Collaborative Interdisciplinary Studies of p53 and Other Predisposing Genes in Li-Fraumeni Syndrome

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A workshop on LFS2 and inherited p53 mutations was held under the sponsorship of the National Cancer Institute in Bethesda, Maryland, December 6–7, 1993. Clinical and laboratory investigators summarized their studies on affected cases and families evaluated at Villejuif, Manchester, Boston, Houston and Toronto. Other participants considered patient management issues and described experimental models. The workshop helped to identify areas for future collaborative research.

J. F. Fraumeni, Jr. (National Cancer Institute, Bethesda, MD) outlined the goals and purposes of the meeting. p53 is the most common site of somatic (acquired) mutations in human cancers, and often plays a fundamental role in the carcinogenic process. In contrast, germline (inherited) p53 mutations are rare experiments of nature in a few cancer-prone families and individuals. Collaborative studies are needed to maximize the gain of information from this model of inherited susceptibility to multiple forms of cancer. Molecular mechanisms revealed through these studies should have broad implications for cancer etiology and biology. The workshop can help develop enduring collaborations to address currently unanswered questions and pursue new opportunities for research.

L. Brugieres and J. Feunteun (Institut Gustave Roussy, Villejuif, France) have identified 9 Li-Fraumeni families (classical LFS) and 50 incomplete LFS families from among 1557 childhood cancer cases (1); 10 of these children had developed secondary primary cancers. In Li-Fraumeni families, the proband has a sarcoma before 45 years of age, a first-degree relative with cancer in this age interval, and another first or second degree relative in the lineage with either cancer under age 45 or a sarcoma at any age (2). Incomplete LFS families display several but not all of these features. Analysis for inherited p53 mutations have, to date, identified 7 germline p53 mutations: 4 in Li-Fraumeni families; 2 incomplete LFS families; and 1 in a child with multiple primary cancers. Most of the abnormalities are missense mutations, as reported by other groups. Five men in these families are obligate carriers who have not developed cancer, whereas all female carriers are affected. The explanation might be the high rate of breast cancer in adult female carriers. One family had the p53 mutation transmitted through an unaffected father, whereas multiple maternal relatives developed cancers that are phenocopies. The family illustrates the pitfall of inferring transmission solely from pedigree data.

J. Birch and A. Kelsey (CRC Paediatric and Familial Cancer Research Group, Manchester, UK) discussed the results of p53 analysis of 22 families: 13 with LFS and 9 with incomplete LFS (3). Germline mutations were detected in 7 of 13 Li-Fraumeni families, and only 1 of 9 incomplete LFS families. p53 mutations were found in all 3 LFS families with a case of adrenocortical carcinoma in infancy. Germline p53 mutations have been reported not only in cancer families but also in sporadic cases of breast cancer, childhood sarcomas, adrenocortical carcinoma, and brain tumors. Compared with families with no detectable mutation, families with a p53 mutation appear to have younger probands and perhaps more cases with multiple primary cancers. In a family with no p53 mutation, tissue specimens from multiple relatives were used for linkage analysis of the MDM2 (mouse double minute 2) gene, which can modulate p53 expression. Initial studies yielded a LOD score of 1.7 for linkage to MDM2 at a τ of 0.001. However, analyses of two polymorphisms within the MDM2 gene have excluded this locus as the inherited defect in the cancer family.

In one Li-Fraumeni family in which a germline p53 mutation has been excluded by linkage, Kelsey described increased p53 expression in nontumor cells (4). The observation was made in paraffin-embedded tissues by immunostaining with the CM1 antibody that reacts with both wild-type and mutant p53 protein. The finding suggests that the mutated gene is upstream in the p53 regulatory pathway and drives p53 overexpression in the germline. Normal cells of controls have consistently shown no CM1 staining. This novel observation of overexpression of wild-type p53 in germline cells might be pursued by Western blotting or immune precipitation to quantitate the p53 protein level.

F. Li and S. Friend (Harvard Medical School, Boston, MA) have collected 48 Li-Fraumeni families and 57 incomplete LFS families. During prospective observation, the development of multiple primary cancer in family members has emerged as an important feature of the syndrome. Among a small member of survivors of childhood sarcoma, the cumulative probability of a second cancer is 50% at 20 years of follow-up observation. Studies of 15 Li-Fraumeni families have identified 8 germline mutations in the coding region of the p53 gene. Fibroblast lines in affected members of several families were found to have acquired p53 mutations in vitro. In one instance, a CC to TT transition suggested in vitro exposure of the cell line to UV radiation.

To examine the possibility of mutations outside the p53-coding region, Friend used the codon 72 polymorphism within the p53 gene to compare the genomic DNA and cDNA of affected members of the 7 families with no mutations. The genomic DNA was heterozygous at codon

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2 The abbreviations used are: LFS, Li-Fraumeni syndrome; cDNA, complementary DNA; LOD, logarithm of the odds.
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72 in the germline of affected members of 4 families; their corresponding germline cDNA had lost heterozygosity. These preliminary findings suggest that one p53 allele produces an unstable transcript, possibly resulting from a defective p53 promoter. Consequently, high risk of cancer is due to the presence of only one transcribed wild-type p53 allele. This novel mechanism, together with germline mutations in the p53-coding region, might account for nearly all Li-Fraumeni families.

Identification of germline p53 mutations by conventional methods (screening gel electrophoresis and DNA sequencing) is labor intensive, and can produce erroneous results. These methods are not suited to the analysis of large numbers of subjects in whom mutations might be widely scattered in the p53 gene. Friend and others have developed functional assays to detect mutations that alter wild-type p53 function (5). One functional assay involves insertion of a p53 cDNA-containing expression vector into yeast cells by homologous recombination, and assaying of p53 function by the growth of yeast colonies.

L. Strong and M. Hanson (M. D. Anderson Cancer Center, Houston, TX) have analyzed family history data and biological specimens from several cohorts totaling more than 700 childhood sarcoma cases (6). Studies include segregation and linkage analyses, DNA testing for p53 mutations, assessment of p53 as a major gene or modifying locus, and phenotypic characterization of cultured fibroblasts of p53 carriers. A subset of kindreds show evidence of a major gene by segregation analysis. Genetic analyses of dominant, recessive, multifactorial and mixed models reveal that several pedigrees fit the dominant model, including families with germline p53 mutations. Evidence exists for additional non-random aggregation. DNA studies have identified phenocopies within p53-positive families, new p53 polymorphisms, and unexpected germline mutations and later cancer occurrences in previously unaffected branches of large families. Some families acquired the features of LFS over time, which illustrates the pitfalls of interpreting family histories from cross-sectional data.

Hanson discussed approaches to finding other candidate genes in p53-negative families. Allelotype of osteosarcomas shows nonrandom deletion at not only the p53 locus on chromosome 17p, but also chromosomes 3, 7, 10, and 13q, which might harbor tumor suppressor genes. Additional candidate genes include those that regulate p53, including ATM, MDM2, GADD45, and P21. Linkage analysis is one approach to studying these genes, but informative families are difficult to collect.

D. Malkin (Hospital for Sick Children, Toronto, Canada) noted that health care in Canada is nationalized and opportunities are available for population-based studies. His work began with cases presenting to The Hospital for Sick Children, and is expanding to encompass referrals from other centers in the Province of Ontario, as well as across the country. Accrual of samples and studies should be imminently facilitated by a Federal Government chartered childhood cancer cooperative group effort which would establish tissue and data banks. Of 325 samples from patients and families with a wide variety of clinical phenotypes, including classical LFS, incomplete LFS, as well as sporadic cases of childhood cancer, 288 have been screened to date by single-strand conformational polymorphisms analysis and DNA sequencing. Twenty-eight germline p53 mutations have been identified, the majority of which occur in classic LFS families. Multidisciplinary studies of the psychosocial impact of predictive genetic testing in families at risk based on p53 status or family history are being initiated.

A. Goldstein (National Cancer Institute, Bethesda, MD) discussed the importance of linkage analysis in LFS. Linkage can rigorously test for heterogeneity and demonstrate cosegregation of phenotypic traits with p53 mutations in families. However, obstacles to linkage analysis persist even after a germline p53 mutation is found in one or more affected relatives. The difficulties include small families that contribute little to the LOD score, missing data due to high mortality among affecteds, uncertainties about age-specific penetrance, the definition of affection, and other factors which can substantially alter results. In one report of a large family in which 28 relatives were typed, genetic analysis showed very strong evidence for linkage (LOD score of 4.25 at $\tau = 0.05$ and 1.2 at $\tau = 0$) (7). In the other reported families, however, the cumulative LOD score was barely above zero. Linkage analysis of LFS requires informative families, accurate diagnoses, data on age, and marker typing on all available relatives. Selection of families should be based on defined diagnostic criteria rather than p53 mutation status.

M. Kastan (Johns Hopkins Medical Center, Baltimore, MD) described biological mechanisms by which germline p53 mutations might predispose to cancer (8). He has analyzed cell cycle arrest at G1 after exposure to DNA-damaging agents, particularly ionizing radiation. In normal cells, p53 protein levels rise soon after irradiation and return to normal in 24–48 h. However, G1 arrest fails to occur in cells with mutant p53 genes or with oncogenic viral proteins that ablate p53 function. Mutations in genes involved in the p53 pathway might account for the Li-Fraumeni phenotype when no p53 mutations can be detected. p53 has other important functions, including apoptosis, and studies in progress to determine how cells select between the alternative pathways of cell cycle arrest or apoptosis.

T. Jacks (Massachusetts Institute of Technology Center for Cancer Research, Cambridge, MA) has produced and studied mice bearing germline p53 mutations (9). Virtually all mice with homozygous p53 mutations die of cancer, mostly T-cell lymphomas, by 6 months of age. p53 heterozygous animals develop tumors after 1 year of age, and penetrance is incomplete. Cancer development often involves loss of the second p53 allele. More than 50% of the tumors in heterozygous animals are sarcomas, 25% are T-cell lymphomas, and the remainder are lung adenocarcinoma, brain tumors, and other cancers. The fewer lymphomas in older heterozygotes might be due to reduced number of thymocytes in the older mice. Cross-breeding of p53 and Rb mutants shows that the offspring heterozygous for Rb and homozygous mutants for p53 have earlier cancer onset, and a tendency to develop multiple primaries that include both germine Rb- and p53-associated cancers. p53-deficient cells expressing the EIA oncogene are resistant to killing by ionizing radiation and drugs such as 5FU, etoposide, and Adriamycin. A possible explanation is that apoptosis after damage by these agents requires increased expression of normal p53 protein.

J. Garber (Dana-Farber Cancer Institute, Boston, MA) discussed genetic predisposition testing to identify carriers of p53 mutations. An important benefit of testing is the identification of noncarriers who are not at increased cancer risk despite a strong family history of tumors. Family members found to be carriers can be targeted for increased medical surveillance, risk avoidance, and chemopreven-
tion. The p53-testing program at Dana-Farber is modeled on the experiences of Huntington disease predisposition (predictive) testing. After a germline p53 mutation has been detected in a family, testing is offered to unaffected relatives who are at 50% risk of carrying the gene. Candidate subjects are provided written information, an informational videotape, and psychological tests to complete. At the first visit, the subject sees a physician, genetic counselor, and psychologist. Subjects who elect to proceed will sign the first of a series of consent forms for participation, and subsequently, blood collection and disclosure. At the second visit, results are disclosed. After disclosure, arrangements are made for medical surveillance, risk-avoidance counseling, access to a telephone hotline to the investigators, and a newsletter. An outside Ethical Advisory Board has been assembled to help ensure autonomy, beneficence, confidentiality, and justice (10).

A. Patenaude (Dana-Farber Cancer Institute, Boston, MA) discussed psychosocial issues in genetic predisposition testing. Among those found to be carriers, predisposition testing reduces uncertainty about susceptibility but elevates uncertainty regarding time and site of cancer development, if any. Likewise, cancer risk for subjects found not to be carriers is reduced.

Psychological and genetic counseling are both important components of any testing program because anxiety is omnipresent. Research on predisposition testing needs to be longitudinal and the impact of testing will change with age, life situation, health status, and social norms. The unit of testing should be individuals rather than families, and testing of children poses special problems. An interview study of parents of sporadic childhood cancer cases shows that most parents would have testing for both themselves and their unaffected children, even if testing has no effect on prognosis and longevity. Most parents said that they would reveal results to the older children. If a mutation were found in the child, most parents expected that their anxiety would increase, though some would feel empowered by the knowledge to become a better informed parent. Parents expect to be reassured by a normal result. One-third said they would not tell their pediatrician of the testing results and the remainder would tell.

In the general discussion, participants indicated that the interest in Li-Fraumeni families lies in not just the inherited defect, but also environmental influences and somatic mutations that determine tumor type, age at onset, and other manifestations of the syndrome. The broader research objectives also include data collection on management of affected individuals and families and new understanding of the biological basis of cancer susceptibility.

Several investigators commented on the relationship between the phenotype described as LFS and the corresponding p53 genotype. The syndrome features diverse tumor types, early ages at onset, and tendency to develop multiple primary cancers. However, no combination of features has been shown to predict the presence of germline p53 mutations with certainty. Accrued data permit estimates of the likelihood of finding an inherited p53 mutation in several clinical settings. In Li-Fraumeni families that fulfill the original "definition" of the syndrome (2), the majority have germline p53 mutations but some families do not. Childhood cases of adrenocortical carcinoma or choroid plexus tumor might carry p53 mutations, particularly if they have relatives with early onset cancers. The mutation frequency appears to be less than 10% among childhood sarcoma cases and patients with multiple primary cancers of early onset. The rate is less than 1% among breast cancer cases in general. These figures can be useful in selecting candidates for germline p53 analysis.

Priorities for future collaboration were discussed. Epidemiological studies are needed on cohorts of consecutive cases and families who are followed to determine the phenotypes of germline p53 mutations, age-specific penetrance, cumulative cancer incidence, and gender differences in gene expression. In addition, effects of radiotherapy and chemotherapy on risk of second cancers among p53 carriers should be evaluated. A larger collaborative study can help determine the frequency of p53 carriers among childhood adrenocortical carcinoma and choroid plexus tumor cases. C. Bonat-Pellie (Institut Gustave Roussy, Villejuif, France) suggested methods for calculating cancer risk among carriers that might overcome certain obstacles to pedigree analysis. The need for psychosocial and cross-cultural studies of genetic predisposition testing was also reviewed. In collaborative laboratory investigations, p53 mutations outside the coding region of the gene should be further evaluated in all Li-Fraumeni families without a detectable germline p53 mutation. The report of p53 overexpression in nontumor tissues of some families can be pursued by a variety of laboratory methods. Additional candidate genes in Li-Fraumeni families remains a strong possibility, and the search can be facilitated by having a bank of normal cells and tumor specimens that are available to laboratory investigators. The biological basis for the heterogeneity of tumor types among p53 carriers remains to be explained. Animal models have already been informative, and future studies might help to identify effective chemopreventive agents and other interventions which could delay or reduce cancer occurrence.

References
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