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p53 gene mutations in multiple myeloma

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Abstract

***Aim*—To assess whether p53 gene mutation is important in the pathogenesis and progression of multiple myeloma.**

***Methods*—Thirty eight DNA samples (derived predominantly from bone marrow) obtained from 31 patients with multiple myeloma were examined for mutations in p53 exons 5–9 by polymerase chain reaction single strand conformation polymorphism. Twenty three samples were analysed at the time of diagnosis (one patient had plasma cell leukaemia), three in plateau phase, and 12 at relapse (one plasma cell leukaemia and one extramedullary relapse).**

***Results*—One p53 mutation was detected in this group of patients (3.2%). This was seen in the diagnostic bone marrow sample of a 35 year old man with stage IIA disease and occurred in exon 6 as a result of a silent A to G transition at codon 213 (CGA → CGG), a polymorphism that has been reported in about 3% of breast and lung tumours.**

***Conclusions*—p53 gene mutations are rare events in multiple myeloma and would seem to be of limited value as a prognostic factor.**

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p53 is a nuclear phosphoprotein encoded by a gene located on the short arm of chromosome 17 (17p13) which is capable of blocking cells at the G1/S transition, thereby halting the proliferation of DNA damaged cells and promoting apoptosis. Point mutations of the p53 gene have been demonstrated in a wide variety of solid tumours[1–5] where they are usually associated with loss of the other allele. The resultant loss of p53 function mediates resistance to chemotherapy induced apoptosis, an event often associated with a poor clinical outcome. A vast number of p53 mutations has been described. They occur throughout the genomic sequence but most seem to be clustered in the central hydrophobic core of the molecule coded for by exons 5–9.[6]

The incidence of p53 mutation in haematological malignancy is variable: a relatively high incidence has been reported in high grade lymphoma, Hodgkin disease, and acute myeloid leukaemia but mutations are rare events in myelodysplastic syndrome, acute lymphoblastic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML).[7] p53 mutations are, however, frequently associated with progressive disease with a poor prognosis, such as CML blast crisis, Richter syndrome, and transformation of low grade lymphoma.[7]

The role of p53 gene mutations in the pathogenesis of multiple myeloma and their potential use as a prognostic indicator remain uncertain. Therefore, we have used the widely accepted technique of polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) analysis to detect p53 mutations in tumour samples obtained from 31 patients with multiple myeloma.

Methods

The study population comprised 31 patients (median age 56.5 years) with multiple myeloma (15 IgG, 11 IgA, one IgD, and four light chain) as defined by standard criteria. Twenty three patients were studied at diagnosis: 20 had symptomatic myeloma necessitating subsequent chemotherapy, two patients had plasma cell leukaemia, and the remaining patient had smouldering myeloma.[8] Bone marrow aspirates containing a minimum of 10% plasma cells were obtained in each case. A further three bone marrow samples were obtained from three patients in plateau phase. Twelve samples were also obtained from 10 patients at time of relapse; these consisted of bone marrow in 10 cases, peripheral blood in a case of plasma cell leukaemia, and a skin biopsy specimen from a patient who relapsed with extramedullary disease. In each case, the tumour specimen contained a minimum of 10% plasma cells.

PREPARATION OF DNA

High molecular weight DNA was obtained from tumour samples by proteinase K digestion, phenol-chloroform extraction and cold ethanol precipitation.

PCR-SSCP ANALYSIS

The oligonucleotide primers used to amplify p53 exons 5–9 are listed in table 1. PCR reactions were performed in 50 µl volumes with 20–50 pg DNA, 50 pmoles sense and antisense primers in buffer containing 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl (pH 9.0), 0.01% Tween, 1.5 mM $MgCl_2$, and 50 mM each dNTP. The reaction mixtures were held at 95°C prior to the addition of 0.5 units of Taq polymerase (Thermoprime; Advanced Biotechnologies, Surrey, UK). PCR amplification conditions for exons 5, 6, 8, and 9 were 95°C for 40 seconds, 62°C for 40 seconds, and 72°C for 40 seconds for 35 cycles followed by a further 90 second extension at 72°C. PCR amplification of exon 7 differed slightly in that the annealing temperature was increased to 65°C.

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Table 1 Primer sequences used for the PCR amplification of p53 gene exons 5–9

Exon (base pairs)	*Primer sequence (5'–3')*
5 (220)	F: CTTCCTCTTCCTACAGTACTC
	R: GCCCCAGCTGCTCACCATC
6 (148)	F: CTCACTGATTGCTCTTAGGTC
	R: CCAGTTGCAAACCAGACCTC
7 (170)	F: GTCTCCCCAAGGCGCACTGG
	R: CAAGTGGCTCCTGACCTGGA
8 (163)	F: CAATCCTGAGTAGTGGTAATC
	R: TGTCCTGCTTGCTTACCTCG
9 (111)	F: CCTTGCCTCTTTCCTAGCAC
	R: GAGGTCCCAAGACTTAGTACC

F = forward primer; R = reverse primer.

For SSCP analysis, 1.5–3 µl each PCR reaction product was added to 10 µl loading buffer and denatured at 80°C for 10 minutes before quenching on ice for several minutes. The denatured samples were then loaded onto FMC Mutation Enhancement Gel (FMC BioProducts; Rockland, Maine, USA) and electrophoresed at a constant 10 W for two to six hours depending on the fragment size. The gels were then fixed and the PCR fragments stained with silver.

DIRECT SEQUENCING OF PCR PRODUCTS

Abnormal bands were excised from the gel, eluted into 100 µl distilled H_2O, and 2 µl aliquots were then reamplified with identical primers and conditions. This PCR product was run on 2% agarose gel, purified with a Qiaquick gel extraction kit (Qiagen GmBH, Hilden, Germany) and then sequenced in both directions using an Applied Biosystems (Warrington, UK) fluorescence ddNTP sequencing kit according to the manufacturer's instructions. The products of the sequencing reactions were then analysed using an Applied Biosystems DNA sequencer (Model 373A), equipped with sequence collection and analysis software, and compared with the known p53 sequence.

Results

Thirty eight samples of genomic DNA were obtained from 31 patients with confirmed multiple myeloma and analysed for p53 mutations by PCR-SSCP for exons 5–9. In each case, the tumour sample contained a minimum of 10% plasma cells. Normal SSCP patterns were observed in 37/38 samples, whereas an abnormal exon 6 band was observed in the remaining sample. This was a diagnostic bone marrow sample obtained from a 35 year old man with stage IIA disease who is currently alive and in complete remission 42 months following autologous bone marrow transplantation. Direct sequencing demonstrated a silent A to G transition at codon 213 (CGA → CGG both coding for arginine) which was also demonstrated in somatic DNA samples (data not shown).

Discussion

The aim of this study was to investigate the degree to which p53 gene mutation contributes to the pathogenesis and progression of multiple myeloma, and whether their detection should be included in routine prognostic factor assessment. We studied 38 DNA samples obtained from 31 patients with PCR-SSCP for p53 exons 5–9 and found a single mutant band. This was demonstrated in the diagnostic bone marrow samples of a 35 year old man with stage IIA disease. Direct sequencing demonstrated this to be caused by a silent A to G transition at codon 213. This polymorphism has been described previously in about 3% of lung and breast tumours,[9] and was also demonstrated in the somatic DNA in our patient. This finding clearly emphasises the importance of sequencing abnormal SSCP bands, particularly as this polymorphism results in the loss of a Taq1 restriction site.

This low frequency of mutation is likely to be a real phenomenon as mutations occurring outside exons 5–9 are rare in other tumour types,[6] and all tumour specimens analysed in this study contained a sufficient proportion plasma cells—that is, a minimum of 10%. It is possible, however, that we missed some mutant bands whose mobilities were similar to that of wild type p53. Our results are in accordance with other PCR-SSCP based studies which have reported a mutation frequency of 2–4%.[10–12] Interestingly, germline mutations have not been demonstrated in individuals with two or more relatives with multiple myeloma.[12]

PCR-SSCP, however, is not sufficiently sensitive to demonstrate mutations occurring in minor subclones which can subsequently be responsible for relapse. This phenomenon has recently been demonstrated in acute leukaemia[13] but is unlikely to be major factor in multiple myeloma as we were unable to demonstrate any mutant bands in the 12 samples obtained at relapse. p53 gene mutation may, however, have a role in a certain proportion of patients; Mazars *et al*[14] demonstrated mutations in eight of 10 human myeloma cell lines derived from patients in the terminal phases of their disease, although, of course, these may have been acquired in vitro. Analysis of clinically derived material with PCR-SSCP and DNA sequencing have also demonstrated mutations in up to 40% of patients with end stage disease and plasma cell leukaemia.[15–17] We were, however, unable to demonstrate mutations in three patients with plasma cell leukaemia and a further patient with end stage extramedullary disease.

We conclude that p53 mutation is a rare event in multiple myeloma and would seem to be of limited value as a prognostic indicator. When detected, mutations seem to be confined to patients with end stage and leukaemic forms of the disease, a situation analogous to CML blast crisis, Richter syndrome, and transformation of follicular lymphoma.

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