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Review Article

Molecular biology: an early detector of oral cancers

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Abstract Oral cancers have been one of the leading causes of deaths particularly in the developing countries. Prime reason for this high mortality and morbidity is attributed to the delay in diagnosis and prompt treatment. Relentless research in the field of oncology has led to advent of novel procedures for the early detection of oral cancers. Molecular biology is highly promising in this regard. It is a procedure that detects alterations at a molecular level much before they are seen under a microscope and much before clinical changes occur. Molecular studies serve as basis by which we will eventually be able not only to augment clinical assessment and classification of oral lesions but also predict malignant potential of oral lesions, thus reducing incidence and increasing the scope for early diagnosis and treatment of oral cancers. However, making such sophisticated tools available for the common man in developing countries is one of the most important challenges faced today.

Keywords: Oral cancer; Early detection; Molecular biology; Developing countries

1. Introduction

Oral cancer is the sixth most common cancer of both sexes in the general population and the third most common cancer in developing nations. About half of patients afflicted die within 5 years of diagnosis, whereas surviving patients may be left with severe esthetic and functional compromises [1-3]. India has one of the highest incidence of oral cancer in the world [4]. Oral cancer ranks number 1 among men and number 3 among women in India [5].

Such high impact diseases are challenging to diagnose with clinical evaluation and existing laboratory testing. Even with the advent of newer technologies and sophisticated laboratory tools, definitive diagnosis has always been elusive. More than two thirds of oral cancers documented are diagnosed only at an advanced stage. To address this fatal problem, an accurate, qualitative, convenient, and noninvasive diagnostic tool needs to be devised. Molecular biological analysis is one such promising platform that can be used for early detection of oral cancers, their management, and thus improve prognosis for the patients. When considering these tools for diagnosis, cost is also a very important factor to be considered, especially in developing nations like India where estimated per capita income is $275.

Molecular biology is defined as the study of biology at a molecular level. It chiefly concerns itself with understanding the interactions between the various systems of a cell including interactions between DNA, RNA, and protein biosynthesis and learning how these interactions are regulated. Extensive research has been carried out in the field of oncology, and molecular tools have assumed great importance. Because our area of concern is the oral and perioral structures, it is of importance to highlight the molecular analytical tools that have been used and that could be used in future in diagnosis and treatment of oral cancers.

Molecular tools most commonly used are the following:

- Chromosome in situ hybridization
- Cytomorphometry
- Immunohistochemistry
- Polymerase chain reaction
- DNA image cytometry
- DNA miroarrays
- Proteomics
- Gene therapy.

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2. Chromosome in situ hybridization

Chromosome in situ hybridization (CISH) is a cytogenic technique that is used to detect and localize the presence or absence of specific DNA sequence or chromosome. It uses fluorescent probes that bind to only those parts of the chromosomes with which they show a high degree of sequence similarity. Fluorescence microscopy is used to find out where the fluorescent probe is bound to the chromosome, thus providing an ability to directly visualize the genetic change in tissue sections or exfoliated cells.

In oral cancers, this technique is used to form the diagnosis and evaluate prognosis and remission of the disease. Analyses of normal and premalignant lesions adjacent to tumors have demonstrated that chromosome instability can be detected in the field of the tumor (ie, in normal and premalignant cells in a tissue at 100% risk of tumor development), and the degree of chromosome instability increases with the degree of histologic progression toward cancer. Analyses of premalignant lesions, for example, oral leukoplakia and erythroplakia from individuals at risk for cancers by CISH have uncovered varying degrees of chromosome instability [6]. Studies indicate that most leukoplakia lesions contain an abnormal number of chromosomes 7 and 17, and lesions with greater than 3% proportion of cells with trisomy 9 have a significant higher likelihood of progression to cancer [7,8]. Studies have resulted in detection of new molecular markers like HER-2/neu at the protein level in salivary duct carcinomas (SDCs). Tissue sections from 12 previously diagnosed SDCs were evaluated by immunohistochemistry (IHC) and CISH for HER-2/neu status. A total of 4 SDCs were positive by IHC; all 4 cases showed amplification with CISH. The remaining 8 cases were negative by IHC and showed no gene amplification with CISH. Salivary duct carcinomas in this study show HER-2/neu overexpression on both the protein and gene levels in approximately 30% of cases [9]. Use of CISH technique can prove to be useful as a molecular diagnostic tool for oral cancers, and its relatively lower cost when compared to techniques like microarrays makes it more affordable for the low socioeconomic groups of the society.

3. Cytomorphometry

It is a quantitative technique that evaluates parameters such as nuclear area (NA), cytoplasmic area (CA), and nuclear/cytoplasmic ratio (NA/CA). Papanicolaou smears are prepared, and the NA, CA, and NA/CA are recorded.

In oral cancers, malignant changes are detected through estimation of NA/CA. They typically show a reduction in CA before the reduction in NA. Cytomorphometric techniques are used to assess nuclear diameter and cytoplasmic diameter in dysplastic lesions and oral squamous cell carcinomas. Cytoplasmic diameter was highest in normal mucosa, lower in dysplastic lesions, and lowest in squamous cell carcinomas. By contrast, nuclear diameter was lowest in normal mucosa, higher in dysplastic lesions, and highest in squamous cell carcinoma. Thus, it suggested that reduced nuclear size and increased cytoplasmic size are useful indications of malignant transformation [10-12]. Cytomorphometric techniques have also been used to assess the effects of smoking crack cocaine on the oral squamous epithelial cells. The study revealed increase in the NA and decrease in the CA and consequent increase in NA/CA, thus concluding that crack cocaine was able to induce significant changes on the oral epithelial cells. Because this illicit drug is normally used in association with other risk factors for oral cancer (tobacco and alcohol), crack cocaine abusers should have frequent preventive oral exams [13].

4. Immunohistochemistry

Immunohistochemistry is a popular technique that has been used as a part of histopathological examination. It can also be used in assessing molecular alterations in premalignant and malignant oral lesions.

p53 mutations are one of the most common events in tumorogenesis of oral cancers [7]. The gene is located at 17p 13.1. It functions as a ‘guardian of cell’ by providing the molecular brake and maintaining the genomic stability. When DNA damage occurs, cell produces p53, which stops cell division by arresting cells at G,-S boundary, induces DNA repair and triggers programed cell death or apoptosis. Tumor suppression of p53 is negated by point mutation, deletion of alleles, and binding to viral protein (E6 or E7 of HPV 16/18). Mutant p53 demonstrates a longer half-life than wild type, and its mutant form is often detectable by molecular biology techniques [14]. p53 protein expression has been detected by IHC in 90% of oral leukoplakia, whereas it is absent in normal mucosa [15]. Several studies have demonstrated that p53 detection by IHC alone or with other markers appears to be associated with greater risk for malignant progression [16-22]. Parabasal detection of expression of p53 by IHC has been shown to have a stronger correlation with progression to cancer in several studies [15,23-25], lending credence to the potential application of p53 detection by IHC for stratification of risk of malignant transformation in oral leukoplakia [7,26-29].

The immunohistochemical investigations have also been employed in detection of human papillomavirus (HPV) infection in oral precancerous lesions and oral cancers. The study suggest that HPV type 16/18 has a close association with the development of oral squamous cell carcinoma and that the infection is maintained even after neoplastic change of the infected cells [30].

Immunohistochemistry is widely accessible and easy to perform at a reasonable cost. However, this semiquantitative procedure is beset by technical artifacts, sensitivity differences between different antibodies, and subjective interpretation, resulting in interobserver variability between pathologists.

5. Polymerase chain reaction

Polymerase chain reaction (PCR) is an extremely versatile technique used for copying of DNA. It allows a single DNA sequence to be copied millions of times or altered in a predetermined way. PCR technique is used for quantitative measurements of DNA or RNA molecules. It has also been used to introduce restriction enzymes sites or to mutate particular bases of DNA.

Polymerase chain reaction technique has been used to amplify DNA in samples from oral carcinomas and has been analyzed with restriction fragment length polymorphism. It detects the commonly implicated molecular alterations in oral cancers such as loss of heterozygosity, microsatellite instability, and changes in methylation pattern [12].

The causal link between HPV and a subset of head and neck squamous cell carcinoma (HNSCC) cases has also been established in recent years. Human papillomavirus DNA has been identified in approximately 15% to 26% of HNSCC cases. The most commonly detected HPV in HNSCC is HPV-16, which has been demonstrated in 90% to 95% of all HPV positive HNSCC cases, followed by HPV-18 and HPV-33. To identify this, PCR technique has been used [31-35].

Another interesting trend currently being investigated is the use of the PCR to determine if surgical margins obtained at the time of surgery that are histopathologically free of tumor contain a small amount of histologically undetectable tumor cells. Specifically, the use of PCR to detect specific mutations identified in the primary tumor in the histopathologically negative surgical margin could be very useful because these mutations would indicate the presence of residual (histopathologically undetected) tumor cells [36]. It will be very important to establish whether the presence of submicroscopic tumor cells contributes to prognosis and clinical outcome.

Use of versatile techniques like PCR must be made readily available and more affordable to the general population by getting third parties to cover them. These techniques enable the clinicians to identify the pathology at the earliest, thus contributing to the future therapeutic improvements.

6. DNA Image cytometry

DNA image cytometry is a technique that permits quantification of nuclear DNA content, thereby assessing the DNA ploidy status.

Molecular and genetic changes arise during carcinogenesis and could lend themselves to be markers of transformation. Mutations in p53, loss of heterozygosity, and chromosomal polysomy are all associated with progression to carcinoma and may be predictive when used and analyzed in combination [37-40]. Routine use of these techniques is, however, hampered by the complexity of the tests, the lack of facilities in many routine laboratories, and the high cost involved [41,42]. As a surrogate for such individual molecular markers, measurement of gross genomic damage, in the form of aberrant DNA content, could be a valuable method for prognostication of malignant and premalignant lesions [43-49]. Relativity recently, there has been a major advancement in this area with the use of automated image cytometry to measure ploidy in nuclei extracted from routinely processed paraffin sections. This system may be more sensitive in the evaluation of oral potentially malignant lesions into ‘low’ and ‘high’ risk [50,51].

Evaluation of ploidy status in oral leukoplakia and oral lichen planus allows the identification of gross genomic alterations and has been shown to be a useful tool in identifying lesions with high risk of malignant transformation to oral cancers. Studies show that anueploid lesions have the maximum incidence of malignant transformation [34,52,53]. DNA image cytometry has also been used to detect cancer cells with abnormal DNA content at the invasive tumor front, which is associated with poor prognosis of the patients with oral carcinomas [54,55]. Thus, it could help to find the appropriate treatment option for the patients. The sensitivity of cytological diagnosis combined with DNA cytometry is 98% and specificity 100% when compared with gold standards of histology [12].

7. DNA microarrays

DNA microarray is a high throughput technology used in molecular biology and helps in the study of sequence of genes. It consists of an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing a specific DNA sequence. This is a short section of gene or other DNA element that are used as probes to hybridize DNA or RNA samples. Because an array can contain tens of thousands of probes, a microarray experiment can accomplish many genetic tests in parallel [56,57].

Gene profiling method has been used for comparing the normal and cancerous oral mucosa, and a distinct pattern of gene expression was observed when normal and cancerous cells were compared in genome-wide analysis of oral cancer [58]. Gene profiling by DNA microarrays is capable of identifying up-regulated or down-regulated genes correlated with oral tumor recurrences and lymph node metastasis, thus helping clinician to plan a treatment that prevents recurrence and reduce chances of metastasis [34].

Microarray platforms have to be developed to accomplish the procedures. A number of issues must be addressed before establishing a microarray platform and beginning expression profiling studies, in particular, the overall cost. For a cDNA microarray platform, one must purchase a clone set, robot, printing pins, and reagents needed for DNA amplification and purification. The cost of these materials can vary significantly, but one can expect to need at least $100 000 to establish such a platform. However, once the process of printing and hybridizing microarrays has been optimized, the cost per experiment will fall dramatically. Thus, one must

decide if the number of planned experiments is enough to warrant the time and cost of establishing a microarray platform. Another challenge is the efficient management and analysis of the large volume of data generated by microarray approaches. However, increasingly sophisticated computational methods continue to be developed that are amenable to large data sets generated from microarray experiments. Certainly, as key disease pathways are identified, custom arrays containing relevant subsets of genes may eventually be integrated into clinical settings for more widespread use [59].

8. Proteomics

The term "proteomics" indicates PROTEins expressed by a genOME and is the systematic analysis of protein profiles of tissues, paralleling the related field of genomics [57]. The major workhorse of proteomics-based expression profiling is still the combination of high resolution 2-Dgel electrophoresis and mass spectrometry. Laser Capture Microdissection, protein biochips, and isotope-coded affinity tag peptide labeling are among the technologies that are currently having an important impact on cancer research [60]. A new protein analysis system, based on the surface enhanced laser desorption/ionization has been recently applied for the separation, detection, and analysis of multiple proteins in very small amounts (10 ng) of microdissected cancer tissue. This system facilitates protein capture, purification, analysis, and processing from complex biological mixtures directly onto protein chip array surfaces, and the detection of the purified proteins is performed by time-of-flight mass spectrometry [56]. For a better understanding of how patterns of protein expression shape the tissue microenvironment, protein expression in tissue derived by Laser Capture Microdissection from squamous cell carcinoma of the oral cavity was analyzed using a high-throughput antibody microarray approach [61]. A reproducible correlation was found between the expression patterns of multiple proteins within epithelial cells and the progression of oral cavity tumor. A comparison of the protein maps of normal and malignant prostate were used to identify 20 proteins lost in malignant transformation, including PSA, a-1 antichymotrypsin, haptoglobin, and lactylglutathione lyase [62].

Hence, proteomic technologies have the potential to greatly aid the development of molecular diagnostics and serve as markers for the early detection of cancer. These technologies will also accelerate the anticancer drug target discovery and validation. Furthermore, proteomic technologies will be used to design rational drugs according to the molecular profile of the cancer cell and thus facilitate the development of personalized cancer therapy [57].

9. Gene therapy

Gene therapy is a promising new approach for the management of oral cancers. It is the insertion of gene into an individual's cells and tissues to treat a disease in which a definitive mutant allele is replaced with a functional one. Gene therapy uses an adenovirus vector, which is used to introduce modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

Gene therapy approaches to oral cancers and precancers include the following:

- Addition gene therapy: Aim of approach is to regulate the tumor growth by introducing tumor suppressor gene that inactivates the carcinogenic cells.
- Gene therapy using oncolytic viruses: This approach uses viruses that replicate only in the tumor cells and thus kills them.
- Suicide gene therapy: In this therapy, enzyme encoding gene is introduced into the tumor cell that stimulate the generation of products that are toxic for the cells.
- Immunotherapy: The aim of immunotherapy is to increase the patient's immune response to the tumor.
- Introduction of genes to inhibit tumor angiogenesis: This technique uses microencapsulated cells for the release of therapeutic proteins to encapsulate recombinant cells [63-70].

Use of molecular biology for management of oral cancers is still at its infancy. In the future, it may be the treatment of choice that will overcome all the complications associated with the present trend of management of oral cancers, thus proving to be a blessing to the patients.

10. Conclusion

Molecular analysis is a fast emerging and fascinating technique that can replace the present available diagnostic procedures in the field of oral oncology for early detection of oral cancers, thus saving the patients of physical and mental trauma they go through when oral cancers are detected at an advanced stage. However, practical problems such as affordability of these tests have to be considered when such tools are used for common man living in developing and underdeveloped countries. Governments of such countries in association with organizations like the World Health Organization and the United Nations Educational, Scientific, and Cultural Organization should conduct nationalized health program schemes for regular screening of high-risk patients with molecular analytical tools at subsidized rates so that oral cancers can be detected at the earliest and hence reduce morbidity and mortality in countries like India, where the incidence of oral cancers is high.

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