Germline p53 Gene Mutations and Cancer—Pandora’s Box or Open Sesame?

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p53—Master of Disguise

Fifteen years have passed since the original discovery of a nuclear phosphoprotein with a molecular mass of 53 kd that reacted with antisera from animals with tumors induced by simian virus 40 (SV40) (1,2). This protein, p53, was shown to be bound to the large T-antigen (T-Ag) component of these viral particles. It was initially thought that because T-Ag was required to maintain the transformed phenotype, the interaction of the novel protein with this antigen was important for transformation (1,2). Therefore, p53 came to be identified as a tumor antigen. The following three subsequent observations led to a redefinition of p53: 1) Large quantities of p53 were detected in a variety of tumor-derived and transformed cultured cell lines (1,2); 2) the protein in transformed cells was found to have a much longer half-life than that in nontransformed cells (3); and 3) a variety of genomic and complementary DNA (cDNA) clones of p53 were found to immortalize cells in culture and to cooperate with the ras oncogene to transform rat embryo fibroblasts in culture (4). These properties were more reminiscent of those of an oncogene—and so, p53 was identified as such.

Ultimately, however, this master of disguise was found out, after it was determined that all the transforming cDNA p53 clones represented mutant forms (5). At about the same time, a decade after it was originally identified, several functional properties of the wild-type form of p53 were observed. It was shown to negatively regulate the cell cycle (6,7); it required loss of function mutations to form tumors in both animals and humans (8); expression of either cDNA or genomic clones of wild-type p53 suppressed the transformation of cultured cells by other oncogenes, the growth of transformed cells in culture, and the tumorigenic potential of cells in animals (9); and point mutations or other gene alterations of wild-type p53 alleles were demonstrated in some fraction of almost all human tumors (10,11). In fact, it is now well established that alterations of the p53 gene (also known as TP53) and its encoded protein are the most frequently encountered genetic events in human malignancy (11). More than 1300 mutations have been reported in almost 50% of tissue samples of all sporadically occurring tumor samples that have been analyzed (12). Between different tumor types, the frequency of mutations varies (10,11).

Now, 15 years after its first appearance, p53 is classified in the family of tumor suppressor genes. From its modest entrance onto the scientific stage, p53 has attained the status of a molecular superstar. In 1992, it garnered the honor of second most significant scientific trend of the year in Time magazine (13); in 1993, p53 was named Molecule of the Year in Science magazine (14).

The p53 gene spans a moderately sized segment of DNA, located on the short arm of human chromosome 17, that is ultimately translated to a protein consisting of 393 amino acids contained in 11 exons—the first of which is noncoding. Five evolutionarily conserved domains within the coding regions are regarded as essential to the functional activity of p53. In addition to the properties of p53 supporting its ultimate function as a growth suppressor as outlined above, studies (15-19) have implicated p53 as a checkpoint control factor at the G1/S phase of the cell cycle. In the presence of DNA damage induced by gamma-irradiation or chemotherapeutic drugs, intracellular levels of p53 rise and promote the expression of a downstream gene, WAFI/Cip1, whose protein product p21 binds to cyclin-dependent kinases and inhibits their activity (20,21). In this manner, the cell cycle is arrested prior to DNA synthesis, and the cell is given the opportunity to repair the damaged DNA. If such repair does not occur, the presence of normal p53 induces the cell through a pathway of apoptosis or programmed cell death. When one evaluates the known properties of p53, it appears likely then that its inactivation could increase the pool of proliferating cells and similarly could increase the probability of their neoplastic transformation by inhibition of apoptosis (12). Although p53 inactivation most commonly occurs through point mutations, small deletions, or insertions within the gene, alterations of the protein through binding of exogenous viral antigens or cellular oncoproteins prevent wild-type p53 molecules from binding DNA and activating transcription of other growth regulatory elements.

p53—Predictor of Trouble

In this issue of the Journal, Kyritsis et al. (22) report their findings on germline p53 mutations in specific subsets of patients with gliomas. Previous studies [reviewed in (23)] initially described similar germline p53 mutations in rare families with the Li–Fraumeni family cancer syndrome (LFS). These families are recognized by the presence of a proband with a sarcoma, a first-degree relative who has

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developed a sarcoma before the age of 45 years, and a second first-degree relative who has developed any type of cancer under the age of 45 years or a sarcoma at any age. Other characteristic features of the syndrome include the occurrence of multiple primary neoplasms in affected individuals, the early age of the patient at onset of most tumors, and the autosomal pattern of inheritance of the disorder as determined by classical segregation analysis. Component tumors of the syndrome include soft-tissue sarcomas, osteosarcomas, breast carcinomas, leukemias, brain tumors, and adrenocortical carcinomas. Following the initial reports of germline p53 mutations in classic LFS families, studies reported the occurrence of these mutations in patients with multifocal osteosarcoma, in children and young adults with second malignant neoplasms in the absence of a positive family history of cancer, in children with adrenocortical carcinoma in the setting of LFS, and in a very small fraction of women with breast cancer (23).

The study by Kyritsis et al. (22) is important in that it confirms the observations of these other studies that germline p53 mutations may occur outside the classically ascertained LFS families. Furthermore, it addresses an important group of patients (i.e., those with brain tumors) who make up a significant proportion of both the adult and childhood cancer population and a significant segment of affected members in LFS families. In the evaluation of cancer families, it is crucial to have a complete and accurate history of as many close relatives as possible. This point is exemplified by the extensive methods, including telephone interviews with relatives, by which this group obtained data. Ascertainment bias is therefore minimized in this study. The germline p53 mutations observed in this patient population are primarily found in patients with multifocal disease or unifocal disease with a second primary malignancy or a family history of cancer (22). The risk of germline mutations is particularly high in those patients with all three factors, as might be expected in kindreds closely resembling LFS families.

Implications of Germline p53 Mutations

The p53 gene is one of several genes associated with predisposition to cancer. The others, including RB1, WT1, APC, and HNPCC, are associated with retinoblastoma, Wilms' tumor, adenomatous polyposis coli, and hereditary nonpolyposis colon cancer, respectively. These genes, however, differ from the p53 gene in that they are primarily associated with tumors of specific histopathology. All of these diseases can be recognized by standard clinical screening procedures, including funduscopy for retinoblastoma, abdominal ultrasound for Wilms' tumor, and sigmoidoscopy or colonoscopy for colon cancer. Furthermore, early therapeutic intervention may be effective in limiting or preventing progression of these diseases. The story of germline p53 mutations presents a much more complex situation. These mutations predispose to a highly unpredictable constellation of tumors within any one pedigree, and many of the classic tumors of LFS cannot be effectively screened with current clinical diagnostic techniques.

Also, all of these component tumors are aggressive and very often lethal. We are therefore placed in a position where the technical ability to identify germline mutations has preceded our clinical ability to prevent disease in unaffected carriers or to definitively diagnose early disease in affected individuals. It is likely that in the near future other cancer-predisposing genes will be isolated that will place a similar burden on the scientific and medical community. Nevertheless, studies such as those by Kyritsis et al. (22) will allow us to better define the populations who might benefit from screening and to improve our understanding of the biology of these mutations and of the ultimate role of p53 in carcinogenesis.

The identification of germline p53 mutations in cancer patients and in their families continues to present a Pandora's box of issues regarding the potential risks and benefits of predictive genetic testing for cancer. With the exception of possibly effective screening tests for breast cancer, recognized effective screening tests are generally not available for the other component cancers typically associated with germline p53 mutations. Although chemoprevention may be of some value in certain cancers, there is no evidence that it is of universal benefit. In an individual patient, one would not know what specific cancer one is protecting against, as there is no known association yet between specific mutations and tumor histology. Despite the many drawbacks of predictive testing, the possibility of reducing the marked loss of human potential resulting from the death of a child or young adult makes pilot research efforts for early intervention in p53 mutation carriers worthwhile. Further studies to develop more accurate testing, to understand the effect of germline mutations on cell transformation, and to perhaps develop models to evaluate methods of genetic intervention will be important. In addition, it becomes critical to further evaluate cancer risk notification techniques and to study the impact on knowledge, attitudes, emotions, and practices of those affected by such testing. Ultimately, one would hope that these studies lead through screening to the early detection and successful treatment of cancer.

References

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Why Monitor Angiogenic Factors in Patients’ Urine?

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Polypeptide growth factors serve as signals in the modeling of tissues during embryonic development as well as in the maintenance of tissue homeostasis in the mature organism. During the growth and metastasis of solid tumors, these growth factors function as autocrine stimulators of the tumor cells and/or play a role in recruiting stromal tissue and angiogenesis during embryonic and postnatal development. The most prominent angiogenesis factors are members of the fibroblast growth factor (FGF) family and are currently known to induce proliferation of endothelial cells in vitro and/or angiogenesis in vivo. The most prominent and best studied angiogenesis factors are members of the fibroblast growth factor (FGF) family, but transforming growth factor-α (TGF-α), epidermal growth factor (EGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), pleiotrophin (P10), and interleukin 4 (IL-4) also qualify as potential tumor angiogenesis factors. Any combination of these proteins may be found expressed in tumor tissues or in tumor cell lines. Thus, it is difficult to decipher which ones are only innocent bystanders and which ones actively drive the rate-limiting tumor angiogenesis and are therefore not only diagnostic markers but also potential therapeutic targets.

Unfortunately, the answer to this question will remain very complex. Tissue-specific regulation of vasculogenesis and angiogenesis during embryonic and postnatal development as well as maintenance of blood vessels in the adult requires the biological activities of different growth factors. In principle, tumor cells can resort to any of these angiogenesis factors and upregulate the respective genes during their transformation. A conservative estimate indicates that more than a dozen distinct protein products are currently known to induce proliferation of endothelial cells in vitro and/or angiogenesis in vivo. The most prominent and best studied angiogenesis factors are members of the fibroblast growth factor (FGF) family, but transforming growth factor-α (TGF-α), epidermal growth factor (EGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), pleiotrophin (P10), and interleukin 4 (IL-4) also qualify as potential tumor angiogenesis factors. Any combination of these proteins may be found expressed in tumor tissues or in tumor cell lines. Thus, it is difficult to decipher which ones are only innocent bystanders and which ones actively drive the rate-limiting tumor angiogenesis and are therefore not only diagnostic markers but also potential therapeutic targets.

It can be expected that in the next few years the tools will be available to specifically target individual angiogenesis factors in vivo and thus demonstrate their relative contribution to angiogenesis in a given set of tumors. This approach is exemplified by recent experiments that used specific antibodies against VEGF and demonstrated that this factor was rate limiting for the growth of selected model tumors.

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