Oncogenes in ENT and Head and Neck - A Review

Abstract

With the exploration of new horizons in the frontiers of genetic linkage with head and neck cancer by advanced molecular biological technique it may be possible to know the various steps involved in the cancer development and new prognostic markers for not only predicting prognosis but also progression and predisposition including routine diagnostic tests for mutant genes for a better diagnosis and treatment of squamous cell cancers of head and neck. Various oncogenes and their products so far identified are ras, myc, c-erb, B1, PRAD-1, int-2, hst-1 and p 53 etc with linkage to head and neck malignancy. The HLA gene association is the most convincing evidence for the role of genetics in the aetiology of nasopharyngeal cancer. EGFR looks to be a promising prognostic indicator as its interaction with the polypeptide epidermal growth factor stimulates proliferation of target cells.

Key Words

Oncogen, Squamous cell cancer, Head and neck, Immunogenetics

Guest Editorial

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Advanes in molecular biology in recent times has brought revolutionary changes in the understanding of genetic mechanism involved in the development and progression of squamous cell carcinoma of head and neck which may be ultimately helpful for better diagnosis, treatment and prognosis.

The basic concept of the genetics of cancer lies in the analysis of these oncoming which are responsible for contributing towards concern formation at three different levels, namely 1) DNA level, with hydrogen bonding between complimentary basis of adenine, guanine, cytosine and thymine (A-T & G-C) which makes up the 50000-100000 genes that contribute the human chromosomes. Double stranded DNA templates within the genes that make up chromosomes produce single stranded mRNA templates themselves for protein synthesis. The chromosomal alteration occurs by translocation, inversion, deletion or mutation, leading to oncogen activation. The latest technology using polymerase chain reaction (PCR) can detect many sites on many chromosomes to be studied.

The traditional way to study DNA is southern blotting and for mRNA is northern blotting with similar principles. After the DNA is extracted from the cells, it is run on an agarose gel, through which an electric current is passed. The DNA is cleaved with a restriction endonuclease.

an enzyme which cuts the DNA into hundreds of thousands of fragments of various sizes at specific sites in the DNA sequence. By using a strong salt solution the DNA is then transferred to a nylon membrane, known as the blotting process. The membrane is then exposed to probes of known fragments of DNA labeled with radioactive isotopes which will find or hybridize to complementary sequence of DNA. When this membrane is exposed to radiographic film, a characteristic band is seen. Restriction fragment length polymorphism (RFLP) analysis is commonly used for tumours when DNA is cut with one or more restriction enzymes and a pattern band representing the two different alleles is seen on the autoradiograph.

Various carcinogens like tobacco, alcohol, chemicals, dyes and viruses like HPV have been linked to cancers of head and neck. Peyton Rous first demonstrated in 1911 that cell-free filtrates of chicken sarcomas could cause sarcoma on other chickens. These filtrates were found to contain a virus whose genome contained an oncogene named V-src, derived from a normal cellular proto-oncogene which are nothing but normal forms of the genes involved in cell growth and differentiation. This proto-oncogene is called C-src and many others have since been isolated.

It is important to note that cancer is not caused by one altered proto-oncogene alone, rather a multistep process, which is evident from the fact that there is an interval of several years between exposure of carcinogens such as tobacco and cancer development. Also some chemicals promote and some initiate cancer formation by synergistic action, the typical examples of which are tobacco and alcohol, in squamous cell carcinoma as those individuals who drink as well as smoke may increase the risk of developing cancer as much as up to 30 times more. Again some individuals are born with a genetic predisposition to cancer like having an abnormal tumour suppressor gene (Tass) such as p53 who can develop cancer at a very young age.

Various oncoming and their products have been identified in SCC of the head and neck, namely ras, myc, c-erb, B1, PRAD-1, including bcl-1, int-2, hst-1 and suppressor gene p53. Oncogenes which cooperate together include ras with myc, ras with p53 and ras with fos etc.

RAS : RAS mutations are commonly found in benign lesions as keratoacanthoma and in verrucous form of SCC in the Indian subcontinent. This may be due to the chemicals in betel-nut and tobacco which are frequently and extensively used in India which are initiating and promoting agents for malignancy when held for a long time in oral cavity. The three types of ras are known as k-ras, H-ras and N-ras. The first two are named after their discoverer Kirsten and Harvey respectively and the last one is named after the tumour in which it was discovered (i.e., neuroblastoma).

MYC : This group of genes includes C-myc, b-myc, L-myc and N-myc. The C-myc is found to be amplified in SCC of head and neck. Elevated level of C-myc mRNA and protein are associated with survival factor. There is neither any difference between groups of untreated patients and treated patients with recurrence nor any correlation with the UICC (TNM) staging. Though it is still not yet proved whether an elevated c-myc protein is a cause or result of the cancer but it may have the potential as a prognostic indicator.

C-ERB B1 : This is associated with a glycoprotein cell surface receptor known as epidermal growth factor receptor (EGFR). It is stimulated by epidermal growth factor which is a polypeptide. When interact with EGFR, it strongly stimulates cell division. EGF was originally isolated from the mouse's submandibular gland and is known to suppress terminal differentiation of keratinocytes. In SCC of head and neck, overexpression of EGFR is found commonly which correlates with poor prognosis. Loss of expression of blood group antigens A, B & H occurs in SCCs along with overexpression of EGFR. However, the assays used to measure expression of the EGFR are not always sufficiently quantitative.

PARD-1 : This encodes for a cyclin, D1 which is important in the regulation of the cell. This

oncogen, on chromosome 11, is part of an amplicon including *bcl*, *int-2* and *hst-1* oncogens. Amplification of *int-2*, *bcl-1*, *hst-1* oncogens have been in SCCs of the head and neck, It is also suggested that in small number of cases, they are linked to poor prognosis. *Int-2* amplification may correlate with tumour recurrence but not stage or histological grade.

P53 : This is a suppressor gene, abnormalities of which is common in SCCs of head and neck, and oesophagus including oral cavity. Abnormalities of P53 have been found in premalignant lesions also like Barrett's oesophagus and dysplastic oral mucosa. Elevated levels of P53 protein may indicate the presence of a tumour virus. When functioning normally, there is a rapid turnover of P53 protein which is undetectable by the conventional immunocytochemical staining technique. However, certain mutant forms of P53 protein which are known to malfunction as tumour suppressor genes, gel stabilized by forming complexes with heat shock protein 70. This inactive complex paradoxically leads to as much as 100 times P53 protein being present in the cell which renders the mutant P53 easily detectable by conventional immunocytochemistry. A similar stabilization occurs when P53 is activated by SV40 tumour virus T antigen.

Immunogenetics & viruses

Patients with nasopharyngeal carcinoma (NPC) have a prominent genetic susceptibility for NPC. EBV is linked with NPC in more than 90% of T3 tumours containing the viruses. EBV in these tumours may act by inducing genetic mutation, leading to oncogen formation or knocking out suppressor gene. Or else, it may be involved in reducing tumour recognition by the immune system. HLA is the only genetic system so far shown to be strongly associated with NPC. This histocompatibility locus antigen lies on the short arm of chromosome 6 and the major histocompatibility of gene complex comprises six recognized loci called HLA-A, -B, -C, -DR, -DQ and -DS. Further, there are

at least 18 recognized distinct alleles at the HLA- A locus and 32 distinct alleles at HLA - B locus. Each of these alleles is capable of determining a product or antigen. The combination of alleles at each locus on a single chromosome is usually inherited as a unit called haplotype like A-2 BW46 and A11-B40. There are three well recognized association between HLA and NPC involving A2, BW46 and B17. Also there is a relationship in the survival pattern and clinical behaviour between the HLA types and NPC. The HLA pattern AW19-B17 is associated with short term survival and A2-BW46 with intermediate survival. Similarly, there is a young onset of NPC in AW19 - B17 pattern but older onset in case of A2 - BW46. Also there is a high VCA/EA titres and low ADCC titres in AW19-B17 but reverse is the case in A2 without BW46 or B17. BW46 is confined to older patients of more than 30 years whereas B17 / BW58 is associated with both young and old patients but more particularly in younger patient. But B11 and B13 are associated with decreased risk. There is not only a differential HLA frequency distribution but also a differential distribution or relative risk in various HLA type or haplotype. The relative risk in HLA A2 : 1.5, BW46 : 1.9, B17 : 2.1, AW 19 -B17 : 2.2. and A2 - BW46 : 3. 4 which shows a linkage disequilibrium as the risk is associated more with the haplotype rather than individual antigen which is found in the general Chinese population. Though there are more than 50 alleles in the HLA – A and B loci but only a few associated with NPC. All is rarely bound with B17 or BW46 but A2 with BW46 in patients with NPC is stronger than in control patient. BW46 is not found in the Caucasian population. The HLA gene association is the most convincing evidence for the role of genetics in the aetiology of NPC.

Human papilloma viruses (HPVs) are also associated with SCCs of head and neck, particularly in recurrent laryngeal papillomatosis. The different oncogenic types of HPVs are HPV6, 11, 16 and 18. The last two oncogens in vivo can contribute to malignant transformation of the keratinocyte including those of oral cavity. The HPV 16 and 18 found on SCC of head and neck are episomal in nature as they are present in small circles of DNA that are not integrated into the cell genome and that could be diluted out by rapid cell division. The two proteins E6 and E7 produced by the oncogen HPV16 and 18 for increasing the proliferative

life span of keratinocytes responsible for cancer development. These proteins E6 and E7 bind the proteins of the tumour suppressor genes P53 and RB, respectively. The bondage of E6 degenerate the P53 and RB, respectively. The bondage of E6 degenerate the P53 for which the function of the tumour suppressor gene may be lost. Also the E7 binding with RB1 correlates the ability of the virus to promote experimental tumour development.

Summary and Conclusion

With the exploration of new horizons in the frontiers of genetic linkage with head and neck cancers, it may be possible to perform on small biopsies of human tumour materials leading to new discoveries by advanced molecular biological techniques about the various steps involved in cancer development and new progression and predisposition. With more knowledge about the individual steps involved on the development

of cancer, it may be possible to have diagnostic testing or evaluation for mutation in certain genes. EGFR looks to be a promising prognostic indicator as its interaction with the polypeptide epidermal growth factor (EGF) stimulates proliferation of target cells. Also EGFR shows a marked structural homology with the product of the avian erythroblastosis virus V-erb B1 oncogen and EGFR is therefore thought to be a prooncogen associated with the development of tumours. EGFR gene amplification has been demonstrated in some of the head and neck SCCs cell lines and also it is reported to show a patchy expression in certain cells of salivary gland neoplasms. P53 is the latest suppressor gene to be investigated which may be helpful in predicting prognosis, progression and predisposition. Smoking, pollution, diet, alcohol and other environmental factors which may not be easy to manipulate for an individual today but a reality in future.

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Congratulation !

Dr. V Phaniendra Kumar, Associate professor Guntur Medical College A.P. was invited to deliver guest lecture on phono surgery from AOI-chennai branch in June 98.