p53 in the Diagnosis of Human Neoplasia

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The traditional methods used to diagnose cancer and predict its course have been increasingly aided by molecular genetics. Unique, molecular genetic tumor markers are a consequence of the clonal expansion of cells which harbor one or more altered genes. Converging lines of evidence now firmly document that cancer is a type of genetic disease with genomic changes that are often somatically acquired (1). Not only are these genetic lesions likely involved in the pathogenesis of cancer, but also they provide useful targets for cancer diagnosis.

The application of molecular genetic markers to cancer diagnosis has now begun through the detection of chromosomal rearrangements, gene amplification, point mutations, and tumor viruses. Another class of potentially diagnostic markers is genetic loss and disabling genetic mutations, which cause a reduction or loss of function of a critical protein. Loss of functional protein may play a role in the pathogenesis of cancer when it involves tumor-suppressor proteins such as retinoblastoma (Rb) and p53 (2). Bi-allelic rearrangement, deletion, or mutation of the tumor-suppressor genes rb and p53 is seen in a wide variety of cancer types, ranging from solid tumors to leukemia and lymphoma (2,3). Depending on the type of neoplasm, tumor-suppressor gene mutation may play a role in early, intermediate, or even late stages of tumor progression (4).

Detection of tumor-suppressor gene mutations may become a valuable prognostic marker for certain cancers. However, detection of mutations for routine clinical use requires improvements in the laboratory techniques involved in testing routinely obtained specimens from patients. In this issue of the Journal, Kovach et al. (5) have designed a polymerase chain reaction-based direct sequencing approach on routinely obtained touch preparations of patient tumor samples to detect point mutations of the p53 gene. By the selection of primers which flank mutational hot spots within the p53 locus, mutations were identified among small groups of carcinoma cells simply scraped from a touch-preparation slide. This technique adds the power of polymerase chain reaction to the morphological identification of malignant cells so they can be specifically isolated and tested. The success of this approach as a diagnostic or prognostic tool will depend on what is learned concerning the role of p53 in tumor development and progression. The p53 protein is unique among cellular regulatory proteins in having been originally considered an oncprotein, prior to the discovery of its role as a tumor-suppressor protein. Approximately 12 years ago, p53 was identified as a cellular protein which bound to the large T antigen of simian virus 40 (6,7) and was present in simian virus 40-transformed cells at levels five to 100 times those present in nontransformed cells. Studies with a temperature-sensitive mutant of large T antigen revealed a positive correlation between the transformed state and the intracellular level of p53 protein. High levels of p53, which were necessary for cellular transformation, were achieved apparently by the stabilization of p53 following its binding to large T antigen (8,9). In addition, complementary DNAs of p53 were able to convert established cell lines to the tumorigenic state (10), immortalize rat embryo fibroblasts (11), and cooperate with the ras gene in transforming and converting primary rodent cells to the tumorigenic state (12-16). However, the interpretation of all of the above studies was dramatically altered when it was discovered that the original p53 complementary DNAs were mutated. Wild-type p53, in contrast to the mutated isolates, was nontransforming (15,16).

The suggestion that wild-type p53 could potentially function as an anti-oncoprotein or tumor-suppressor protein derives from several independent experimental approaches: 1) wild-type p53 inhibits the transformation of rodent cells by known oncogenes such as ras and Ela (17,18) and, more importantly, wild-type p53 also inhibits the growth of human colon carcinoma cells and osteosarcoma cells; 2) human malignancies derived from the breast, colon, lung, and esophagus often contain a mutated p53 gene (and exhibit elevated levels of mutant p53 protein); and 3) the familial cancer syndrome Li-Fraumeni is characterized by the presence of germ line mutations in p53 (19). As might be anticipated from earlier studies of p53, mutant p53 genes derived from human tumors are transforming.

Human p53 is a 393-amino-acid phosphoprotein which localizes to the nucleus [reviewed in (2,20,21)]. The gene encoding this protein is located on the p arm of human chromosome 17, and it is composed of 11 exons, the first of which is noncoding. In cross-species comparisons, the p53 proteins reveal five highly conserved regions between amino acid residues 13-19, 111-136, 165-175, 230-252, and 264-280, which are frequently the site of mutation in many human tumors, including cancers of the breast, colon, lung, and brain, as well as some leukemias and lymphomas. Indeed, mutation appears to cluster within these conserved domains and to result in a protein with altered biological activities, including cooperating with the ras oncprotein, binding to heat shock protein (hsc 70), and extension of half-life.

While p53 is clearly involved in the regulation of cell proliferation, its exact biochemical function is unknown. However, structural analysis based upon the p53 amino acid sequence predicts that its carboxyl terminus contains a helix-turn-helix domain characteristic of DNA-binding proteins. Very recently, p53 has been shown to function as a transcriptional activator and, potentially, this role may be the etiology of its effects on cell growth (21). Several possibilities exist for the molecular consequences of p53 inactivation and its effects on cell growth as depicted in Fig. 1.

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While p53 mutation is a frequent event in human neoplasia, it is not clear whether its presence is significant for the prediction of tumor behavior or the degree of tumor progression. Mutation of p53 appears to be a later event in the development of colon carcinoma (corresponding to the transition from late adenoma to carcinoma), although it has also been reported to occur earlier during adenoma formation in patients with familial polyposis coli (4). In regard to cervical carcinoma, the interaction of the papillomavirus E6 oncoprotein with p53 is an early event which is associated with the immortalization of epithelial cells, but not with their tumorigenic conversion. Similarly, p53 is found to be mutated in the earliest phase of breast carcinoma (in situ carcinoma) which precedes the development of invasive carcinoma.

In an effort to define the precise temporal relationship between mutant p53 expression and human malignancy, Kovach et al. (5) have used an approach that combines touch preparation of surgical specimens with polymerase chain reaction of DNA to evaluate the p53 gene in morphologically isolated tumor cells. The advantage of such an approach is that it permits the characterization of p53 in the tumor cells themselves and reduces the probability of contamination with germ line p53 contributed by admixed normal cells from the tissue specimen. Although polymerase chain reaction amplification is sensitive, contamination and the potential for false results remain problems. Furthermore, the technique is labor intensive, especially when coupled with DNA sequencing, and, for the time being, it is too complex for routine hospital use. Other diagnostic approaches may emerge which directly examine the p53 protein by immunohistochemistry with epitope-specific antibodies that distinguish mutant and wild-type p53. Functional assays may be developed to measure DNA-binding capacity, which is often reduced or absent in mutated p53. However, the technique described by Kovach et al. represents an important step toward the time when DNA typing becomes an integral and conventional component of cancer diagnostics.

References

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