transcribing key upstream and downstream regulatory proteins. The combination of genetic predisposition and epigenetic/environmental factors (gene methylation, tobacco and alcohol use, HPV infection) exerts, moreover, a modifying influence on the genetic background. Despite recent updates in our knowledge of OSCC developing process and therapeutic improvements, the prognosis of this malignancy still remains poor. Ultimately, we can envision that the demarcation of events underlying the oral malignant transformation may offer better prediction of biological behavior, more accurate clinical prognosis estimation and new targeted treatment options. This review focuses on the critical genetic alterations that characterize oral cancer and elucidates their proposed mechanism of contribution to the tumorigenic process. Emphasis has been given to the role of loss of heterozygosity in stem (progenitor) cells, the "field" cancerization hypothesis of OSCC genesis, and furthermore to data regarding the cell cycle dysregulation and cell aneuploidization. Disruption of certain signaling pathways (EGFR, NFkB, STAT, Wnt/β-catenin, TGF-β, and PI3K-AKT-mTOR) plays, also, a documented role in OSCC pathogenesis and certain molecules have been suggested as fine targets for molecular-targeted therapy.

Key words: Oral Squamous Cell Carcinoma, genetics, molecular pathways.

INTRODUCTION

Squamous Cell Carcinoma of the Head and Neck (SCCHN) represents the sixth most common cancer and may involve the oral cavity (OSCC) as well as the larynx and pharynx, resulting in high mortality (5-year survival rate is approximately 50%) despite molecular and therapeutical advances (Parkin *et al.*, 2005). Although genetic predisposition, tobacco, alcohol, betel nuts use and Human Papilloma Viruses (HPV) infection are well recognized risk factors, the lack of established markers for early detection of OSCC in precancerous dysplastic lesions and the limited response to chemotherapy does not allow improvement of overall prognosis (Bagan & Scully, 2008).

In fact, the mapping of genetic and epigenetic mechanisms underlying the malignant progression of OSCC is incomplete (Kim & Califano, 2004) and the exact turning point for the oral epithelium remains to be clarified. Current data support the hypothesis that neoplastic cells derive from the clonal expansion of a single (somatic epithelial) stem cell or de-differentiated epithelial tumorigenous cells with newly acquired self-renewal capacity (Lobo *et al.*, 2007). The accumulated genetic and epigenetic alterations (i.e. decreased function or inactivation of tumor suppressor genes, activation of oncogenes, modifications of intracellular molecular pathways) and the abnormal influence of growth factors in the cell cycle cause dysregulation and switching towards a neoplastic cell phe-

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notype characterized by mesenchymal drive and increased tumor invasiveness (Choi & Myers, 2008). Recent advances in molecular techniques has deepened our understanding of certain signaling networks enhancing our ability to synthesize biomarkers for diagnosing cancerous lesions and creating individualized treatment options, improving the survival rate of oral cancer patients (Campo-Trapero *et al.*, 2008). Molecular analysis is highly promising in detecting alterations at a molecular level earlier than they are seen under a microscope and much before clinical changes occur (Ahmed *et al.*, 2009). The identification of specific biomarkers appointed, respectively, to the early, intermediate and late stages of oral tumorigenesis would prove very helpful in the management of OSCC (Schwartz, 2000).

A. GENETIC ALTERATIONS AND EPIGENETIC/ENVIRONMENTAL FACTORS ASSOCIATED WITH OSCC

Worldwide epidemiological studies suggest that the development of OSCC is influenced by geographic, genetic, cultural and environmental risk factors. The etiology of oral cancer is a complex issue with a variety of genetic, epigenetic, toxic and viral agents implicated (Bagan & Scully, 2008).

A1. Microsatellite Instability and Loss of Heterozygosity

Microsatellites are stretches of DNA in which a short motif (usually 1-5 nucleotides long) is repeated 5-100 times. During DNA replication, these regions are at high risk for variations caused by slippage of the DNA polymerase and inadequate repair of errors, a defect redeemed by the Mismatch Repair System (MMR) (De Schutter *et al.*, 2007). In tumors, defects in the MMR system may be present leading –with gain or loss of repeat units– to somatic changes (Microsatellite Instability – MSI) and finally to cancer (mutator phenotype) (Field *et al.*, 1995) (Fig. 1). MSI seems to have a high prevalence in sporadic colorectal cancer, its implication in HNSCC appears, however, to be controversial as both high and low frequencies of MSI have been reported. A recent study (De Schutter *et al.*, 2009) used the most definitive techniques to resolve controversy and concluded that MSI has a very low (< 2%) prevalence in HNSCC.

A cell, whose genome has undergone mutations, may encompass loss of genomic material ranging from a few hundred nucleotides to a whole chromosome. As a result, numerous alleles are silenced, while their function is being sustained by the remaining active alleles (the cell is named heterozygous for that mutation) (Zhang & Rosin, 2001). Loss of heterozygosity represents the loss of normal function of one allele of a gene in which the other allele was already inactivated and marks a suppressor phenotype that is characterized by a wide variety in chromosomal numbers (aneuploidy) and extensive loss of genetic material (allelic imbalance – AI) (Sparano *et al.*, 2006; De Schutter *et al.*, 2007) (Fig. 1).

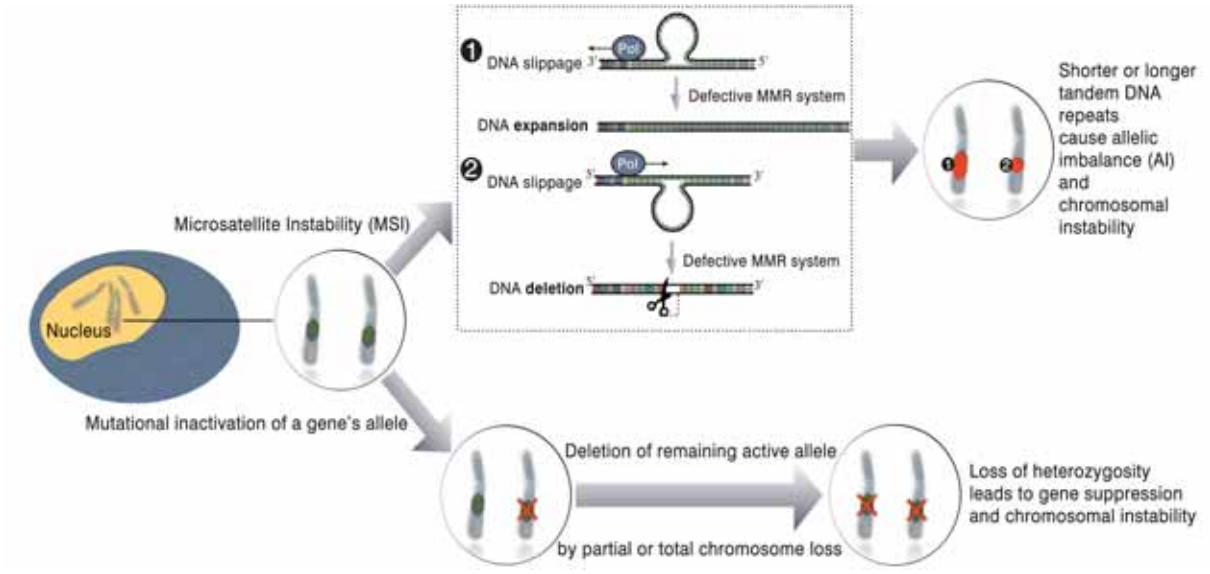

FIG. 1. Mechanisms underlying chromosomal instability in oral cancer.

TABLE 1. Common chromosomal alterations that characterize OSCC

Gene region	Affected genes	Type of alteration
3p14.2	FHIT (Fragile Histidine Triad)	Mutation
3p21	RASSFIA (Ras Association Domain Family AI)	Deletion
3p22.3	MLHI (DNA Mismatch Repair Gene)	Deletion
3p25	XPC (Xeroderma Pigmentosum Group C)	Deletion
3p25-26	VHL (von Hippel-Lindau)	Deletion
3q26	PI3KCa (Phosphatidylinositol 3 Kinase)	Amplification
5p15.33	TeRT (Telomerase Reverse Transcriptase)	Amplification
5q21	APC (Adenomatous Polyposis Coli)	Deletion
7p12	ErBbI (Epidermal Growth Factor Receptor)	Amplification
8q24.11	EXTI (Exostosin I)	Amplification
8q24.21	C-myc	Amplification
8q24.23	PTK2 (Protein Tyrosine Kinase 2)	Amplification
9p21-22	INK4a, ARF (p16, p14)	Deletion
11q13	CCND1 (Cell Cyclin D1)	Amplification
13q13.3	BRCA2 (Breast Cancer 2 Susceptibility Protein)	Deletion
13q14.3	RB1 (Retinoblastoma Protein)	Deletion
17p13	TP53 (p53)	Mutation
17q21.2	BRCA1 (Breast Cancer 1 Susceptibility Protein)	Amplification

Oral cancer and precancerous lesions are dominated by chromosomal quantitative alterations in various genetic regions (Table 1). The presence or absence of specific DNA sequence or chromosome can be detected with the use of hybridization techniques (chromosome *in situ* hybridization – CISH, DNA microarray, polymerase chain reaction – PCR) and fluorescence microscopy. In oral cancer, chromosome instability can be found early in tumor-adjacent normal and premalignant cells thus marking the tissue at 100% risk of tumor development (Ahmed *et al.*, 2009). LOH is also found more frequently (compared to MSI) and occurs early, progressively increasing at each histopathological step (De Schutter *et al*., 2007, 2009). Partridge *et al.* (1998) used a microsatellite assay to screen 31 potentially malignant oral lesions for genetic abnormalities and found AI in 24 (77%) of them. They also observed that the presence of lesions with AI at two or more (> 2) relevant loci is correlated with high risk for them evolving into squamous cell carcinoma. The same researchers (Partridge *et al.*, 1999) later proposed that allelic imbalance at 3p22-26, 3p14.2, 9p21 (MLH-1, XPC, VHL, FHIT, p16INK4a) should be considered a better predictor of outcome than the TNM system, while a recent study (Uzawa *et al.*, 2001) reports that the loss of alleles 3p14 and 9p21 occurs early on in the development of OSCC and can even occur in simple keratosis. Sparano *et al.* (2006) accomplished to increase the predictability by performing a genome-wide analysis that allowed the exact mapping of the candidate altered genes. The use of available techniques to detect LOH can, therefore, prove to be useful as a molecular diagnostic tool of chromosomal instability enabling better evaluation of prognosis and remission of the disease (Ahmed *et al.*, 2009). A staging system that will include the combined molecular marker score and results from histology, will enable us to accurately determine the risk of a premalignant oral lesion developing into cancer (Lee *et al.*, 2000; Zhang & Rosin, 2001).

A2. Methylation of promoter DNA regions

Methylation occurs when certain methyl groups (CH_3) bind to promoter regions of genes (usually CpG nucleotides) affecting their function without changing the structure or sequence of the selected gene (Campo-Trapero *et al.*, 2008). Both hypermethylation (by suppressor gene inactivation) and hypomethylation (by inappropriate oncogene activation) can induce carcinogenesis (Auerkari, 2006). These aberrations and their implication in oral cavity tumors have been studied relatively recently (Rosas *et al.*, 2001; Ogi *et al.*, 2002; Kulkarni & Saranath, 2004) and results showed that methylation pattern changes may play a very important role because they are frequently related to the loss of gene expression (Barros & Offenbacher, 2009). Aberrant hypermethylation patterns in the promoter regions of p16 (CDKN2A) and E-cadherin (CDH1) are reported to influence cell division and cell-cell adhesion, respectively. Methylation of the p16 promoter in particular, has been considered a very important predictive marker for detecting malignant transformation, since it depicts uncontrolled cell division (Hall *et al.*, 2008). DNA methylation can also inactivate DNA-repair genes (i.e. O6-methylguanine-DNA methyltransferase – MGMT) and therefore contribute to the the multistep row of genetic alterations required for tumor progression (Ha & Califano, 2006).

Taking under consideration that methylation is considered as an early event in oral carcinogenesis (Lopez *et al.*, 2003), selective screening of certain genes methylation state could be proved extremely useful in the early detection of OSCC. Viswanathan *et al.* (2003) successfully detected OSCC in 38 out of 51 Indian patients using a gene methylation panel of gene-specific markers. Serum analysis via methylation-specific PCR in patients with HNSCC has proved successful in detecting primary or recurring oral malignancies (Sanchez-Cespedes *et al.*, 2000; Rosas *et al.*, 2001). In the future, the methylation status of cells within surgical margins could be assessed intraoperatively via real-time methylation-specific PCR analysis (Goldenberg *et al.*, 2004) and new targeted treatment options (such as 5-azacytidine/5AC) will eventually be developed for safe use to reverse hypermethylation state of certain genes (Shaw, 2006).

A3. Tobacco and alcohol use

The most important risk factor for the development of oral cancer in the Western countries is the consumption of tobacco and alcohol (Parkin *et al.*, 2005). There is also compelling evidence correlating the use of smokeless tobacco products and the development of OSCC in Asia (Warnakulasuriya, 2002; Proia *et al.*, 2006). Tobacco and alcohol consumption are implicated in 75% of all HNSCC and they are thought to have a multiplicative combined effect that greatly increases overall risk (Blot *et al.*, 1988; Franceschi *et al.*, 2000; Johnson, 2001; Reibel, 2003). Additionally, the risk of cancer of the oral cavity and pharynx was found to be associated with heavy alcohol intake in non-smokers and with heavy tobacco smoking in non-drinkers thus indicating that smoking and drinking are also independent risk factors (Talamini *et al.*, 1998; Hashibe *et al.*, 2007). Oral leukoplakia, in addition, appears to be far more common in smokers than in non-smokers (Banoczy *et al.*, 2001).

The polycyclic aromatic hydrocarbons (PAHs) and tobacco specific nitrosamines (TSNA) are the major carcinogens in cigarette smoke (IARC, 1986). These factors act locally, on oral mucosa keratinocyte stem cells, causing genetic damage ultimately interfering with DNA replication (Báez, 2008). Key events that characterize malignant transformation in tobacco-associated tumors are mutations affecting the ras oncogenes and the p53 tumor suppressor gene (17p), the latter associated with early recurrence and development of second primary tumors (Shin *et al.*, 1996). Paterson *et al.* (1996) reviewed the spectrum of molecular changes found in OSCC from Western and Eastern (India, SE Asia) countries. p53 mutations were common in tumors from the West (47%) but infrequent in the East (7%). Tumors from India and SE Asia were characterized by the involvement of ras oncogenes, including mutation, loss of heterozygosity (H-ras) and amplification (K- and N-ras), alterations which were associated with areca nut chewing. The above observation simply confirms the complexity and multiplicity of factors and pathways implicated in the pathogenesis of OSCC.

There is a lack of clear experimental evidence, on the other hand, for pure ethanol to be carcinogenic (Kato & Noruma, 1994; Wight & Ogden, 1998); it is presumed to act in concert with other, more direct, carcinogenic mechanisms (generation of free radicals, solvent effect on tobacco, nutritional deficiency, induction of microsomal enzymes involved in the metabolism of xenobiotics) (Riedel *et al.*, 2005). Alcohol may influence the proliferative stem cells by both intracellular and intercellular (permeability) pathways regulating their exposure to carcinogens (Ogden, 2005).

However, there is increasing evidence that points to acetaldehyde –also a product of tobacco smoke– as the major contributor for the carcinogenic effect of alcohol (Seitz *et al.*, 2001; Burton, 2005) since it has the ability to induce DNA mutations by interacting covalently with DNA (Báez, 2008). Further review of the literature and the publication of homogenized data from various control groups will enable researchers to shape a global protocol in order to raise awareness of the association between alcohol and OSCC and identify certain patients at increased risk.

A4. Human Papilloma Virus (HPV) infection

More that one hundred HPV types have been identified with several, including HPV16 and HPV18, classified as "high risk" due to their oncogenic potential once integrated in the genome (zur Hausen, 1996). The viral DNA codes for two oncoproteins, E6 and E7, that promote tumor progression by inactivating key tumor suppressors (p53 and retinoblastoma protein, respectively) resulting in uncontrolled DNA replication and apoptotic impairment (Munger & Howley, 2002).

Studies questioning the involvement of HPV in oral carcinogenesis have yielded ambiguous results (Yeudall & Campo, 1991; Bouda *et al.*, 2000; Gillison & Lowy, 2004; Kreimer *et al.*, 2005; Syrjänen, 2007), with the reported infection percentages in potentially malignant and cancerous lesions ranging from 0 to 90%. In a meta-analysis of data from 94 studies, Miller & Johnstone (2001) reported a frequent association between high risk HPVs and OSCC lesions compared to normal oral mucosa, with HPV16 being generally the prevailing type (75%) (Herrero *et al.*, 2003). A newer study, however, exhibited only a weak causal association for HPV in the case of OSCC (Hobbs *et al.*, 2006). Furthermore, HPV16 immortalized keratinocytes demonstrated no tumorigenic activity in nude mice (Oda *et al.*, 1996) unless subjected to chronic exposure to the tobacco carcinogen benzo(α)pyrene or other chemicals (Park *et al.*, 1995). Additionally, HPV16 infection and integration is seldom found in oral premalignant lesions and invasive carcinomas (Ha *et al.*, 2002). Taken together, these observations suggest that HPV alone is incapable of inducing malignant transformation and the nature of the association for OSCC is yet unclear (Feller *et al.*, 2010). It is of certainty, however, that substantial differences in cytogenetic profiles, clinical characteristics and disease outcome between HPV-positive and HPV-negative tumors exist (Koch *et al.*, 1999; Gillison *et al.*, 2000; Slebos *et al.*, 2006) thus suggesting the latter may constitute a distinct class of disease. Generally, genomic instability in cytopositive oral SCC is thought to be initiated and maintained by high risk E6/E7 oncoprotein-induced dysregulation of cell cycle control mechanisms (Feller *et al.*, 2010). The clarification of the exact mechanism and timing (early stage or "hit and run" action) of the HPV infection will enable us to precisely determine risk and shape preventive and protocols. HPV-positive tumors are generally thought to present with a more favorable outcome; patients with oropharyngeal cancer have exhibited a better response to chemo- and radiotherapy, as well as longer overall survival (Worden *et al.*, 2008; Fallai *et al.*, 2009). The efficacy of the quadrivalent vaccine in preventing oral infection by HPV16 and 18 warrants further evaluation (Gillison *et al.*, 2008).

B. FIELD CANCERIZATION – DISTINCT PATTERN OF LOH & STEM PROGENITOR CELLS

It is well established that most solid tumors result from a multistep process of accumulated genetic alterations (Renan, 1993). HNSCC is often associated with widespread epithelial histopathological alterations that remain undetected. That observation was first made by Slaughter *et al.* (1953), who proposed the concept of "field cancerization"; the hypothesis that there are changes throughout the mucosa of the head and neck cancer patients. The theory was an effort to explain the origin of the second primary tumors frequently seen in the oral cavity tissues.

Califano *et al.* (1996) noted –by examining 87 cases with head and neck lesions– that the spectrum of chromosomal loss increased at each histopathological step (benign hyperplasia-dysplasia-carcinoma *in situ*-invasive cancer) via accumulation of genetic changes that affect both oncogenes and tumor suppressor genes and occur in a distinct order. Their suggested model for cancer progression demonstrates 9p21 or 3p as earliest detectable events. That was also confirmed by Mao *et al.* (1996), who –by studying 84 cases of oral leukoplakia (OPL)– demonstrated that losses of the 9p21 and 3p14 regions may be related to early processes of tumorigenesis in HNSCC and their existence in premalignant tissues may serve as a marker for cancer risk assessment. On the same subject, Rosin *et al.* (2000) examined 116 biopsies from patients with OPL and found not only a massive amount of

LOHs but a characteristic pattern as well, confirming the earliest findings of Califano *et al.* (1996). In addition, 3p and/or 9p were present at virtually all progressing lesions thus suggesting that loss in these arms is a prerequisite for progression (Van Der Riet *et al.*, 1994; Bockmühl *et al.*, 2000; Choi *et al.*, 2004; Coon *et al.*, 2004). The risk of progression to cancer is low when no genetic change is seen, intermediate if there is genetic loss on the short arms of chromosomes 3 and 9 and high if there is 3p and 9p loss accompanied by genetic loss on additional chromosome arms (4q, 8p, 11q, 13q, and 17p) (Rosin *et al.*, 2000). High-risk lesions may progress to cancer in up to 50% of cases, whereas low-risk lesions progress to cancer in only 2% (Zhang *et al.*, 2001). Early detection of premalignant lesions becomes, therefore, of high importance with adjuncts to available methods including vital tissue staining (with toluidine blue) and exfoliative cytology (Epstein *et al.*, 2002).

Most tumors are clonal in origin and it has been estimated that five events are required in humans to transform a normal cell into a cancer cell (Hahn & Weinberg, 2002). As a result, only long time residents of the epithelium, most likely the stem cells, have the ability to accumulate the number of necessary genetic hits that will result in cancer formation (Reya *et al.*, 2001). Stem cells are defined as having the capacity to divide both symmetrically, producing two daughter stem cells, and asymmetrically, producing one daughter cell that terminally differentiates and another that maintains "stemness" characteristics. Therefore within the concept of initiation of a cancer stem cell, the first step of carcinogenesis is to block asymmetric cell division without interfering with the cell ability to divide symmetrically.

A tumor should be regarded as a whole organ whose various cells have different goals and distinct roles in its economy (Graziano *et al.*, 2008). Recent studies in oral lesions described the existence of only a subpopulation of cells with characteristics that correspond to the stem and amplifying cells of normal oral epithelium (somatic stem cells) (Mackenzie, 2004; Costea *et al.*, 2005; Locke *et al.*, 2005). Prince *et al.* (2007) tested the tumorigenic potential of different populations of cancer cells from HNSCC samples; a minority population of cells with primitive cellular morphology that typically comprises less than 10% of the cells in a HNSCC tumor, gave rise to new tumors *in vivo*. Moreover, the new tumors had the original tumor heterogeneity and could be serially passaged. It is therefore possible for somatic stem progenitor cells in normal epithelium to drive tumor formation (Owens & Watt, 2003). That finding led Braakhuis *et al.* (2004) to propose a genetic progression model for OSCC. During the initial phase, a stem cell acquires a genetic alteration (i.e. on p16 locus) that is transferred to its daughter cells thus forming a patch. At the next phase, the patch converts into an expanding field as a result of accumulation of genetic alterations. These fields vary in size and may remain undetected or appear as leukoplakia/erythroplakia. Ultimately, a dominant clone prevails and develops into carcinoma.

C. CELLULAR SENESCENCE

Cell division processes are characterized by a specific counting mechanism that forces a limit upon the number of times that a normal cell duplicates (Hayflick, 1965). That limit has been found to correlate with the length of telomeres; cells irreversibly arrest and become senescent when telomeres are critically shortened (Harley, 1991).

According to classical paradigm of tumor initiation-progression, evolving pre-malignant cell populations exhaust their endowment of allowed doublings and accomplish their tumorigenic program by breaching the mortality barrier and acquiring unlimited replicative potential (Sumida & Hamakawa, 2001). However, this model has been recently disputed, since according to the cancer stem cell theory, the stem cells or their early progenitors have been suggested to be the real target in the initiation event (Pannone *et al.*, 2007). The stem cells are naturally immortal and become mortal only when they are induced to terminally differentiate and lose their telomerase activity. Consequently, the initiation checkpoint should be appointed as one that stably and irreversibly inhibits the mortalization of stem cells (Trosko & Tai, 2006).

C1. Human reverse telomerase transcriptase (hTeRT) and cell immortalization

Telomeres are protein-DNA structures at the ends of eukaryotic chromosomes (Blackburn, 1991) that consist of tandem repeats of the highly conserved G-rich sequence TTAGGG (Moyzis *et al.*, 1988). At the end of each cell division, the telomere of the 3′ end is incompletely replicated due to DNA polymerase inability to commence synthesizing DNA when an RNA primer is not present (Feng *et al.*, 1995). The end replication problem is resolved by telomerase, a reverse transcriptase (TeRT) that uses an RNA template to

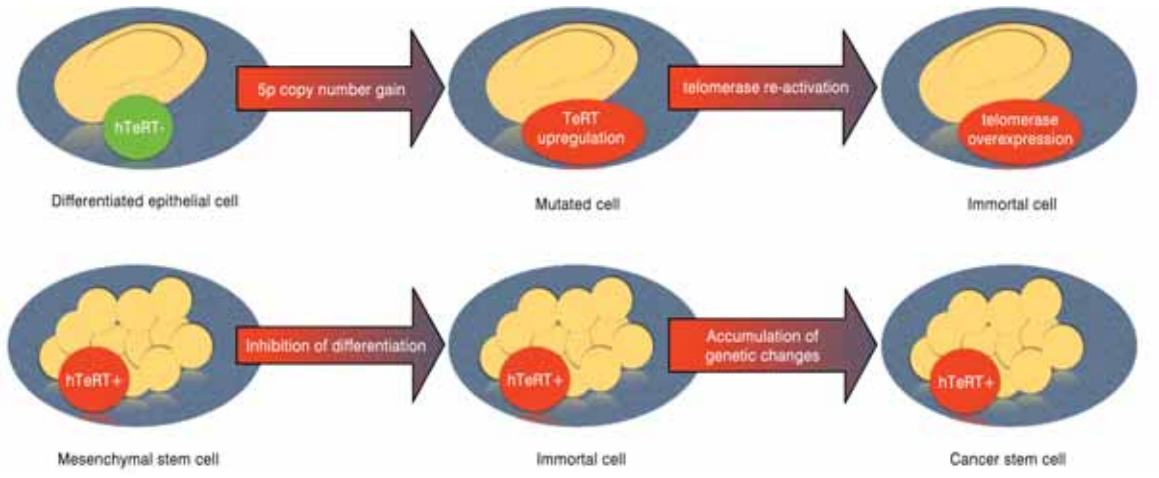

FIG. 2. Target cells of cancer initiation process: A mortal cell represses telomerase activity and finally loses it during the normal differentiation process. A differentiated cell that has re-established telomerase activity can restore telomere length and become immortal thus transforming into a cancer cell.

add DNA repeat units in a progressive manner (de Lange, 1994; Greider, 1996).

The locus that controls the expression of the enzyme lies on chromosome 5p. Among others, gene copy number gain of 5p was frequently found suggesting that proto-oncogenes localized in this region might be activated contributing to OSCC initiation and progression (Okafuji *et al.*, 1999). Freier *et al.* (2007), checked the association between gain in TeRT (gene encoding for hTeRT) and telomerase level of expression; the results suggested that other proto-oncogenes residing in the vicinity of TeRT might be target genes of the common 5p gains in OSCC (Fig. 2).

There is an overall agreement on the critical role played by telomerase in the mechanism of cell immortalization during oral carcinogenesis supported by research conducted both *in vitro* (Muntoni *et al.*, 2003) and *in vivo* (Goessel *et al.*, 2005). Moreover, findings from researching telomerase activity in oral tumors and premalignant lesions (Kim *et al.*, 1994; Kannan *et al.*, 1997; Curran *et al.*, 1998; Sumida *et al.*, 1998; Miyoshi *et al.*, 1999) suggest telomerase re-activation as the major mechanism behind cell immortalization. Furthermore, Liao *et al.* (2000) showed that telomerase activity directly correlates with lymph node extracapsular spreading and overall survival. However, there is supporting evidence for normal oral epithelia to express telomerase as well (Kannan *et al.*, 1997; Sumida *et al.*, 1999; Pannone *et al.*, 2007) thus demonstrating that the original target in cancer initiation process may be an immortal cell (likely a stem cell) (Fig. 2). The conceptual problem of whether the telomerase activity in cancer is restored in a differentiated (or commited cell) or if it is preserved in the clonal expansion of a mutated stem cell, is crucial to understanding the carcinogenic process and to the future of cancer therapy (Fujita *et al.*, 2004; Yajima *et al.*, 2004; Sebastian *et al.*, 2005; Zhong *et al.*, 2005).

D. CELL CYCLE DYSREGULATION IN OSCC

D1. Centrosome abnormalities and Spindle Assembly Checkpoint (SAC) impairment

To establish bipolarity of the mitotic spindle and therefore, symmetric chromosome segregation, it is important for only two centrosomes to be generated during each cycle. To guarantee centrosome duplication and only once per cycle, the cell division and centrosome duplication cycles are synchronized (Hinchcliffe & Sluder, 2001) by factors that also trigger the G1/S transition (Meraldi *et al.*, 1999). Along with the microtubules, centrosomes regulate the formation of the mitotic spindle and the polarity of cell division being therefore, crucial for chromosome segregation and cytokinesis (Brinkley, 2001).

Centrosome abnormalities are a frequent finding in many tumors (Duensing & Munger, 2001) and they have been implicated in chromosome missegregation and the generation of aneuploid cells (Wassmann & Benezra, 2001). Centrosome defects are thought to drive chromosomal instability via their ability to mediate multipolar spindle formation (Fig. 3). Thirthagiri *et al.* (2007) checked oral epithelial dysplasias and

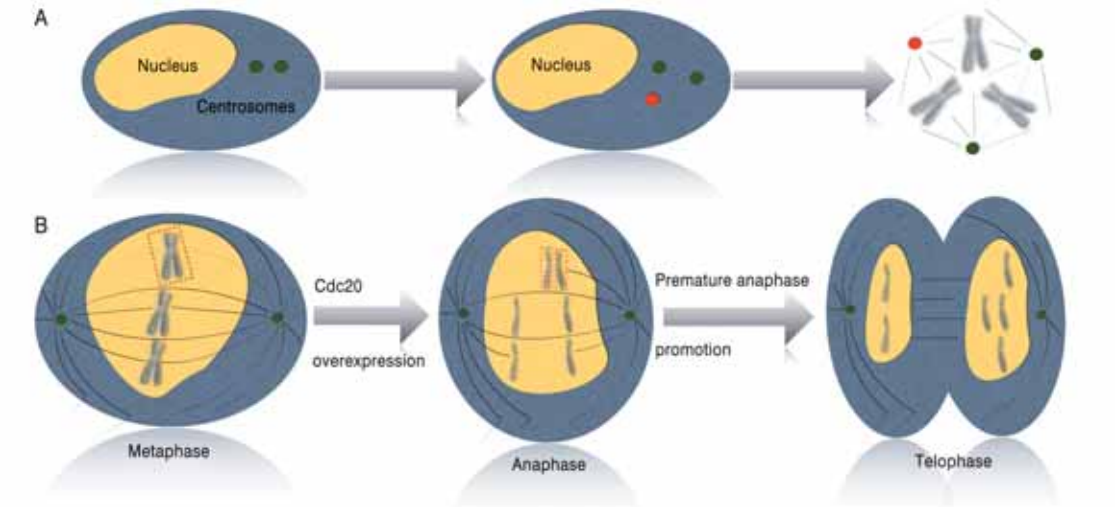

FIG. 3. Mechanisms underlying aneuploidy in OSCC: Abnormal centrosome number by abnormal centrosome duplication (shown in red) in early stages of neoplastic progression can potentially drive to chromosome desegregation by the formation of multipolar mitotic spindles (aneuploidy). The mitotic checkpoint monitors the integrity of kinetochore capture of metaphase chromosomes by spindle microtubules during mitosis. The majority of spindle checkpoint defects may occur as a result of altered expression of known checkpoint genes or mutations in as yet unidentified checkpoint genes. In OSCC, Cdc20, a spindle checkpoint protein, is often overexpressed causing premature anaphase promotion and aneuploidy.

carcinomas for centrosomal abnormalities monitoring disorders in diameter, size and number of centrosomes, the latter being higher in carcinomas rather than the dysplasias. They suggested that the presence of centrosome abnormalities in oral dysplasias raises the possibility of such defects contributing to malignant progression. In addition, Reiter *et al.* (2009) exhibited that the number of centrosomal abnormalities is strongly associated to tumour size, occurrence of distant metastasis and disease-free survival of HNSCC patients confirming that centrosome alterations are often linked with aneuploidy, cell transformation, and tumor progress.

A growing body of evidence suggests that defects in the spindle assembly checkpoint (SAC), a surveillance mechanism crucial for proper segregation of chromosomes during mitotic cell division (Musacchio & Salmon, 2007), are responsible for an increased rate of aneuploidization during tumorigenesis (Minhas *et al.*, 2003; Bharadwaj & Yu, 2004; Rajagopalan & Lengauer, 2004; Kops *et al.*, 2005) (Fig. 3). An important SAC protein, Cdc20 that normally activates the anaphase promoting complex (APC/C) (Wasch & Engelbert, 2005), was examined recently for its role in aneuploidy (Mondal *et al.*, 2007). The researchers observed overexpression of Cdc20 in several OSCC cell cultures and primary head and neck tumors providing evidence for an association with premature anaphase promotion resulting in mitotic abnormalities.

D2. Upstream cell cycle regulators: Cyclins and cyclin-dependent kinases

The cyclins and cyclin-dependent kinases (CDKs) form the core of cell cycle regulation (Sherr & Roberts, 1999). CDKs family members are constantly available during the various phases of the cell cycle, their on-time activation, however, is triggered by interaction with cyclins and results in the formation of complexes that are required for the cell to pass through specific phases of the replicative sequence. D-type cyclins, for example, interact with CDK4 and CDK6 and are necessary for G0/G1 transmission. This type of cyclins, in particular, has been thoroughly investigated as of central importance in governing entry into the cell cycle through phosphorylation of the retinobastoma protein (pRb) as a response to upstream mitogenic stimuli (Sherr, 1993) (Fig. 4). Rb lies hypophosphorylated (thus active) and binds to E2F, S-phase genes transcription factor, causing cell arrest at G1 phase. Cellular response to upstream mitogenic stimuli leads to phosphorylation of Rb (pRb) by cyclin D1/CDK4 complex and the subsequent release of E2F leading to S-phase entrance. Cyclin-D1 is considered as a proto-oncogene (Bcl-1 or PRAD-1), mapped to 11q13, often overexpressed in oral cancer (Miyamoto *et al.*, 2003). Recent findings (Sartor *et al.*, 1999; Redon *et al.*, 2001; Rousseau *et al.*, 2001) suggest that cyclin-D1 gene amplification is an early event during oral car-

cinogenesis. Furthermore, the overexpression of cyclin D1 is linked up to increased tumor aggressiveness and therefore a higher risk of occult metastases and poor prognosis (Capaccio *et al.*, 2000; Mineta *et al.*, 2000).

D3. Downstream cell cycle regulators: Cyclin-dependent kinase inhibitors

Cyclin-mediated pRb phosphorylation is counteracted by the activity of another regulating group of proteins, the cyclin-dependent kinase inhibitors. Two CDKI families are known, the prototype genes of these being the p16/CDKN2A/MTS1 (Serrano *et al.*, 1993) and p21/WAF1/CIP1 (El-Deiry *et al.*, 1995), respectively.

D3a. p16

p16/MTS1 binds with cyclin-D1 CDK4/6 complexes leading to their dissolution, thus inhibiting pRb phosphorylation and causing cell cycle arrest at the G1 phase (Hall *et al.*, 1995) (Fig. 4). The gene that encodes the inhibitor is mapped on chromosome 9p21-22, a region that has been studied extensively for loss of genomic material resulting in the total acceptance of aberrations in the MTS1 tumor suppressor locus being very common (Papadimitrakopoulou *et al.*, 1997; Sartor *et al.*, 1999; Kresty *et al.*, 2002; Piboonniyom *et al.*, 2002; Sailasree *et al.*, 2008). Towards identifying the actual causes of that loss, Ohta *et al.* (2009) concluded that p16, while certainly possesses an important role in the genesis of OSCC, is mainly inactivated by DNA hypermethylation (an epigenetic mechanism), rather than gene mutation and allelic deletions (Lopez *et al.*, 2003; Kulkarni & Saranath, 2004). The HPV E7 oncoprotein also leads to p16 overexpression, which is to date one of the best biomarkers for active HPV-related oropharyngeal carcinogenesis (Campisi *et al.*, 2007; Allen *et al.*, 2010). The observed lack of p16 expression demonstrates that its inactivation occurs early and is one of the major events during oral carcinogenesis (Buajeeb *et al.*, 2009) serving as a prognostic marker of poor clinical outcome particularly when correlated with pRb and cyclin-D1 levels (Jayasurya *et al.*, 2005; Suzuki *et al.*, 2006; Sailasree *et al.*, 2008).

D3b. p21, p53

p21 inhibitor has been described as the critical downstream mediator of the p53 gene, a tumor suppressor which has been implicated in a variety of cellular processes including G1 arrest, apoptosis, senescence and differentiation (Nylander *et al.*, 2000). The p53 gene acts as a transcription factor of p21 and prevents the cell from going beyond phase G1, permitting DNA repair ("guardian of the genome") (Lane, 1992) (Fig. 4). If this is not possible, p53 induces apoptosis of these cells to avoid the transmission of potentially carcinogenic material (Bartkova *et al.*, 2005; Gorgoulis *et al.*, 2005).

The gene that harbors p53 (TP53) is located in chromosome 17p13 (Scully *et al.*, 2000) and has been found inactivated or altered in most oral tumors (Og-

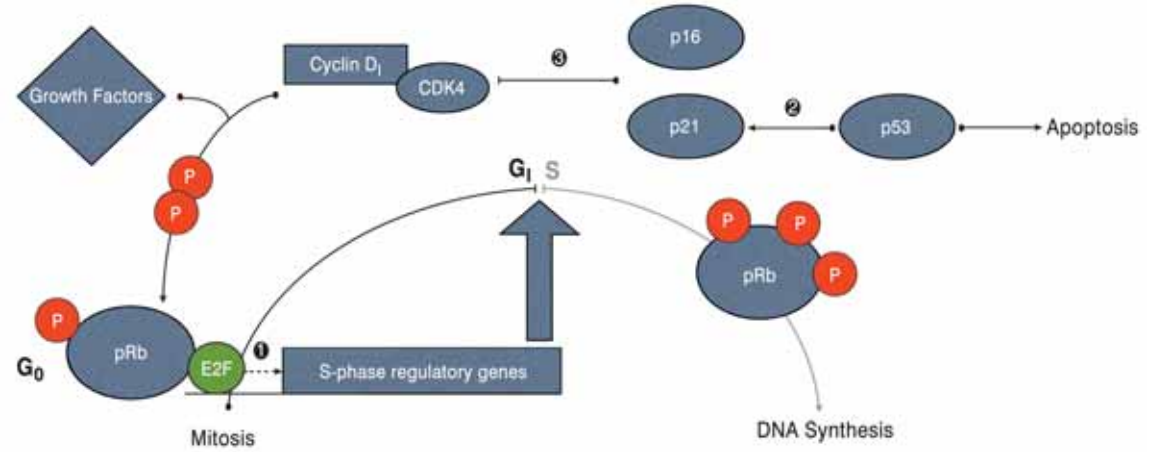

FIG. 4. Upstream and downstream regulation of pRb in oral cancer: The Rb and p53 related pathways, are two major, interconnected biochemical pathways frequently perturbed in oral cancer (1, 2). p16 protein indirectly correlates with pRb expression by causing the cyclin D1/CDK4 to dissolve (3). p16 encoding gene suppression and the resulting lack of p16 expression is among the earliest detectable findings that characterize the oral tumorigenic process.

den *et al.*, 1992; Sakai *et al.*, 1992). However, there is much debate on the timing of this change and the effect on survival (Boyle *et al.*, 1993; Wood *et al.*, 1994; Regezi *et al.*, 1995; Shintani *et al.*, 1995). The accumulation of p53 is found more frequently in high grade rather than low grade leukoplakias (Liu *et al.*, 1999), while meta-analysis concluded that 47% of oral premalignant lesions expressed p53 (Warnakulasuriya *et al.*, 1998). Many recent studies have focused on the TP53 gene, analyzing its gene status and protein expression in HNSCCs. When restricting analysis to tumors with alterations causing an obvious change in protein (mostly tobacco and alcohol related tumors), TP53 mutation was found to be a strong and independent variable for prognosticating survival (Nylander *et al.*, 2000). Despite that fact, it is unclear whether this expression represents mutant p53 or stabilized wild type protein (Mithani *et al.*, 2007). A possible confounding variable in these studies is HPV, whose E6 gene product induces the degradation of the p53 protein whereas the TP53 locus remains wild type (Westra *et al.*, 2008). Head and neck carcinomas are very rarely characterized by both disruptive TP53 alterations and HPV positivity thus suggesting that TP53 mutations cannot offer prognostic information independently from HPV status (Gold & Kim, 2009). On the other hand, loss of pRb and accumulation of p53 are associated with the histological progression of oral tumors and the acquisition of invasive potential (Soni *et al.*, 2005).

p21 gene somatic mutations, on the other hand, have not been described in oral cancers. Only recently, Ralhan *et al.* (2000) were able to identify a $p21^{WAF1}$ polymorphism (A→G substitution in codon 149) and document its vital role in oral tumorigenesis. Agarwal *et al.* (1998) showed that expression of p21 is increased in premalignant and malignant oral lesions regardless of whether they incorporate wild-type or mutated p53. Also, Yook & Kim (1998) suggested that the pathway may be induced by p53 indepedent mechanisms. The incidence of the p21 overexpression in oral tumorigenesis has not been fully confirmed and requires further investigation.

D3c. The Retinoblastoma protein

The cell cycle progresses through certain turn of events defined by the existence of checkpoints that need to be triggered in order for DNA synthesis to be accomplished. When internal (i.e. genomic damage) or external (i.e. metabolic disruption) stimuli apply, that cascade of events can be interrupted through certain self-regulatory mechanisms. A critical example of such a mechanism is (likely) that of the retinoblastoma protein (pRb), a tumor suppressor that normally rests hypophosphorylated, forming a complex with the transcription factor E2F causing cell arrest at the G1 phase (Nevins, 1992; Alberts *et al.*, 1998). In stimulated cells, cyclin-D1/CDK4 phosphorylates pRb causing the E2F-pRb complex to dissolve (Oswald *et al.*, 1994; Cordon-Cardo, 1995; Weinberg, 1995) allowing E2F to transcriptionally activate genes that regulate DNA synthesis (S-phase regulatory genes, Fig. 4) (Goodger *et al.*, 1997). Also, pRb function may be abrogated as a result of either mutations in the Rb gene or in components that regulate the Rb product (i.e. the HPV E7 oncoprotein triggers pRb proteolytic degradation). While pRb expression levels in OSCC have been studied extensively, results exhibit heterogeneity (Pande *et al.*, 1998; Sartor *et al.*, 1999; Schoelch *et al.*, 1999; Nakahara *et al.*, 2000; Soni *et al.*, 2005). More than 90% of oral tumors exhibit at least one abnormality, including Rb, cyclin D1 or p16. This suggests that in order to understand pRb role in the control of cell growth, it is important to examine not only the level but also the phosphorylation status of the pRb protein. Therefore, a precise demarcation of the relationship between pRb and its upstream (cyclin D1) or downstream (p16, p21) regulators needs to be set for enabling better prognostication and clinical outcome (Jayasurya *et al.*, 2005).

E. SIGNALING PATHWAYS & GROWTH FACTORS

E1. Epidermal Growth Factor Receptor (EGFR) and related molecular pathways

The epidermal growth factor receptor (a trans-membrane protein of the ErbB family of receptor tyrosine kinases) is activated when bound with peptide growth factors of the EGF-family of proteins (Normanno *et al.*, 2006). These extracellular ligands induce the formation of receptor homo- or heterodimers and subsequent activation of the intrinsic tyrosine kinase domain (Olayioye *et al.*, 2000), linked with site-specific intracellular signaling pathways that correlate directly with cellular proto-oncogenes and epithelial cells autonomous proliferative ability (Tsantoulis *et al.*, 2007). The genetic locus that harbors ErbB1 resides at the short arm of chromosome 7 (7p12) (Patmore *et*

al., 2005) and has been found amplified in 80-90% of HNSCC (Temam *et al.*, 2007), suggesting that protein overexpression rather than mutation might be responsible for activation of the EGFR pathway in HNSCC (Sheikh Ali *et al.*, 2008). Gebhart *et al.* (2004) found OSCC to exhibit frequent gain of 7p12 but no significant differences with respect to class or grade of tumors were observed. Disease free survival of tumors without 7p gain clearly exceeded that of tumors with 7p gain. However, lymph node affection and relapse of the 7p positive tumors was found to occur more frequently. Conclusively, it was shown that genomic gain in 7p12 must be regarded as embedded in the sum of all genomic alterations present in OSCC. EGFR overactivity can also be achieved either via autocrine stimulation through the co-expression of the receptor with one of its ligands, TGF-a, or by the expression of mutant truncated forms (EGFR variant III) of the receptor (Quon *et al.*, 2001; Sok *et al.*, 2006). Clinically, the overexpression of ErbB1/EGFR is associated with low rate of cell differentiation, increased cell proliferation, inhibition of apoptosis and neoangiogenesis. All these alterations are consistent with poor prognosis, aggressive tumor behaviour and increased size, accompanied with regional lymph node spread, resistance to radiation and greater risk of metastasis (Ulanovski *et al.*, 2004; Zimmermann *et al.*, 2006).

The importance and impact of EGFR overactivity on OSCC prognosis has been the rationale for the development of anti-EGFR therapies, which target either the extracellular (monoclonal antibodies – cetuximab) or the intracellular (tyrosine kinase inhibitors – erlotinib) domain of the receptor (Razak *et al.*, 2010). So far, only cetuximab has shown benefit for OSCC patients, when given in combination with radio and chemotherapy (Moon *et al.*, 2010). EGFR and its downstream molecules create a signaling cascade (Fig. 5) in which the mitogen-activated protein kinases (MAPK), the Akt-mTOR and the STAT proteins possess a dominant role. Consequently, the low response rate to EGFR inhibitors is probably due to the constitutive, EGFR independent activation of

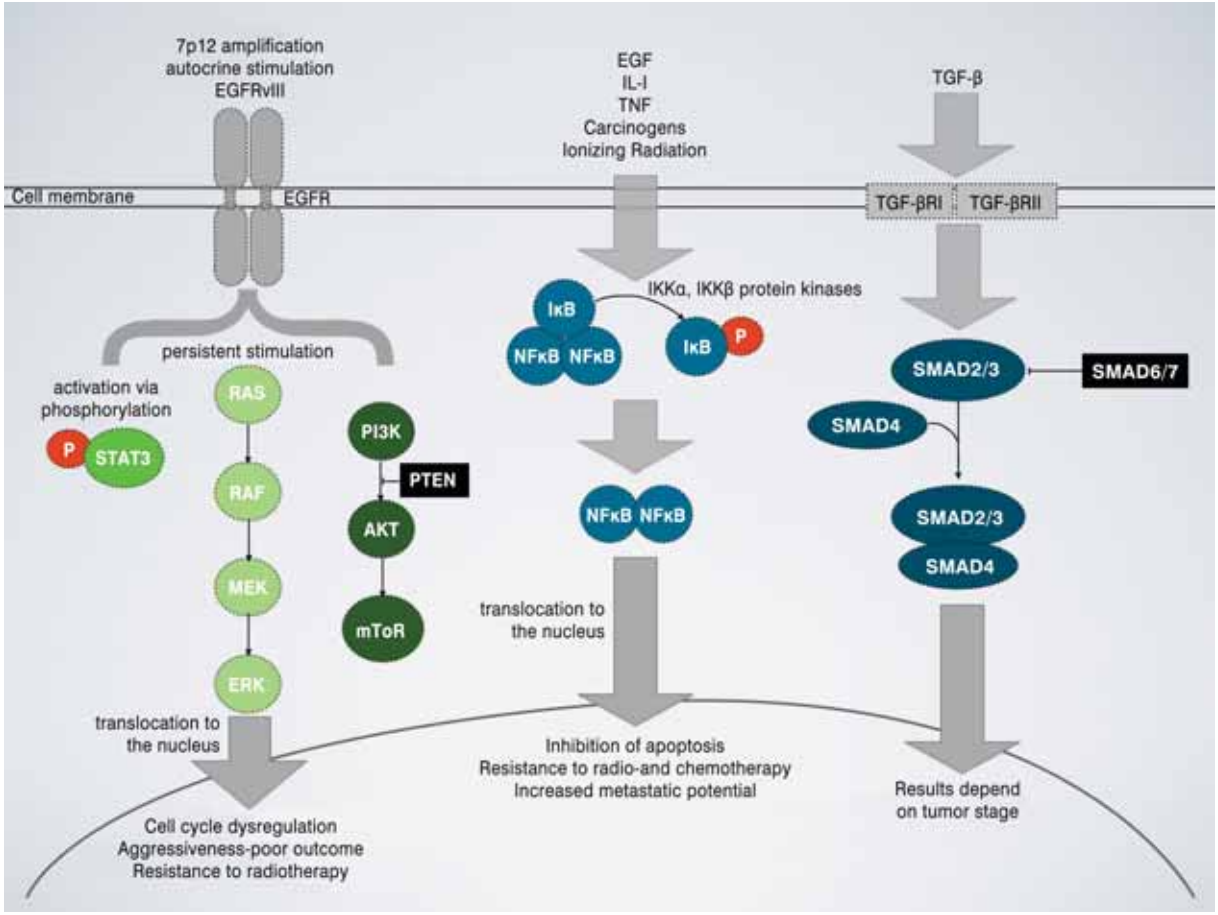

FIG. 5. Aberrant molecular pathways and their contribution to the genesis of OSCC.

these pathways. The molecular progress of carcinogenesis depends on the aberrant expression of a combination of different agents, which explains why a multidisciplinary approach, rather than monotherapy, may offer the best clinical results (Caponigro *et al.*, 2006).

E1a. MAPK Signaling Pathway

The MAPK signaling pathway is a potent regulator of survival, cell proliferation and differentiation. The activation of EGFR sets in motion a plethora of molecules that interact with proteins –most importantly the ras proteins– found at the internal side of the cytoplasmic membrane. They initiate a multistep phosphorylation cascade that leads finally to the activation of the ERK1/2 proteins. These are consequently translocated to the nucleus, causing alteration in the expression of various nuclear and cytoplasmic molecules, i.e. cyc-D1 (Beeram *et al.*, 2005; Roberts & Der, 2007). ERK1/2 levels are elevated in carcinomas with regional lymph node spread and also in relapsing tumors. In addition, the ras proteins play a crucial role in regulating angiogenesis in tumor development, while ERK1/2 can also activate EGFR through an autocrine mechanism (Richter *et al.*, 2005).

E1b. Akt-mToR Signaling Pathway

The serine/threonine protein kinase Akt is a downstream target of phosphatidylinositol-3-kinase (PI3K), which is activated in response to the EGFR elevated levels (Reuter *et al.*, 2007). In addition, the Akt signaling pathway can be triggered by EGFR-indepedent mechanisms: amplification of the 3q26 chromosomal region harboring the PI3KCa oncogene (catalytic subunit of PI3K) is considered a frequent event in HNSCC (~40%) resulting in PI3K overexpression and Akt activation (Woenckhaus *et al.*, 2002; Yu *et al.*, 2007). The same effect can be achieved by the decreased expression of PTEN (phosphatase and tensin homologue – an AKT inhibitor), which is observed in ~30% of OSCCs, and is considered a fine prognostic indicator of poor clinical outcome (Lee *et al.*, 2001; Squarize *et al.*, 2002; Kurasawa *et al.*, 2008).

Akt is a key regulator for the proliferation of both normal and cancer cells and even for angiogenesis (Amornphimoltham *et al.*, 2004; Randis *et al.*, 2008). It is strongly related to progression from dysplasia towards carcinoma *in situ* and invasive carcinoma (Segrelles *et al.*, 2006). Overactivation of Akt is observed in a majority of head and neck tumors (Tosi *et al.*, 2005; Mandal *et al.*, 2006) as well as several other cancer types such as in breast and colorectal cancer (Ghayad & Cohen, 2010; Johnson *et al.*, 2010). Akt activation is considered an early event in oral carcinogenesis (Massarelli *et al.*, 2005) and is consistent with poor patient outcome (Yu *et al.*, 2007).

The most important molecule in the Akt signaling pathway is that of mToR (mammalian Target of Rapamycin). The early presence of mToR in HNSCC has been verified and it is believed that it gradually becomes more intense as the process of oncogenesis continues. Another significant finding from various studies is that mToR can be EGFR-independently activated (Amornphimoltham *et al.*, 2005; Molinolo *et al.*, 2007) thus demarcating the molecule as a potential target for therapy. Rapamycin has been used to inhibit mToR signaling in HNSCCs (Wang *et al.*, 2009a). Recently, a study in mice exhibited promising results, reducing the overall size of premalignant lesions and preventing their further progression (Czerninski *et al.*, 2009).

E1c. STAT Signaling Pathway

Seven members of the STAT (Signal Transducer and Activator of Transcription) family have been discovered to date. They are latent cytoplasmic transcription factors that can be activated by a variety of molecules, such as growth factors, cytokines, hormones and peptides. EGF has been shown to activate STAT1 and STAT3 in EGFR-overexpressing cells (Bowman *et al.*, 2000). The activated proteins translocate into the nucleus, where they interact with a variety of DNA loci (Siavash *et al.*, 2004; Choi & Myers, 2008), such as the VEGF which codes for the Vascular Endothelial Growth Factor, as well as other genes, responsible for cell cycle and death (cyc-D1, c-Myc and Mcl-1) (Onishi *et al.*, 2008).

Studies that have investigated the relationship between HNSCC and STAT proteins, suggest that the expression and tyrosine phosphorylation of STAT proteins, and mainly that of STAT3, is elevated during oncogenesis (Grandis *et al.*, 2000). Furthermore, direct interaction between STAT proteins (STAT3 in particular) and the activated EGFR leads to STAT phosphorylation and subsequent activation (Grandis *et al.*, 2000). The highest levels are found in T1 and T2 stages of tumor development, whereas they are considerably reduced in T3-T4 stages (Nagpal *et al.*, 2002). It seems that this major pathway is inactive in normal oral mucosa, but it is considered as one of the

early phenomena in HNSCC (Song & Grandis, 2000). Since STAT3 is activated by EGFR, EGFR inhibition in combination with STAT decoy oligonucleotides (that inhibit STAT3) could be a potential synergetic therapy protocol (Sen *et al.*, 2009).

E2. TGF-β

The Transforming Growth Factor (TGF-β) shows a remarkable diversity of biological functions and is the generic term for a family of proteins that constitutes of several members with high similarity and homology (Kaminska *et al.*, 2005). There are three subforms, TGF-β1, TGF-β2, TGF-β3, and each one is accompanied by its own receptor (TGF-βRI, TGF-βRII and TGF-βRIII, respectively) (Fujii *et al.*, 1986). TGF-βRI and TGF-βRII are transmembrane proteins involved in signaling pathways, while TGF-βRIII –even though it outnumbers the other two receptor forms– is thought to regulate the binding of TGF with the other two receptors (Massagué, 1998). Through a cascade of phosphorylating events, TGF finally interacts with the Smad proteins (Fig. 5), forming complexes that cause the transcription of genes such as the PAI-1, JunB and type VII collagen gene (Dennler *et al.*, 1998; Vindevoghel *et al.*, 1998; Miyazawa *et al.*, 2002; Pring *et al.*, 2006; Molinolo *et al.*, 2009; Wang *et al.*, 2009b). The biological function of TGF varies depending on the tissue (normal or neoplastic). Physiologically, TGF-β regulates growth, healing process, tooth development and induces fibrosis. In addition, TGF functions to elaborate the extracellular matrix (Prime *et al.*, 2004a).

TGF is thought to possess a dual role in the development of HNSCC; at early stages, it suppresses the epithelial tumor progression via its ability to negatively regulate cell proliferation. However, in later stages of carcinogenesis, TGF enhances invasion and metastatic potential, by promoting angiogenesis and by suppressing the immune responses (Pring *et al.*, 2006). Cancer cells with altered TGF expression, also gain immunity against apoptosis (Prime *et al.*, 2004b).

Structural mutations of the TGF receptors, and mostly TGF-βRII, play an important role (Chen *et al.*, 2001b; Paterson *et al.*, 2001). Another critical point is the TGF-βRII/TGF-βRI ratio. This ratio determines the balance between growth and inhibition. A decreased ratio is responsible for the suppression of the protective role of TGF (Noma *et al.*, 1991). The most crucial mechanism in HNSCC is the decrease of TGF-βRII expression in cancer cells, caused by the hypermethylation of TGF-βRI, post-transcriptional modifications or mutations of the promoter of the TGF-βRII gene (Bae *et al.*, 1995; Munoz-Antonia *et al.*, 1996).

The reduced levels of TGF-βRII, regardless of the way they are achieved, contribute greatly to the progression and development of oral carcinomas. Many studies have suggested that that there is a clear and gradual decrease of TGF-β, TGF-βRI and TGF-βRII expression (from oral normal mucosa – hyperplasia – dysplasia – early and later stages of neoplasia – metastases) (Paterson *et al.*, 2001; Mincione *et al.*, 2008). A distinctive example is that of TGF-βRII; its expression is greatly reduced at the invasive fronts (Mincione *et al.*, 2008). Furthermore, TGF-βRII expression is associated with the histologic degree of differentiation of cancer cells. The lower the TGF-βRII expression, the less differentiated the cancer cells are. Thus, decreased levels of TGF-βRII are linked with more aggressive behavior of the cancer cells in HNSCC (Huntley *et al.*, 2004).

E3. NF-κB

Members of the nuclear factor kappa B (NF-κB) family of dimeric transcription factors (TFs) regulate expression of a large number of genes involved in immune responses, inflammation, cell survival, and cancer. In non-stimulated cells, the NF-κB TFs are physiologically bound to inhibitory IκB proteins and are thereby sequestered in the cytoplasm. External stimuli (Fig. 5) trigger phosphorylation and degradation of IκB proteins, liberating the TFs, which subsequently translocate to the nucleus and drive the expression of target genes that are related with cell proliferation (TNF-a, IL-6), apoptosis (Bcl-2) and immune responses (TNF-a, IL-6, IL-1β) (Sethi *et al.*, 2008). Two protein kinases, IKKα and IKKβ, mediate phosphorylation of IκB proteins and represent a convergence point for most signal transduction pathways leading to NF-κB activation. Most of the IKKα and IKKβ molecules in the cell are part of IKK complexes that also contain a regulatory subunit called IKKγ or NEMO (Häcker & Karin., 2006).

The increased and aberrant expression of NF-κB affects various cellular functions responsible for neoplastic transformation (Allen *et al.*, 2007). Increased levels of NF-κB cause the up-regulation of cyclin D, which elongates the duration of cell cycle (Guttridge *et al.*, 1999; Joyce *et al.*, 2001). NF-κB is also associated with the tumor suppressor protein p53, through

the Mdm2 molecule. High levels of NF-κB dysregulate the expression of p53, resulting in the loss of its protective role (Tergaonkar *et al.*, 2002). Studies have also shown that NF-κB is strongly related with the MMPs (MMP = Matrix Metal Proteinases), thus promoting invasion and metastatic potential (Bond *et al.*, 1998).

There is strong evidence implicating NF-κB in the development of HNSCC. Squarize *et al.* (2006) suggested that NF-κB plays a central role in the expression of IL-6, creating a cross-talk between NF-κB and the STAT proteins, through an autocrine mechanism. Specifically, high levels of NF-κB lead to elevated levels of IL-6 and successively to an increase of STAT expression. Another target-molecule for NF-κB is Cyclooxygenase-2 (COX-2), a key regulatory enzyme in the synthesis of prostaglandins (PGs) from arachidonic acid. Overexpression of COX-2 has been linked with HNSCC, due to the fact that it inhibits apoptosis, promotes cell proliferation and new vessel growth, and disrupts intracellular junctions (Sawhney *et al.*, 2007). Furthermore, the aberrant expression of NF-κB provides cancer cells with resistance to both radio- and chemotherapy (Dong *et al.*, 2002; Kim *et al.*, 2004a; Callaghan *et al.*, 2006) (Fig. 5).

The up-regulation of NF-κB expression is an early event in the development of HNSCC as it is present even from the epithelial hyperplasia stage. While the progression of oncogenesis reaches its final stages, the raise in the NF-κB level is gradually becoming more intense (Sawhney *et al.*, 2007).

E4. Wnt/β-catenin molecular pathway

Another important signaling pathway is that of the Wnt (Wingless Type 1) family (Rhee *et al.*, 2002). The Wnt family consists of nineteen secretory glycoproteins located at the cellular membrane (Uraguchi *et al.*, 2004). The key regulated Wnt effector is β-catenin, which either forms a complex with E-cadherin, contributing to the intracellular junctions and the preservation of tissue architecture, or combines with other molecules stimulating a pathway that might contribute to the carcinogenic process, as its upregulation correlates with inhibition of apoptosis and cell proliferation.

The activation of Wnt results in the release of β-catenin from its complexes and finally the translocation into the nucleus, where it interacts with target genes (Hinck *et al.*, 1994; Papkoff *et al.*, 1996; Orford *et al.*, 1997; Lo Muzio, 2001). Most of the targeted genes code for oncogenetic proteins like COX-2, cyclin D-1 and c-Myc (Chen *et al.*, 2001a; Lo Muzio, 2001; You *et al.*, 2002) that inhibit apoptosis or increase the rate of cell proliferation (Behrens *et al.*, 1996; Huber *et al.*, 1996; Van de Wetering *et al.*, 1997). While the expression of β-catenin is found altered in OSCC (Ueda *et al.*, 2006; Mahomed *et al.*, 2007), no activating mutations in this molecule have yet been identified (Lo Muzio *et al.*, 2005).

Several other components of the Wnt pathway are found deregulated in oral cancers. It has been verified that the activation of Wnt is linked with the inhibition of apoptosis caused by the c-Myc protein, in association with the Tumor Necrosis Factor (TNF). In addition, the up-regulated Wnt affects the expression of MMPs, which are responsible for the degradation of extracellular matrix molecules associated with tissue destruction and – as far as HNSCC is concerned – with invasion (Imai *et al.*, 1996; Ueno *et al.*, 1997; Shimada *et al.*, 2000). The latter is confirmed by various studies demonstrating that the expression of Wnt is more intense at the invasive fronts (Uraguchi *et al.*, 2004; Yang *et al.*, 2006). Recently, Iwai *et al.* (2010) exhibited that certain alterations of the Wnt signaling pathway cause the cell to lose its epithelial morphology and obtain mesenchymal characteristics (epithelial-mesenchymal transition – EMT) resulting in the creation of a more aggressive, invasive cell phenotype. Despite documented observations to date, however, our knowledge on the pathway contribution in the development of OSCC is still rather limited and exhibits small clinical relevance.

CONCLUSIONS

The genesis of OSCC is a very complicated phenomenon, involving countless interacting molecules that can be combined in an abundance of ways. This paper reviews critical molecular events and factors known to be involved in the development of oral and oropharyngeal squamous cell carcinoma. Distinct expression patterns and aberrant signaling pathways can now be identified through the use and progress of molecular biology. The exact mapping of altered genetic loci and role of their products provides us with an invaluable diagnostic tool for the identification of precancerous lesions, thus contributing immensely to the successful treatment of cancer patients.

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