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Oral Pathology

SINGLE NUCLEOTIDE POLYMORPHISMS IN ORAL CANCER: A REVIEW

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ABSTRACT Oral cancer is a major health concern as it can cause significant morbidity and mortality. A dose response relation has been established between oral cancer and tobacco usage. Though tobacco and alcohol have been long associated with the disease, genetic factors also play an important role in its pathogenesis. Various genetic mutations and epigenetic regulations are associated with the etiology of Oral Cancer. The focus of the current review is to emphasize the role of most common genomic variants known as single nucleotide polymorphisms in oral cancer. Single Nucleotide Polymorphisms also known as SNPs are precise nucleotide sites in the human genome where in, it is possible to have two or more different nucleotides at a specific position on a chromosome. These are the most common type of variations occurring in the genetic constitution. SNP studies in various populations emphasize association of SNPs with risk predisposition or susceptibility to oral cancer.

KEYWORDS : Single Nucleotide Polymorphism, Oral Cancer, Polymorphisms

INTRODUCTION:

Single Nucleotide Polymorphisms also known as SNPs are precise nucleotide sites in the human genome where in, it is possible to have two or more different nucleotides at a specific position on a chromosome. These are the most common type of variations occurring in the genetic constitution. Each SNP represents a difference in a single DNA building block called nucleotide. Single Nucleotide Polymorphisms are base changes present in >1% of an ethnic population, present in the exonic coding region of the gene or in the non-coding intronic regions, directly or indirectly affecting gene expression and function These SNPs occur one in every 300 nucleotides. Hence there are roughly 10 million SNPs in Human Genome.[1]

SNPs are being extensively studied to explain the pathogenesis of various diseases and conditions. Single-nucleotide polymorphisms (SNPs) have become an integral part of a large number of research studies which are designed to identify critical differences in DNA sequence, which contribute to phenotypic variation for specific traits.

SNPs can be used as biological markers which can be used in locating the genes associated with the specific disease[1]. Studies on SNPs have also highlighted the relation between the polymorphisms and oral cancer.

Oral Cancer is a multifactorial complex process driven by genetic and epigenetic factors. Among the epigenetic factors, major risk is attributed to tobacco users. But amongst the tobacco habituates, a percentage of population only develop persistent lesions and 3-8% transform into malignancy[2]. The rest of the population do not develop cancer and some don't have any such habits, yet develop cancer. Thus there is a need to focus on genetic variations as well. The present review highlights the role of SNPs, the most common genetic variations in oral cancer. SNPs in genes that regulate DNA mismatch repair, cell cycle regulation, metabolism and immunity are associated with genetic susceptibility to cancer[3]. SNPs can be used as genetic markers to follow the inheritance patterns of chromosomal regions from generation to generation and as powerful tools in the study of genetic factors associated with many diseases.

Oral cancer is the 11th most common human cancer world wide. In India, it is a major health concern with an annual incidence of 77,003 constituting 26% of the global burden. More than 90% of oral malignancies are squamous cell carcinomas, (OSCC) which accounts for more than 95 % of all head and neck cancers. According to World Health Organization, there are an estimated 529,000 new cases of oral cavity and pharynx cancers, and more than 300,000 deaths caused by oral cancer every year.[3]

Despite advancements in treatments over the past few decades, the 5-year survival rate of OSCC remains less than 50 %. This low survival rate has been attributed to a lack of understanding of the etiopathogenesis of OSCC. Both environmental risk factors, including cigarette and alcohol consumption, and genetic risk factors, including genetic polymorphisms, are strongly associated with oral carcinogenesis.[3] Studies in various populations emphasize the association of SNPs susceptibility to oral cancer.

In the present review, we attempt to summarize the importance of SNPs in oral cancer. The keywords used for the analysis were Single nucleotide polymorphisms and Oral Cancer in pubmed search engine, from past 5 years. These included various case control clinical studies and reviews. Full text articles were only included and the abstracts and duplicate articles were excluded.

Structure Of SNPs

The SNPs are single base changes seen in the exonic coding region of the gene or in the non-coding intronic regions, directly or indirectly affecting gene expression and its function. (Figure 1).Variations in the gene expression and their effect on cancer susceptibility differ depending on the location of the SNPs . SNPs are located in different regions of genes such as promoters, exons, introns as well as 5′- and 3′ UTRs. Therefore, alterations in gene expression and their effect on cancer susceptibility vary depending on the location of the SNPs.

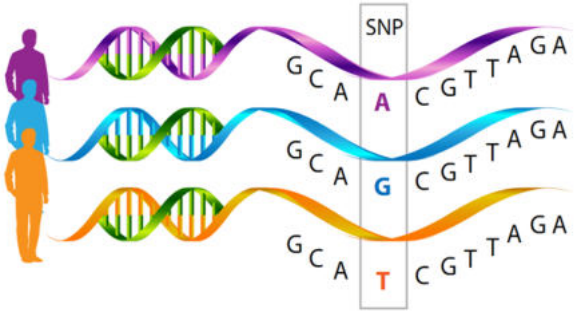

Figure 1: Basic structure of SNP

The **promoter region SNPs** affect genotypic expression by varying promoter activity, transcription-factor binding, DNA methylation and histone modifications .

The **exonal SNPs** affect cancer susceptibility by suppressing gene transcription and translation In the **intronic regions,** SNPs may alter the three dimensional structure of DNA resulting in changes in characteristics of the molecule such as Gibbs free energy affecting stability of the molecule, and may impact DNA polymerase activity and transcription factor binding.

SNPs in regions that are located far from the actual genes reduce or enhance gene transcription through long-range cis effects. SNPs may be present in a single allele or both alleles resulting in heterozygous or homozygous genotypes. The ancestral allele is the wild-type (WT) allele and the altered allele is the SNP allele. [4]

Oral Carcinogenesis involves the disruption of the normal cell cycle. There are seven types of genes and proteins that participate in controlling the normal cell cycle[5].

i)Growth factors, (ii) growth factor receptors, (iii) signal-transduction, (iv)transcription factor, (v) pro-or anti-apoptotic proteins, (vi) cell cycle control proteins, and (vii) DNA repair proteins. Cancer can result from the expression of mutant forms of these proteins.[6]

Types of Polymorphisms in Single Nucleotide.

Promoter-related polymorphisms

A good number of genes mutated in human cancers encode components of the cell cycle processes**.** Variations in the promoter region of the related genes may end in abnormal expression, thus predisposing the individuals carrying these genetic variants to cancer.[7] Promoter-related polymorphisms affect transcription factor binding, that differ promoter activity, gene transcription, mRNA stability as well as translation. As a result, protein levels are altered that potentially determine the individual's susceptibility to diseases including cancer. It also affects the DNA methylation which serves as the epigenetic factors.[4]

Exonal SNPs

Exonal SNPs generally influence cancer susceptibility by genetic mechanisms, classified as non-synonymous and synonymous coding SNPs (cSNPs) based on their ability to replace the encoded amino acid. These changes alter cell signaling pathways and also levels of oncogenic and tumor suppressor proteins. The non-synonymous SNPs affects the protein-coding sequences while the synonymous SNPs do not affect the protein-coding sequences. The non-synonymous SNPs can be a missense mutation or nonsense mutation. It has been estimated that each person has about 24,000–40,000 nonsynonymous SNPs and that there are 100,000 –300,000 nonsynonymous SNPs constituting about 1% of the total SNPs in the entire human genome.[8]

Non-synonymous cSNPs result in amino acid substitution that can affect protein function. Alterations in the amino acid sequence can alter the secondary structure of the protein by increasing or decreasing hydrogen bonding and phosphorylation, which affects protein interactions and functions. As a result, these changes alter cell signaling pathways as well as levels of oncogenic and tumor suppressor proteins.

Synonymous cSNPs do not change the amino acid sequence of the encoded protein. Synonymous cSNPs affect gene function and expression by changing the expression of neighbouring genes. Synonymous polymorphisms may also affect messenger RNA splicing, stability, and structure as well as protein folding. These changes significantly affect function of proteins resulting in changes in cellular response to therapeutic targets, which explains differential responses of individual patients to medications.[4]

Intronal SNPs

Intronal SNPs alter the genetic susceptibility to cancer by both genetic and epigenetic mechanisms. Introns are involved in regulating tissue-specific gene expression, mRNA transcription, and translation. Introns are also involved in alternative splicing and genome imprinting. Genomic imprinting means differential expression from maternal and paternal alleles due to differences in DNA methylation and histone acetylation. Polymorphisms in the these regions alter gene expression.[4]

UTR-Related SNPs

The 5′ and 3′ UTRs of mRNAs control translation. The 5′-UTR regulates translation initiation, whereas the 3′-UTR determines mRNA stability. Specific regulation of mRNA translation is an essential part of gene expression and can be modulated by sequence variations in the 5′ and 3′ UTRs. Single nucleotide Polymorphisms (SNPs) causes changes in the secondary structure and miRNA target sites within UTRs . These changes alter the expression of known cancer related genes and signaling pathways. Sequence changes in the UTR regions affect mRNA folding that impacts transcript stability, mRNA processing and/or translational control. Thus, UTR-SNPs (non-coding SNPs located in the UTR) may have functional consequences on mRNA stability and/or expression.[4]

SNPs In Undefined Genetic Regions

SNPs in non-coding regions control specific genes through long-range chromatin interactions. Most of these interactions are located in sites with active histone modifications and transcription factor binding sites.

In some cases, changes in tRNAs and rRNAs are associated with cancer susceptibility. Mutations in the mitochondrial tRNA genes alter the secondary and tertiary tRNA structure, leading to transcriptional and translational defects in the mitochondrial respiratory chain components.[4]

Methods Of Detection Of SNPs

Single nucleotide polymorphism (SNP) detection technologies are used to find new polymorphisms and to locate the allele(s) of a known polymorphism in target sequences. SNP detection technologies have evolved from extensive laboratory work, highly automated, efficient, and relatively inexpensive methods. [9] Technologies has evolved ways in both SNP discovery and genotyping areas. The nearly completed human genome sequence provides the reference, against which all other sequencing data can be compared.[9]

Over the past twenty years, many different methods have been developed for SNP genotyping which include Polymerase Chain Reaction(PCR), hybridization, allele-specific PCR, primer extension, oligonucleotide ligation, direct DNA sequencing and endonuclease cleavage after amplification of the subjected genomic region by PCR. Recent technologies for SNP genotyping are Taq Man method, Invader method, MALDI-TOF method.

The first method in identifying SNPS was done by scanning of restriction sites in the genome by Otstein eta al in 1980. It was then known as Restriction Fragment Length Polymorphisms(RFLP). This simple and sensitive enzymatic technique for the amplification of DNA fragments has been used for various purposes, but especially for the detection of nucleic acid polymorphisms to find biological meaning in genetic variation and molecular divergence in living organisms. The majority of polymorphisms in the human genome are single nucleotide polymorphisms (SNPs). Though it was quite efficient, it involved extensive work. The application and refinement of the PCR method since its invention in the 1980s has lead to advancement in molecular genetic research. Polymerase chain reaction(PCR) came into picture but this also needed DNA sequence synthesis and oligonucleotide synthesis which were an costly act . The search for an understanding of the causes of genetic variants and mutations has resulted in the development of a simple laboratory technique, known as the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, for the detection of single nucleotide polymorphisms (SNPs).PCR-RFLP is based on endonuclease digestion of PCR-amplified DNA. The specific restriction endonuclease recognizes and cleaves the DNA in the region of the point mutation of the PCR products. The mutation is discriminated by digestion with specific restriction endonucleases and is identified by gel electrophoresis after staining with ethidium bromide (EtBr). This convenient and simple method is inexpensive and accurate for SNP genotyping and especially useful in small basic research studies of complex genetic diseases. The limitations of this protocol are in the cases such that the sequences of target SNPs are not suitable.[10] This issue was solved by the Human Genome Project which increased its production rate in order to sequence the human genome by shotgun sequencing. High quality genome sequences then became available for comparison.

With the development of better DNA polymerases and sequencing

chemistries, Direct DNA sequencing is very efficient method as a scanning tool for identification of SNPs when compared to all the methods. This method needs high quality amplified DNA samples which involves high cost. Microarray can also be used for detection of Single Nucleotide Polymorphisms. The development of microarray technology has had a significant impact on the genetic analysis of human disease. The recently developed single nucleotide polymorphism (SNP) array can be used to measure both DNA polymorphism and dosage changes. Due to the complexity of genetic alterations in cancer cells, high density SNP array analysis is a very demanding technique. The application of SNP arrays in combination with PCR, tissue array and functional analysis technologies will eventually lead to the full revelation of the complex genetic alterations in human cancers.

Various methods are discussed, but the ideal method should be easy to carry out , at the same time reliable and cost efficient. It should produce data that can be easily analyzed. Although no such ideal genotyping method exists, a number of promising SNP genotyping methods are currently available and further improvements in biochemistry, engineering, and analytical software will bring the existing methods closer to the ideal.[12]

By using suitable techniques, studies have been done extensively to identify SNPs associated with Oral Cancers. Below mentioned are few SNPs associated with Oral cancer since last 5 years. (Table: 1)

Table 1: SNPs Associated With Oral Cancer

Gene	Cases	Controls	Inference
Fatty Acid Desaturase (FADS1)[13] rs174549	300	305	FADS1 gene serves as protective mechanism for oral cancer
COL9A1[14] rs550675	360	486	COL 9A1 can be used as a risk prediction for oral malignancy
Fibroblast Growth Factor Receptor 4 (FGFR4)[15] rs351855	955	1191	FGFR4 is associated with higher risk of Oral Cancer
Insulin-like growth factor 2 mRNA-binding protein 2(IGF2BP2)[16] rs 11705701 rs 4402960 rs 1470579	1349	1198	IGF2BP2 is associated with less favourable clinical presentation of oral cancer
Long Pentraxin[17] rs 3816527	865	1189	Long Pentraxin has an association with the occurrence of oral cancer
Maspin[18] rs 2289520	741	601	Maspin is associated with increased susceptibility to oral cancer
Tissue inhibitor of metalloproteinase-3(TIMP31)[19] rs 9862	747	1200	Polymorphism with TIMP31 makes an individual more susceptible to oral cancer
miRNA [20] miR605 rs2043556 miR608 rs491510	576	1552	SNPs in these Genes increases the susceptibility to oral cancer
NFKB1[21] rs 28362491 rs 72696119	425	485	NFKB1 is associated with increased risk of Oral Cancer
Resisten Gene[22] rs 3219175			Resisten Gene is Associated with high risk for advanced tumor size
SerpinB5[23] rs 1071138 rs 8089104	741	601	SerpinB5 is associated with increased susceptibility to oral cancer
Sex-determining region on the Y-chromosome-related high-mobility-group box(SOX 11)[24] rs 77996007	1196	1200	SOX11 is associated with progression of oral cancer
Survivin[25] rs 9904341	47	101	Survivin is strongly associated with oral cancer
WW domain containing oxidoreductase rs 11545028[26]	761	1199	WW domain containing oxidoreductase can be used as predictive marker in oral cancer
XPA gene[27] rs 1081738	362	350	XPA gene can be used as biomarker for poor prognosis.
Transforming Growth Factor(TGF-β1)[28] rs334348	356	350	TGF-β1can be used as a novel biomarker for predicting oral cancer.
Retinobalstoma gene (RB)[29]	311	350	Retinoblastoma gene can be used as prognostic biomarkers to stratify patients based on disease-free survival and therefore maybe helpful in therapeutic decision-making.
CyclinD1[29]	311	350	CyclinD1 can be used as prognostic biomarkers to stratify patients based on disease-free survival and therefore maybe helpful in therapeutic decision-making.

SNPs As Biomarkers:

Biomarkers can be used for individualized assessment in multiple clinical settings, including disease risk management and distinguishing benign from malignant tissues. Biomarkers can be classified based on the disease state, including predictive, diagnostic, and prognostic biomarkers. A prognostic biomarker informs about a likely cancer outcome. **SNPs** are one of the most used **biomarkers** indicating genetic variation, associated with susceptibility and prognoses of **oral cancer.**

Biomarkers can be used for various purposes such as

1. Disease risk management – Predictive markers
2. Distinguishing benign from malignant tissues- prognostic markers.
3. Diagnosis of the lesion- Diagnostic Biomarkers

Oral cancer biomarkers may be molecular such as genetic mutations, polymorphisms of genes, DNA copy number variance, telomere instabilities, and cell-cycle signaling pathways that are involved in oral cancer. These biomarkers allows individualization of treatment,and quantitative real-time RT-PCR is frequently used to measure the expression of these markers.

CONCLUSION

Despite extensive research being carried out on role of various SNPS, the mechanism remains complex. No single SNP study is complete within itself. A panel needs to be created of the various SNPs to bring it into therapeutic and prognostic application. Analysis of SNPs via high throughput genomic analysis as reported in genome-wide association studies (GWAS) and next generation sequencing have emerged as a powerful approach to identify susceptibility loci enabling information on thousands of SNPs simultaneously. These platforms generally use smaller samples and are rather expensive and need to be validated in larger samples using alternative technology including nucleotide sequencing and Real-time PCR.

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