Molecular Genetics of Cancers (Part I)

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Advances in the molecular genetics of cancer in the last 15 years have made significant impact on patient management. The application of new technologies in molecular genetics and immunology may provide the clinicians with the tools to enhance patient survival. The focus of this review is to provide a brief simplified description of the basic science of molecular genetics using head and neck cancer as a model and to outline potential applications and limitations of our current knowledge.

The Conceptual Basis of the Molecular Genetics of Cancer

A) Proto-oncogenes and Oncogenes

i) Proto-oncogenes

Proto-oncogenes are normal cellular genes that are thought to be crucially involved in normal growth regulation and cell differentiation.[1] The signals for mitogenic stimulation of cells may come from different sources including growth factors, growth factor receptors, cytoplasmic signal transduction proteins and nuclear proteins. Proto-oncogenes that function at each step of this pathway have been discovered and thus are thought to play an important role in a regulatory network that extends from the cell surface into the cell nucleus. When mutated or deregulated these genes attain the capacity to destabilize normal cell growth and regulation and, in this state, are referred to as oncogenes.

ii) Oncogenes

The resultant oncogene is a gene that can contribute to malignant transformation of cells and cause tumours in animals and humans. Like all genes, the protein product that they direct to be synthesized by the cell mediates the effect of oncogenes. The differences between the oncogene-directed protein product and that which would have been formed by the normal cellular gene can be qualitative e.g. novel proteins secondary to mutations in the gene sequence or quantitative e.g. too many copies of a normal protein product.

iii) Oncogene Activation

Oncogene activation is known to occur in at least four ways

1. Gene acquisition of a novel transcriptional promoter.

This results in gene over-expression with a resultant increased gene product.

2. Chromosome translocation with resultant deregulation of a proto-oncogene close to the chromosomal breakpoints.

An example of this is the Philadelphia chromosome commonly found in chronic myelogenous leukaemia (CML). In CML, the *c-abl* proto-oncogene on chromosome 9 is moved (translocated) to a region on chromosome 22 called the breakpoint cluster region (bcr) as a result of a reciprocal chromosomal movement. The resultant *bcr-abl* combination codes for a novel protein product resulting in unregulated stimulation to growth and thus chronic myelogenous leukaemia.

3. Gene amplification due to increased gene copy number.

This process, called gene amplification, has been implicated as playing a causative role in a number of malignancies. For example, amplification of the N-*Myc* proto-oncogene occurs in approximately 40% of human neuroblastomas.[2]

4. Point mutation in the gene resulting in an altered protein product.

An oncogene may be formed by a single nucleotide base pair substitution in a proto-oncogene, which can result in the production of a mutant protein with a loss of normal cell regulation. Analysis of a series of human pancreas tumour biopsies demonstrated a 95% incidence of K-*Ras*-2 codon 12 mutations.[3] Other members of the *ras* gene family, such as H-*Ras* and N-*Ras*, have been implicated in a wide range of human malignancies. Mutagenic agents such as the components of tobacco smoke; alcohol and radiation appear to play an important role.

B) Tumour Suppressor Genes

There is another class of genes, called tumour suppressor genes whose expression inhibits malignant transformation of cells. Mutation of such genes may lead to neoplastic transformation. Tumour suppressor genes were first recognized in the aetiology of the inherited forms of retinoblastomas and Wilm's tumours. It is now evident that mutation in the p53 tumour suppressor gene occurs in a wide variety of human cancers including lung, bone, colon, mammary and various haematological neoplasms.[4] Li-Fraumeni families, who have a germ line p53 mutation, can develop cancer at any of these sites emphasizing the role of p53 as the "guardian of the genome".

C) The Multi-stage Models of Malignancy

Human cancers develop through a process involving several stages. The classical model is a successive passage of the cell through the three stages of initiation, promotion and progression.[5]

1. Tumour initiation

A rapid, irreversible process that presumably results from genetic changes within the cell as a result of the cell's interaction with a carcinogenic agent. Initiated cells may be thought of as being primed for the development of malignancy, although they themselves do not express this neoplastic potential unless they undergo promotion.

2. Tumour promotion

Unlike initiation, the process of promotion is reversible with a prolonged latency period. Initiated cells develop into viable neoplastic lesions under the stimulus of the promoting agent. Progression refers to the characteristic of malignant cells to gradually attain increasingly unbridled growth characteristics. While the generation of a tumour cell appears to be a multi-step event, the activation of a single oncogene by the various mechanisms described earlier is a single discrete event. This apparent discrepancy may be explained by the fact that activation of single oncogenes may only be one facet of a complex process leading to the eventual development of a fully malignant cell.

3. Tumour Progression

The characteristic of already established malignant tumours is to successively acquire more aggressive grades of malignancy. These acquired qualities include such properties as propensity to tumour metastasis and development of radiation and chemotherapy resistance. Studies from *in vitro* models, experimental animals and studies of human cancer have strongly supported the concept that several molecular events must occur during carcinogenesis.

Molecular Genetics of Head and Neck Cancer

Genetic susceptibility

Among genes that activate or eliminate tobacco carcinogens, the null genotypes of the carcinogen-metabolizing genes glutathione-S-transferase (GST)T1 and GSTM1, the GSTP1 (AG or GG) genotype has been shown to represent risk factors for head and neck squamous cell carcinoma (HNSCC) and are markers for genetic susceptibility to tobacco-induced carcinogenesis.[6] Mutagen sensitivity (a higher rate of spontaneous chromosomal aberrations in lymphoblastoid cells) induced by the tobacco-associated cancer mutagen, benzoapyrene diol epoxide is also associated with risk of HNSCC development.[7]

Cytogenetic (Chromosomal) Changes

The most frequent chromosomal abnormalities in HNSCCs are unbalanced translocations leading to chromosomal deletions affecting 3p13-q24 (in >60% of tumours), 4p (43%), 5q12-q23 (30%), 8p22-p23 (65%), 9p21-p24 (43%), 10p (39%), 13q12-q24 (30%), 18q22-23 (>60%), and 21q (52%).[8] Loss of the inactive X and loss (or rearrangement) of Y occur in 70% of tumours from female and male patients respectively. Less common consistent changes found in 30-40% of tumours are gains affecting 3q21-qter, 5p, 7p, 8q, and 11q13-23. Allelic losses at marker loci in specific genomic regions, indicating the inactivation of critical tumour suppressor genes within the regions, have been demonstrated at 3p, 5q, 9p, 11q, 13q, 18q, and 17p. A genetic progression model for HNSCC has been postulated[9] with certain genetic events indicated as early (loss of heterozygosity (LOH) at 3p, 9p21, and 17p13), intermediate (11q, 13q11, and 14q), and late (6p, 4q, and 8) (Fig 1). The accumulation of genetic events is associated with histopathological progression although there may be a prolonged latency period during which clonal genetic alterations may be present before an invasive phenotype is produced. Some studies have correlated chromosome deletion or mutation with poor clinical outcome (LOH at 2q, 3p, 9p, 11q, 18q)[10] or biological behaviour such as radiation therapy resistance (1p, 3p, 8p 14q).[11]

Cellular Oncogenes in Head and Neck Cancer

ErbB Receptor Proto-oncogenes

The protein products of the erbB proto-oncogenes belong to the transmembrane type I receptor tyrosine kinase family.[12] The *erb*B receptors have four homologous members in the family: the epidermal growth factor receptor (EGFR or *erb*B-1 or HER-1 for human EGF receptor-1), *erb*B-2 (*neu* or HER-2), *erb*B-3 (HER-3) and *erb*B-4 (HER-4). Head and neck tumours analysed for RNA expression, gene amplification and structural alteration of the EGFR gene, *erb*B-1, showed that 13% displayed *erb*B-1 gene amplification and/or gene rearrangement. Over expression of the *erb*B-1 gene has been observed in 67% of the head and neck tumours studied whereas a stepwise increase of EGFR expression in histologically normal epithelium, premalignant and cancerous lesions has been observed following the histological progression. Those patients expressing high levels of EGFR or showing EGFR gene amplification had tumours that were clinically more advanced. Although there is no evidence for gene amplification or increased RNA transcript of *erb*B-2 in HNSCC, increased *erb*B2 oncoprotein expression has been noted.[12] Overexpression of *erb*B-3 and *erb*B-4 mRNA and protein have been found in some HNSCC lines, whereas no gene amplification was detected.[13] In clinical settings, the roles of *erb*B-3 and *erb*B4 in HNSCC progression are less clear.[12] However, their ability to form heterodimers among the *erb*B family members upon stimulation by their direct ligands i.e. heregulins (HRG)-β1 has been shown to enhance the signalling pathways of EGFR or *erb*B-2 and the invasive properties in HNSCC cells.[14,15]

At least six distinct peptides can bind directly to EGFR including EGF, transforming growth factor-alpha (TGF-α), amphiregulin, betacellulin, heparin-binding-EGF-like growth factor, and epiregulin. The direct ligands of *erb*B-3 and *erb*B-4 are isoforms of the HRGs, and no exogenous ligand for *erb*B-2 has yet been characterized. In HNSCC, the expression of multiple *erb*B ligands apart from EGF and TGF-α has only recently been described.[16,17] In addition, all *erb*B ligands can interact with each other by a mutual amplification mechanism as well as by autocrine induction.[17] The *Erb*B ligand-receptor complex recruits a unique set of signalling proteins activating multiple distinct pathways that elicit specific biological responses associated with carcinogenesis and/or metastasis.[14-16]

Normal mucosa	Hyperplasia	Dysplasia	Carcinoma in situ	Squamous cell carcinoma
	9p21 deletion	3p deletions	11q13	18q deletion
	p16/p14 inactivation	17p13(p53 mutations)	13q21	10q23
	Trisomy 7	Tetraploidy	8p deletion	3q26
	EGFR		Aneuploidy	pTEN inactivation
	Telomerase activation		Cyclin D1 amplification	

Fig 1. Genetic Progression Model of Head and Neck Cancer Progression.

Ras Proto-oncogene

The ras family of oncogenes (N-*ras*, H-*ras*, K-*ras*) encode the membrane-associated G-proteins, guanosine triphosphatases (GTPases), that constitutively maintain an activated GTP-bound state and serve as signal transducers for cell surface growth factor receptors. Conflicting results have been reported regarding the importance of the ras family in HNSCC. NIH/3T3 transfection assay uncovered activated c-H-ras-1 proto-oncogenes in two squamous cell carcinoma cell lines established from human oral carcinoma patients.[18] Anderson and Irish found that approximately 15% of oral and oropharyngeal malignancies demonstrate point mutations in the H-ras gene at codon 12 while K-ras mutations were rare.[19]

CCND1 (PRAD1)

Transition from the Gl to the S phase (the DNA synthesis phase) and from the G2 to the M phase (the mitosis phase) are critical control points in the cycle of a growing cell. A group of proteins called cyclic-AMP-dependent protein kinases (cdks) participate in the regulation of these transitions. These cdks form complexes with proteins called cyclins, including cyclin D1. The *CCND1* gene that encodes the cyclin D1 protein is located on chromosome 11q13, and this locus is amplified in 20-52% of HNSCC. LOH in this region represents allelic imbalance via amplification of *CCND1*. Overproduction of cyclin Dl may push a tumour cell through the G1/S transition, resulting in uncontrolled cell division and perpetuation of other genetic alterations. *CCND1* gene amplification and overexpression have been shown in HNSCC and premalignancy and have been correlated with poor outcome.[20]

EMS1

The *EMS1* oncogene is located in the same region as the *CCND1* gene on chromosome 11q13. *EMS1* encodes a cytoskeletal protein homologous to the avian F actin-binding protein (cortactin), which is involved in regulating the interactions between components in the adherens junctions. *EMS1* amplification in tumours results in overexpression of cortactin leading to a redistribution of cortactin from the cytoplasm into cell-matrix contact sites. This might affect the functioning of cytoskeleton and cell-adhesion structures and contribute to the invasive behaviour of tumour cells. In HNSCC, *EMS1* amplification occurs in 20% of patients and predicts early recurrence and reduced survival independent of other known risk factors.[21]

MPP11

Genetic high-copy amplification of the 7q22-31 genomic region has been recognized as an important event in the progression of HNSCC. A novel human candidate oncogene, *MPP11*, encoding a phosphoantigen with a regulatory role in the mitotic phase of the cell cycle, has been mapped to the critical region of 7q22-31.1.[22] An increased copy number of MPP11 and overexpression in the majority of primary HNSCC tissues and cell lines examined suggest that this gene is activated during malignant progression and may play an oncogenic role in HNSCC.

Tumour Suppressor Genes in Head and Neck Cancer

p16

The most commonly deleted chromosome site in HNSCC is located at 9p21-p24 which is the locus for the *CDKN2/MTS1/INK4A* tumour suppressor gene.[23] The $p16^{INK4A}$ gene product is an inhibitor of the cyclin/cyclin-dependent kinase complex and inactivation of the INK4A gene leading to absence of the p16 protein is found in 80% of HNSCC patients.[24] The $p16^{INK4A}$ gene is proposed as the earliest known tumour suppressor gene to be inactivated in HNSCC, whereas deregulation of p53 and cyclin D1 occurs later. Although only 10-15% of HNSCCs demonstrate point mutation at the p16 gene, frequent homozygous deletion in this region and transcriptional silencing through promoter methylation are major inactivation mechanisms in these tumours. Recently, a significant relationship between loss of $p16^{INK4A}$ expression and an adverse disease outcome in HNSCC has been shown.[20]

p53

Loss of 17p13 occurs in up to 60% of invasive SCCs and is the locus of the p53 tumour suppressor gene.[25] Alterations of the p53 gene by allelic losses, point mutations, deletion, or inactivation disrupt its role as a guardian of the genome by impairing the cell's ability to repair and undergo apoptosis in response to DNA damage and thus leads to genomic instability. An overall p53 mutation incidence of 40-50% has been reported in a study of invasive tumours studied by direct sequencing of exons 5 through 8.[26] A higher incidence of p53 mutation has been found in the tumours of smokers and drinkers than in the tumours of patients who developed HNSCC without exposure to these agents. It would appear that p53 gene mutation could be an early event in HNSCC carcinogenesis as it has been observed in premalignant lesions.[25] In addition, an increasing pattern of p53 alterations has been demonstrated in premalignant lesions following the histologic progression. Overexpression of p53 in tumour-distant epithelia of HNSCC patients has been reported to correlate with an increased incidence of second primary

tumours.[27] The findings of p53 mutations in histologically negative margins of completely resected tumours and the distinct p53 genotypes in synchronous primary tumours suggest that p53 can be used as a marker for molecular staging and fingerprinting in HNSCC.[26]

FHIT

The *FHIT* (Fragile Histidine Triad) gene, located at 3p14, contains FRA3B, the most common fragile site in humans, and is frequently the target of allelic loss, homozygous deletions, and genetic rearrangement in many human tumour types. Alterations of *FHIT*, such as homozygous deletions of exons at the genomic level, insertions of intronic sequence and aberrant transcripts at the mRNA level, and lack of detectable protein can be found in up to 66-68% of patients with HNSCC 28 and the loss of *FHIT* has recently been shown to be an independent adverse prognostic factor for disease-free survival in oral tongue cancers. Overexpression of the *FHIT* gene has been shown to induce cell apoptosis and altered cell cycle processes, although the underlying mechanism is still not well understood.

Evidences suggest that several additional putative tumour suppressor genes may reside at chromosomes 3p, 6p, 8p, 9p, 13q21, and 18q. For example, there are at least two candidate tumour suppressor genes at 9p21-22 in addition to the p15/p16/p19 genes.[29] Further study will be required to identify new markers and to find the possible candidate genes within the consistently lost regions.

Diagnostic and Therapeutic Applications of Molecular Genetics

I. Diagnostic Applications

A. Identification of Premalignant Lesions at Risk of Progression

Identifying patients with lesions at risk of transformation or progression is important so that they can limit their exposure to risk factors and can be given more aggressive treatment strategies such as chemoprevention. Increased polysomies, defined as the presence of three or more copies of the chromosome, of chromosomes 7 and 17 were associated with progression from normal epithelium to dysplastic mucosae and with the risk of development of oral cancer.[30] Using the technique of microsatellite assay, which provides information about the frequency of allelic imbalance (AI) at polymorphic markers within chromosomal regions that harbour the tumour suppressor genes, Mao *et al.*[31] demonstrated that the presence of AI at 3p and 9p can identify patients with dysplastic lesions at risk of progression. Further studies incorporating markers at additional chromosomal arms revealed that patients with dysplastic lesions with AI at two or more of the key chromosomal loci have a 75% chance of developing an upper aerodigestive tract tumour in five years.[32] Identical allelic losses at 9p21, 11q13, 17p13, 3p, and 13q21 were identified in histologically benign mucosal specimens and cervical nodal metastases of patients with unknown primary HNSCC, suggesting the foci of clonal precancerous cells within these sites are presumed to harbour the primary tumour.[33]

B. Cancer Detection

Failure to diagnose HNSCC in its earliest stages is the most important factor contributing to the poor treatment outcome. Early detection is also crucial to effective surveillance after treatment of HNSCC. Head and neck cancers are among the most antigenic tumours and several potential tumour markers for HNSCC have been suggested such as squamous cell carcinoma antigen (SCCAg)[34] and cytokeratin fragment 19 (CYFRA 21-1).[35] High urinary levels of TGF-α have been detected in patients with advanced head and neck cancers and preliminary findings indicate that the quantity of marker detected is proportional to the clinical extent of the malignancy.[36] Recent studies using quantitative real-time PCR have also shown that viral sequences of EBV[37] and human papillomavirus (HPV)[38] may be detectable in the sera of patients with advanced HNSCC and may represent novel markers for disseminated disease. Innovations in molecular technology such as microcapillary array may allow more rapid and efficient screening of large numbers of samples.[39]

C. Cancer Localisation

Tumour cells expressing oncogene products, which differ qualitatively from their normal counterparts, offer situations where it may be possible to perform new tests for cancer diagnosis and imaging. These proteins in turn have the potential to act as antigen targets that specifically designed antibodies can recognize. An oncoprotein must satisfy three criteria to be of maximal use for *in vivo* studies:

a) the protein must be easily accessible by the injected antibody (i.e. it must be expressed on the cell surface)

b) the protein target should be specific to the cancer cell.

c) the target protein should be significantly over expressed relative to background levels

In HNSCC, radioimmunoscintigraphy using ^{111}indium-labelled antibodies directed against the EGFR or ^{99m}Technitium-labelled antibody against the keratinocyte-specific CD44 splice variant 6 have been evaluated in a Phase I clinical trial.[40] The techniques can be safely administered and seem to be useful in imaging patients due to high and selective tumour uptake.

II. Prognostic Applications

As our knowledge of oncogenes increases it seems apparent that some oncogene products are necessary at the earliest stages of carcinogenesis. However, other genes are turned on late in the malignant process and seem to be required to stimulate the cancer to advanced stages of malignancy. In addition, it appears that still other genes are necessary for the development of certain tumour characteristics such as angio-/lymphangiogenesis and propensity to metastases. Overexpression of EGF-R seems to be an important marker in HNSCC. Advanced clinical lesions (T4 stage) had significantly different EGFR RNA levels from those lesions of the T3 or T1/2 stages. In addition, those tumours with nodal metastases have statistically significantly EGFR overexpression levels compared with those with no evidence of nodal disease.[41] Another group has recently demonstrated in patients with oral SCCs that the expression of all four *erb*B receptors was significantly associated with shortened survival and the combination of *erb*B-2, B-3, and EGFR, but not *erb*B-4 significantly improved the predicting power.[42]

HNSCC is characterized by its capacity to invade

adjacent tissues and metastasise loco-regionally. The cooperation of multiple proteolytic enzymes which are secreted by tumour cells and/or host cells and whose substrates include extracellular matrix (ECM) components are required for cancer cells to invade the ECM and penetrate the lymphatic or blood vessel wall where they may grow and metastasise to distant sites. ECM proteolysis is also involved in the angiogenesis necessary for the continued growth of solid tumours. Recent studies demonstrated that the expression of multiple key molecules involved in HNSCC invasion, angiogenesis and metastasis including matrix metalloproteinases (MMPs) and vascular endothelial growth factors (VEGFs) are a common feature in both experimental [14,15] and clinical models of HNSCC[43,44], and that the analysis of specific MMPs and VEGFs may be useful to evaluate the malignant potential in an individual HNSCC.

III. Therapeutic Applications

(i) Anti-Oncogene Therapeutics

a) Anti-DNA/RNA Therapeutics

Theoretically, by preventing the flow of abnormal cellular information from the oncogene DNA to RNA to protein, one could inhibit the expression of the malignant phenotype. Specific anti-sense molecules could be designed to block replication or expression of oncogene DNA. Similarly, blocking molecules could be directed to prevent translation of the oncogene RNA into its abnormal onco-protein. *In vitro* experiments have shown promising results. It can be difficult, however, to regulate the amount of blocking which occurs due to *in vivo* mechanisms such as feedback regulation.

b) Anti-Oncoprotein Therapeutics

Immunotherapy is one particular application of this technology that may be a promising approach to cancer therapy. The created monoclonal antibody is able to recognize a specific tumour target such as an oncogene protein product. Anti-EGFR monoclonal antibody (mAb) has been shown to be an effective anti-proliferative agent for HNSCC via several mechanisms including induction of G1 cell cycle arrest and apoptosis, direct terminal differentiation, inhibition of the production of proteolytic enzymes[45] and angiogenic factors,[15] and enhancement of the antitumour activity of chemotherapeutic drugs and radiation therapy. Currently, Phase III clinical trial evaluation of combining anti-EGFR mAb, C225, with radiation or chemotherapy for patients with advanced HNSCC is underway. Another approach using immunotoxin is composed of an antibody or a cytokine and a conjugated molecule capable of destroying the cancer cell such as a toxin, drug or radio-nucleotide. Based on the uniform expression of interleukin-4 (IL-4) receptor on HNSCC cells, a chimeric protein composed of circular permuted IL-4 and a truncated form of a bacterial toxin called *Pseudomonas* exotoxin was produced and found to be highly and specifically cytotoxic to HNSCC cells via the induction of apoptosis.[46]

ii) Gene therapy

The aim of gene therapy is to introduce new genetic material into cancer cells that will selectively kill cancer cells with no toxicity to the neighbouring non-malignant cells. Gene therapy uses a vector to deliver a DNA sequence into cells and then the DNA incorporates itself into the cellular genome and produces proteins that have a therapeutic effect. A variety of gene therapy approaches have been described as follows.

a) Replacing or compensating for a tumour suppressor gene

Active tumour suppressor genes that are lost or altered through acquired genetic mutations can be reintroduced into tumour cells to control progression through the cell cycle. Transfection of human HNSCC cell lines with functional p53 temporarily arrested the growth of cells in tissue culture and reduced tumour growth in nude mice. A Phase I study using human adenovirus/p53 gene transfer as a surgical adjuvant in advanced head and neck cancers has been completed and revealed a survival benefit compared with that reported in chemotherapy trials.[47] The phase II clinical trial for anti-tumour efficacy is currently underway.

b) Inserting genes that produce cytotoxic substances

"Suicide" gene therapy or genetic prodrug activation therapy inserts a gene into the tumour that encodes for a protein that will convert a non-toxic prodrug into a toxic substance. In HNSCC, the herpes simplex virus thymidine kinase (HSV-tk) gene therapy has been investigated the most.[48] Tumour cells are genetically modified with HSV-tk gene and are then treated with the prodrug ganciclovir. HSV-*tk* enzyme phosphorylates ganciclovir to ganciclovir monophosphate and then triphosphate. Ganciclovir triphosphate inhibits DNA synthesis and results in apoptosis (or programmed cell death).

c) Future Directions in Gene Therapy

Genes currently under investigation include suicide gene (HSV-*tk*-gancyclovir, immune-modulatory cytokine genes (IL-2, GM-CSF, IL-12) and tumour suppressor genes (p16, p21, p53, FHIT). Delivery vehicles under investigation include viral and non-viral systems.[48] Viral vector systems include those based on adenoviruses, retroviruses, defective adenoviruses, adeno-associated virus, and herpes virus. Various techniques are being developed to enhance the viral infection efficiency and selectivity of tumour cells such as using bispecific antibodies that recognize domains of a viral vector as well as the EGFR and using a modified viral vector that recognizes the integrins of the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ class frequently overexpressed in HNSCC. Non-viral gene delivery systems include liposomes and naked DNA. Other modality is 'non-gene gene therapy' using agents to restore the DNA binding function of key growth-regulatory elements or to target pathways that affect the ability of the p53 binding protein, Mdm2, to degrade p53. Targets under investigation include the inhibition of tumour angiogenesis as this will decrease the vascular support required for tumour growth and support. A novel gene therapy approach uses an adenovirus called ONYX-015 which lacks the E1B region of the virus. The E1B region normally binds and inactivates p53 and is needed for viral replication in normal cells. The ONYX-015 lacks E1B and therefore only infects cells that lack functional p53 leading to apoptosis.[49]

The escape of malignant cells from the local host tissues, metastasis, penetration and then the successful acquisition of the distant host environment by a sequential enzyme activation is also coded by specific proto-oncogene activation and tumour suppresser gene deactivation. Overcoming the extra-cellular barriers to tumour

spread is fundamental to the definition and spread of malignancy, these spreading factors are coded and can be detected at enzymatic, cytoplasmic, m-RNA and DNA levels. It is hoped that the "profile" of spreading factors for a tumour 43 may provide an index to later prognosis and survival, and perhaps even provide therapeutic interventions with anti-metastatic factors to supplement the growing armamentarium available to the surgical oncologist.

CONCLUSION

The understanding of how the cancer cell progresses has increased substantially in the last two decades. With this increased understanding of molecular cancer genetics, our ability to apply new technologies to control and prohibit the growth of cancer is coming closer to reality.

REFERENCES

1. Weinberg R. The molecular basis of oncogenes and tumor suppressor genes. Ann N Y Acad Sci 1995;758:331-8.
2. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 1984; 224:1121-4.
3. Almoguera C, Shibata D, Forrester K, Martin J, et al. Most human carcinomas of the exocrine pancreas contain mutant c-K-rad genes. Cell 1988;53:549-54.
4. Masuda H, Miller C, Koeffler HP, Battifora H, Cline MJ. Rearrangement of the p53 gene in human osteogenic sarcoma. Proc Nat Acad Sci (USA) 1987;84:7716-9.
5. Fearon E, Hamilton S, Vogelstein B. Clonal analysis of human colorectal tumours. Science 1987;238:193-7.
6. Cheng L, Sturgis E, Eicher S, Char D, Spitz M. Glutathione-S-transferase polymorphisms and risk of squamous-cell carcinoma of the head and neck. Int J Cancer 1999;84:220-4.
7. Wang L, Sturgis E, Eicher S, Sitz M, et al. Mutagen sensitivity to benzo (a) pyrene diol epoxide and the risk of squamous cell carcinomas of the head and neck. Clin Cancer Res 1998;4:1773-8.
8. Van Dyke D, Worsham M, Benninger M, Krause C, et al. Recurrent cytogenic abnormalities in squamous cell carcinoma of the head and neck. Genes Chromosomes Cancer 1994;9:192-206.
9. Califano J, van der Riet P, Westra W, Nawroz H, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996;56:2488-92.
10. Partridge M. Current status of genetics for prediction, prognosis, and gene therapy. Curr Opinion Otolaryngol & Head Neck Surg 2000;8:69-79.
11. Cowan J, Beckett M, Weichselbaum R. Chromosome changes characterising in vitro response to radiation in human squamous cell carcinoma lines. Cancer Res 1993;53:5542-7.
12. O-charoenrat P, Rhys-Evans PH, Archer DJ, Eccles SA. The role of c-*erb* B family of receptors in head and neck squamous cell carcinomas. Oral Oncol 2002;38:627-40.
13. O-charoenrat P, Rhys-Evans P, Eccles SA. Characterization of ten newly-derived human head and neck squamous carcinoma cell lines with special reference to c-erb B proto-oncogene expression. Anticancer Res 2001;21: 1953-63.
14. O-charoenrat P, Rhys-Evans P, Court WJ, Box GM, Eccles SA. Differential modulation of proliferation, matrix metalloproteinase expression and invasion of human head and neck squamous carcinoma cells by c-*erb* B ligands. Clin Exp Metastasis 1999;17:631-9.
15. O-charoenrat P, Rhys-Evans P, Modjtahedi H, Eccles SA. Vascular endothelial growth factor family members are differentially regulated by c-*erb* B signaling in head and neck squamous carcinoma cells. Clin Exp Metastasis 2000;18:155-61.
16. O-charoenrat P, Modjtahedi H, Rhys-Evans P, Court WJ, et al. Epidermal growth factor-like ligands differentially upregulate matrix metalloproteinase-9 in head and neck squamous carcinoma cells. Cancer Res 2000;60: 1121-8.
17. O-charoenrat P, Rhys-Evans P, Eccles SA. Expression and regulation of c-*erb* B ligands in human head and neck squamous carcinoma cells. Int J Cancer 2000;88:759-65.
18. Tadokoro K, Ueda M, Ohshima T, Fujita K, et al. Activation of oncogenes in human oral cancer cells: a novel codon 13 mutation of c-H-ras-1 and concurrent amplification of c-erb-B-1 and c-myc. Oncogene 1989;4: 499-505.
19. Anderson J, Irish J, Ngan B-Y. Prevalence of Ras oncogene mutations in head and neck carcinomas. J Otolaryngol 1992;21:321-6.
20. Bova R, Quinn D, Nankervis J, Cole I, et al. Cyclin D1 and $p16^{INK4A}$ expression predict reduced survival in carcinoma of the anterior tongue. Clin Cancer Res 1999;5:2810-9.
21. Rodrigo J, Garcia L, Ramos S, Lazo P, Suarez C. *EMS1* gene amplification correlates with poor prognosis in squamous cell carcinomas of the head and neck. Clin Cancer Res 2000;6:3177-82.
22. Resto V, Caballero O, Buta M, Westra W, et al. A putative oncogenic role for MPP11 in head and neck squamous cell cancer. Cancer Res 2000;60:5529-35.
23. Nawroz H, van der Riet P, Hruban R, Koch W, et al. Allelotype of head and neck squamous cell carcinoma. Cancer Res 1994;54:1152-5.
24. Reed A, Califano J, Cairns P, Westra W, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Res 1996;56: 3630-3.
25. Boyle J, Hakim J, Kock W, Hruban R, et al. The incidence of p53 mutations increases with progression of head and neck cancer. Cancer Res 1993;53:4477-80.
26. Brennan J, Mao L, Hruban R, Boyle J, et al. Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332:429-35.
27. Homann N, Nees M, Conradt C, Dietz A, et al. Overexpression of p53 in tumor-distant epithelia of head and neck cancer patients is associated with an increased incidence of second primary carcinoma. Clin Cancer Res 2001;7:290-6.
28. Tanimoto K, Hayashi S, Tsuchiya E, Tokuchi Y, et al. Abnormalities of the FHIT gene in human oral carcinogenesis. Br J Cancer 2000;82: 838-43.
29. Nakanishi H, Wang X-L, Imai F, Kato J, et al. Localization of a novel tumor suppressor gene loci on chromosome 9p21-22 in oral cancer. Anticancer Res 1999;19:29-34.
30. Voravud N, Shin D, Ro J, JS L, et al. Increased polysomies of chromosomes 7 and 17 during head and neck multistage tumorigenesis. Cancer Res 1993;53:2874-83.
31. Mao L, Lee J, Fan Y, Ro J, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nature Med 1996;2:682-5.
32. Partridge M, Emillion G, Pateromichelakis S, A'Hern R, et al. Allelic imbalance at chromosomal loci implicated in the pathogenesis of oral precancer; cumulative loss and its relationship with progression to cancer. Oral Oncol Eur J Cancer 1997;34:77-83.
33. Califano J, Westra W, Koch W, Meininger G, et al. Unknown primary head and neck squamous cell carcinoma: molecular identification of the site of origin. J Natl Cancer Inst 1999;91:599-604.
34. Snyderman C, D'Amico F, Wagner R, Eibling D. A reappraisal of the squamous cell carcinoma antigen as a tumor marker in head and neck cancer. Arch Otolaryngol Head Neck Surg 1995;121:1294-7.
35. Yen T, Lin W, Kao C, Cheng K, Wang S. A study of a new marker, CYFRA 21-1, in squamous cell carcinoma of the head and neck, and comparison with squamous cell carcinoma antigen. Clin Otolaryngol Allied Sci 1998;23:82-6.
36. Fazekas-May M, Suen J, Yeh Y, Yeh H, Milligan L. Investigation of urinary transforming growth factor alpha levels as tumor markers in patients with advanced squamous cell carcinoma of the head and neck. Head Neck 1990;12:411-6.
37. Lo Y, Chan L, Lo K, Leung S, et al. Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. Cancer Res 1999;59:1188-91.
38. Capone R, Pai S, Koch W, Gillison M, et al. Detection and quantitation of human papillomavirus (HPV) DNA in the sera of patients with HPV-associated head and neck squamous cell carcinoma. Clin Cancer Res 2000;6:4171-5.
39. O-charoenrat P, Rusch V, Talbot S, Sarkaria I, et al. Casein kinase II alpha subunit and C1-inhibitor are independent predictors of outcome in patients with squamous cell carcinoma of the lung. Clin Cancer Res 2004;10:5792-803.
40. Stroomer J, Roos J, Sproll M, Quak J, et al. Safety and biodistribution of ^{99m} Technitium-labeled anti-CD44v6 monoclonal antibody BIWA 1 in head and neck cancer patients. Clin Cancer Res 2000;6:3046-55.
41. O-charoenrat P, Rhys-Evans PH, Archer DJ, Eccles SA. C-erbB receptors in squamous cell carcinomas of the head and neck: clinical significance and correlation with matrix metalloproteinases and vascular endothelial growth factors. Oral Oncol 2002;38:73-80.
42. Xia W, Lau Y-K, Zhang H-Z, Xiao F-Y, et al. Combination of EGFR, HER-2/neu, and HER-3 is a stronger predictor for the outcome of oral squamous cell carcinoma than any individual family members. Clin Cancer Res 1999;5:4164-74.
43. O-charoenrat P, Rhys-Evans P, Eccles SA. Expression of matrix metalloproteinases and their inhibitors correlates with invasion and metastasis in squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 2001;127:813-20.
44. O-charoenrat P, Rhys-Evans P, Eccles SA. Expression of vascular endothelial growth factor family members in head and neck squamous cell carcinoma correlates with lymph node metastases. Cancer 2001;92:556-68.
45. O-charoenrat P, Rhys-Evans P, Modjtahedi H, Court W, et al. Over-expression of epidermal growth factor receptor in human head and neck squamous carcinoma cell lines correlates with matrix metalloproteinase-9 expression and in vitro invasion. Int J Cancer 2000;86:307-17.
46. Kawakami K, Leland P, Puri R. Structure, function, and targeting of interleukin 4 receptors on human head and neck cancer cells. Cancer Res 2000;60:2981-7.

47. Clayman G, Frank D, Bruso P, Goepfert H. Adenovirus-mediated wild-type p53 gene transfer as a surgical adjuvant in advanced head and neck cancers. Clin Cancer Res 1999;5:1715-22.
48. Yu Z, Chan MK, O-charoenrat P, Eisenberg DP, et al. Enhanced nectin-1 expression and herpes oncolytic sensitivity in highly migratory and invasive carcinoma. Clin Cancer Res 2005;11:4889-97.
49. Heise C, Sampson-Johannes A, Williams A, McCormick F, et al. ONYX-015, an E1B gene-attenuated adenovirus, causes cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nature Med 1997;3:639-45.