Recent Advances in the Molecular Biology of Head and Neck Tumours

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Abstract

Our knowledge about the genetic basis of head and neck tumours is accumulating fast. This article reviews the important advances in this area over the last decade and its implications for the future. Newer aspects of gene therapy, molecular staging and understanding head and neck carcinogenesis on a molecular level are outlined.

Key Words

Oncogenes, Tumour Suppressor Genes, Molecular Biology, Head and Neck, Squamous cell carcinoma

INTRODUCTION

The last decade has seen an explosion in our knowledge about the molecular mechanisms involved in cancer. Head and Neck Squamous cell Carcinoma (HNSCC), accounting for about 95% of all head and neck cancers has received a lot of attention. A tumour progression model for HNSCC is rapidly emerging; one can outline how a normal upper aerodigestive tract epithelial cell undergoes malignant transformation in a series of steps.

A large number of gene and chromosome abnormalities are found in tumour cells. It is likely that malignancy results from the accumulation of about 6 to 10 independent genetic events¹. The individual pattern of these in a particular tumour determines its behaviour.

It is now clear that there are 3 main mechanisms involved in Head and Neck carcinogenesis: (a) Cellular oncogenes and oncoproteins; (b) Loss of Tumour Suppressor Gene activity through deletions or mutations and (c) Viral oncogenes which infect human

cells and are incorporated into the human genome and alter cell regulatory pathways. Each of these will be reviewed in turn. Figure 1 shows the genes and products involved in HNSCC. Many more such genes have been postulated to exist in specific locations, and will be identified in the near future. This leads us to the question of the practical utility of our knowledge of molecular biology in the management of head and neck cancers. Much of current research addresses earlier diagnosis, accurate prognosis and control of cancer progression by gene therapy. Many trials have been approved and are being conducted on patients, and initial results appear promising in some cases. Clinical oncobiology, has just begun yielding its fruits.

Cellular Oncogenes, Gene Amplification and Protein Expression

Normal DNA replication and cell proliferation requires several steps² in sequence, beginning with the binding of growth factors (e.g. Epidermal growth factor, EGF) to their specific cell

surface receptors (e.g. EGFR). This is followed by amplified transmission of these signals by membrane-bound proteins (e.g. G-proteins, TRK-Proteins with Tyrosine Kinase activity) to the

nucleus. Specific nuclear proteins (e.g. cyclins) are expressed, which activate other genes for cell division after binding to DNA. These steps are outlined in Figure 1.

Figure 1 : Genetic and cellular regulation factors involved in head and neck squamous cell cancers

The genes which control this regulated, orderly cell proliferation are called 'proto-oncogenes'. Cancer results partly from abnormal activation of these genes and their products through carcinogen-induced mutations (e.g. base deletions, inversions, point mutations in the DNA sequence) and these genes are transformed to 'oncogenes'.

Since 1986, several groups of oncogenes have been found in HNSCC, and the list is ever-growing. The most important of these relevant to HNSCC are discussed below.

The CCND1 Amplicon

A critical group of proto-oncogenes (called 'oncogenes' hereafter, implying that they are transformed in malignant cells) are found on the long arm of chromosome 11, locus 13 (11q13). This is found to be amplified (multiple copies) consistently in HNSCC. As shown in Figure 2, this group contains several oncogenes *bcl-1*, *int-2*, *hst-2* and *PRAD-1*. Only the *PRAD-1* gene codes for a protein, and this is *cyclin D1*, whose function is mentioned above.

Figure 2 : The CCND1 Amplicon

Overexpression of *cyclin D1*, or amplification of CCND1 oncogenes is currently thought to be an early event in a large proportion of HNSCC^{4,5}, concomitantly with p53 abnormalities (see below). Recent^{6,7} studies have shown that cyclin D1 overexpression correlates with HNSCC progression. The tumor suppressor genes p53, p16 and Rb all inhibit the cyclin D1 pathway-mediated cell division (negative regulation).⁸

Although amplified areas have been found on virtually every chromosome in transformed cancer cells, it seems very likely that chromosomes 3q and 5p are harbouring two crucial, yet undiscovered oncogene systems for HNSCC initiation.⁹

Myc

Cellular myc (c-myc) is another family of regulatory nuclear proteins expressed in HNSCC; but its mutation and amplification is common only in India (39%)¹⁰, but uncommon in the West.¹¹ The expression of this protein is also common in pre-cancerous oral lesions¹², indicating a possible role in early tumour progression.

Ras

The Ras family of oncogenes (H, K and N) code for proteins which stimulate the cytoplasmic pathways with tyrosine kinase activity, leading to increased cell division. They are expressed quite frequently in HNSCC (40-70%), and according to recent studies¹³, at relatively late stages (T3 and T4) of a tumour. Ras expression therefore reflects disordered cell division, rather than playing any significant role in HNSCC initiation.¹¹

Epidermal Growth Factor Receptor (EGFR)

Several growth factors, including the tumour growth factor α (TGF- α), stimulate the EGFR to initiate cell division. In HNSCC, there are increased levels of TGF- α , which can be used as a useful tumour marker. A recent¹⁵ study

found EFGR and TGF- α levels correlating with prognosis and tumour recurrence in a subgroup of GNSCC. In addition, EFGR has a strong link with metastasis, hypopharyngeal cancer in particular and diminished survival.¹⁶

Recently, retinoids (vitamin-A like substances) have been advocated for protection against oral cancer, because they decrease EFGR and TGF- α levels.¹³ Anti-EFGR monoclonal antibodies (genetically engineered) are undergoing clinical trials as anti-tumour therapy, and have been recently shown to reduce the dose of cisplatin required in combination chemotherapy.¹⁷

There are numerous other oncogenes, and no doubt, many more to be discovered. A few currently interesting oncogenes being studied, relevant to HNSCC include *cdc* genes and *kinases*¹⁸, the *MDM-2 gene*^{19,20} and the Ki-67 oncoproteins^{20,21}, and are being evaluated as prognostic markers.

Tumour Suppressor Genes

As evident from their name, tumour suppressor genes play a vital role in controlling cell proliferation. When they are mutated, or deleted, a tumour cell population proliferates rapidly. The p53 is the most well-studied in this group, others of importance in HNSCC being the p16 and the RB (*Retinoblastoma*) genes.

The p53 gene

Located on the short arm of chromosome 17 (17p), the p53 is the commonest gene to be involved in human cancer. Cigarette smoking has been shown to cause mutations in the gene⁸, or a breakage in 17p, thus leading to a loss in its function as a “guardian of the genome”, as popularly referred to. Point mutations of p53 represent the commonest genetic change in human cancer.²¹

The mechanism of p53 action, uncovered only recently, is two-fold. Firstly, it activates the nearby p21 gene to inhibit cyclin-dependant kinase pathways and thus block cell division

in the G1 phase of the cell cycle.²⁰ Secondly, it has been recently shown in the presence of irreparable DNA damage, it promotes progressive cell death (apoptosis). This it does by inducing the expression of a family of apoptosis-related genes (*bcl-2*, *bcl-x*, *mcl-1*, *bax*).^{22,23} Thus the natural anti-cancer role of p53 could be to prevent malignancy by killing DNA-damaged cells. Indeed, it has been shown that p53 expression increases in smoking and dysplastic lesions.²⁴

Recent⁸ evidence suggests that the p53 gene mutations are secondary to the loss of parts (deletions) of several other chromosomes, including 9p and 3p, which contain other several vital tumour suppressor genes. Mutant p53 genes produce p53 proteins which are functionally useless, but unusually stable, and therefore are overexpressed.

The p53 mutations occur in upto 60% of HNSCC, and indicate a bad prognosis²⁵, and is investigated clinically by several oncology units worldwide. Recently²⁶ it has been shown that introduction of p53 genes through retroviruses (gene therapy) controls tumour growth in HNSCC, and has a synergeistic effect with paclitaxel (*Taxol*), a new anti-cancer drug being used in HNSCC.

Retinoblastoma (RB) gene

In about 20% of HNSCC, a loss or mutation of 13q causes inactivation of the tumour suppressor RB gene. This may serve as a molecular marker of HNSCC in the future.

The p16 is another tumour suppressor gene on chromosome 9p.²⁶ Other postulated suppressor genes are awaiting identification on 3p, 4, 8, 14q and 19q.

Viral Oncogenes

Apart from the established link between the Epstein-Barr virus (EBV) and more than 90% of T3 nasopharyngeal carcinoma (NPC), the causative link between Human Papilloma Virus (HPV types 16 and 18) is also fairly strong, it

has been shown that HPV DNA incorporates itself near the myc locus²⁹, expressing E6 and E7 proteins that bind with and inactivate the p53 protein.

In a recent study³⁰ of 1205 head and neck tumours, HPV DNA was detected in 416(34.5%). The oral cavity was the most frequent site, followed by the pharynx and larynx respectively. HPV DNA has also been isolated from the nuclei of normal surrounding cells in these cases, raising the possibility of HPV infection being a tumour initiation event.

OTHER RECENT ADVANCES

Metastasis

From a wide array of abnormal proteins expressed in malignant tumours it is difficult to isolate the ones selectively responsible for metastasis. It seems likely however that metastasis results from expression of mutated intercellular signalling proteins.

Thus underexpression of E-Cadherin³¹, a cell adhesion molecule (CAM) results in clumps of tumour cells breaking off, promoting metastasis. Other strong candidate genes include the *nm23*³² and the *p21-ras*³³. Anti-metastasis agents like carboxyamidotriazole³⁴ are undergoing clinical trials.

Tumour Angiogenesis

Depriving a malignant tumour of its blood supply by inhibiting new blood vessel growth (angiogenesis) has been an important area of cancer research for the last few years. Any growth above 1-2 cm diameter requires its own blood supply. However, it has been realized that some oncogenes (e.g. *Ras*, *Src*, *Raf* etc) can induce the expression of angiogenesis growth factors³⁴, and anti-oncogene strategies have not been very successful.

However, the discovery of nitric oxide (NO)molecule as an important vasoregulator in the last decade has triggered a new line of enquiry. It has been recently³⁵ discovered to

be a key mediator in head and neck tumour angiogenesis and metastasis. Inhibiting its production has demonstrated significant tumour control in vitro, although results of clinical trials are awaited.

Clinical Applications of Molecular Biology in Cancer

It is now clear that there are multiple genetic pathways operating in the transformed cell. Though we are still far from controlling these pathways, partly because there are many more still undiscovered, we have already begun to utilize some of our knowledge in the diagnosis, prognosis and the treatment of head and neck cancer.

HNSCC Markers in Diagnosis

The use of tumour markers in HNSCC is mainly restricted to research at present. This is partly because of the lack of a universally expressed oncogene or oncoprotein in all head and neck tumours. Moreover they would be much more expensive and time-consuming compared to current histopathologic techniques.

Recently³⁶ p53 mutations from cells in saliva have been reported to be sensitive in detecting neoplastic clones of early HNSCC. Because they are relatively common, p53 mutations are increasingly being used in clinical practice. It is particularly useful for detecting recurrence, since p53 mutation is a late event in most cases- a recent²⁷ study on laryngeal cancers has demonstrated p53 expression (Mutated, stable) in 82% of recurrent tumours compared to 29% of primary cancers. Other research groups^{3,24,27} have also found p53 expression to be the best among current prognostic markers. More assays are being developed³, and are likely to be used in the near future.

Molecular Staging in HNSCC

Surgery and radiotherapy has not improved survival rates of metastatic HNSCC substantially in the last few decades despite signifi-

cant improvement in technique. This is primarily because of failure to prevent local recurrence and micrometastasis.

The new Polymerase CHAIN Reaction (PCR) genetic technology is sensitive enough to detect one mutant cancer cell among 10,000. By comparison, current histopathologic techniques cannot detect small numbers of tumour cells at resection margins or cervical nodes in HNSCC - a 5 μm section through a 1 cm node samples only 1/2000th part of the node. Since the most important prognostic factor in treated patients with HNSCC is the completeness of primary tumour eradication, molecular staging may help the objective considerably.

In an ongoing study¹ at the John Hopkins University, Baltimore, 52% of HNSCC tumours were shown to have neoplastic cells in the resection margins by the p53 PCR assay, that were not detected by routine histopathology. Within 27 months, about 39% of this subgroup developed local recurrences. A prospective multi-institutional trial has been launched to assess the efficacy of molecular analysis of surgical margins and nodes, based on p53 and other assays. It is

likely that molecular assays based on p53, cyclin D1, Ras etc would enter routine clinical head and neck practice in the near future.

It is important to realize that such technology would in no way replace histopathologic techniques - they are designed to detect micrometastases undetectable by conventional technology. Molecular staging may necessitate major revisions of IUCC /AJC classifications, and significant changes in treatment policies. Whether this will improve survival rates, remains to be seen.

Gene Therapy

Gene therapy can be broadly defined as a range of therapeutic strategies based on molecular biology. The ultimate goal of gene therapy in cancer is difficult to define at present, because there is so much we do not know about cancer biology yet. But in the near future, it is certain that any breakthroughs in cancer therapy is most likely to come throughs genetic technology. At present many major centres are gradually adopting gene therapy as part of a combined modality with traditional methods, often as clinical trials.

Table 1. Gene Therapy for Cancers (HNSCC)-Current Clinical Trials

I Gene Transfer	<ul style="list-style-type: none"> • Adenovirus mediated recombinant p53 and K-ras transfer • Liposome-mediated anti-sense raf oncogene transfer
II Monoclonal Antibodies to Oncoproteins	<ul style="list-style-type: none"> • Intratumour injection and liposome- mediated administration of anti-Epidermal Growth Factor Receptor Antibody (anti- EFGR)
III Adjuncts to Chemotherapy	<ul style="list-style-type: none"> • Anti-EFGR and cisplatin synergy • P53 gene and Taxol synergy • Carboxyamidazole (anti-metastatic agent) • High-dose chemotherapy with Bone Marrow support by stem cell proliferating factors e.g. GM-CSF • Anti-tumour angiogenesis factor: L -NAME (NO synthetase inhibitor)
IV Immunogenetherapy	<ul style="list-style-type: none"> • Genetically modified anti-tumour lymphocytes (with GM-CSF genes) • Interleukin-2 gene transfer • Suicide-gene transfer and chemotherapy • Increasing tumour antigenicity
V Gene Markers	<ul style="list-style-type: none"> • Molecular staging • Monoclonal antibodies in hybridoma culture.

Table 1 provides a summary of current gene therapy trials under way in the treatment of HNSCC. Some of them have been approved for wider based population trials, and already, some results²⁷ appear to be promising for HNSCC. Although immunogenetic therapy is the commonest (70%) form of gene therapy in cancer in general, its role in HNSCC is presently limited. Gene transfer is being used increasingly, but because our current vectors - adenoviruses and liposomes, are relatively inefficient, newer vectors are being actively sought for. Newer antineoplastic agents are being developed which utilize genetic strategies. Gene therapy is a fast-growing area in clinical oncology.

Conclusion

Molecular genetics of cancer has grown out of infancy, and we are close to understanding a complete tumour progression model of HNSCC, as we have done with colon cancer. The last few years have seen a surge in research in head and neck cancer genetics. Since HNSCC is a relatively localized tumour and considered curable in Stages I and II, efforts should now concentrate on early diagnosis and prevention of micrometastases and recurrence; and using gene therapy to increase cure rates of advanced tumours. Interrupting the stepwise genetic progression of HNSCC and metastases could completely change the scenario in the coming years.

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