Abstract. The global increase in incidence and mortality, as well as the poor prognosis of oral squamous cell carcinoma (OSCC), has intensified efforts in the field of prevention and early detection of this disfiguring disease. Prevalence of OSCC is common in areas with high consumption of tobacco products and alcohol. Understanding the carcinogenicity of this cancer, using innovative techniques in genomic and proteomic analysis, is the main focus of current research in OSCC, and the hunt for potential molecular biomarkers is accelerating. Although recent advances in preventive, diagnostic and therapeutic techniques related to OSCC have yielded novel molecular targets, partially uncovered signal pathway dominance and advanced early neoplasia detection, the number of deaths attributed to this disease exceeds that reported for cervical cancer, malignant melanoma and Hodgkin’s disease. Application of advanced molecular biology techniques for classification, profiling of tumour tissues and/or identification of potential markers of OSCCs is on the rise. This review aims at outlining the available knowledge on epidemiology, aetiology, molecular biology, and genomics and proteomics in relation to OSCCs.

Epidemiology of oral squamous cell carcinoma (OSCC). OSCC, a malignancy of the lip, mouth or tongue, is predominantly of squamous cell nature with multiple heterogeneous genetic and epigenetic changes that might be involved in the development of this disease (1). The majority of these cancers occur primarily in three anatomical locations: floor of the mouth, soft palate and the ventrolateral aspect of the mobile portion of the tongue (1). The tumour is histologically divided into well-, moderately- and poorly differentiated patterns, with six histological variants recognised in the current WHO classification (1).

The disease ranks 11th in frequency and 13th in cancer-specific mortality, being a major cause of cancer morbidity and mortality in the world (2, 3). The overall incidence attributed to OSCC is increasing worldwide, with current estimates of age-standardized rates of 6.6/100,000 and 3.1/100,000 in men and 2.9/100,000 and 1.4/100,000 in women, respectively (2, 3). Over 500,000 new cases of OSCCs are reported annually with three-quarters of these occurring in patients from developing countries, defined as Africa, Central and South America, Caribbean, China, Asia (Outer-Eastern, South-Eastern, South-Central and Western), Melanesia and Micronesia/Polynesia (2-5). This geographical variation probably reflects the prevalence of specific environmental influences, rather than genetically-determined or ethnically-related risk factors (3-6).
An estimated 30,000 new cases of this cancer are expected to be diagnosed in the United States in 2003, and approximately 7,200 people will die of the disease (7). In Eastern and Central European countries, a 2 to 3-fold mortality increase has been recorded in the last 3 decades (8).

Several factors point to a progressive increase in the incidence and mortality of OSCCs worldwide, with additional factors that might contribute to this increase in the developing countries. Among these are the continuous deterioration in oral health care, lack of diagnostic techniques and methods of early detection, lack of proper coding systems, ambiguity of terms sometimes used as causes of death, inaccuracy of demographic information, lack of autopsy data, urbanization and the changes in lifestyle recently witnessed in many of these countries. In addition, there is very little information on the prevalence of OSCC in many of the developing countries, with other factors leading to lower reporting of OSCCs as compared to...
the developed world. These include cultural influences, lack and delays associated with the provision of oral health services, low rate of publications in peer-reviewed journals, under reporting of OSCCs patients in health centres, deficiency of patient records or OSCC registries, short life-span, and the fact that poor people (the mostly affected ones) do not have access to general oral health services which are mainly designed for well-off individuals.

**Aetiology of OSCCs.** In the West, epidemiological studies suggest that the social habits of tobacco use and/or alcohol consumption are associated with the increased risk of OSCC (9). In South-East Asia, increased risk for this type of cancer is associated with chewing of tobacco (5, 6, 9). In addition to OSCCs, tobacco use, either smoked or non-smoked, is a well-known risk factor for multiple neoplasia, including those of the oesophagus, lung and bladder (10). Co-factors in several of these malignancies, in particular OSCCs, include alcohol, dietary factors, immunodeficiency and viral infections (11). Despite the possible role of tobacco/or alcohol exposure in the development of OSCCs, the majority of people who smoke, chew tobacco and/or drink alcohol do not develop the disease, and not all OSCC patients are users of any of these substances (9). These observations point to possible genetic factor(s) that might be involved in the development of OSCCs (9). In head and neck SCCs, epidemiological evidence from case–control studies indicated that a family history of this type of cancer is considered to be a risk factor for the development of the disease with individuals having a positive family history having 3.5–3.71 times the risk of developing the cancer, albeit when tobacco smoking and alcohol consumption are allowed for (12, 13).

**Genetic changes involved in OSCCs**

**Role of tumour suppressor genes (TSGs)/oncogenes.** Although the mechanism(s) underlying the development of OSCC remains unclear (14-16), carcinogenesis of this disease has been suggested to be a multi-step process involving interaction(s) between several factors like tobacco-associated intra-oral carcinogens, alcohol consumption and/or viral infections (reviewed in 14-16). In carcinogenesis, tumour cells proliferate excessively without coordination with the microenvironment and the cells continue to divide as a result of loss of either cell cycle check-points, other cell cycle/growth control regulators, apoptosis, oncogene/TSGs, DNA damage response and/or other cellular regulators (Figure 1). In the literature, a series of reviews have critically summarised findings related to genes/or their protein products that might be involved in the development of OSCCs (reviewed in 17-25). TSGs, representing loss-in-function and defined as gene products whose loss contribute to the development of cancer, are classified as "gatekeepers" and "caretakers" (26, 27). Gatekeepers, like p53, pRB, ABC and NK1, function to directly suppress cell proliferations and, upon re-introduction of the gene into tumour cells, suppression of tumourigenicity might be achieved (26, 27). Caretakers, however, function to maintain the integrity of the genome through excision repair, mismatch repair and homologous recombinations (26, 27). As a result of the functions of the caretakers, mutations in other genes, including oncogenes or gatekeeper TSGs, might occur (26, 27). TSGs are known to play a crucial role in cell cycle regulation, arrest and apoptosis, and failure of the role(s) played by any/or all of these genes will force the protective system to collapse and enable for a cancer to develop (Figure 1). By now, there are about 2200 genes with known or unknown functions, that have been detected through the Cancer Genome Anatomy Project (CGAP), suggested to be involved in the carcinogenesis of OSCCs (28). Among these genes are known TSGs (like p53, p16, p15, p18, p19, p21, p27, p53, p14ARF, p19INK4A, p16INK4B, p15INK4B, p14AFR, p15ARF, p21, p27, p53, pRB) and oncogenes (like hst-1, int-2, EGFR/erbB, c-erbB-2/Her-2, sis, Cyclin D1, ras, raf, stat-3, myc, fos, jun, c-myc, bcl-2, Bax), which were found to be involved in the carcinogenesis of OSCCs, though with conflicting results (reviewed in 14-25). One of the most studied TSGs in cancers, including OSCCs, is the p53 gene, which has been called the "Guardian of the Genome" or "The Molecular Policeman", that has a central role to play in genome integrity and stability, cell cycle progression, cellular differentiation, DNA repair and apoptosis (27, 28). The gene is known as a transcription factor and it exerts its TSG function, in part, by activating target genes after upstream stress signals, such as DNA damage, have been relayed (Figure 1). The p53 gene can be inactivated by several mechanisms including point mutations, deletions and/or binding with other cellular and/or viral protein like the E6 protein of the human papilloma virus types 16/18 (27-29). Estimates suggest that up to half of all human cancers carry some sort of inactivation of the p53 gene via point mutations, overexpressions, deletions and/or binding to viral proteins (27, 29, 30, 31). In oral/head and neck SCCs, the reported incidence varied from 43-96% (reviewed in 32-34) – probably as a result of substantial differences in the detection techniques used (32-34). Mutations of this gene were also found in a minority (range of 22-45%) – also subject to the method used – of oral dysplastic lesions, showing that the gene can be mutated before development of the invasive neoplasia (32-36). However, results on p53 gene expression in oral/head and neck SCCs were conflicting regarding the relationship between the expression/or mutations of the gene, clinical as well as the patient's histopathological parameters (32-34). The role played by mutations of this gene in the progression and vital
inactivation of other TSGs like $p_{16}^{ink4a}$, $p_{15}^{ink4b}$, $p_{19}^{ink4c}$, $p_{14}^{ARF}$, $p_{21}^{waf1}$, $p_{27}^{Kip1}$ and pRB, which are directly or indirectly linked to the transcriptional activity of the gene, have also been examined in these cancers (17-25).

Oncogenes, defined as genes with a "gain-in-function", contribute to converting a normal cell into a cancer cell when a normally present proto-oncogene becomes inappropriately activated through either mutation or abnormal expression at high levels (26, 27). These can be represented by growth factors or growth factors receptors (hst-1, int-2, EGFR/erbB, c-erbB-2/Her-2, sis, Cyclin D1), intracellular signal transducers (ras, raf, stat-3), transcription factors (myc, fos, jun, c-myb) and apoptosis (bcl-2, Bax) (26, 27). Activation of oncogenes might affect the gene product itself, leading to direct activation or alteration of its regulatory mechanism, which then leads to increased expression and/or failure to switch off transcription at appropriate times. So far, several studies have examined the expression/or mutations in over a hundred oncogenes in dysplastic epithelium and oral/head and neck SCCs (reviewed in 14-25). In many studies, several known oncogenes were consistently detected in these cancers, some being found to be more strongly associated than others and, interestingly, few being found to be limited to specific cancers associated with tobacco habits (reviewed in 14-25). It has been argued that oncogenes, either directly or indirectly, might be connected to development and/ or growth disturbances in oral/head and neck SCCs, possibly by acting as cellular ‘switches’ at key biochemical points to affect a whole series of phenotypic changes, thus leading to tumour transformation in these cancers (reviewed in 14-25). Therefore, understanding the functional variety of these oncogenes might provide valuable insights into the complex network of events that are involved in the tumourgenesis of OSCCs, and it is hoped that this might lead to both diagnostic and therapeutic benefits for patients (reviewed in 14-25).

Most of the studies that have described TSGs/or oncogenes in OSCCs, using either cell culture experiments/or archival formalin-fixed, paraffin-embedded tissues or fresh frozen tissue specimens, have examined either amplifications, deletions/or mutations of a gene at the DNA level, over- or under-expression of the gene product at the RNA/or protein level using traditional methods in molecular biology like PCR, PCR-SSCP, PCR/direct DNA sequencing, RT-PCR, CDGE, blotting, DNA cloning, in situ hybridizations, ELISA, EIA, RIA or immunohistochemistry which generally work on a "one gene in one experiment" basis, indicating that the throughput is substantially limited and the "whole picture" of the gene function(s) is hard to obtain. The fact that multiple TSGs/or oncogenes have been reported in oral dysplastic and malignant epithelium, and the "whole picture" of the gene function(s) is hard to understand of the reported in oral dysplastic and malignant epithelium, and the "whole picture" of the gene function(s) is hard to understand of the role played by $p_{53}$ gene in oral/head and neck SCCs, reviewed in 32-34, 37).

For a better understanding of the reported in oral dysplastic and malignant epithelium, and the "whole picture" of the gene function(s) is hard to understand of the role played by $p_{53}$ gene in oral/head and neck SCCs, reviewed in 32-34, 37). Nevertheless, it is now possible to identify over/or under-expressed molecular targets in OSCCs using the new approaches of genomic and proteomic research.

**Genomic instability.** Cytogenetic analysis of OSCCs to detect chromosomal alterations either at the short (p) or long (q) arm of a chromosome following evaluation of the human genome, has become quite efficient using techniques such as restriction landmark genomic scanning (RLGS), fluorescence in situ hybridization (FISH), cross-species colour banding (RxFISH), loss of heterozygosity (LOH), restriction fragment length polymorphism (RFLP), comparative genomic hybridization (CGH), microsatellite instability (MI), chromosome painting and trypsin-giemsa banding (G-banding), which are frequently used to detect patterns of chromosomal imbalances and gross chromosomal regions involved in structural rearrangements (39-41). The most valued findings, thus far, in oral/head and neck SCCs are gains of 3q, 8q, 9q, 20q, 7p, 11q13 and 5p and losses of 3p, 9p, 21q, 5q, 13q, 18q and 8p (reviewed in 22). It has, therefore, been suggested that cytogenetic analysis may be useful for examining clinical differences in tumour behaviour and response to therapy. Numerous studies are now underway to examine the biology of, and genetic changes in, OSCCs that might lead to additional markers for use as rapid, non-invasive screening methods for individuals at high risk for primary or recurrent cancers (reviewed in 22).

**Immortalization by telomerase activity.** The structures at the ends of eukaryotic chromosomes, "termed telomeres", are random arrays of hexamers TTAGGG, bound to proteins and are located on the ends of each chromosome (42). Since telomeres are lost during cell divisions, the chromosomal ends are no longer protected, which leads to the fusion of chromosomes and karyotypic abnormalities that eventually cause cell death (42). The ribonucleoprotein enzyme telomerase extends the telomeric repeat sequences at the chromosomal ends; it is active in about 90% of human neoplasia, but inactive in most of the normal cells (42). In a study by Mao et al. (43), head and neck SCC cell lines, tumour specimens and adjacent normal and dysplastic mucosa were analysed for telomerase activity. No telomerase activity was found in any of the normal tissues, but it was found in 100% of the cell lines, 90% of the dysplastic lesions (43). However, recent developments suggest that telomere maintenance might not be an obligatory requirement for initial tumour formation in some settings and that telomerase activation contributes to tumourigenesis involving a number of aberrant molecular events affecting a range of genes, pointed to a great for large scale analysis of genes that might be involved in this type of cancer. Nevertheless, it is now possible to identify over/or under-expressed molecular targets in OSCCs using the new approaches of genomic and proteomic research.
independently of its role in maintaining telomere length (42). However, the understanding of telomere biology remains incomplete and implicates additional complexity in the relationships among telomere, telomerase and oral/head and neck SCCs, as suggested by others (43-46).

Angiogenesis. As normal cells progress toward malignancy, they must switch to angiogenic phenotype to attract the nourishing vasculature that they depend on for their growth, in a process known as angiogenesis. Angiogenesis, defined as the growth of new blood vessels (neovascularization) from pre-existing ones, is a multi-step process, which appears to be regulated by both stimulatory and inhibitory factors and is vital for the continued growth and survival of solid neoplasms (47, 48). The vast majority of highly-vascularized tumours showed a poor prognosis and the influence of tumour angiogenesis proved to be independent of conventional prognostic indicators. The angiogenesis-indicating signals are exemplified by vascular endothelial growth factor (VEGF), some matrix metalloproteinases (MMPs), interleukin-8 (IL-8) and acidic as well as basic fibroblast growth factors (FGF 1/2) (49). Expression of VEGF family members, i.e. VEGF-A and VEGF-C has been reported in head and neck SCCs and has been found to be associated with metastasis (50). Although the quantification of microvessels in tissue sections is mainly used to evaluate angiogenesis, it appears to be subject to biases, as reflected in contradicting research findings where, in particular, the choice of the staining methods seems crucial. However, since studying tumour angiogenesis offers a uniquely attractive therapeutic target, the next decade may produce better methods that can be used to produce a

Figure 2. Schematic illustration of the cDNA and protein microarrays, 2-D PAGE and MALDI/SALDI-TOF-MS techniques in oral squamous cell carcinomas and their pair-wised normal controls.
Dissecting the genome by advanced new technologies

Completion of the Human Genome Project (HGP) more than three years ago led to a surge in use of genomic and proteomic technologies for identification of markers for early detection and molecular-targeted therapy in neoplasia (51, 52). The number of defined human genes and expressed sequence tags (ESTs) continues to grow rapidly and new tools are being developed for interrogation of these databases (51, 52). High-throughput innovative screening techniques in genomics and proteomics are now widely available, allowing researchers to rapidly screen and confirm new genes, mRNA transcripts and/or proteins. In particular, differential expression of these molecules between malignant and pair-wised normal tissues allows researchers to identify genes and critical pathways that are involved in cancer development.

Laser capture microdissection (LCM). The tumour microenvironment of a carcinoma consists not only of the malignant epithelial component, but also the surrounding stroma and normal tissue. These distinct microcompartments use receptors, cell junctions, and inter- and intracellular signalling molecules to allow tumour cells to communicate with their surroundings, playing an active role in their control/or progression (53). Removing a subpopulation of these cells for growth in an in vitro system interrupts potentially important cell–cell and cell–matrix interactions that might affect the neoplastic behaviour, therefore giving scientists a false impression of the in vivo tumour composition and physiology. The advent of LCM has enhanced our ability to specifically dissect and remove target subpopulations of cells from frozen/or ethanol-fixed tissues, as well as cell culture populations under direct microscopy, which can further be stained or unstained and used for any kind of study that involves changes in DNA, RNA/or proteins (54). High-throughput analysis of microdissected specimens allows for clean discrimination of events occurring in- and between each of these tissue micro-compartments and, accordingly, researchers are now able to profile low molecular weight proteins in patient serum samples and query them with new, powerful bioinformatics tools to cluster unaffected and cancer patients into their respective groups. To take a more realistic "snapshot" of a tumour’s in vivo inherent properties using LCM, Alevizos et al. (55) carried out large-scale gene expression profiling on tumour and normal oral epithelial cells and identified about 600 genes to be associated with OSCCs. These OSCC-associated genes included oncogenes, TSGs, transcription factors, xenobiotic enzymes, metastatic proteins, differentiation markers and genes that have not, so far, been implicated in OSCCs (55).

Genomic array technology. Microarray, or DNA chip, is a new powerful tool for studying gene expression profiles of biological samples to determine the molecular basis of interactions on a large scale that is impossible using conventional analysis (56). Since the start of its application on biological samples in 1995, the fundamental concepts behind the array, the technology needed for making and using these chips, and the multitude of statistical tools needed for analysing the data have been extensively reviewed and critically evaluated (56). This technique makes it possible to address fundamental research questions by examining the expression of thousands of genes simultaneously, with a promise to help in the development of rational approaches to therapy, as well as to facilitate disease diagnosis and prognosis, assuring its entry into clinical practice in specialist centres and hospitals within the next few years (56). The technology is now a well-established method for comparing the expression of genes and ESTs in laboratory and clinical samples, where thousands of genes can be examined simultaneously through amplification of RNA with fluorescent labels and applying the labelled transcripts to array slides containing large numbers of oligonucleotides or cDNAs (Figure 2). Advances in array technology provide a high-throughput approach to monitor the c-DNA arrays for mRNA or total RNA expression, genomic single nucleotide polymorphism (SNPs) and CGH (56). This technology is being used to identify patterns of gene expression, SNPs and CGH that may be indicative of certain diseases, including OSCCs, where a detailed comparison of gene expression profile with respect to normal tissue is possible (Figure 2). After completion of the HGP, there has been a dramatic increase in the amount of genomic information now available in databases, with the prediction that more updated information will be available in databases, thus giving rise to changes in disease research (51, 52). With over 30,000 known human genes, the application of innovative techniques is important for understanding the function and structure of genes involved in a disease process (51, 52). The complete sequence of the human genome will also provide a new starting point for understanding our basic genetic makeup and how variations in our genetic instructions result in disease, in particular, OSCCs.

To date, comparison of gene expression profiles between oral/head and neck SCCs and normal tissues, that showed altered expression levels of genes involved in the control of cell growth and differentiation, angiogenesis, apoptosis, cell cycle and signalling, has been shown in a few, well-controlled, published microarray studies from the West with significant findings (57-63). Schmalbach et al. (59) reported
the expression profile of 101 genes between metastatic and non-metastatic oral/oropharyngeal SCCs, with a subset of 57 genes that showed significant expression differences between metastatic tumours and normal mucosa. The profile found included genes related to the extracellular matrix, adhesion, motility, inflammation and protease inhibition suggesting that the knowledge gained might facilitate early detection of aggressive tumours and targeted therapeutic investigations (59). Belbin et al. (60) identified 375 genes that showed significant expression differences, and divided the patients with head and neck SCCs into clinically distinct subgroups based on the gene expression patterns, suggesting that gene expression profile can be used as a predictor of outcome (60). In another work by Ginos et al. (60) the gene expression profile was studied in 41 cases of head and neck SCCs, and 2890 genes were found to be associated with proliferation, extracellular matrix production, cytokine/chemokine expression, immune response, invasion and metastasis, which showed significant differences in their expression, suggesting evidence for a new gene expression-based biomarker of local treatment failure in these cancers (61). On the other hand, Leethanakul et al. (62) successfully applied LCM to procure cells from representative sets of head and neck SCC and their matching normal tissues to be used in array experiments. A consistent decrease in expression of differentiation markers like cytokeratins, and an increase in the expression of a number of signal transducing and cell cycle regulatory molecules, as well as growth and angiogenic factors and tissue degrading proteases, were found between cancer and normal cells (62). It has been suggested that the LCM approach might facilitate the identification of candidate markers for early detection of premalignant lesions, as well as novel targets that can be applied for pharmacological intervention in this type of cancer (62).

In a recent report, Ibrahim et al. (64) studied the differential expression of 588 genes by cDNA macroarray analysis in Caucasians and Africans, and found that alterations of 123 genes are common in OSCCs, regardless of ethnic differences, other socio-cultural risk factors and clinicopathological parameters. Among the genes found were several interesting keratin genes, p15

\[\text{ink4a}\], Bax, CASPs, MMPs, TOP1, p73, DNA-repair protein XRCC1 and DNA excision repair protein ERCC5 (64).

Proteomics technology. The term "proteomics" indicates proteins expressed by a genome, and is the systematic analysis of protein profiles of tissues paralleling that of genomics (65) (Figure 2). The evaluation of protein structure, function and regulation has evolved rapidly over recent decades, and large amounts of information about a particular protein’s activation status and interactions can now be obtained in a matter of minutes, employing the new high-throughput approaches (65) (Figure 2). The mainstay of proteomics–based expression profiling is still the combination of high-resolution 2-D PAGE gel electrophoresis and mass spectrometry (MS) (66). Recent
breakthroughs in protein microarrays have produced commercially available protein pathway arrays, that are able to test cell lysates for both their phosphorylated and non-phosphorylated forms using specialized protein chips. Protein microarrays also depend on specific antibody ligand interactions, but avoid some of the complications of immunohistochemistry by allowing rapid, quantitative comparison of the proteins of interest using fluorescence-based imaging programs (65, 66). Matrix-assisted laser desorption and ionization time-of-flight (MALDI–TOF) and surface-enhanced laser desorption and ionization (SELDI)-TOF are two of the methods currently being employed for this purpose (65, 66). The general principle of the MALDI-TOF revolves around the rapid photovolatization of a sample embedded in UV-absorbing matrix, followed by TOF-MS analysis (67). MALDI-TOF-MS has become a popular and versatile method to analyse a wide range of macromolecules of biological origin, from cells to tissues (67). Due to its ability to desorb high-molecular weight thermolabile molecules, its high accuracy and sensitivity, combined with its wide mass range (1-300 kDa), MALDI-TOF-MS is becoming a promising method in the clinical chemistry laboratory for identification of biomolecules in complex samples, including peptides, proteins, oligosaccharides and oligonucleotides (67). The other new protein analysis system, SELDI, has recently been applied for the separation, detection and analysis of multiple proteins in very small amount (~10ng) of micro-dissected cancer tissue (67). This system facilitates protein capture, purification, analysis and processing from complex biological mixtures directly on to protein chip array surfaces, and the detection of the purified proteins is performed by TOF-MS. A major feature in SELDI-TOF-MS is its ability to provide a rapid protein expression profile from a variety of biological and clinical samples. The use of these methods for biomarker identification, as well as the study of protein–protein and protein–DNA interaction, makes it suitable for studies involving OSCCs, since it has been used in projects involving identification of potential diagnostic markers for prostate, bladder, breast and ovarian cancers (66). There are very few studies, all from Western countries, which have applied these techniques in OSCCs to search for related tumour proteins that might have potential clinical applications as biomarkers, enabling tumour identification at an early stage in high-risk individuals, prediction of treatment response and detection of residual or recurrent carcinoma (68-71). He et al. (68) examined the protein expression profile in OSCCs/their corresponding normal resection margins by MALDI-TOF-MS, and found a number of tumour-associated proteins including heat-shock protein (HSP) 60, HSP27, alpha B-crystalline, ATP synthase beta, calgranulin B, myosin, tropomyosin and galectin 1 to be significantly altered in their expression levels in cancers, compared with their paired normal tissues (68). However, due to the complex and heterogenous components of the tissue samples, proteins extracted from tissues are often mixed with many other kinds of molecules such as lipid and carbohydrate, etc. which are often from undesired cells and/or components and can hardly be removed completely during the protein sample preparation, which results in poor separation and low resolution of protein spots on 2-D PAGE. To obtain a homogeneous cell population, Knezevic et al. (69) analysed protein expression in tissue derived from OSCCs, using an antibody microarray approach and LCM to procure total protein from specific microscopic cellular populations. Although the cellular quantities obtained by LCM are generally low, which makes the detection of low abundance proteins difficult, and the fact that fixation and staining procedures involved in LCM can possibly cause some artifacts, they have demonstrated that quantitative, and potentially qualitative, differences in expression patterns of multiple proteins within epithelial cells reproducibly correlate with progression of OSCCs, where most of the proteins identified in both cell types were involved in signal transduction pathways (69). In an attempt to map proteins present in saliva by 2-D PAGE, Ghafoori et al. (70) identified 100 proteins representing 20 different identities according to accession numbers. Abundant proteins found expressed in different forms were: alpha-amylase, immunoglobulin A, prolactin-inducible protein, zinc-alpha (2)-glycoprotein and cystatins (S, SA, D and SN), while other proteins found were interleukin-1 receptor antagonist, von Ebner’s gland protein (lipocalin-1) and calgranulin A and B (S100A8 and A9) (70), apolipoprotein A-I, beta (2)-microglobulin, glutathione S-transferase P and fatty acid-binding protein, showing that human saliva contains a large number of proteins that are involved in inflammatory and immune responses (70). Wu et al. (71) performed 2-D PAGE/SELDI-TOF-MS to identify proteins differentially expressed in two head and neck cell lines (one derived from the primary tumour and the other from a metastatic lymph node) obtained from the same patient. Two membrane-associated proteins, annexin I and annexin II, and glycolytic protein enolase-alpha were found to be up-regulated, and calumenin precursor down-regulated, in the metastatic cell line, suggesting that these proteins might be important molecules in head and neck SCC invasion and metastasis (71).

In most of the developing countries, tumour data banks exist only in either formalin/or ethanol-fixed paraffin-embedded tissues, which produce excellent histomorphology and good preservation of macromolecules. However, and for proteomic research initiatives to take place in these countries, the use of available data banks needs to be tested and evaluated first in Western research laboratories. In a recent study, it has been shown that proteins were
Molecular markers in OSCCs. In a recent review of 169 published articles, Schliephake et al. (73) identified 29 suggested molecular markers of relevance in OSCCs although the prognostic relevance of these tumour markers is still not quite clear. Only 12 out of 23 reports on the prognostic relevance of markers for cell cycle acceleration and proliferation indicated a significant association with prognosis, while 20 out of 29 studies on markers for tumour suppression and anti-tumour response showed prognostic relevance (73). Markers of angiogenesis exhibited only minor importance for the prognosis and treatment of OSCC (73). Results on markers of tumour invasion and metastatic potential appeared to be too premature for a statement regarding their prognostic value. In general, the location of markers within the malignant lesion, and not their quantitative assessment, is important. The analysis of the invasive front of the neoplastic lesion with regard to the occurrence of molecular markers is supposed to be of great importance for prognostication, and it is expected that genomics and proteomics research in OSCCs might provide better and sensitive molecular biomarkers that can be used in these cancers.

Gene therapy. The approach of gene therapy is important to the treatment of many diseases and, to achieve promising gene therapy strategies for OSCCs, there is a need to understand the molecular mechanism(s) involved in the development of the disease, yet no single molecule characterises all types of OSCCs. In oral/head and neck SCCs, replacement of TSGs, specially the p53 TSG, in cancer cells has been studied and clinical trials have shown evidence of gene transduction and expression, mediation of apoptosis and clinical responses including pathological complete responses (reviewed in 74-78). However, efforts to improve gene delivery systems, design of immunogenic and antiangiogenesis gene therapy, as well as adjuvant use of gene therapy with conventional chemotherapy, radiation therapy and surgery in oral/head and neck SCCs are on the increase, and further refinement of these efforts are to achieve clinical success (73-78).

Future directions

A progressive increase in incidence and mortality of OSCC patients is expected in developing countries. Although the disease is among the few preventable human cancers, the identification, development and validation of markers related to OSCC is challenging oral cancer research laboratories in both developed and developing nations. Despite some promising molecular markers, to find a useful molecule that can be used in early diagnosis of the disease in otherwise asymptomatic individuals consuming excessive amounts of tobacco/or alcohol has proven to be an elusive goal. Most of the current markers in OSCC are directed towards formulating a clinical decision-making process, following an initial suspicion raised by more conventional means. This points to a need for targets that can be of practical value to the healthcare system, where use of such markers in diagnosis, prognosis and therapeutic selection can become more common and the type(s) of samples to be tested can be expanded. Expected information from these technologies might soon exert a dramatic change in the pace of OSCCs research and may have a positive impact on the care of OSCC patients. There is a need for a new age of OSCCs molecular target discovery and combined proteomic and genomic information will be mined for markers useful to the five main known categories of tumour biomarkers: screening, diagnosis, prognosis, therapeutic monitoring and prediction of OSSC recurrence. Biomarkers, as competing technologies for cancer screening, may be judged on their effectiveness in reducing cancer mortality by the early detection of precancerous lesions, which are amenable to surgical removal or treatment by chemotherapy and chemoprevention. Identification of biomarkers is increasingly being used for investigation and is producing an enormous amount of information, and with the rapid advances in genomic and proteomic technology, the discovery of useful biomarkers has arisen to unravel the unique signatures of cancer cells. These signatures are enabling investigators to pose scientific questions and address problems that, until recently, were inconceivable. For instance, the focus on biomarker-based disease detection has now shifted from one biomarker to a panel of biomarkers, in distinguishing normal cells from precancerous or cancerous cells.

Statistical methodologies are being developed to analyze multivariate data in reference to disease outcome, with sophisticated bioinformatics tools and computation algorithms developed to extract knowledge from data generated by high-throughput technology in genomics and proteomics. The role of MALDI-TOF-MS/SELDI–TOF-MS in cancer fingerprinting is very promising and the widespread availability of this expertise will go a long way in helping diagnosis and monitoring of several malignancies, including OSCCs. New signal pathway-
targeted pharmacotherapeutics, such as trastuzumab for breast carcinoma, as well as imatinib for gastrointestinal stromal tumours and chronic myelogenous leukaemia, have come from an enhanced understanding of the genetic and protein alterations within a tumour. It is high time that oral cancer becomes the next candidate for targeted therapies, since genomic searches for novel genetic alterations and proteomic profiling of OSCCs are just the beginning of the changes yet to come in diagnosis and treatment of this disease. The co-evolution of genomics and proteomics as complementary approaches to neoplasia will allow us to move closer to the goals of earlier detection, improved prevention and institution of a molecular-based, tumour-specific approach to the treatment of OSCC of each individual patient. Following the era of the human genome and the emergence of innovative technologies, there is a good chance for oral cancer researchers to collaborate and conduct comprehensive analysis of the genome, transcriptomes and proteomes in health and disease by sharing knowledge between developed and developing world. In the former scenario, although prevalence of OSCC is generally low, substantial advances have been made in the application of advanced techniques for early detection, effective treatment and molecular understanding of OSCCs. In the developing part of the world, however, where OSCC is a pressing problem, other types of cancers like lung, breast, colon and leukaemia might overshadow the problem, given the limited resources available. Nevertheless, it is predicted that soon the ever-increasing body of translational research will move from the laboratories to the clinics and revolutionise the gloomy scenario currently facing the increasing numbers of patients with OSCCs.

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