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Approaches of Oncogenomics and Bioinformatics in OSCC and HNSCC detection of novel genetic alterations

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ABSTRACT

Cancer, a genetic disease which is caused due to genetic mutation like point mutations in genome. Genome is the total count of chromosome. Oral squamous cell carcinoma is the 2nd while Head and neck cancer is the 6th death causing disease worldwide. The arrival of new genomic era provides a valuable sources of knowledge to investigate new technologies for cancer detection and treatment. Oncogenomics is the sub-branch of genomics. Onco-genomics is also known as cancer genomics. Oncogenomics applies high throughput technologies to characterize cancer associated genes and to identify new oncogenes or tumor suppressor genes, which may provide new insights for cancer diagnosis, predicting clinical outcome of cancers and new targets for cancer therapies. It includes the study of genomes and their associated interactions in cancer cells by genomic technologies and investigate its potential to revolutionize clinical practice, including cancer diagnosis. Over the past decade, the development of new genomic technology has revolutionized the modern biological research and drug discovery. In this review article, we provide an overview of the current and emerging tools involved in genomic studies, including expression arrays, microRNA arrays, array CGH, ChIP-on-chip, methylation arrays, mutation analysis, genome-wide association studies, proteomic analysis, integrated functional genomics analysis and related bioinformatic and biostatistical analyses. This genetic paradigm now motivates intensive efforts in cancer gene discovery and validation. High-resolution genome scanning technologies, such as array based comparative genome hybridization (array-CGH), have uncovered highly re-arranged human cancer genomes harboring strikingly large numbers of recurrent copy number alterations (CNAs). Oncoproteomics, application of proteomic technologies in oncology and parallels the related field of oncogenomics. With a wealth of information that can be applied to a broad spectrum of biomarker research projects serves as a reference for biomarker researchers, scientists working in proteomics and bioinformatics, oncologists, pharmaceutical scientists, biochemists, biologists, and chemists

Key word: Oncogenomics, Oncoproteomics, OSCC, HNSCC, array-CGH, CNAs, Bioinformatics.

INTRODUCTION

Oncogenomics, a high throuput technology which characterizes different genes associated with cancer cells to identify new oncogenes or tumor suppressor genes, which may provide new insights for cancer diagnosis, predicting clinical outcome of cancers and new targets for cancer therapies. The outcome of targeted cancer therapies such as Gleevec, Herceptin and Avastin raised the hope for oncogenomics to elucidate new targets for cancer research[1]. Oncogenomics study can identify new drug targets for genotype-specific treatments and also provides strategies to validate the targets and to develop drugs. Oncogenomics research has progressed logically from molecular profiling to model systems, cancer pharmacology and clinical trials. Its study covers cutting-edge issues such as array-based diagnostics, pharmaco proteomics, pharmaco genomics and molecularly targeted therapeutics includes discussions of ethical, legal, and social issues related to cancer genomics and clinical trials[2].

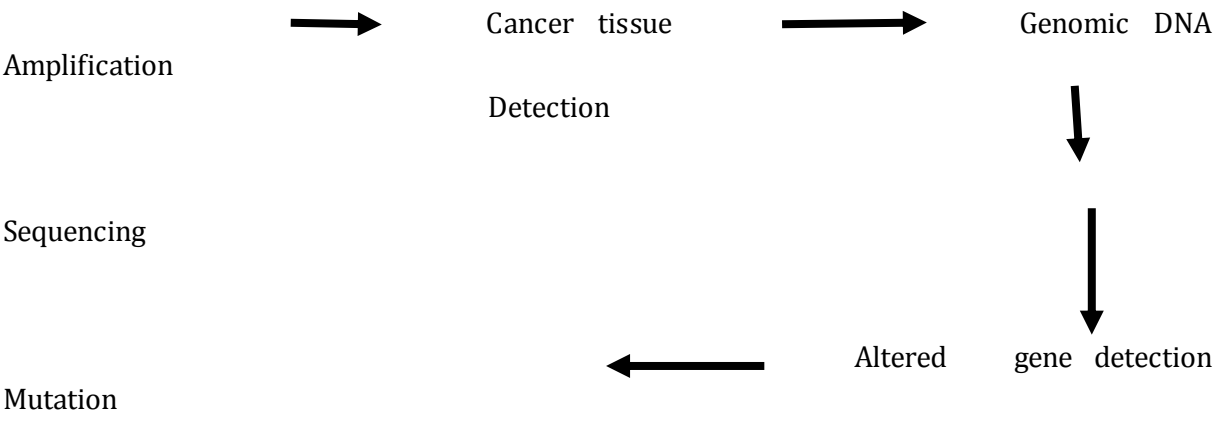
Alcohol and tobacco also acts like carcinogens which are responsible for the alteration in pathway like DNA repair, carcinogen metabolism and cell cycle. Tobacco use has steadily declined over the last four decades[3]. In parallel, there has been a decline in cancers of most in incidence of HPV-positive head and neck squamous cell carcinoma (HNSCC) has been dramatic, causing the rates of tonsillar cancer to increase by as much as threefold in some countries[4,5]. HNSCC mostly found on areas like larynx, pharynx, oesophageous and these are different histologically, biologically and pathologically[6]. OSCC and HNSCC patients are drastically increasing due to infection of human papilloma virus which effects oral cavity and esophagus[7,8] .

HPV-positive patients markedly experience a better survival , their tumors are molecularly distinct from traditional head and neck cancers[9]. Human papillomavirus-related (HPV+) head and neck squamous cell carcinoma (HNSCC) is a subgroup of HNSCC where the incidence is continuously increasing in most developed countries[10]. The vast majority of HPV+ HNSCC originates from the oropharynx, and in particular the tonsillar beds[11]. These tumors are almost exclusively associated with HPV-16 which have integrated and functionally active E6 and E7 viral oncoproteins while in comparison to HPV-negative tumors appear to have an overall better outcome, independent of treatment modality[12]. Oral squamous cell carcinoma(OSCC) a multistep process which results from several epigenetic, genetic and metabolic alterations due to exposure of oral mucosa to tobacco carcinogens, alcohol or human papilloma virus This genetic alterations which transforms normal cell to cancer cell occur only in certain genes which are also known as cancer-causing genes and these genes have been traditionally classified as either proto-oncogens (e.g., the genes for MYC, ERBB2,[HER-2/neu], and EGFR) or tumor suppressor genes such as the genes that encode TP53, CDKN2A, and RB. P^{53} is a tumor suppressor gene, imbalance in the P^{53} cause the pre-malignant lesions. Pre-malignant lesions indicates the early stage of oncogenesis [13,14,15].

Protooncogenes normally function as proliferative agents, and when mutated or misregulated in cells, they promote uncontrolled cell growth resulting in transformation to cancer cell. In cell cycle alteration the most commonly altered gene in HNSCC are CDKN2A, CCND1, and RB1. NRF2, KEAP1, CUL3 are the oxidative response of HNSCC which are altered by mutation[16,17,18]. EFGR,

NOTCH1, P53, FBXW7 are HNSCC genes. PIK37, AKT, mTOR in between these genes PIK37 is the 2nd most commonly mutated gene[19,20]. Usually these genes are phenotypically dominant and require a gain-offunction mutation or chromosomal gain to become oncogenic.

The availability of the human genomic sequences has made easier to locate alterations in genes such as chromosomal aberrations, translocations, deletions, amplifications and methylation[21,22,23,24,25,26]. Now a days DNA sequencing techniques are used to reduce the cost and efficiency. Here in the given diagram strategies of Oncogenomics work are shown.



BIOINFORMATICS AS A TOOL IN ONCOGENOMICS STUDY:

Oral squamous cell carcinoma (OSCC) and HNSCC (Head and neck squamous cell carcinoma) are the most common tumors originating from abnormal squamous cell in oral cavity and oesophageous . Although many immeasurable contributions have been done but the molecular mechanism of OSCC and HNSCC seems to be less clarified. Use of bioinformatics methods can explore differentially altered expressed genes (DEGs) between Oral, Head and Neck squamous carcinoma tissueswith normal tissues as these differentially expressed genes demonstrated the potential to serve as prognostic biomarkers[27] and therapeutic targets. GO (Gene Ontology) analysis also helps to investigate the critical genes in the progression of OSCC and HNSCC[28] . Furthermore, protein-protein interaction (PPI) networks and pathway enrichment analysis can also be done to estimate the significant pathways. Thus all theseprovides a systematic perspective to understand the underlying mechanism in OSCC and HNSCC development. One of the most commonly used methods to annotate the gene function is through Gene Ontology (GO, http://www. geneontology.org/)[29,30]. GO classifies gene function according to three organizing principles molecular function, biological process and cellular component. When certain GO terms are statistically enriched in a cluster, it may suggest possible functional significance of the cluster of genes. Another commonly used bioinformatic analysis tool is Gene Module Analysis[31,32] .On the basis of GO term analysis builds on preexisting knowledge for the interpretation of microarray data, one can interrogate the global gene expression profile with respect to known sets of genes by gene module analysis.

BIOINFORMATICS DATABASES FOR ONCOGENOMICS ANALYSIS

Databases and software are already established for the study and analysis of gene mutations. COSMIC (Catalogue of somatic mutation in cancer) database assemble curate, organize and provides information for somatic cancer mutations[33]. cBIOportal (http://www.cbioportal.org/) visualize the TCGA data features including many data sets which makes availability of a copy number of alteration and mutation. OncoPrint also displays the same alteration data as in network viewer in a matrix heatmap. A feature which makes it different is its easy use. Oncomine includes cancer microarray database and integrated data mining tool. The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/) has characterized an extensive number of human tumors to search for molecular alterations in particular gene. TCGA an evolving technology mainly provides information of tumor types and can be apply to identify the Oncogenomics alterations[34]. Progenetix (http://www.progenetix.org/) database provides information about copy number variation of human cancer[35]. Cytoscape (http://www.cytoscape.org/) Software are used for visualizing the complex networks, for integration of these with any type of genomics data and patients clinical information available. A wide library has been made to focus on developed community for cancer data analysis[36].

Approaches to oncogene Identification

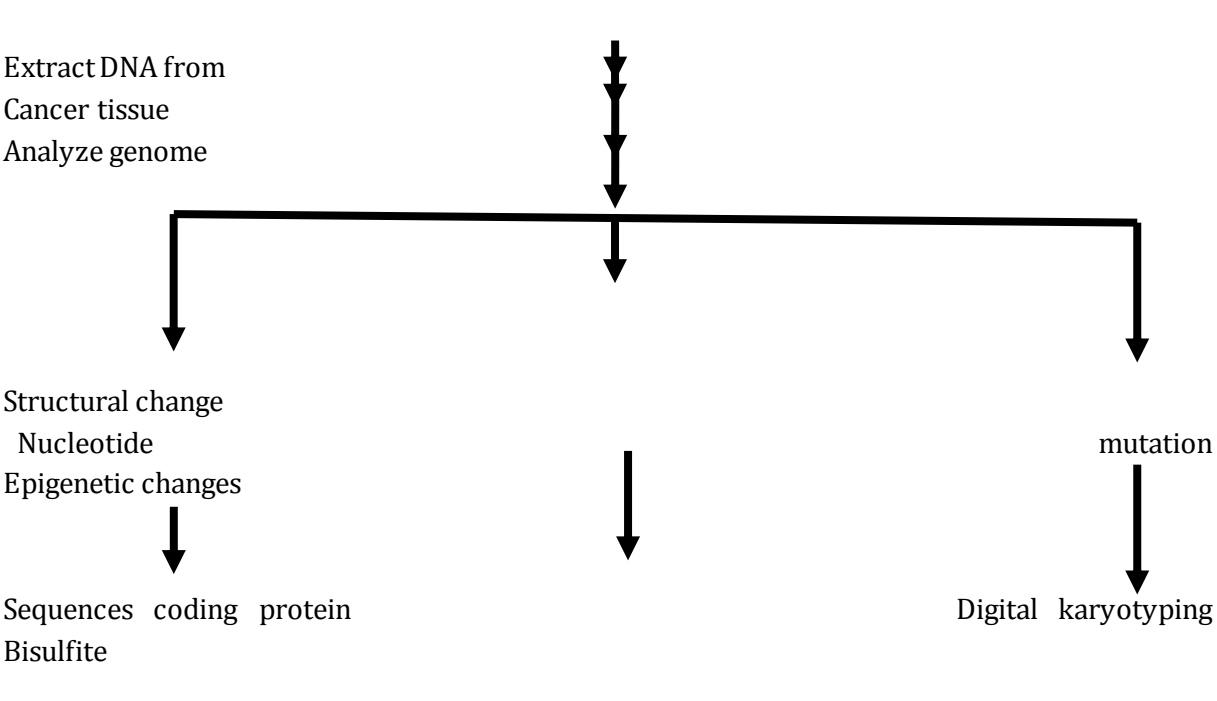
New high-throughput genomic analysis techniques such as massively parallel sequencing and ultra high-resolution CGH are much beneficial in identifying the remarkable heterogeneity in cancer genomes by implicating a multitude of genes and pathways in oncogenesis and cancer progression. Presently comparative oncogenomics is a powerful approach in oncogene identification. It is done by performing cross- species comparisons to identify oncogenes and research involves studying cancer genomes, transcriptomes and proteomes in other model organisms, i.e., mice, identifying potential oncogenes, and reffering back to human cancer samples to find whether homologues of these oncogenes are also important in causing cancer in humans. Comparative genomic hybridization (CGH) is a technique that is capable of measuring CNAs (copy number alterations). The term CGH was firstly used for competitive hybridization of differentially labeled DNA on metaphase chromosomes[37]. Profiling allelic imbalances found in human tumors is a powerful tool for identifying cancer gene–containing loci, the most commonly used approach being array comparative genomic hybridization although the resolution of this technique has improved dramatically. Copy number of gains and losses in human tumors are usually large, rearrangements often encompass many genes that do not contribute to tumorigenesis. Therefore, differentiating “driver” cancer genes from “passenger” genes requires a validation in other systems. Various microarray platforms have enabled high-resolution genome-wide analysis of CNAs by array based CGH (aCGH). High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays(Nat. Genet., 20, 207–211) cDNA clone. Many different platforms are currently available for a CGH analysis, such as bacterial artificial chromosome (BAC) clone. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat. Genet., 23, 41–46.), single nucleotide polymorphism (SNP)[38]. Representational oligonucleotide microarray analysis: a high-resolution method to detect genome copy number variation Genome[39]. Most of the sample platforms of genome at specific positions denotes certain distance between the measurement points (probes) and this

distance is referred to as the resolution of the platform. Array-CGH has been used extensively in cancer research comparative genomic hybridization and its applications in cancer. Nat. Genet., 37, 11–17.) and careful analysis of CNAs can facilitate cancer gene discovery[40,41].

Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene (Nat. Genet., 25, 144–146). Various automated methods have been described to analyze the results obtained from a CGH measurement. They typically either smooth the data and/or try to estimate the location of the aberration by defining 'break-points' at which the CNA is defined to start or end[42]. Mutational analysis of all gene families is a powerful approach to oncogenomics.

TECHNIQUES FOR IDENTIFICATION OF ONCOGENES

With the introduction of new technology some approaches are used to detect or identify the new gene or altered gene. Here is the diagrammatic representation of the process and techniques.



Digital karyotyping is a sequence based techniques[43,44] which is a high-throughput technology to identify DNA copy number and allow amplification and deletion. Its main concept is genomic DNA cleavage by restriction endonuclease, amplification of these segments, cloned and sequenced. Recent implementation of CGH to microarrays containing genomic DNA sequences provides improved resolution, but is currently limited by the number of sequences that can be assessed[45,46]. CGH is mainly applied for the detection of copy number variation of the cancer cell. Through this technique one can distinguish the normal cell and cancer cell. It is helpful to

identify the segmental alteration in chromosomal regios which are related to disease[47] and is used to detect chromosomal alteration or variation. BAC (Bacterial artificial chromosome) is an E.coli F factor based cloning. The use of BAC end sequences gives minimally overlapping clones for sequencing large genomic regions. This method is applicable to decline the toxic effects and deletions of E.coli cells. Inverse PCR or end rescue PCR are multiple steps methods with which BAC clone end sequences can obtain. Bisulfite genomic sequencing should be done for the identification of DNA methylation. This sequncing method makes availability of a quantitative and qualitative, an efficient approach to detect 5-methylcytosine at single base-pair resolution[48]. Bisulphate genomic sequencing method was first introduced by Frommer et al.

TECHNOLOGIES IN ONCOGENE DETECTION

The microarray can be defined as an ordered collection of microspots (the probes), each spot containing a single species of a nucleic acid and representing the genes of interest. The microarray assay is a powerful molecular technology that allows the simultaneous study of the expression of thousands of genes or their RNA products, giving an accurate picture of gene expression in the cell or the sample at the time of the study. For example, the expression of all the genes for drug resistance and metabolism or all the known oncogenes in a cell can be detected and measured in the same timeframe[49]. Microarray analysis can also be used to detect SNPs. In tumor profiling, microarrays are used to compare gene expression in tumor cells with that of normal cells[50]. These comparisons provide the underlying genomic pathways involved in carcinogenesis in diverse populations. The use of microarray analysis has also led to the development of molecular signatures for predicting prognosis in several cancers. DNA microarray technology allows profiling of the expression of thousands of genes at once, possibly representing the whole genome. Usually a microarray consists of a selection of probes representing several genes applied onto a solid surface. This technology is based on hybridization between labeled free targets derived from a biological sample and an array of many DNA probes that are immobilized on a matrix. The targets are produced by reverse transcription and the simultaneous labeling of RNA extracts from a biological sample hybridized with DNA fragment probes.

GENOMICS AND PROTEOMICS AS A NOVEL APPROACH IN OSCC AND HNSCC

Oncoproteomics is the study of proteins and their interactions in a cancer cell by proteomic technologies and has the potential to revolutionize clinical practice, including cancer diagnosis and screening based on proteomic platforms as a complement to histopathology, individualized selection of therapeutic combinations that target the entire cancer specific protein network, real-time assessment of therapeutic efficacy and toxicity, and rational modulation of therapy based on changes in the cancer protein network associated with prognosis and drug resistance. This technology can alsobe applied to discover new therapeutic targets and to study the effects of drug. The study of oncoproteomics provides mankind with a better understanding of neoplasia. Proteomics is a promising approach in the identification of proteins and biochemical pathways involved in carcinogenesis. Proteomic technologies are now being incorporated in oncology in the post-genomic era. New research in post-genome era, proteomics provides a powerful approach for the analysis of normal and transformed cell functions, for the

identification of disease-specific targets, and for uncovering novel endpoints for the evaluation of chemoprevention agents and drug toxicity[51]. Oncoproteomics refers to the application of proteomic technologies in oncology and parallels the related field of oncogenomics. Understanding how genomics is currently influencing cancer prevention, screening, diagnosis, treatment, and survivorship is essential for optimal nursing care of patients and their families. This article aims to introduce nurses to how genomics is currently integrated into cancer care from prevention treatment and the influence on oncology nursing practice. Oncogenomic and proteomic analyses offer the opportunity to accelerate the pace of discovery for clinically relevant targets. A variety of high-throughput technologies including expression profiling and mass spectrometry technologies are being used to analyze cancer genomes and proteomes with the ultimate goal of identifying new cancer genes and therapeutic targets. Potentially, the identification of disease-associated proteins and protein signatures could be used as tumor markers for early detection, response to therapy, or relapse. A greater understanding of the molecular events underpinning clinical outcomes will provide useful tools in the identification of new targets for future therapy. These advances have already begun to manifest in several key areas of treatment including early detection of cancer, evaluation of surgical margins, determination of necessary extent of surgery, and predictions of outcome and recurrence. In this chapter we review key technological advances leading to these recent changes as well as many of the studies helping to implement these technologies and apply them to patients with head and neck cancer.

DISCUSSION

OSCC and HNSCC treatment has advances in surgical techniques, radiation therapy, chemotherapy, and molecular targeted strategies. As well as there is a wide increment in our understanding of tumor biology through research focusing on individual genes. OSCC and HNSCC is a heterogeneous disease with deregulation of cellular pathways, including differentiation, apoptosis, angiogenesis, and metastasis. However, poor overall survival persists despite this progress, in large part because treatment decisions continue to be based on traditional parameters, such as tumor size, tumor site, and presence of regional or distant metastases. OSCC and HNSCC represents an extremely heterogeneous disease with misregulation of multiple interrelated cellular pathways, including differentiation, apoptosis, angiogenesis, and metastasis. The complexity of interactions between genes and proteins and the environment and the difficulty of finding the right combinations of targets to study pose fundamental problems with successful identification of therapeutic targets and predictive elements. Oncogenomics is new research sub-field of genomics, which applies high throughput technologies to characterize genes associated with cancer and synonymous with cancer genomics. Recent advances in preventive, diagnostic and therapeutic techniques related to OSCC and HNSCC have yielded novel molecular targets. Abnormalities of many genes critically involved in the regulation of the cell cycle are found in OSCC and HNSCC. Detection of these genetic changes may assist in both the diagnosis and treatment of OSCC and head and neck cancer in the future. Tumor formation is in part driven by DNA copy number alterations (CNAs), which can be measured using microarray-based Comparative Genomic Hybridization (aCGH). Bioinformatics tools, software and databases are helpful to get the information of gene related pathways, frequencies of gene

alteration. In short oncogenomic approaches can help to accelerate the cancer-gene discovery and treatment. As oncogenomic approaches are fast, cost-effective, efficient, more systematic and broad.

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