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The p53 Gene in Human Cancer

M. R. Stratton

INTRODUCTION

CERTAIN DNA viruses can induce tumours in experimental models. The transforming activity of the small DNA virus SV40 is attributable to a portion of the viral genome that encodes a protein termed the large T antigen. p53 was initially detected as a host cell protein that associates with the large T antigen in cells infected by SV40 [1, 2]. It was independently discovered as an antigen overexpressed in chemically transformed mouse cells [3].

STRUCTURAL ALTERATIONS IN THE p53 GENE AS SOMATIC EVENTS IN HUMAN CANCER

The first indication that mutation of the p53 gene might be a common step in the development of human cancer emerged from investigations into the role and location of tumour suppressor genes (recessive oncogenes). Comparison of germline and tumour DNAs using polymorphic probes revealed that loss of one or other parental allele (loss of heterozygosity) on the short arm of chromosome 17 occurs at high frequency in many types of neoplasm. This type of result is usually interpreted as indicating the presence of a tumour suppressor gene in the vicinity. The p53 gene had previously been localised to this region and fine mapping of deletions in colon carcinoma indicated that the common deleted area includes the p53 locus [4]. Prompted by this clue, Vogelstein's group sequenced the p53 gene in two colon carcinomas and subsequently in other tumours showing loss of heterozygosity on chromosome 17p. In most cases single base substitutions were detected in the remaining p53 allele [4, 5].

A substantial body of data contributed by several groups now indicates that the p53 gene is the most commonly mutated gene known in human cancer. Most of the mutations are missense single base substitutions resulting in replacement of one amino-acid by another in the p53 protein. A minority result in abnormalities of mRNA processing, frame shifts or premature termin-

Correspondence to M.R. Stratton, Institute of Cancer Research, The Haddow Laboratories, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, U.K.
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ation. Less commonly, complete or partial deletions and other gross rearrangements of the gene are detected. Point mutations of p53 are scattered through several codons but are mainly confined to four regions of the gene located in exons 5–8 that are conserved through evolution. There are four codons which are "hotspots" and which together account for approximately 30% of mutations in the gene [6]. Because most p53 proteins with single aminoacid substitutions have a much longer half life and are present at higher levels in the cell than the wild type protein, indirect evidence of a mutated gene can be obtained from immunohistochemical studies using antibodies to the protein [7].

Mutations of the p53 gene are present in all major histogenetic groups and indeed in most subtypes of cancer. Thus they are found in epithelial, mesenchymal, haemopoietic and lymphoid neoplasms and in tumours of the central nervous system. The proportion of tumours carrying p53 mutations may be high, but it appears that this is not an obligate step in oncogenesis. Mutation of the p53 gene constitutes an intermediate or late step in the sequence of genetic alterations required for tumour development (in the models examined so far). For example, mutations usually occur around or during the transition between adenoma and carcinoma of the colon [8]. Similarly they are found in blast crisis of CML but not in the earlier chronic phase [9]. However, exceptions to this rule exist and it may well be that mutation of p53 occurs at different times in different tumour types and that adherence to a predetermined sequence of genetic events is not as important as the final accumulation of the necessary set of mutations.

There is evidence that the sites and types of p53 mutation differ between tumours [6]. These differences may reflect variable constraints imposed by the biological characteristics of different tissues or exposure to different types of carcinogen. There is some support for the latter notion in the unique case of hepatoma in which mutations cluster at a particular codon and the nucleotide substitution is consistent with that induced *in vitro* by aflatoxin, a strong candidate carcinogen for hepatoma in certain parts of the world [10, 11].

GERMLINE MUTATIONS OF THE p53 GENE IN THE LI-FRAUMENI SYNDROME

The Li-Fraumeni syndrome (LFS) is a rare familial predisposition to cancer that is transmitted in an autosomal dominant manner [12]. The syndrome is characterised clinically by sarcomas in children (of bone and soft tissue) associated with a high incidence of breast cancer in female relatives. Leukaemia, brain, lung and adrenocortical tumours constitute less common features of the disease. It transpires that some LFS patients carry one mutated and one wild type p53 allele in their germline and that the disease is transmitted with the mutant allele [13, 14]. Many of these mutations are at the same sites as those arising as somatic events in sporadic tumours, including one of the "hotspots" described above. It is therefore thought that LFS patients are predisposed to cancer because one p53 allele is inactivated in the germline and therefore only the remaining allele needs to be altered by somatic mutation before a cell can escape from the tumour suppressor activity of the p53 protein. In normal individuals developing a sporadic tumour both p53 alleles must be inactivated in the same cell by somatic mutation. On this model the genetics of p53 are rather similar to those of the prototype tumour suppressor, the retinoblastoma gene (however, see below for complications).

Further studies are now required to ascertain the proportion of LFS families that carry p53 mutations. Moreover, given the variable presentation, moderate penetrance, unknown new mutation rate and significant mortality at an early age it is conceivable that many patients carrying a mutated p53 allele do not form part of a classical LFS kindred. It is therefore now of considerable medical importance to ascertain the prevalence of germline mutant p53 alleles in patient groups such as other types of familial breast cancer, apparently sporadic premenopausal and bilateral breast cancer or sporadic rhabdomyosarcomas. Clinicians need to consider what they may be able to offer such individuals. Cancer screening to provide early diagnosis and prophylactic treatment may be appropriate for some. However, the beneficial effects of these may have to be weighed against detrimental effects of testing upon lifestyle and economic status. Moreover, in the case of antenatal screening, ethical factors have to be given careful consideration.

IS P53 A DOMINANT TRANSFORMING GENE OR A TUMOUR SUPPRESSOR GENE?

Early in the 1980s several groups demonstrated that overexpression of p53 could immortalise or transform cells either on its own or in collaboration with other oncogenes such as *ras* [15–17]. This pattern of activity is similar to that of genes such as *myc* which are conventionally regarded as dominantly acting oncogenes (i.e. they can transform cells in the presence of a normal allele and the proto-oncogene usually requires activation to contribute to onocogenesis). Doubt was cast upon this interpretation when it emerged that the cDNA clones used in all these experiments were mutated rather than wild type. Moreover, the pattern of structural alterations found in tumours (widely scattered point mutations and various typs of homozygous deletion) is more suggestive of a tumour suppressor gene (or recessive oncogene in which both alleles require inactivation) than of a dominantly acting gene. Further experiments have recently revealed that wild type p53 does indeed suppress the transformed phenotype in a number of cell systems [8, 18, 19].

However, if p53 were to behave as an orthodox tumour suppressor gene, then mutated versions should theoretically be inactivated and thus not affect the phenotype when introduced into normal cells. Nevertheless p53 clones with several different mutations stubbornly act to transform cells [20, 21]. Reconciling the respective transforming and suppressor activities of mutated and wild type p53 remains problematic. One possibility is that p53 protein functions in the form of protein oligomers. Oligomers composed exclusively of wild type protein function normally. Oligomers composed of mutant protein are inactivated. However, hybrid oligomers of mutant and wild type protein take the mutated (i.e. inactivated) phenotype [22]. On this model a mutation in a single p53 allele may provide the cell with a poliferative advantage without alterations of the remaining allele. Nevertheless, the overall effect is inactivation of the protein (a dominant negative or dominant loss of function effect).

To complicate matters further, however, there is preliminary evidence to suggest that mutated p53 can enhance the transformed phenotype even in the absence of normal p53 protein. This data suggests that some mutations of p53 may confer a gain of function on the p53 protein rather than the loss of function usually associated with tumour suppressor genes [23].

FUNCTION OF THE p53 PROTEIN

p53 is a nuclear phosphoprotein of short half-life that is modified by or interacts with other proteins that are known to

be involved in regulation of the cell cycle [24]. Many mutated versions of p53 protein exhibit a much longer half-life and bind to heat shock proteins [25]. Documented effects on cell proliferation include regulation of the transition from G1 to S phase of the cell cycle [26] and a role in determining cell death through apoptosis [27]. These regulatory functions may be mediated by the interaction of p53 protein with specific DNA sequences [28, 29] which in turn may allow regulation of other genes at the transcriptional level [30] or perhaps by a role in initiating DNA replication [31]. DNA binding capacity, transcriptional activator function and initiation of DNA replication are all altered in mutated p53 proteins [28, 29, 31].

INTERACTIONS OF p53 AND RB PROTEINS WITH DNA VIRUS ANTIGENS

p53 was discovered through its association with SV40 large T. It also binds to other DNA virus transforming proteins including the E1b of adenovirus and E6 of papillomavirus [32, 33]. Interestingly enough, it is now known that the protein encoded by the retinoblastoma tumour suppressor gene also binds to SV40 large T, to the E1a protein of adenovirus and the E7 of papilloma virus. There is therefore a remarkable symmetry between p53 and RB1. RB was detected and isolated as a gene involved in a familial cancer syndrome, was subsequently found to be a target for somatic mutation in many tumours and finally was shown to be bound to DNA virus transforming proteins. Conversely p53 was originally discovered as a protein bound to DNA virus products, was subsequently shown to be a target for somatic mutations and finally was implicated in a familial cancer syndrome.

FUTURE PROSPECTS

The investigation of the cell and molecular biology of p53 in tumours should provide further insights into the regulation of cell proliferation and its disturbance in tumour cells. The fact that p53 mutations are so common in many different types of tumour may allow detailed examination of mutational spectra and hence provide clues to the carcinogens reponsible. Finally investigation of the prevalence of p53 germline mutations may alter our perspective of the balance between inherited and environmental factors in cancer and directly contribute to the care of patients.

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