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Review

Point mutations of the *P53* gene, human hepatocellular carcinoma and aflatoxins*

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The tumor suppressor *p53* exerts important protective functions towards DNA-damaging agents. Its inactivation by allelic deletions or point mutations within the *P53* gene as well as complex formation of wildtype *p53* with cellular or viral proteins is a common and crucial event in carcinogenesis. Mutations increase the half-life of the *p53* protein allowing the immunohistochemical detection and anti-*p53* antibody formation. Distinct *G* to *T* point mutations in codon 249 leading to a substitution of the basic amino acid arginine by the neutral amino acid serin are responsible for the altered functionality of the mutant gene product and were originally identified in 8 of 16 Chinese and 5 of 10 African HCC patients. Both groups are frequently exposed to mycotoxin contaminations of their food. Today an average *P53* gene mutation rate of 25% is assumed for high-aflatoxin B_1-exposure regions. This is double the rate observed in low-aflatoxin B_1-exposure countries. Although many HCC patients displaying *P53* mutations also suffer from HBV infection, which itself can lead to rearrangements of *P53* coding regions or induce the synthesis of viral proteins possibly interacting with *p53*, the specific *G* to *T* transversion within codon 249 of the *P53* gene seems to directly reflect the extent of aflatoxin B_1 exposure.

Key words: Tumor suppressor genes; Mycotoxins; Liver cancer

Hepatocellular carcinoma (HCC) represents one of the most common lethal tumors worldwide (1) and accounts for up to 30% of all types of malignant tumors in South East Asia and south and equatorial Africa. Chronic hepatitis B and C virus (HBV and HCV, respectively) infection, cirrhosis and aflatoxin B_1 exposure are considered the major aetiologic factors of HCC development (2). Molecular biological techniques have increasingly elucidated the pathogenic mechanisms which induce or maintain malignant hepatocyte transformation, and have provided evidence that inactivation of the tumor suppressor *p53* is a common and crucial event in carcinogenesis.

The *P53* gene — normal and deranged functions

The *P53* gene, located on the short arm of chromosome 17 (17p13), encodes a nuclear phosphoprotein

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which was originally identified as forming complexes with the large *T*(umor) antigen of the Simian Virus 40 (3). Wildtype *p53* protein contains at least three functionally active domains (Fig. 1). The carboxyterminus harbours an oligomerizing function that allows *p53* tetramer formation (4) and a DNA-binding activity. A transcriptional *trans*-activator function resides in the aminoterminus (5,6), which is involved in the control of transcription by regulating the interaction of DNA polymerase-alpha with other components of the DNA replication complex (7). Whereas wildtype *p53* is not required for normal ontogenic development in mice (8), it seems to exert pivotal protective functions as "guardian of the genome" (9): DNA-damaging agents like ultraviolet light, gamma-irradiation or certain chemotherapies (10) stimulate intracellular accumulation of *p53*. In high concentrations *p53* inhibits DNA replication and cell growth thus providing sufficient time for DNA repair or allowing cytolysis, if the damage exceeds the restoring capacity of the cellular DNA repair systems (9).

Inactivation of the tumor-suppressing potential can be achieved by (1) allelic deletions or (2) point mutations within the *P53* gene as well as by (3) complex formation of wildtype *p53* with cellular or viral proteins (Fig. 2). Abnormal structure and expression of the *P53* gene are observed in various hepatoma cell lines (11). HBV DNA integration can take place in the short arm of human chromosome 17 (12) near *P53* sequences (13) and structural rearrangements and subsequent aberrant transcription of the *P53* gene were found in both hepatocarcinoma cell lines and HCC tissues (14). In clonogenic assays using human fibroblast cell lines it was recently demonstrated that the disruption of one *P53* gene, with the concomitant reduction of *p53* protein levels, was not sufficient to induce increased endogenous gene amplification considered to represent a preneoplastic condition in this system. However, the loss of both *P53* alleles in these cells resulted in an increased rate of amplifications in association with the failure to arrest growth (15).

Point mutations are present in *P53* cDNAs derived from astrocytomas, breast cancers, small cell lung cancers, esophageal cancers, osteosarcomas, rhabdomyosarcomas and colon cancers (7). Germ line *P53* mutations occur in families with the *Li-Fraumeni* syndrome with autosomal dominantly inherited risk of diverse myesenchymal and epithelial neoplasms at multiple sites (16,17). Almost 90% of these mutations are clustered in four regions of exons 5–8 of the *P53* gene (18; Fig. 1). Point mutations affect distinct base pairs and result in the substitution of single amino acids. *G* to *T* transversions detected in codon 249 in human HCC (19,20) lead to a substitution of the basic amino acid

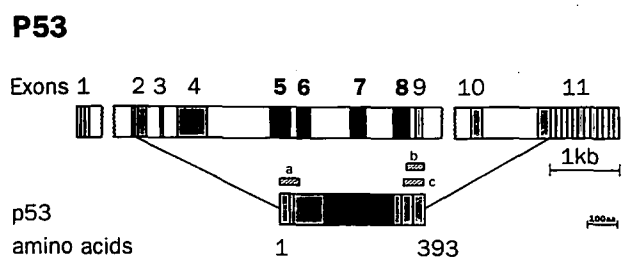

Fig. 1. Genomic organisation of human *P53*, which consists of 11 exons (numbers above) and 10 introns (white boxes). The hatched grey bars represent non-coding regions from which mRNAs are transcribed. The exons in black reflect preferred regions for point mutations. Interrupted white bars indicate a distance of 10 (intron 1) and approximately 2.5 kb (intron 10), respectively. The encoded *p53* protein is given below the gene sequence. a: *trans*-activating domain, b: oligomerizing domain, c: DNA-binding domain of *p53* protein. Size markers in kb (kilobases) and aa (aminoacids), respectively. The same pattern of coloration was chosen for correspoding exons and protein domains.

arginine by the neutral amino acid serin and may explain the functional alteration of the mutated protein. These mutations are all the more important since no mutations are observed in any other region of exons 5–8 of *P53* or in non-tumorous tissues of the same patients. Furthermore, while no *P53* mutations were detectable in early HCC stages as classified by histological evaluation, abnormalities of the *P53* gene were shown in eight of 22 advanced HCCs (21). In six of seven cases of these advanced tumors, the *P53* gene aberrations were significantly associated with the loss of heterozygosity or alteration of a second tumor suppressor gene, the retinoblastoma *Rb* gene (21). This emphasizes the relevance of structural *P53* aberrations in advanced stages of disease and supports the hypothesis that an accumulation of mutations in tumor suppressor genes is important for tumorigenesis.

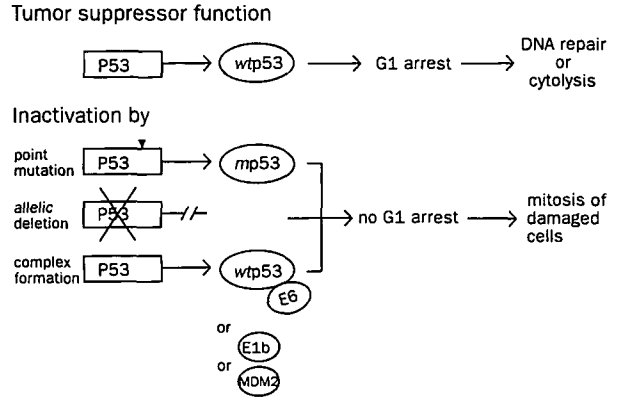

Fig. 2. Schematic illustration of the tumor suppressor function of wildtype *p53* (wtp53). Possible modes of inactivation of *p53* by point mutation (▲, *m*p53), allelic deletion or complex formation with viral (E6, E1b) or cellular (MDM2) proteins and the consequences for the cell cycle are depicted below.

Mutant *p53* proteins have lost the ability to act as tumor suppressors in transfection assays. While wildtype *p53* can suppress transformation in oncogene cooperation assays between c-*myc* and c-*ras*, mutated *p53* can gain transforming potential (22). Mutations increase the half life of the normal *p53* protein several times and result in the translation of a more stable protein that can be detected with immunohistochemical methods and induces anti-*p53* antibody formation in more than 25% of HCCs (23). The diagnostic efficiency of anti-*p53* antibody determination in detecting *P53* gene mutation, however, remains to be elucidated. When monoclonal anti-*p53* antibodies were used to stain tissue sections, overexpression of *P53* was shown in 9 of 58 HCC specimens (24). This indirect detection of *P53* mutations may, however, underestimate the actual frequency of gene mutations, because it will not recognize allelic deletions or codon stop introductions which lack protein translation. On the other hand, *p53* protein analysis may also overestimate the prevalence of gene mutations since other mechanisms of protein stabilization such as inactivation of *p53*-degrading enzymes or complex formation may play an important role. By forming heterologous oligomeric complexes with wildtype *p53*s mutated *p53* inactivates wildtype *p53* and antagonizes its tumor suppressing potential (25,26). The oncoproteins *E1b* and *E6* of the adeno- and papilloma viruses, respectively, are further examples for *p53* inactivating binding partners (27), which are derived from DNA tumor viruses. Recently, co-immunoprecipitation experiments identified the MDM2 protein, which was encoded and originally described on a *m*ouse *d*ouble *m*inute chromosome but overexpressed in human sarcomas, as the first cellular binding counterpart of *p53* (28, Fig. 1). Preliminary data suggest that no *p53* mutations occur if MDM2 gene amplification is present. As with viral oncoproteins, large amounts of MDM2 protein may complex and inactivate wildtype *p53* (28).

Aflatoxins and HCC

There seems to be a geographical correlation between *P53* codon 249 mutation in HCCs and aflatoxin B_1 uptake. The described point mutation was originally found in eight of 16 Chinese and five of 10 African patients (19,20) with both groups living in regions with traditionally high exposure to mycotoxins. None of these mutations were detectable in 20 patients with HCCs recently studied in Great Britain (29). Only two of 13 HCC DNAs from Germany displayed a *C* to *T* and a *T* to *A* transversion, respectively, in codons 257 or 273, but not in codon 249 (30). In all of the latter countries the environmental aflatoxin uptake is low. More recent studies claim a *P53* gene mutation rate of 25% for high-aflatoxin B_1-exposure regions (31). This is twice the rate observed in low-aflatoxin B_1-exposure countries, but still considerably less than previously assumed. Data from 167 patients with HCCs from various geographic areas differing in daily mycotoxin exposure also support the notion that codon 249 mutations of the *P53* gene are directly associated with high aflatoxin uptake (32). Aflatoxin B_1, the main food contaminating mycotoxin in China and Africa, is a fungal metabolite, mostly from inappropriately stored grain and is known as a hepatocarcinogen and epidemiologically defined risk factor for HCC development in different species (32,33). The involvement of aflatoxins, bioactivated by P_{450} isoenzymes (34), in the observed *G* to *T* point mutations seems even more likely, because they specifically induce *G* by *T* substitutions by reacting almost exclusively with DNA guanines at the N7 position (35).

Since many HCC patients investigated for *p53* mutations also suffer from HBV infection, the relative impact of these factors in the development of HCC is difficult to establish. Integration of HBV DNA during infection leads to manifold rearrangements of cellular DNA that can also affect *P53* coding regions. Furthermore HBV DNA integration induces the synthesis of *HBV* transactivator proteins (36–39) which may inactivate *p53* by complex formation. However, the specific *G* to *T* point mutation within codon 249 of the *P53* gene in HCC DNAs is not pathognomonic for all HCCs but seems to directly reflect the extent of aflatoxin B_1 exposure.

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