Diagnostic omics based molecular techniques in oral pathology: Current perspectives and future prospects

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Abstract

The completion of human genome project has been a landmark event in the development of diagnostic pathology and personalized medicine. Like other fields of medicine, oral pathology also tends to show a paradigm shift from conventional diagnostic techniques to a molecular based format. Omics based molecular techniques can be accurately used for diagnosis, prognosis, risk analysis and treatment of various oral lesions especially oral cancer. This will go a long way in providing a more precise diagnosis which in turn will give the patient a risk free treatment protocol tailored for his individual needs. The paper analyses the evolution of oral pathological diagnostic techniques with an impetus into the current omics based techniques and a peep into the future development prospects.

Keywords: Oral cancer, Omics based, Molecular.

Introduction

Basic biomedical laboratory techniques are often the bed rock on which clinical practice is based. Compared to conventional diagnostic techniques newer methods exhibit more accuracy and depth which helps the clinician in justifying his decision making.[1] The Flexner report published in 1910 linked the scientific principles underlying human biology and disease to the clinical decision making that is central to effective medical practice.[2] There is a trend to apply omics based techniques to improve human health by accelerating drug discoveries and motivating life styles.[3] Even though omics based diagnostic techniques hold great promise several factors discourage their use over conventional diagnostic methods especially in third world countries. These include lack of skilled man power,[4] lack of infrastructure like clinics and laboratories,[5] poor computer connectivity, unfavorable health policies,[6] lack of data and even religious beliefs.[7] Hence it is imperative that newer techniques are made more cost effective so that better clinical care can be provided to the patient.

Pre-omics Diagnostic Techniques in Oral Pathology

Oral cancer has always been an extremely challenging diagnostic dilemma for oral pathologists as its prognosis depends mainly on its staging. Almost half of the cases of oral cancer have been diagnosed in TNM stages III and IV.[8] Oral and orophryngeal cancers usually have a survival rate of less than 50% after 5 years but show great improvement if diagnosed early.[9] Cancer mortality is affected by delay in diagnosis especially if it is done in advanced stages beyond the sequelae of treatment.[10] Hematoxylin & Eosin staining backed by use of special stains and good clinicopathologic acumen is often considered the gold standard in diagnosis of oral lesions. The next innovation was the use of immunohistochemistry which is rarely used for a primary diagnosis but is most significant when applied for poorly differentiated neoplasms and in categorization of lymphomas.[11] However there are severe limitations of immunohistochemistry like variable antibody reactivity, staining, poor quantification and subjective interpretation.[12] Traditional biochemistry has used several approaches for diagnosis including ELISA, gene cloning, conventional and real time qualitative PCR, chromosomal cytogenetic analysis, comparative genomic hybridization, karyotyping, electrophoresis and fluorescence in situ hybridization (FISH).[13] These techniques have enabled identification of molecular markers of several diseases. The angiogenic marker cluster of CD34 serves as an important predicting tool for recurrent OSCC.[14] Saliva has been used as a potential agent in detection of oral diseases and several studies have identified small ncRNAs in them which is a biomarker for cancer.[15] Elevated expression of TGF and EGFR in premalignant lesions with dysplasia was used as an intermediate marker of malignancy.[16] Kang et al[17] showed an enhanced BMI (B lymphoma –MLV insertion region 1 homolog),an oncogene expression in premalignant lesions.

Omics based Diagnostic Techniques

Genomics

Cancer based genomics is a rapidly growing discipline in which the genetic molecular basis of malignancy is studied at the scale of whole genomes and successfully identified specific oncogenes and tumor supressors.[18] Genomic alterations have been identified for leukoplakia as well as in the process in sequential cancer tumerogenesis.[19] Even though specific oncogenes and tumor suppressor genes have been identified, therapy for cancer management strategies' is still at its infancy.[18]

Genome wide Association Studies (GWAS)

The genome wide association study attempts to discover novel genes affecting an outcome by testing a large number of genetic variants for association. GWAS is usually hypothesis generating rather than hypothesis testing. While GWAS discoveries can be directly used for clinical risk prediction on rare occasions, the more typical scientific path is for GWAS to generate gene that may be associated with a

disorder. GWAS studies in oral health including periodontal diseases, salivary flow rate and dental fear are currently under way. Notable association for oral health include PVT1/GSDMC locus on chromosome 8q24 associated with nonsyndromic CLL with or without CLP. IRF6 gene associated with cleft lip/palate, ACTN2 gene with regulatory ameloblasts during enamel formation and TFIP11, associated with extracellular enamel matrix has been identified by GWAS.[20]

Whole Exome Sequencing (WES)
Sequencing the entire protein coding regions is called exome sequencing. It is a promising omics tool for rapid identification of functional variants.[21] Al-Hebshi et al.[22] used WES technique in addition to confirming known genes of oral squamous cell carcinoma identified several novel driver events and pathways providing further evidence for genetic heterogenicity of oral cancer. Stephens et al (2013)[23] and Nichols AC(2012)[24] used whole exome sequencing in study of adenoid cystic carcinoma and HPV positive an negative head and neck squamous cell carcinoma

Next Generation Sequencing (NGS)
Next generation sequencing, massively parallel or deep sequencing are related terms that denote a DNA sequencing technique where an entire human genome can be sequenced within a day.[25] Progress in genome sequencing technologies post the genome project completion in 2013 has led to decreased cost per megabase and increase in number and diversion of sequence genomes. Probing genomes in greater depth can lead to enhanced understanding of how genome sequence variants underlie phenotype and disease.[26] Use of NGS has enabled researchers in identifying alterations in OSCC. Whole genome sequencing studies have identified alterations with TP53, CDKN2A, PIK3CA and HRAS genes.NGS was used to identify NOTCH 1 gene involved in squqmous differentiation.[27] Sweeney et al[28] used NGF metod and found that mutations in SMO gene coded smooth protein was more common in maxillary ameloblastomas while BRAFV600E mutations were commoner in mandibular ameloblastomas.

Microbiomics
Microbiotic cells amounting upto 10 to 100 trillion within a single human form a significant propotion of the total living cells in the body. The population of our microbial occupants is known as our microbiome.The greater uniqueness of individual microbiome can help us tackle health issues pharmacogenetically with greater ease.[29] The adoption of an ecological approach for maintanence of oral health with a shift to personalized stratergies to refrubrish health of helpful oral microbiota subsequent to the dysbiosis after treatment of oral ailments like periodontitis.[30]

Proteomics
Chi et al[31] used mass spectrometry based quantitative proteomic analysis by way of labeled mass spectrometry completed to high performance liquid chromatography system (HPLC) to reveal signaling pathway for oral cancer cells in vitro. Their findings reveal that interferon signaling pathway is significantly altered in OSCC lesions. The role of Interferon in pathogenesis of OSCC as a potential biomarker and for targeted therapy should be explored further.

Transcriptomics
Saliva is a noninvasive accessible and efficient diagnostic media. Salivary transcriptomic analysis can detect a large panel of human DNA which has a diagnostic value in evaluation of OSCC .Li et al[32] analysed potential salivary RNA biomarkers IL8, IL1B, DUSP1, MA3, OAZ1, S100P and SAT and yielded 91% specificity and sensitivity in detecting OSCC from controls proving that the novel clinical approach can be a very robust reproducible tool for early cancer detection..Studies analyzing the role of HPV virus in oropharyngeal squamous cell carcinoma using different transcriptomics profiling using RNA scope to stratify subtypes of head and neck cancer.[33]

Epigenomics
Genetic information such as DNA sequences have been limited to fully explain mechanism of gene regulation and disease process. Epigenetic mechanisms which include DNA methylation, histone modification and non-coding RNAs can regulate gene expression and affect disease progression. Epigenetic mechanism play important role in gene expression during developmental and pathological processes of dental diseases. Epigenetic modification can lead to dental abnormalities during teeth development. Inflammatory reaction in dental pulp and periodontal tissues affect epigenetic modifications causing change in gene expression.[34] Oral cancer has also been subjected to epigenetic analysis with numerous studies revealing that the development and progression of this malignancy are partially induced by altered epigenetic substrate together with genetic alterations and prolonged exposure to environmental risk factors.[35]

Lipidomics
Lipids play critical functions in cellular survival, proliferation, interaction and death since they are involved in chemical energy storage, cellular signaling and cell-cell interactions. These cellular processes are strongly related to carcinogenesis pathways particularly to transformation, progression and metastasis suggesting the mediator role of bioactive lipids in oncogenesis.[36] Rapid intraoperative mass spectrometry (REIMS) is an emerging technique that allows near real time characterization of human tissue in vivo by analysis of aerosol released during electrosurgical dissection. The coupling of REIMS technology with electro surgery for tissue diagnostics is called intelligent knife (iknife) The use of iknife which is a fast, real time mass spectrometry based identification of surgical margins of tissues introperatively by differentiating tumor and normal areas and hence waiting for frozen sections can be avoided.[37]

Metabolomics

Metabolomics is a core descriptive science of system biology focusing on the study of low molecular weight compounds in biological system. Analysis of human metabolome which is composed of diverse group of metabolites can aid in diagnosis and prognosis of oral squamous cell carcinoma.[38] Breathomics, a branch of metabolomics measures total amount of volatile organic compounds (VOCs) in exhaled air. VOCs produce in the body and released into blood and transported into the lungs where they are exhaled. Measurement of unique VOCs may indicate the presence of chronic inflammation and oxidative stress that can be used as potential biomarkers for head and neck cancer detection.[39]

Ancillary Omics based Molecular Techniques
Molecular Imaging

Imaging techniques can be used as non invasive approach to detect molecular and cellular techniques in living cell and organisms. Techniques like CT, MRI and PET can be used to screen patients with oral tumors particularly OSCC in early stages.[40] Antibody coated gold nanoparticles target and illuminate cancer cells under a reflectance based optical imaging system. Gold nnoparticles provide an optical contrast to discriminate normal and cancerous cells.[41] Oral cavity when exposed to light in UV-visible regon, flourophones absorb a portion of photons and gets excited and the lower energy photons can be detected as floorescence. Cancerogenesis changes the concentration of fluorescence emitting properties of native flourophores which is helpful in detecting cancer sensitivity. Autoflourescence spectroscopy is also useful to separate normal, premalignant and malignant lesions.[42] By using Fourier transform (FT)-Raman spectroscopy changes in vibrationary band of normal, dysplastic and OSCC seem to arise from the structural, conformational and compositional structure of proteins.[43] Principal component analysis (PCA) of the Raman spectral date can discriminate between normal, inflammatory, premalignant and malignant conditions of oral tissues.[44] Conofocal Reflective microscopy is a non invasive optical tool where image contrast is determined by differences in refractive indices of organelles and other subcellular structures within the tissue. It differs from conventional microscope as it illuminates a small spot within tissue with laser.[45] It can be used noninvasively to image tissue without fixation, sectioning and staining and can be used for pre surgical determination of cancer margins and intrasurgical guidance of patients in real time thus being valuable diagnostic tool for precancer and cancer.[46]

Microfluidic System

Microfluidic system, proposed by Manz and Wedmer in 1990 also called micrototal analysis systems (μTAS) or "lab on a chip" have shown tremendous potential in the field of single cell analysis as the microfluidic systems have the advantages of high analysis efficiency, easy operation, small sample and reagent composition and dimensional matching between channel sizes and cell diameter.[47] Microfluidic evices utilize cellular biophysical properties and devices have been developed to measure single cell mechanical or electrical properties distinguishing normal cells and their malignant counterparts. Cancer cells can by identified from clinical samples by cell affinity chromatography, magnetic activated cell sorting microfiltration and On Chip DEP (Dielectrophoresis).[48]

Stem Cell Therapies

In head and neck OSCC, cancer stem cells have an integral role in tumor intiation, disease progression and treatment. It has features like self-renewal, high migration capacity, drug resistance and high proliferation ability.[49] Various techniques for cancer stem cell detection proposed include marker expression and cell expression by flow cytometry, side population assay, ALDH activity test, spheres test, soft agar assay and cryopreservation of tumor cells.[50]

Conclusion

Due to the exponential increase in genome based techniques with declining costs it is not far when genome related visits by patients to clinics becomes the norm rather than the exception. The individual ownership of a persons genome and its formation and its rights to informed consent and privacy over sequencing, testing and disclosing genomic information is yet to gain legal recognition.[51] It is imperative that these molecular based techniques should be used as in an adjunct role for in depth diagnosis while maintaining conventional pathologic techniques as a gold standard.

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