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Oncogenes and Tumour-suppressor Genes in Squamous Cell Carcinoma of the Head and Neck

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Cancer is now considered to be a multi-hit process which involves a number of aberrant genetic events culminating in malignant transformation. In squamous cell carcinoma (SCC) of the head and neck the action of both oncogenes and tumour-suppressor genes has been identified during the course of the disease. Cytogenetic analysis of these carcinomas has demonstrated chromosomal breakpoints, particularly in the regions of 1p22 and 11q13 together with frequent amplification of the proto-oncogenes in the 11q13 amplicon; *int*-2, *hst*-1 and *bcl*-1. *Ras* mutations have been infrequently identified in the Western World whereas *ras* over-expression has been a common finding and may be associated with the early development of head and neck cancer. C-*myc* over-expression appears to correlate with a poor prognosis for these patients. The tumour-suppressor gene p53 is also thought to be involved in the development of SCC in head and neck tumours and its aberrant expression is associated with a history of heavy smoking and heavy drinking. E-cadherin, a putative tumour-suppressor gene is down-regulated in poorly differentiated head and neck SCC and maybe important in nodal metastasis. A recent study has indicated that the Human Papilloma Virus (HPV 16 and 33) has a role in the aetiology of tonsillar carcinomas and HPV has been shown to produce transforming proteins which bind to and inactivate the p53 tumour suppressor gene. This evidence suggests that the possibility of a viral mechanism for the development of SCC in the head and neck should be considered. This paper proposes a series of genetic events to explain the development of SCC of the head and neck.
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INTRODUCTION

CANCER IS a genetic disease and the development of molecular biology techniques over the past 10 years has opened up a whole new dimension in our understanding of neoplasia. Normal genes or proto-oncogenes may become activated oncogenes by a mutation caused by chemical carcinogens, irradiation or possibly viruses. The activated oncogenes may produce an abnormal gene product as a result of a mutation or an abnormal amount of the gene product due to amplification or over-expression of the gene. Oncogenes have been demonstrated at the level of growth factors and their receptors, as well as genes controlling signalling from the cell surface to the nucleus [1]. A range of techniques have been developed to evaluate oncogenic activity in cancer cells. DNA can be investigated for chromosomal aberrations at the cytogenetic level, and at the molecular level for mutated or amplified genes. Also, assays have been developed to investigate abnormal RNA transcripts and over-expressed oncoprotein levels using molecular hybridisation, immunohistochemical and enzyme linked immunosorbent assay (ELISA) techniques [2].

Oncogenes represent one major class of cancer genes involved in human cancer, and are most likely involved in both initiation and progression of neoplasia [3]. A second class of cancer genes are the tumour suppressor genes, which produce tumours following their inactivation or loss of expression [4]. The tumour suppressor genes were discovered through the study of inherited cancer susceptibilities. Knudson [4] postulated that the genetic changes in the germ line which give rise to inherited cancer susceptibility, may also be an important step in the development of sporadic cancer at the somatic level. Recent evidence indicates that the human papillomavirus produces transforming proteins which can form complexes with the retinoblastoma gene and also with the p53 tumour-suppressor gene [5, 6]. This opens up a new approach in understanding a viral aetiology for head and neck cancer.

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ONCOGENES IN HEAD AND NECK CANCER

There is now a large body of evidence suggesting that both qualitative and quantitative changes in oncogenes are involved in uncontrolled cellular proliferation. In a number of human cancers amplification of oncogenes has been found in association with or without over-expression of their respective gene. The precise action of these genes in either normal or tumour cells is uncertain, and in many cases the method of activation is unknown. Alterations in expression of oncogenes are usually attributed to mutations, amplification or rearrangements. However, concomitant genetic alterations are not always found with elevated levels of expression of these oncogenes [7–9]. Other mechanisms must exist to explain over-expression of certain oncogenes without obvious genetic alterations.

Oncogene activation and expression has been investigated in head and neck cancers over the past 7 years, and a number of studies have been correlated with clinicopathological parameters and survival. The oncogenes thus far studied have included members of the *ras* and *myc* gene families, *erb*B-1, *erb*B-2, *int*-2, *hst*-1, *bcl*-1, TGF, c-*mos* and *raf*.

ras GENE FAMILY

The *ras* gene family (H-*ras*, K-*ras* and N-*ras*) contains genes which are structurally related and encode for a protein of 21 kD molecular weight. *ras* p21 proteins are homologous to G proteins and possess GTPase activity and are located on the internal part of the cytoplasmic membrane [10]. Activation of the *ras* oncogene has been shown to be caused by specific base mutations in the *ras* gene and elevated levels of RNA transcripts and p21 *ras* oncoproteins have been demonstrated in many human tumours [9].

Mutations in the *ras* gene family have been shown to play an important role in the development of a number of human cancers. High levels of *ras* mutations have been found in adenocarcinoma of the pancreas (90%), colon (50%), thyroid (50%) and lung (30%) [11]. Ki-*ras* mutations have been demonstrated in both precancerous colonic polyps and in the adjacent carcinoma in the same patients, thereby providing evidence of a genetic link between these two stages of colonic cancer development [12, 13].

The role of the *ras* genes in the initiating stages of carcinogenesis was first demonstrated in chemically induced tumours in the mouse model system and mutations in the Ha-*ras* oncogene were found in both the pre-neoplastic papillomas and in subsequent carcinomas [14, 15]. However, evidence for the involvement of the *ras* gene in the early stages of solid tumours is unclear as reports on both *ras* mutations and *ras* overexpression are contradictory in a range of tumours. Ki-*ras* and N-*ras* mRNA and *ras* p21 levels were found to be specifically elevated in benign and malignant breast cancers [16, 17] and it is of particular note that high levels of *ras* p21 expression were found in hypoplastic specimens of the breast in patients who subsequently developed cancer [18]. However, contrary results have been reported by a number of groups [19–21]. Certain reports indicate that there is a general trend for high levels of *ras* p21 to be associated with poor prognostic factors [21–24] and Watson *et al.* 1990 [21] found a correlation between increased *ras* p21 expression and poor prognosis in breast cancer.

In colorectal carcinomas, *ras* mutations have been identified in about 50% of all carcinomas and large adenomas [25]. However, *ras* mutations were found in less than 10% of adenomas under 1.0 cm in diameter [26]. This may indicate that the adenomas with *ras* mutations are more likely to progress into a more advanced adenoma which may subsequently develop into a colonic carcinoma. Thus *ras* mutations may be considered to be involved in the early stages of colonic carcinomas but not the initiating event. Further evidence for the role of *ras* in the early stages of human cancer comes from the work on human neuroblastomas where high levels of *ras* p21 expression were found to correlate with a favourable prognosis [27, 28]. However, recent studies have demonstrated that *ras* mutations in non-small cell lung cancers are associated with a shortened survival [29] and may be therefore considered late events in the development of certain lung tumours, contrary to the findings in colonic carcinomas. Clearly the timing of the alteration in the *ras* gene, be it by mutation or over-expression, cannot be considered to be a common theme in all human cancers and this may be explained by differing carcinogenic agents that are involved in these tumours.

In head and neck cancer, a very interesting geographic split has been demonstrated regarding the incidence of *ras* mutations. In India, 35% of oral squamous cell carcinomas were shown to have a H-*ras* mutation [30], whereas the findings of four recent studies in western Europe and in the USA have shown that *ras* mutations are found in less than 5% of head and neck cancers [8, 31–33]. In India the prevalent use of betal quid chewing and reverse smoking, are probably initiating agents in oral cancer and these agents may be responsible for the high incidence of *ras* mutations in the Indian population compared with western Europe (Table 1).

It is also of note that amplification of the *ras* gene has not been recorded in head and neck patients in the Western world [8, 34–37], whereas K- and N-*ras* amplification have been reported in oral cancer in India [38].

Apart from the Indian study there is little evidence to suggest that mutations or amplification of the *ras* gene play a role in the development of head and neck cancers. However, overexpression of this gene family has been demonstrated by a number of research groups in squamous carcinoma of the head and neck [8, 39–42] (Table 1). It is of note that Sheng *et al.* [8] found that the level of Ha-*ras* expression in head and neck cancer was not determined by genetic alterations of the Ha-*ras* gene, in the form of loss of an allele, mutation or the presence of a rare allele, thereby indicating that other, as yet unknown mechanisms must also be responsible for the overexpression of this gene.

Furthermore a relatively high frequency of Indian patients with oral carcinomas at differing stages of the disease were found to have loss of heterozygosity at the H-*ras* locus and thus these authors suggested that H-*ras* maybe involved in the early stages of oral cancer [43].

The relationship between p21 *ras* expression and prognosis was analysed by two groups in Japan [40, 41] and in the UK [42]. We demonstrated [42] that over-expression of the p21 *ras* correlated with a favourable prognosis in squamous cell carcinoma in the disease free group of head and neck patients, thereby indicating that over-expression of the *ras* oncogene maybe an important event in the early stages of the development of this cancer. Although this finding was contrary to those found by the two Japanese groups [40, 41], their results were at variance with each other. This may indicate that different environmental factors inter-relate in the initiation of head and neck cancers in these two different geographical regions.

Heavy use of tobacco and high alcohol consumption have been proposed as possible environmental factors in head and neck cancer [44, 45]. Azuma *et al.* [40] found a correlation between elevated p21 *ras* expression and tobacco use, when the patients were separated into smokers or non-smokers in a Japanese series. Whereas in a more detailed investigation into the smoking history in a series of head and neck cancer patients in the UK, no direct correlation was found with p21 *ras* expression [46]. However, when smoking history was assessed with over-expression of both the tumour suppressor gene p53 and p21 *ras*, a correlation was found with a history of heavy smoking [46].

Table 1. ras mutations, amplification and over-expression in head and neck cancer

Oncogene	No. of patients	Percentage mutations	Nucleotide substitution (number)	Ref.
ras mutations				
Ha-*ras*	54	3.4	G–T codon 12 (2)	8
Ki-*ras*		0		
N-*ras*		0		
Ha-*ras*	37	5.4	G–A codon 12 (1) G–T codon 12 (1)	31
Ki-*ras*		0		
N-*ras*		0		
Ha-*ras*	57	35	G–A codon 12 (1) G–T codon 12 (7) G–A codon 13 (1) A–G codon 61 (10) G–T codon 61 (3) A–T codon 61 (1)	30
Ki-*ras*		0		
N-*ras*		0		
Ki-*ras*	42	0		33
Ki-*ras*	21	0		32
Ha-*ras*		0		
N-*ras*		0		
Ki-*ras*	20	0		*
Ras amplification				
Oncogene	No. of patients	Amplification (%)		Ref.
Ha-*ras*	14	0		34
	23	0		38
	85	0		8
	21	0		35
	21	0		36
	66	0		37
Ki-*ras*	14	0		34
	23	17		38
	21	0		35
N-*ras*	23	30		38
	21	0		36
Ras over-expression				
Oncogene	No. of patients analysed	Percentage over-expressed		Ref.
Ha-*ras*	14	70		39
	79	19		8
Ki-*ras*	14	64		39
p21 *ras*	121	59		40
	56	55		41
	69	62		42

*Field *et al.* (in preparation).

Table 2. c-myc amplification and over-expression in head and neck cancer

Oncogene	No. of patients analysed	Amplification (%)	Ref.
Amplification			
c-*myc*	23	17	38
	21	0	35
	21	0	36
	66	10	37
N-*myc*	23	39	38
	21	0	35
	21	0	36
L-*myc*	23	0	38
	21	0	35
	21	0	36
Over-expression			
Oncogene	No. of patients analysed	Percentage over-expressed	Ref.
c-*myc*	7	29	130
	14	70	39
	27	30	49
	44	48	47

c-*myc* GENE FAMILY

c-*myc* gene family is made up of c-*myc*, L-*myc* and N-*myc*, these genes code for a nuclear protein of 62 kD molecular weight, which appears to be involved in cell growth and differentiation in normal cells [7]. Amplification and over-expression of these genes have been implicated in the development of a number of human cancers [7, 9].

Amplification and rearrangements of the *myc* gene family have not been shown to be an important feature of head and neck cancer in the western World, [35–37], whereas in India both c-*myc* and N-*myc* were amplified in 17 and 39% of the cancers, respectively [38] (Table 2). It is of note that both Merritt *et al.* [36] and Leonard *et al.* [37] found co-amplification of the *int*-2 oncogene with the c-*myc* oncogene in 2/2 and 2/6 cancers, respectively. It is not yet clear whether amplification of the *int*-2 gene or the associated "amplicon" on chromosome 11, is the important genetic region in the development of head and neck cancers. Possibly, co-amplification of *int*-2 and c-*myc* genes may represent a subset of tumours with a particular clinical outcome.

Over-expression of the c-*myc* gene has been demonstrated in a number of tumours without amplification of the gene [7] and this also appears to be the case in head and neck cancer (Table 2). Elevated levels of c-*myc* RNA transcripts were initially reported by Spandidos *et al.* [39] in head and neck cancer and subsequently in other papers [37, 47–49]. The p62 c-*myc* oncoprotein levels have been measured in head and neck tumours and a correlation was found between elevated c-*myc* expression and a poor prognosis in these patients [47]. A similar correlation was found between elevated c-*myc* expression and clinical outcome using the *myc*1-9E10 monoclonal antibody in an immunohistochemical analysis [50]. C-*myc* genetic alterations have been demonstrated to be associated with a poor prognosis in breast cancer [51] and c-*myc* over-expression has been shown to correlate with a poor prognosis in cervical carcinomas [52].

Therefore the results from breast, cervical and head and neck cancers clearly point to the involvement of the c-*myc* gene in the progression of these cancers.

AMPLIFICATION OF 11q13 IN HEAD AND NECK TUMOURS

Multiple cytogenetic rearrangements have been demonstrated in 31 squamous cell carcinomas of the head and neck [53].

Table 3. Amplification of genes in the 11q13 amplicon in head and neck cancer

Oncogene	No. of patients analysed	Amplification (%)	Reference
int-2	21	52	35
	21	48	36
	10	25	62
(Coamplification)			
bcl-1/*int*-2	66	8	37
	20	35	61
hst-1/*int*-2	5	40	60
bcl-1	23	40	61

Table 4. Amplification and over-expression of erb*B*-1 *and* erb*B*-2 *in head and neck cancer*

Amplification Oncogene	No. of patients analysed	Amplification (%)	Reference
*erb*B-1			
(EGFR)	17	0	70
	21	19	71
	21	9	35
	21	0	36
	66	10	37
*erb*B-2	21	0	35
	21	0	36
	38	0	37
Over-expression			
Oncogene	No. of patients analysed	Percentage over-expressed	Reference
erb-B-1	17	0	70
(EGFR)	15	53	71
	28	14	49
*erb*B-2	14	0	48
	75	0*	75
	15	0	76

*Cytoplasmic staining found in 60% of tumours; see text for details.

However, Jin *et al.* [53] have specifically shown a clustering of breakpoints in these tumours on chromosomes 1 and 11 and in particular, 9 of the 35 tumours had a breakpoint at 11q13. Furthermore, Owens *et al.* [54] have found one of ten tumours to have the same breakpoint site. Recently, Lammie and Peters [55] have reviewed the cancer related genes at the 11q13 site. These include *hst*-1, *int*-2, *bcl*-1, *sea*, *prad*-1 (D11 5287), *men*-1 and *ems*-1. The *bcl*-1, *prad*-1, *hst*-1 and *int*-2 genes are all within a 250 kb. region at 11q13. Chromosomal translocations have also been found at 11q13 in sporadic parathyroid adenomas [56, 57], in lymphocytic malignancies and in B-cell neoplasms [58, 59]. Furthermore amplification of genes in the 11q13 amplicon have been reported in breast cancers, and in transitional cell carcinomas of the bladder [55].

This is pertinent to head and neck cancer as six independent reports have to date demonstrated amplification of genes in the 11q13 amplicon region. Amplification involves the *int*-2, *bcl*-1, *hst*-1 genes, either singly or co-amplified [35–37, 60–62], (Table 3). The clinical correlations of *int*-2/*bcl*-1 amplification in squamous cell carcinomas is uncertain but there maybe an indication that they have a prognostic significance [35, 61]. The biological significance of this region is heightened by the knowledge that the *int*-2 and *hst*-1 genes encode for members of the fibroblast growth factor family [63, 64]. Attempts to locate the *bcl*-1 gene have been unsuccessful so far, however evidence now indicates that the *prad*-1 gene is within 120 kb of the original breakpoint locus of *bcl*-1 and it has been argued by Lammie and Peters [55] that *prad*-1 may be the putative *bcl*-1 oncogene. The *prad*-1 gene [65] has recently been isolated by a number of groups (designated to the HGM locus D11S287 [56]) and has been shown to have sequence homology with the cyclin family, which may control cell cycle progression [66]. Moreover *prad*-1 has been shown to be co-amplified with *bcl*-1 and *int*-2 in a number of reports on breast cancer [55] and as *prad* RNA levels have also been found to be over-expressed in the amplified breast tumours [67], this would point to the fact that *prad*-1 may be the active oncogene at the 11q13 site.

Other candidate genes for the "active" gene at 11q13 may be *ems*-1 or the loci D11S814, D11S97 or D11S147 or even possibly *men*-1, a tumour suppressor gene which maps between *pysm* and D11S146 [55].

EPIDERMAL GROWTH FACTOR RECEPTOR AND c-*erb*B-2 GENES

The epidermal growth factor (EGF) receptor has been proposed to be the proto-oncogene of the *erb*B oncogene [68]. Epidermal growth factor is a polypeptide which stimulates proliferation in a range of cells by interacting with its receptor, a 170 kD transmembrane glycoprotein. Amplification of epidermal growth factor receptor (EGFR) has not been shown to be a consistent feature of head and neck cancer tumours or cell lines [35–37, 69–71], (Table 4). Over-expression of the EGF receptor gene (*erb*B-1) has been found in 53% of 15 squamous cell carcinoma tumours, of which four had an amplified *erb*B-1 gene, and this was shown to correlate with the level of differentiation of these tumours [71].

The c-*erb*B-2 oncogene is known to share considerable homology with the epidermal growth factor receptor [72, 73]. *erb*B-2 is a transmembrane protein with a cell-external ligand-binding domain and a cell-internal domain with tyrosine–kinase activity, involved in signal transduction. The c-*erb*B-2 has been shown to be often amplified in adenocarcinomas of the breast, stomach, renal carcinomas and ovarian carcinomas and over-expressed in carcinomas of the breast, stomach, salivary tissue and pancreas [74, references in 74]. There is no evidence for amplification of *erb*B-2 in head and neck squamous cell carcinomas [35–37] or over-expression of c-*erb*B-2 RNA transcripts [48]. However, in a study of c-*erb*B-2 oncoprotein expression in 75 squamous cell carcinoma of the head and neck, using four different antibodies to c-*erb*B-2, cytoplasmic staining was found in 60% of the specimens [75]. No correlations were found between c-*erb*B-2 positive cytoplasmic staining and any of the clinico-pathological parameters or survival. Even though c-*erb*B-2 membrane staining in breast cancer tumour cells is associated with over-expression of the c-*erb*B-2 gene, the interpretation of cytoplasmic staining in squamous cell carcinomas is open to debate [75, 77–80] (Table 4).

TUMOUR SUPPRESSOR GENES IN HEAD AND NECK TUMOURS

Evidence for the role of possible tumour suppressor genes in head and neck cancer is accumulating from the work with the p53 [81, 82] and E-cadherin genes [83, 84].

p53 *gene*

The proto-oncogene product p53 was initially identified as a host cell protein bound to the large T antigen, the dominant transforming oncogene of the SV40 virus [64]. p53 protein is expressed at low levels in non-transformed cells, however, elevated levels of p53 expression are found in many tumours and cell lines [86]. Normal levels of p53 act as tumour suppressor genes but mutations in p53 may convert it into a dominant gene, however they may remain as recessive or become null mutations [87, 88]. Approximately half of the adult cancers, including lung, breast, colon, oesophagus and skin cancers contain p53 mutations [89] and aberrant p53 expression is now considered to be one of the most common genetic features in a wide range of human cancers [90].

The timing and frequency of p53 mutations in colorectal tumourigenesis has been investigated by Baker *et al.* [91]. They have demonstrated that p53 gene mutations are rare events in adenomas, regardless of size, and p53 mutations are also infrequent in both adenomas and carcinomas that contained both copies of chromosome 17p. Whereas 86% of tumours that had lost one copy of chromosome 17p had a p53 mutation. These authors concluded that alterations in the p53 gene occurred around the transition from adenoma to carcinoma and was therefore a late step in the progression of colonic carcinomas. These findings were supported by immunohistochemical studies on a group of 150 benign and malignant colorectal carcinomas [92]. Similarly p53 over-expression has been shown to be a rare event in benign breast lesions [93, 94], whereas p53 over-expression was found in 50% of breast carcinomas and maybe associated with prognosis [93, 95, 96], indicating that p53 genetic aberrations are probably late events in breast cancer.

The realisation that the mutant p53 gene product is a more stable protein than the wild type p53 protein [97], has enabled many groups to utilise immunohistochemical methods to demonstrate the mutant p53 protein product in tumour tissue. The half-life of wild type 53 protein is of the order of 20 min, compared with the mutant half-life of about 8 h. Expression of the p53 gene product has been analysed in head and neck cancer recently by two groups using a range of antibodies [81, 82] and over-expression of p53 has been found in 49/73 (67%) [81] and 16/47 (34%) [82] of these tumours. Furthermore two head and neck cell lines have been shown to have p53 mutations by sequence analysis at codons 238 and 152 (HN5, codon 238 TGT-AGT and HNRr, codon 152, CCG-CTG) [82].

There is overwhelming evidence for a correlation between heavy smoking and lung cancer [43] and in addition the p53 gene has been found to be over-expressed and mutated in lung tumours that are associated with smoking [98, 99]. It was therefore considered that there maybe a strong possibility for an association between p53 over-expression and smoking in head and neck cancers. This hypothesis was tested by Field *et al.* [81] who demonstrated that there was indeed a correlation between heavy smoking and elevated p53 expression. 6 out of 7 non-smokers did not express p53 whereas 20 of 37 heavy smokers were found to have elevated p53 expression. Furthermore all but one of the patients who had stopped smoking for more than 5 years had high levels of p53 expression, thereby indicating that alterations in this gene maybe one of the early events in the development of these cancers [81].

In another recent study into p53 expression in head and neck cancer, a number of premalignant skin lesions were included and as there was no evidence for p53 over-expression, it was argued that the p53 gene could not be used to identify early genetic damage in these premalignant lesions [82]. However, Brash *et al.* [100] have shown that over half of the squamous cell carcinoma of the skin had p53 mutations and that all of the mutations were at dipyrimidine sites, and 25% of them had CC to TT double base changes which is a characteristic of ultraviolet light induced mutations. As the carcinogenic agents involved in head and neck cancer are most likely different from those in skin lesions and as there is no immunohistochemical evidence at present to determine if the p53 gene plays a role in the premalignant stages of head and neck cancer, the question of whether the p53 gene is involved in the early stages of this disease remains debatable.

The p53 sequence analysis data for head and neck tumours is not as yet available; however, Chiba *et al.* [99], have shown that G to T transversions account for 56% of p53 mutations in lung cancers, unlike other tumours which have mainly G to A transversions. As the type of mutation is often associated with specific mutagens, this data would suggest that the lung cancer may have been caused by a specific mutagen, adding weight to the link between smoking and lung cancer. Furthermore, Chibia *et al.* [99] concluded that mutations in the p53 gene occurred frequently in the early stages of non small cell lung cancers and that p53 had a role independent of tumour progression.

An association has also been recently found between heavy smoking and heavy drinking in head and neck patients with elevated p53 expression [101]. Similarly, in oesophageal carcinomas, which are associated with high tobacco and alcohol consumption, a higher frequency of A to T transversions have been found in tumours with p53 mutations [89, 102], thereby indicating that the synergetic action of tobacco and alcohol may act as a mutagen in oesophageal cancer, and also in head and neck cancer.

There is evidence for a possible interactive role between the p53 gene product and the E6 protein of the human papilloma virus (HPV) [6]. The HPV-16 E6 protein is known to promote *in vitro* degradation of the p53 protein via the ubiquitous dependent proteolytic pathway [103]. HPV-16 has been proposed to be one of the causative agents in the development of cervical carcinomas and as only low levels of p53 protein have been reported in these tumours, it is possible that HPV-16 E6 interacts and inactivates the p53 protein. This mechanism may also operate in head and neck cancers as papilloma viruses have been reported in both benign and malignant lesions [104]. However, even though Gusterson *et al.* [81] reported that there was no cytological evidence of HPV-16 in their study on p53 expression in head and neck cancers, a recent report by Snijders *et al.* [105] has demonstrated a specific association between tonsillar carcinomas and HPV which will be discussed in a later section of this review.

E-cadherin gene

The cadherin gene family is composed of three subclasses, E-, N- and P-cadherins [83, 84]. The E-cadherins are considered to be specifically responsible for epithelial cell–cell adhesion. During metastasis particular cell–cell adhesion

mechanisms appear to operate incorrectly, and even though malignant transformation does not necessarily affect cadherin expression, it has been demonstrated that down regulation of the E-cadherin gene was associated with both invasion and metastasis [108–111].

E-cadherin down regulation may provide a very powerful method to assess the invasive and metastatic potential of human tumours, and has been recently studied in both head and neck squamous cell carcinomas [83, 84] and in gastric carcinomas [111]. Schipper *et al.* [83] have demonstrated that E-cadherin is expressed in well and moderately well differentiated squamous cell carcinoma of the head and neck, but no detectable E-cadherin was found in the poorly differentiated tumours. Moreover, seven out of eight lymph nodes analysed were found to have down-regulated E-cadherin irrespective of the differentiation grade of the primary tumour. This indicates that the E-cadherin gene may play a role in the differentiation of the tumour and also act as a possible tumour suppressor gene. E-cadherin has been assigned to chromosome 16q22 [112] and this gene may represent the new tumour suppressor gene located at 16q22 in hepatocellular, breast and prostate carcinomas [113–115]. It is also of note that Owens *et al.* [54] have recently found chromosomal translocations at 16q22 in two head and neck squamous cell carcinomas.

ONCOGENIC VIRUSES IN HEAD AND NECK CANCER

There is increasing evidence for the role of viruses in the pathogenesis of certain human cancers, in particular: the human papilloma virus in carcinoma of the uterus, cervix, vulva, penis, oesophagus and larynx [116]; and in Epstein–Barr virus in nasopharyngeal tumours [117] and Burkitts lymphoma [118]; hepatitis B virus in hepatocellular carcinoma patients [119].

A number of viruses, including reteroviruses, adenoviruses, herpes simplex and human papilloma virus have been considered to possibly have a role in oral cancer [120]. The human papilloma virus has recently been shown to have a very important interactive role with the tumour suppressor genes p53 and the retinoblastoma gene (RB) and deserves further attention in this review.

There is evidence to indicate that certain DNA tumour viruses exert some oncogenic effects on their host cells by interacting with cellular proteins. In the case of the human papilloma viruses, HPV-16 is a potent *in vitro* transforming agent [121], and produces the transforming proteins E6 and E7 which forms complexes with tumour suppressor gene products. The HPV-16 E7 proteins binds to the retinoblastoma gene product [5] and the HPV-16 E6 protein binds to the p53 gene product [6]. The fact that mutations in both the RB and p53 genes have been reported in a wide range of tumours and HPV produces binding oncoproteins, indicates that there is most likely an interaction between these viral oncoproteins and the tumour suppressor genes in developing tumours. In the case of the p53 tumour suppressor gene the E6 proteins coded by HPV-16 and 18 are known to promote degradation of p53, thereby inactivating it [103].

Human papilloma viruses (HPV) are known to be associated with a variety of oral lesions and oral squamous cell carcinomas [104, 122–125]. Yeudall and Campo [125] demonstrated an altered restriction pattern in HPV 18 E1 region but no sequence alterations were found in either the normal or malignant oral specimens. It is of note that in a Taiwan study [123] into human papilloma virus in oral cancer, an association was found between HPV-16 sequences and chewing/smoking habits of the patients, indicating a possible interaction between both viral and chemical agents in the pathogenesis of these tumours.

There is also evidence for an interactive role between HPV and the c-*myc* gene in cervical carcinomas and in squamous cell carcinoma of the anus [126, 127]. In HPV associated tumours, in particular cervical carcinomas, alterations in the c-*myc* gene have been reported [128, 129], however it is only recently that there has been a direct association between HPV and the c-*myc* gene. HPV DNA sequences have been shown to be integrated into the genome of genital carcinomas at chromosomal regions where the c-*myc* and N-*myc* genes are mapped and furthermore the *myc* genes were found to be structurally altered or over-expressed in these carcinomas [126]. Also in squamous cell carcinoma of the anus, the majority of the tumours with an amplified c-*myc* gene were HPV positive [127].

Recent evidence points to a very specific association between tonsillar carcinoma and a HPV aetiology. Snijders *et al.* [105] have detected HPV DNA in all of the 10 cases of tonsillar carcinomas they investigated, 5 with HPV 16, and 5 with HPV 33, whereas there was no evidence of HPV infection in 7 cases of tonsillitis. This indicates a 100% prevalence of HPV infection in tonsillar carcinomas compared with a figure of about 30% for other sites within the aerodigestive tract. It is of particular note that these authors also detected HPV 16 and HPV 33 E7 encoding spliced E6*1 transcripts in all of the tonsillar carcinomas studied, which supports the argument for HPV having a role in tonsillar cancer.

These findings opens up a new approach in considering the interactive effects of oncogenes, tumour-suppressor genes and viruses in squamous cell carcinomas of the head and neck.

POSSIBLE MOLECULAR MECHANISMS IN HEAD AND NECK CANCER

Cancer is now considered to be a multi-hit process which involves a number of aberrant genetic events culminating in malignant transformation. This process maybe separated into the stages of initiation and progression of the disease in which a number of discordant genetic events may occur [4].

The molecular mechanisms of a number of cancers has been intensively researched with probably the most elegant genetic model being suggested for colonic carcinomas [25]. Fearon and Vogelstein [25] have proposed a series of genetic events which may occur in the development of colonic cancer and possibly take the following order: alterations of a gene on chromosome 5q (possibly MCC, mutated in colon cancer) which may lead to abnormal proliferation of colonic tissue, followed by DNA hypomethylation (inhibits chromosome condensation and responsible for chromosomal loss or gain) and could initiate a benign adenoma; *ras* mutations on chromosome 12p may represent the next step as they are associated with the selective outgrowth of small adenomas; also mutations in the tumour suppressor gene (DCC) on chromosome 18q, may alter cellular adhesion of the cells resulting in clonal expansion of the adenoma, and the ability to invade the basement membrane and eventually metastasise. In addition the role of mutations in the tumour suppressor gene p53 on chromosome 17, have been shown to be associated with the transition from the adenomas stage to a colonic carcinoma.

Despite the fact that these authors have proposed a sequence of genetic events in the development of colonic cancer, they argue that the development of the carcinoma is dependent on the accumulation of various genetic events and may not occur in this precise order.

Research into the molecular mechanisms of head and neck cancer is not advanced as that of the colon, however it is still possible to postulate certain mechanisms that maybe operating in the development of head and neck cancer.

Cytogenetic analysis of squamous cell carcinomas of the head and neck has demonstrated that there is a clustering of breakpoints on chromosomes 1 and 11 at 1p22 and 11q13, which might be implicated in the aetiology in this disease [53, 54]. Furthermore amplification of a number of oncogenes (*int*-2, *hst*-1 and *bcl*-1) in the amplicon region at 11q13 have been reported by a number of groups [36, 37, 60–62], however the precise oncogene within this amplicon still has to be determined as well as its clinical significance.

Studies related to the tumour-suppressor gene p53 in head and neck cancer have demonstrated that this gene is over-expressed [81, 82] and p53 mutations have been demonstrated in two head and neck cell lines [82]. It is also of note that over-expression of this gene correlates with a history of heavy smoking and that all but one of the patients who had stopped smoking for more than 5 years had high levels of p53 expression, thereby indicating that alterations in this gene may be one of the early events in these cancers [81]. In addition over-expression of the *ras* and the p53 genes in these patients also correlated with a history of heavy smoking [46] and p21 *ras* over-expression in a Japanese study on oral carcinomas also showed an association with a history of smoking [40].

When these results are considered in conjunction with the finding that p21 *ras* over-expression correlated with a favourable prognosis in patients with a head and neck cancer [42], it would suggest that the *ras* oncogene maybe also involved in the early developmental stages of head and neck cancer. *ras* mutations were found in 35% of chewing tobacco-related oral cancers in an Indian Study [30], but these mutations are rare in head and neck cancers in the Western World. However, in view of the number of papers that have reported ras p21 over-expression in these tumours outside the Indian Subcontinent, other mechanisms, apart from known ras mutations must exist to activate this gene.

The c-*myc* oncogene has been shown to be over-expressed in four studies of head and neck cancer [39, 47, 49, 130] and amplified in two out of four studies [35–38]. In the largest of these studies which was investigating elevated levels of c-*myc* oncoprotein, it was found that c-*myc* over-expression correlated with a poor prognosis [47]. It may therefore be argued that activation of the c-*myc* gene is a late event in the progression of these cancers [34, 47]. Also it has been recently demonstrated that E-cadherin, a cell–cell adhesion molecule is down regulated in poorly differentiated head and neck cancers and in lymph node metastasis [83, 84], indicating that inactivation of this gene is also most likely a late event in the progression of head and neck squamous cell carcinomas.

Although a number of other oncogenes have been investigated in squamous cell carcinoma of the head and neck, only the *erb*B-1 oncogene has been shown to be a consistent feature of head and neck cancers and is most likely to be involved during the development of this disease.

The involvement of the human papilloma virus in the aetiology of head and neck cancer has been debatable, however recent evidence demonstrates that HPV produces a protein which binds to the p53 tumour-suppressor gene and inactivates it. Moreover HPV DNA (HPV-16 or 33) has been detected in all of the tonsillar carcinomas examined [105] thereby indicating that certainly a subgroup of head and neck cancers do have a viral aetiology.

The discovery of oncogenes and tumour suppressor genes in human cancers generally, has lead to a greater understanding of the disease process. The clinical potential of these molecular markers is beginning to be illustrated in the fields of diagnosis and prognosis and may in future have a possible role in therapy.

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