Molecular etiopathogenesis of esophageal cancers

Ruggero MONTESSANO (a), Monica HOLLSTEIN (b) and Pierre HAINAUT (a)

(a) Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, Lyon, France
(b) Division of Toxicology and Cancer Risk Factors, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Summary. - The occurrence and the relevance in squamous cell carcinomas (SCC) and Barrett's adenocarcinomas (ADC) of genetic alterations, namely mutations in the p53 gene, allelic loss at various chromosomal loci and altered expression of genes involved in the regulation of cell proliferation, are described and discussed with reference to the etiology and pathogenesis of esophageal cancers. Mutations in the p53 gene occur in both SCC and ADC with a frequency of up to ~ 80%, although with a strikingly different pattern of mutations. In ADC, a very high frequency of G>A transitions at CpG dinucleotides was observed, whereas in SCC, mutations at A>T base pairs were comparatively high. In addition, in SCC, the frequency of p53 mutations was related to tobacco smoking. These data therefore indicated that such mutation analysis could provide valuable insight into the etiology of this cancer. It is also apparent that the genetic alterations involving genes other than p53 are also present in the natural history of esophageal cancer. The significance of these genetic changes as well as alterations of expression of cell cycle regulatory genes in esophageal carcinogenesis is briefly discussed.

Key words: esophagus, cancer, p53 gene, genetic alterations.

Introduction

Cancer of the esophagus (squamous cell carcinoma and adenocarcinoma) is among the ten most frequent cancers in the world (over 300,000 new cases/year) with a predominance of cases occurring in developing countries [1] and with dramatic variations in incidence rates between regions of the world [2]. The mortality rates are very similar to the incidence rates [3] due to the relatively late stage of diagnosis of this cancer and the poor efficacy of treatment; the relative survival of these patients at 5 years is less than 10% [4]. Epidemiological studies have greatly contributed to the identification of tobacco smoking, alcohol drinking, betel chewing and some dietary habits as major risk factors in squamous cell carcinomas (SCC) [5]. Tobacco and alcohol are major risk factors in Europe and North America but not in high-risk areas like Lintian (China) and north-east Iran [2], where dietary habits seem more relevant. Papilloma viruses have been detected in specimen of esophageal cancers, but epidemiological and experimental evidence for their role in human esophageal carcinogenesis is still unclear [6].

Adenocarcinoma of the esophagus develops from Barrett's esophagus, a condition in which normal squamous epithelium is replaced by metaplastic columnar epithelium [7]. This condition is the result of a prolonged gastro-esophageal reflux and these patients have more that 100 fold higher risk than the general population of developing esophageal carcinoma [8], a cancer that accounts now for approximately 50% of all esophageal cancer in the USA [9].

In both types of esophageal cancers the role of genetic/familial aggregation as well as the mechanisms underlying the interaction between the various risk factors in a given high risk population are still ill-defined.
Our understanding of the molecular and genetic basis of cancer development has progressed considerably in the last decade and the integration of this knowledge with epidemiology, pathology and clinical disciplines can yield great benefits in studies of etiopathogenesis and in the improvement of the efficacy of screening and therapy of esophageal cancer.

This review will focus on the occurrence of genetic alterations in squamous cell and adenocarcinoma of the esophagus and in the role of these genetic changes, particularly those concerning the p53 tumor suppressor gene, in the elucidation of etiology and pathogenesis of this cancer, that could have a direct bearing on the implementation of primary and secondary preventive measures.

**p53 mutation spectrum in cancer of the esophagus**

**Involvement of p53 in human cancer**

Point mutations in the p53 gene are among the most common genetic abnormalities documented in human cancers [10]. The p53 gene, located on chromosome 17p13, encodes a Mr 53000 phosphoprotein that possesses sequence-specific DNA binding properties. In normal cells, the protein is continuously synthesized but does not accumulate to significant levels due to its very rapid turnover (2 to 15 min). However, when cells are exposed to DNA-damaging agents, the p53 protein is stabilized and becomes capable of transactivating several target genes encoding effectors of cell-cycle arrest in G1 and of apoptosis [11-13]. Thus, p53 is thought to act as a "genomic policeman" to prevent DNA replication after genomic damage, either by arresting cells in G1 and allowing time for proper DNA repair, or by inducing the apoptotic suicide of cells that contain DNA damaged beyond repair. Mutations commonly found in cancer primarily affect conserved regions within the DNA-binding domain of p53 and are thought to act by preventing the molecule from transactivating its normal genomic targets [14]. This "loss of function" phenotype may enhance the genomic instability of cancer cells and favor the rapid accumulation of multiple genetic alterations that characterizes the progression towards full neoplasia.

In addition to loss-of-function, there is growing evidence suggesting that some p53 mutations may also confer an oncogenic, gain-of-function phenotype. First, transfection of several p53 mutants enhance the tumorigenic potential of p53-null cells. Secondly, some mutants affect the transcriptional regulation of genes distinct from those controlled by wild-type p53, including the HIV-1 regulatory gene TAT-1 and the multidrug resistance gene MDR-1. Thirdly, many mutants are actually expressed to high levels in cancer tissues, suggesting this sustained expression is selectively retained by cancer cells [15]. However, the precise mechanisms by which p53 exerts these tumor promoter properties is not known. Understanding these mechanisms may be of crucial importance for the correct interpretation of the histochemically-detectable accumulation of p53 in many cancer tissues.

**Prevalence of p53 mutations in cancer of the esophagus**

A number of studies have analyzed the occurrence of p53 mutations in esophageal cancers [16-29]. Among 240 cases of squamous cell carcinomas of the esophagus (SCC) analyzed in the literature, 110 (45.8%) have been found to contain mutations that have been confirmed and identified by sequencing. The prevalence of p53 mutations is even higher for adenocarcinomas (ADC), with 46 cases of mutant p53 identified in 64 patients screened (71.8%). In most instances, the methodology used is based on PCR-amplification of exons 5 to 8 of the p53 gene, which encompasses the sequences encoding the whole DNA-binding domain of the molecule and their flanking splicing sites. Among 32 SCC samples where all p53 exons (1 to 11) have been analyzed by sequencing, 14 mutations were found in exons 5 to 8 (43.8%), versus none in exons 1 to 4 and only one in exons 9-11 (codon 342, exon 11) [18]. Thus, mutations outside exons 5 to 8 appear to be rather uncommon occurrence in esophageal cancers.

Mutations in p53 do not appear to correlate significantly with tumor grade. However, p53 mutations have been observed in hyperplastic and dysplastic squamous epithelium [16, 24], as well as in dysplastic Barrett's mucosa adjacent to adenocarcinoma [21, 26]. These data indicate that, in contrast with colon or breast cancers, p53 mutation can be an early event in the genesis of both squamous cell carcinoma and adenocarcinoma of the esophagus.

**Distribution and impact of p53 mutations in SCC and ADC**

A survey of the localization of p53 mutations demonstrates striking differences between squamous cell carcinoma and adenocarcinoma of the esophagus (Figs 1a and 1b). In adenocarcinoma, mutations are distributed throughout exons 5-8, with "hot-spots" at codons 175 (exon 5), 248 and 273 (exon 8), representing, respectively, 9%, 16% and 16% of all point mutations in adenocarcinomas. These three codons are the most frequently mutated in all cancers and represent 6.1%, 9.6% and 8.8%, respectively, of the 4,496 mutations recorded in the p53 mutation database. In contrast, no mutation is found at codon 249, another classical "hotspot" that represents 5.6% of p53 mutations in all cancers.
About 70% of the mutations found in cancer affect residues involved in the structure of the DNA-binding surface, including residues directly in contact with DNA (30%). In contrast, mutations in the core domain are relatively rare (6% of all mutations). In SCC of the esophagus, mutations in DNA-binding residues represent only 8%, whereas 30% of the mutations occur at sites encoding the hydrophobic core. Such mutations are expected to be highly disruptive and may lead to the unmasking of protein domains that are normally buried within the tertiary structure of the wild-type molecule. In ADC, p53 mutations occur primarily at residues of the DNA-binding surface. Further studies are needed to determine whether the p53 mutations found in SCC reflect the selection of mutant proteins with defined functional properties.

Spectrum of p53 mutations in SCC and ADC

Mutations of p53 can result from either endogenous biological processes or from DNA attack by exogenous, environmental carcinogens. The nature of p53 mutations in any particular cancer type is indicative of the biochemical mechanisms responsible for the DNA lesions that cause cancer. Thus, analyzing the spectrum of p53 mutation may help to identify particular carcinogens involved in the etiopathogenesis of esophageal cancer [10].

In all cancers, about 25% of p53 mutations are transitions occurring at CpG dinucleotides, a type of lesion which is thought to occur mostly by deamination of 5-methylcytosine generating thymidine (C to T mutations). Such mutations are likely to result from spontaneous, endogenous processes and their fixation in the genome probably reflects the lack of proper DNA repair. In contrast, other types of mutations, such as G:C to T:A transversions, transitions at G:C bases pairs (not at CpG dinucleotides) or mutations at A:T base pairs, are relatively frequent in populations exposed to carcinogens. For example benzo(a)pyrene, a carcinogen present in tobacco smoke, reacts preferentially with guanine to generate G to T transversions, and mutations of this type account for 36% of all mutations found in lung cancers suggesting that the high frequency of this mutation in lung tumors is attributable to smoking. Other examples are tandem transitions at C:G bases pairs, a typical molecular signature of exposure to ultraviolet light that is common in both squamous and basal cell carcinoma of the skin; or mutations at codon 249 in hepatocellular carcinomas associated with aflatoxin B1 exposure [10, 31-33].

SCC and ADC of the esophagus differ strikingly in the pattern of mutations in p53 (Fig. 2). p53 mutations in ADC show a very high frequency of transition at CpG
dinucleotides (63%). To date, this is the highest level of CpG transition found in any cancer type (other cancer types with frequent CpG transitions are colon carcinoma and pancreatic cancer, with, respectively, 45.8 and 36%). G:C to T:A transversions and mutations at A:T base pairs are comparatively rare (taken together, 14%). In contrast, in SCC CpG transitions are rarer than is most tumor types (18%), and mutations at A:T base pairs account for 31% of all mutations. The spectrum of p53 mutations in SCC is indicative of the involvement of exogenous carcinogens, in agreement with epidemiological data supporting the role of environmental agents, in particular tobacco and alcohol. The high frequency of mutations at A:T base pairs may reflect depurination of DNA and/or exposure to chemical carcinogens such as acetaldehyde, a metabolite of ethanol. The pattern of p53 mutations in ADC is more difficult to interpret. Transitions at CpG dinucleotides are generally considered as the hallmark of mutations occurring spontaneously by hydrolytic deamination of 5-methylcytosine. Recent evidence [34, 35] also indicates that the mutability of CpG dinucleotides may also result from enzymatic deamination and methylation by methyltransferases, which bind with high affinity to the premutagenic DNA mismatches G:U and G:T and prevent efficient DNA repair. Further studies are needed to identify the respective role of these mechanisms in the genesis of p53 mutations in ADC.

p53 mutations in SCC in relation to exposure to tobacco and alcohol

In many areas of the world, tobacco and alcohol have been identified as major factors in the etiopathogenesis of squamous cell carcinoma of the esophagus. The literature to date reports 91 SCC patients for whom data on exposure to tobacco and/or alcohol are available. The distribution of p53 mutations among these patients reveals a strong relationship with tobacco smoking (Fig. 3). Only 20% of non-smoker SCC patients have p53 mutations, in contrast with 80% in patients who smoke more than 20 cigarettes/day. Even light smoking (less than 20 cigarettes/day) results in an increase in p53 mutation frequency to 50%. The relationship with alcohol consumption is less clear, since p53 mutations are found in 32% of non-drinkers, 51% in patients reporting a consumption equivalent to less than 1 liter of wine/day and 58% of in heavy drinkers. However, the respective role of each risk factor is difficult to assess since most of the patients with p53 mutations were exposed to both tobacco and alcohol. These data further indicate that both risk factors cooperate in the etiopathogenesis of squamous cell carcinoma of the esophagus. Similar findings have been reported in head and neck squamous cell carcinoma [36]. At present, the data available on p53 mutations in ADC in relation with defined risk of exposure are too limited to be informative.

Fig. 2. - Spectrum of p53 mutations in in squamous cell carcinomas and adenocarcinoma (Fig. 1B) of the esophagus. Data (from the p53 mutation database [10]) are expressed in percent of the total number of mutations found in SCC or in ADC.

Fig. 3. - Incidence of p53 mutations in SCC patients in relation to tobacco and alcohol consumption. 95 SCC patients for whom quantitative data on tobacco and alcohol consumption were available have been used in this study. Patients have been separated in three groups according to tobacco consumption, including non-smokers, moderate smokers (less than 20 cigarettes/day) and heavy smokers (more than 20 cigarettes/day). In each group, the percentage of patients with p53 mutations has been calculated (white histograms). The grey histograms in the background illustrates the proportion of patients in each group who is also reported as having a moderate or high alcohol consumption. The data on tobacco and alcohol consumption are based on patient’s interview at the time of diagnosis.

Allelic loss and mutations in esophageal cancers

Epidemiological studies have shown that the development of a clinically detectable tumor is the result of a series of events (at least two) that occur over a period of many years [37] and observations in humans and in experimental animals show that the development of a neoplasm is the result of the acquisition and accumulation of genetic alterations in the stem cell pool of the esophagus. The median age of death from esophageal cancer is 80 years. At least 40% of patients have a family history of esophageal cancer. Consistent with this pattern, there is a clear association between p53 mutations and the development of esophageal cancer. The p53 gene is located on chromosome 17 and is one of the genes that is most frequently altered in human cancer. The p53 protein is a transcription factor that regulates the expression of genes involved in cell cycle control, DNA repair, and apoptosis. Mutations in the p53 gene can lead to loss of function, which can result in the development of cancer. The p53 protein can also be lost due to epigenetic changes, such as DNA methylation, which can silence gene expression. These changes can also lead to the development of cancer.
population and it is also apparent that the temporal sequence of these genetic changes differs in the different target tissues.

Measurements of loss of heterozygosity (LOH) has proven to be effective as an indicator of tumor-specific genetic loss defining regions of the genome or genes relevant in tumor pathogenesis (and possibly of the occurrence of mutations in these genes) [41]. This approach has been a key element in the elucidation of the tumor-suppressor function of retinoblastoma and p53 genes localized on chromosomes 13p14 and 17p13 [42-44].

Table 1 shows the allelic losses in esophageal cancers (squamous cell and adenocarcinomas) that occur with high frequency (> 40% of informative cases) based on multiple and reproducible studies; in the table the putative candidate genes localized in the area of allelic loss and the occurrence of mutations in these genes are also indicated.

No significant differences were detected in the prevalence of allelic losses at the various loci in the two types of esophageal cancer, squamous cell and adenocarcinoma. Allelic loss of 17p occurs very frequently in esophageal cancer, as in many human solid tumors and is associated with the presence of mutations in the p53 tumor-suppressor gene localized in the 17p13 locus. In colorectal cancers [45] and in non-small cell carcinomas of the lung [46], the loss of an allele involving this locus is also often associated with the occurrence of p53 point mutations in the remaining allele, resulting in the inactivation of the tumor-suppressive activity of this gene. In these two tumor types the prevalence of p53 mutations was low (0-17%) in tumors with two 17p alleles, but it increased significantly (75-86%) in tumors with one 17p allele. These observations and others [39] indicate that these two events (p53 mutation and loss of normal 17p) take place close in time and relatively late in the overall development of these tumors. The pattern seems different in malignant gliomas, where in a large proportion of tumors the prevalence of p53 mutations in tumors retaining two 17p alleles is higher (33%), indicating that p53 mutations occur before and independent of, deletion of the 17p locus [47]. In esophageal cancer, similarly to what is observed in malignant gliomas, the available data [22, 48] indicate a high prevalence of p53 mutations in tumors with two 17p alleles. This evidence suggests that p53 mutation is an early event in esophageal carcinogenesis and that the presence of such mutations favors the occurrence of 17p deletion in the other allele. It should be noted, however,

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>Tumors with allelic loss/informative tumors (%)</th>
<th>Minimal area of loss</th>
<th>Candidate genes</th>
<th>Mutations (*)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3p</td>
<td>25/52 (40-64)</td>
<td>3p21.3</td>
<td>hMLH-1</td>
<td>Very rare</td>
<td>[89, 55]</td>
</tr>
<tr>
<td>5q</td>
<td>50/94 (36-80)</td>
<td>5q21.2</td>
<td>APC/MCC</td>
<td></td>
<td>[55, 48, 53, 56, 49]</td>
</tr>
<tr>
<td>9p</td>
<td>55/95 (45-65)</td>
<td>8/17 (47)</td>
<td>MTS-1, IFNA</td>
<td>0 &lt;= 40%</td>
<td>[89, 56, 59]</td>
</tr>
<tr>
<td>9q</td>
<td>11/18 (60)</td>
<td>9q31</td>
<td>ESS1</td>
<td></td>
<td>[89]</td>
</tr>
<tr>
<td>13q</td>
<td>66/134 (41-54)</td>
<td>13q14.1</td>
<td>Rb</td>
<td>No mutations</td>
<td>[48, 55, 22, 90, 56, 49]</td>
</tr>
<tr>
<td>17p</td>
<td>65/124 (43-65)</td>
<td>17p13</td>
<td>p53</td>
<td>High</td>
<td>[89, 48, 55, 22, 49, 91, 92]</td>
</tr>
<tr>
<td>17q</td>
<td>56/91 (62)</td>
<td>17q21.3</td>
<td>BRCA1, erbB2, CSF-3, NF-1, ITB4</td>
<td></td>
<td>[93]</td>
</tr>
<tr>
<td>18q</td>
<td>28/79 (23-46)</td>
<td>18q23.3</td>
<td>DCC</td>
<td>Rare</td>
<td>[55, 94, 49]</td>
</tr>
</tbody>
</table>

(*) See also text for appropriate references.
that in a number of cases 17p allelic loss occurs in the absence of p53 mutations, pointing to the possible presence in this chromosomal region of a tumor-suppressor gene(s) other than p53.

Allelic loss also occurs with high prevalence (36-75%) in 13q, 5q, and (possibly), to a lesser extent, 18q chromosomes, and in regions of these chromosomes where the Rb, APC/MCC and DCC genes are located. Coexistence of allelic loss at 17p, 13q and 18q has been reported in esophageal cancers and it appears that these allelic losses precede MCC-5q allelic loss [22, 48, 49]. In esophageal cancer, as observed in colorectal [50,51] and lung small cell [52] carcinomas, there is no apparent association between allelic loss at APC and MCC loci even though these two genes are closely linked in this region of the 5q chromosome [49, 53].

The picture emerging from these data is that, in contrast to p53, APC/MCC or DCC genes may not be principal targets for the loss of the heterozygosity occurring on 5q and 18q. No mutations were detected in the Rb gene in tumors with or without 13q allelic loss [48], although absence of Rb protein is found in a significant fraction of esophageal SCC [54]; similarly, mutations in the APC gene were very rarely detected (2 among 163 tumors) [55-57]. In contrast APC mutations and not 5q allelic loss were the most common genetic alterations in colorectal cancer [58]. In the case of the DCC gene mutations were detected in only 2 out of 51 esophageal cancers examined and allelic loss at the 18q21.3 locus specific for the DCC gene occurred at a prevalence of only 23% [55]. It is reasonable to assume that other genes with tumor-suppressor activity are located in these chromosomal regions.

Another frequent allelic loss in either squamous cell or adenocarcinoma of the esophagus involves chromosome 9p21-22 [59], the region in which the gene MTS-1 [60] is located. This gene encodes the p16 protein, a specific inhibitor of cell cycle G1->S phase progression, that when mutated loses this function [61] (see Alterations of cell-cycle regulatory proteins). However, it is still unclear for this cancer as well as other cancers [62] whether MTS-1 gene alone or other tumor-suppressor gene(s) present in the same chromosomal region are the target of the allelic deletion. Homozygous deletions involving MTS-1 sequences appear to be rare [63] in primary esophageal tumors. The data available indicate that the prevalence of MTS-1 gene deletions does not account for the frequency of 9p21-22 allelic loss observed in esophageal cancers, and this points to the presence in this chromosomal region of multiple oncogene tumor-suppressor genes relevant to esophageal carcinogenesis. A similar pattern is present in primary squamous cell carcinoma of the head and neck, a cancer with similar etiology and molecular pathogenesis [64].

Data are also available on allelic losses occurring in 3p21.3, 9q31 and 17q21.3 regions in which genes of relevance to cancer development are known to be located. hMLH-1, a human mismatch repair gene linked to cancer susceptibility in hereditary nonpolyposis colorectal cancer, is located on chromosome 3p21 [65]; ESS1, a gene associated with the autosomal dominant disorders, multiple self-healing squamous epitheliomata and Gorlin syndrome, mapped in chromosome 9q22-31 [66, 67] and BCRA1, a gene associated with susceptibility to breast and ovarian cancer is located on 17q21.

Alterations of genes encoding proteins that signal or control cell-cycle progression

As in many human cancers, alterations of genes encoding proteins involved in growth signaling and in cell-cycle control are common in cancer of the esophagus. These genes include the EGF receptor (HER-1 or EGFR) and its close homologue HER-2 (also named c-erbB-2) (amplifications), int-2 and hst-1 (amplifications), c-myc (amplifications), p16/MTS-1 (deletions and mutations), cyclin D1 (amplifications and/or overexpressions), Rb (deletions) and p53 (mutations and deletions). The significance of p53 alterations is discussed in another paragraph (see p53 mutation spectrum in cancer of the esophagus). Functionally, these genes may be classified into two categories: growth signal transduction genes (EGFR, int-2, hst-1 and c-myc), and cell-cycle control genes (p16/MTS-1, cyclin D1 and Rb).

Alterations of growth signal transduction genes

Alterations in the HER-1 (7p12-13) and HER-2 (17q21) genes have been found in many tumor types, as for example breast and ovarian cancers, gastric cancer and brain tumors. These alterations include mostly amplifications and/or overexpression, and more rarely gene rearrangements [68, 69]. Both genes encode transmembrane receptors with intrinsic tyrosine kinase activities. The HER-1 protein binds EGF and TGFα, whereas the identity of HER-2 ligands is not fully elucidated. In both SCC and ADC of the esophagus, amplification, but not rearrangement, of HER-1 and of HER-2 have been detected in 15-30% of the cases [70-73]. Amplification is not always correlated with overexpression, and the number of tumors also overexpress HER-1 protein without evidence of gene amplification. Interestingly, HER-1 overexpression has been found to be significantly correlated with p53 mutation [28].

Increased expression of HER-2 protein has been observed in Barrett’s mucosa, in adenocarcinoma and in tumors of the cardia, but there is no significant association
between levels of expression and grade, histology or prognosis of the tumor [70]. It is important to note that the q21 region on chromosome 17 also contains the genes encoding retinoic acid receptor and topoisomerase IIIa. In breast cancers, both of these genes are co-amplified in 50% of the cases with HER-2 amplification. Thus, the amplicon on chromosome 17q may contain more than one gene relevant to the pathogenesis of esophageal cancer [74].

The int-2 and hst-1 genes both encode growth factors of the fibroblast growth factor (FGF) family and are also known as FGF-3 and FGF-4, respectively. Both genes are located 35 kb apart on chromosome 11q13 and are frequently co-amplified in various tumor types, including breast, lung and stomach cancer. Hst-1 has originally been isolated as an oncogene in gastric cancer and its activation may be relevant to the neoplastic transformation of the aerodigestive epithelium (see review in Yoshida et al. [71]). In esophageal cancers, co-amplification of int-2 and hst-1 has been observed in 30 to 50% of primary tumors and appears to be correlated with a higher incidence of eventual distal metastasis [75-77]. However, the correlation between 11q13 amplification and overexpression of hst-1 and/or int-2 is unclear. Indeed, the 11q13 amplicon contains a third gene, Cyclin D1 gene, which plays an essential role in cell-cycle progression in G1 and is often over-expressed in esophageal cancers (see Alterations of cell-cycle regulatory proteins).

A remarkable feature of esophageal neoplasms is the absence of mutations in members of the ras gene family [72, 78]. This is in contrast with a number of other cancers in which ras genes are frequently altered by point mutations at specific codons, in particular adenocarcinoma of the lung (30%) and the colon (40%) and pancreatic tumors (70%). In adenocarcinomas of the lung, missense mutations in K-ras often co-exist with mutations in p53. The mutations in both genes are predominantly G:C to T:A transversions, a typical molecular signature of tobacco carcinogens [34]. Why tobacco carcinogens do not target ras genes in esophageal cells is unknown. Interestingly, experimental esophageal tumors induced in the rat by N-nitroso compounds show a high prevalence of mutations in H-ras [79].

The c-myc gene is located on chromosome 8q24 and is amplified in 14 to 25% of esophageal cancers [28, 73]. In 23% of a group of patients from a high-risk area of China, c-myc amplification has also been detected in the normal mucosa adjacent to the tumor [28]. C-myc encodes a nuclear protein which is thought to regulate the transcription of other genes important for cell growth. Activation of the c-myc gene occurs in a variety of human tumors and may contribute to both early and late stages of tumors progression [80].

### Alterations of cell-cycle regulatory proteins

Cell-cycle progression is regulated by the sequential activation of cyclin-dependent kinases (CDK) and their association with specific regulatory subunits, the cyclins. The key cyclin involved in progression from G1 to S is cyclin D1 (Fig. 4). Genetic alterations leading to the constitutive activation of the CDK/cyclin D1 pathway appear to be common in squamous cell carcinoma of the esophagus. These alterations can occur at distinct levels within the pathway, including: a) inactivation of p16/MTS-1 (on chromosome 9p31) by diverse mechanisms, in particular deletion and missense mutation, b) amplification and overexpression of cyclin D1 (on chromosome 11q13) and c) deletion and inactivation of growth control

![Fig. 4 - Interplay between cyclin D1, cyclin-dependent kinases (CDK), cyclin kinases inhibitors (p16/MTS-1 and p21), and retinoblastoma susceptibility gene (Rb) in the regulation of cell cycle progression from G1 to S. Cell cycle progression from G1 to S requires, among others, activation of specific CDKs (CDK4 and CDK6) in association with cyclin D1. The active CDK/cyclin D1 complex phosphorylates sequentially the Rb protein, thus releasing Rb-bound transcription factors of the E2F family. Free E2Fs transactivate genes that are essential for entry into S and DNA replication (S-phase effectors). CDK/cyclin D1 activity is negatively regulated by binding of several cyclin kinases inhibitors, including p16/MTS-1 and p21^{Ntv-1}. p16/MTS-1 is thought to be activated in response to growth control stimuli through pathways which are not fully elucidated. p21^{Ntv-1} is activated in a p53-dependent manner in response to genotoxic stress. Thus, there are at least two distinct pathways for suppression CDK/cyclin D1 activity. Molecules shaded are those which are frequently altered at the genetic level in cancers of the esophagus.](image-url)
the retinoblastoma susceptibility gene \textit{Rb} (on chromosome 13q14). Amplification and overexpression of cyclin D1 is the most common of these genetic events [54, 81, 82].

A study of 50 esophageal tumors from various geographical origin, [54] found amplification and overexpression of cyclin D1 in 32% of the tumors, and loss of Rb protein expression in 17%. The tumors that had cyclin D1 alteration had normal levels of Rb expression, while those that did not express Rb did not show amplification or overexpression of cyclin D1.

The exact involvement of cyclin-dependent kinase inhibitors (CDKis) aberrations in esophageal cancer is still unclear. Several studies have described missense mutations of 16/MTS-1 but reported frequencies that widely differ from one publication to another [69, 83-85]. Recent evidence suggest that the prevalence of p16/MTS-1 mutations is in fact quite low (10%) and that mutations occur both in exon 1 and 2 of the gene [63]. Homozygous deletions of p16/MTS-1, which are very frequently observed in cell lines, are rare in primary tumor samples but the frequency of heterozygous deletions is not precisely known [69].

In other tumors, recent evidence suggests that loss of p16 activity could also result from aberrant or decreased p16/MTS-1 gene expression. The p16/MTS-1 locus has a complex structure and encodes two transcripts with distinct protein coding potential that are differently regulated during cell cycle. Derepression of the activity may thus result from an imbalance between the levels of expression of the two transcripts [86]. Moreover, p16/MTS-1 gene expression is frequently decreased in nasopharyngeal cancers and it is possible that down regulation of the protein rather than point mutation of the gene may be a common mechanism of p16/MTS-1 inactivation [87]. Whether aberrant p16/MTS-1 expression also occur in cancer of the esophagus remain to be determined.

Aberrations of p16/MTS-1, cyclin D1 and Rb all appear to have similar functional consequences: they contribute to inactivate the suppressor function of Rb and to promote the activation of E2F proteins by direct or indirect mechanisms (Fig. 4) [88]. Thus, distinct tumors may use different molecular mechanisms to disrupt a common regulatory pathway and evolve towards an identical tumor phenotype. This model provides a paradigm explaining why tumors of the same histologic type show heterogeneity at the genetic level.

Interestingly, there is no clear correlation between alterations in the CDK/cyclin D1 pathway and \textit{p53} mutation. This suggests that the contribution of \textit{p53} mutation to the carcinogenic process involves the deregulation of other pathways in addition to the \textit{p21}*-dependent regulation of CDK/cyclin D1 (Fig. 4). These other pathways may include the regulation of DNA repair and apoptotic processes, two biological processes in which wild-type \textit{p53} plays a critical role.

Taken together, genetic aberrations in pathways that control G1/S progression are a very frequent event in squamous cell carcinoma of the esophagus. This high frequency may reflect a functional requirement to abrogate normal G1/S control to allow cells to progress towards malignancy. Indeed, the homeostasis of the esophageal mucosa is dependent upon a very delicate balance between cell renewal, differentiation and death. By switching-off the molecular controls integrating these three processes, genetic alterations in G1/S may compromise the normal life cycle of mucosal cells and allow them to escape growth control, an essential step towards the rapid acquisition of further genetic lesions during esophageal carcinogenesis.

\textbf{Conclusions}

The analysis of \textit{p53} mutations in esophageal cancers reveals that ADC and SCC are very distinct pathologies at the molecular level. Indeed, \textit{p53} mutations found in these tumors differ by their frequency, their nature, their distribution and their impact on the protein structure. Such differences reflect not only the involvement of distinct epidemiological risk factors but also the existence of distinct, tissue-specific pathways of progression towards neoplasia. In SCC, exposure to both tobacco and alcohol appear to be a source of \textit{p53} mutations. However, it is interesting to note that the mutation spectrum of SCC significantly differs from that of other tobacco-related cancers such as lung carcinoma, suggesting that the nature, the metabolism of the tobacco carcinogen(s) involved and/or the interaction with other risk factors is not the same. In ADC, the very high frequency of mutations at CpG dinucleotide suggest that \textit{p53} mutations may result from a complex interplay between exogenous risk factors and endogenous biological processes. Similar mechanisms may be at work in the genesis of adenocarcinomas of the cardia, but the number of cases reported at present in the literature [7] is too small to draw significant conclusions.

Beyond these differences, SCC and ADC of the esophagus present similar molecular features that are likely to be essential for the understanding of the etiopathogenesis of both diseases. Mutations in \textit{p53} are frequent and occur early during progression towards neoplasia, suggesting that the \textit{p53} protein must probably play a crucial role in the homeostasis of normal esophageal mucosa. In a manner similar to buccal and airway mucosa, esophageal mucosa is extremely exposed to environmental risk factors and its genetic homeostasis may require constant policing through activation of \textit{p53}-dependent pathways leading to transient cell-cycle arrest or to apoptosis. In this context, cells that have lost \textit{p53} suppressor function through mutation may have acquired an immediate selective advantage since they would be
able to proliferate in conditions where normal cells are growth-suppressed. This may give rise to populations of actively growing cells which are at high risk of becoming the precursor of a neoplastic lesion. In light of this model, the analysis of both p53 protein expression and p53 gene mutation in very early lesions of the esophagus may prove a useful method to identify those which are at high risk of neoplastic transformation, in particular among tobacco smokers. In addition, it is evident that other genetic alterations are involved in esophageal carcinogenesis. The elucidation of their temporal occurrence may have a considerable impact in the assessment of the efficacy of screening procedures or of chemopreventive interventions.

Acknowledgements

We gratefully acknowledge the financial support received from the Commission of the European Communities (grant no. EVSV-C152-0119). We would like to thank Magali Maillet for her secretarial assistance.

Submitted on invitation.
Accepted on 30 November 1995.

REFERENCES


