p53 Overexpression in Squamous Cell Carcinoma of the Esophagus

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Background: Coastal South Carolina has a high incidence of squamous cell carcinoma of the esophagus (SCCE) among black residents. Overexpression and mutations of the p53 tumor suppressor gene have been noted in SCCE from other high-incidence regions. The purpose of this study was to determine the frequency of p53 overexpression in this region both in patients with SCCE and in normal subjects.

Methods: Normal and malignant tissue obtained at esophagoscopy and normal esophageal mucosa (NEM) from random autopsies were studied with monoclonal antibodies to the p53 gene product. Total cellular RNA was extracted from SCCE, reverse transcribed to complementary DNA, and a portion of the p53 gene was amplified via polymerase chain reaction and sequenced.

Results: Immunohistochemical studies on SCCE from nine patients showed that six (67%) were positive, two (22%) were negative, and one was indeterminate for p53 overexpression. The corresponding normal samples showed that three (33%) had p53-positive cells in the basal epithelial layer, whereas six did not. NEM from 18 random forensic cases displayed p53 overexpression in seven (39%). Eight of the nine tumors had p53 mutations.

Conclusions: p53 overexpression and mutations are frequently found in SCCE from patients in coastal South Carolina. Overexpression in normal epithelium from random autopsy cases may indicate an inherited or acquired predisposition in this geographic region.

Key Words: p53—Esophageal cancer—Tumor suppressor gene—Risk factors.
prolonged half-life (5,6). Thus, immunohistochemical staining makes it possible to screen for the prevalence of mutations in both normal and malignant tissue (7).

The goal of this study was to use immunohistochemical techniques to determine the frequency of $p53$ mutations in normal and malignant esophageal tissue from patients with SCCE. Samples of normal esophageal mucosa (NEM) from random forensic cases also were examined in order to determine whether there is evidence of alterations in $p53$ expression in the local population. These data may provide insight into why certain populations in the low country are particularly prone to this deadly disease.

**MATERIALS AND METHODS**

**Tissue and Cell Lines**

Samples of SCCE and adjacent NEM were obtained at esophagoscopy and frozen in liquid nitrogen. These specimens were available because they were in excess of that which was needed for routine histologic evaluation. Portions of normal esophagus also were obtained from forensic cases in which frozen tissue could be obtained within 4 h postmortem.

The tissue culture cell line OVCAR-3, a human ovarian carcinoma line, has been shown to contain a mutated $p53$ gene and express high levels of $p53$ protein (8,9). These cells were used as controls for the purpose of comparing the various anti-$p53$ monoclonal antibodies.

**Immunohistochemistry**

Frozen samples of esophageal tissue were mounted in OCT medium. Cryostat sections (5–10 μm) were thaw-mounted on Histostik-coated slides, dried at 23°C, lyophilized, fixed in 100% acetone, air dried, and then rehydrated with phosphate-buffered saline (PBS). Ten percent goat serum was then applied to block any nonspecific binding. The primary $p53$ mouse monoclonal antibody (10 μg/ml) in normal goat globulin, 0.1% saponin, and PBS (NGG-sap-PBS) was added for 60 min, followed by washing. The secondary goat antimouse immunoglobulin G conjugated to horseradish peroxidase (or rhodamine in cases where immunofluorescence was used) was applied to the slide for another 60 min and then washed. DAB (3,3’-diaminobenzidine), Gill’s hematoxylin, and 1% osmium tetroxide were sequentially incubated with the specimen; dehydration was accomplished with ethanol/xylene; and the sample was then mounted under a no. 1 coverslip with Permount (Fisher).

**Monoclonal Antibodies**

There are a number of commercially available monoclonal antibodies to $p53$, some of which are listed in Table 1. These antibodies were prepared using synthetic peptides as antigens (Fig. 1). The specificity of these antibodies was compared in studies on OVCAR-3 cells, which are known to express high levels of a mutated form of $p53$. An antitransferrin receptor antibody (HB21) and an anti-Lewis’-reactive antibody (B3) were used as controls in cryostat sections. These antigens are known to be expressed in esophageal mucosa in distinctive patterns (10,11).

**RNA Extraction and Complementary DNA Synthesis**

Total cellular RNA was extracted from frozen tumor tissue according to the method of Chomczynski and Sacchi (12). RNA was quantitated by OD at 260 nm and a portion examined by denaturing formaldehyde gel electrophoresis for lack of degradation (13). RNA was reverse transcribed to complementary DNA (cDNA) with random primers and Moloney murine leukemia virus reverse transcriptase.

**Polymerase Chain Reaction**

Oligonucleotide primers were designed according to the published wild-type sequence of $p53$ (14). Among the 11 exons making up this gene, all mutations have been reported to occur within exons 5–9 and between amino acid residues 130 and 290 (of 393) (15–17). The primer pair defined a region between codons 120 and 321, yielding a 606-base pair (bp) polymerase chain reaction (PCR) product.

PCR was performed with the cDNA template, 1× PCR buffer II, 200 μM dNTPs, 2.5 μM of each primer, 3.0 mM MgCl₂, and 2.5 U Amplitaq DNA polymerase (Perkin Elmer Cetus). PCR conditions were 39 cycles at 94°C for 1 min (denaturing), 56°C for 2 min (annealing), and 72°C for 3 min (extension). Resulting PCR products were run on a 2% agarose 1× TAE gel, and the 606-bp fragment was excised and isolated by glass beads.

**Cloning and Sequencing**

In the initial three cases, the PCR product was blunt end ligated into the SMA I site of pBluescript KSII(+) and amplified in XL1-Blue cells (Stratagene). Positive colonies were picked, expanded,