Loss of 1p and rearrangement of *MYC* are associated with progression of smouldering myeloma to myeloma: sequential analysis of a single case

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ABSTRACT

We report serial genetic studies on a young female patient initially diagnosed with asymptomatic smouldering myeloma who progressed to symptomatic myeloma 4.5 years after presentation. An unbalanced translocation, der(14)t(4;14)(p16;q32), was initially found in all plasma cells plus deletions of other chromosomal regions as detected by array-based comparative genomic hybridization. Deletion of chromosome 13 was observed in a minor population of plasma cells (<20%) for the first two years, increasing to 100% of plasma cells by the time of multiple myeloma diagnosis. Loss of 1p and a rearrangement of *MYC* were first observed in a small population of plasma cells one year prior to the clinical diagnosis of multiple myeloma, but these subclones increased rapidly in size to become the major population suggesting that they were directly involved in the transformation process. This case report provides a unique insight into the mechanisms of disease progression from smouldering multiple myeloma to multiple myeloma.

Key words: smouldering multiple myeloma, plasma cell, chromosomal abnormality, progression, arrayCGH.

Citation: Chiecchio L, Dagrada GP, Protheroe RKM, Stockley DM, Smith AG, Orchard KH, Cross NCP, Harrison CJ, and Ross FM on behalf of the UK Myeloma Forum. Loss of 1p and rearrangement of MYC are associated with progression of smouldering myeloma to myeloma: sequential analysis of a single case. Haematologica 2009;94:1024-1028. doi:10.3324/haematol.2008.004440.

Introduction

Smouldering multiple myeloma (SMM) and monoclonal gammopathy of undetermined significance (MGUS) comprise the two forms of asymptomatic plasma cell (PC) disorders. Both conditions are characterized by a variable period of stable disease, which may eventually evolve to symptomatic multiple myeloma (MM). The diagnostic criteria of SMM include paraprotein (PP) level >30 g/L, and/or percentage of PC in the bone marrow (BM) >10%.[1-4] The distinction between SMM and MM is not evident from histopathologic examination of the BM alone, but is influenced by clinical findings indicative of end-organ damage, which are absent from SMM patients. In compliance with the British Committee for Standards in Hematology (BSCH) Guidelines on the Diagnosis and Management of Multiple Myeloma 2005,[5] SMM is not treated unless there is evidence of progression but it is recommended that cases should be closely monitored. The rate of progression has been calculated as 10% per year for the first five years.[4,6]

Many of the genetic changes found in patients with MM have also been found in SMM. Approximately 50% show primary translocations involving the immunoglobulin heavy chain (IgH) locus leading to the dysregulation of oncogenes including the CyclinD, *FGFR3/MMSET* and *MAF* genes. [7-10] These translocations are known to be early events in the disease process. Although some have been associated with a poor outcome in MM, the prognostic significance is less clear in the context of pre-malignant conditions.[10] Some studies showed a decreased incidence of *IgH* translocations involving 4p16 and 16q23 in MGUS, which led to the suggestion that these translocations might be the initiating event in *de novo* MM or that they were linked to rapid progression from MGUS to MM.[11-13] Little information is available on the specific changes associated with disease progression. Both MGUS and MM are highly heterogeneous at the genetic level and progression may be linked to the type, presence or absence of an *IgH* translocation.

In this report, we describe the clinicopathological and genetic findings of a young patient initially diagnosed with SMM. She was followed for a period of four and half years until progression

Funding: research supported by the Leukaemia Research Fund.
Manuscript received on December 4, 2008. Revised version arrived on January 16, 2009. Manuscript accepted on February 16, 2009.
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to symptomatic MM. The unique aspect of this study represents the complete description of BM morphology in association with cytogenetic and molecular cytogenetic analysis for each year of the follow-up period.

Design and Methods

Case report

A 30-year old caucasian woman was incidentally found to have protein in her urine in September 2001. Full blood count was normal. Her BM aspirate (sample 1) showed 9% PC with atypical morphology and the trephine displayed a degree of architectural disorganization, with low level increase in the number of PC, and occasional focal aggregations. There was an IgAk PP of 32.4 g/L, with no evidence of end-organ damage although β_2M was 3.4 mg/L and renal function was slightly impaired (creatinine 132 mmol/L; reference range, 60-125 mmol/L); a renal biopsy showed minor glomerular abnormalities, but no changes that are typically associated with MM related renal damage. She was diagnosed with SMM and no treatment was given. Subsequent bone marrow investigations were performed in June 2003 (sample 2), June 2004 (sample 3) and May 2005 (sample 4). PC levels were 13%, 3% (hemodilute) and 28%, respectively, with trephines indicating low level PC infiltration in normocellular marrows. PP levels fluctuated between 25.5 and 33.2 (Figure 1). Creatinine did not increase, and was 113 mmol/L in 2005, but β_2M increased to 5.6 mg/L at that time. Repeat MRI scans showed no change from diagnosis.

In January 2006 the patient remained asymptomatic, despite a slight increase in PP (35.7 g/L) and a marginal decrease in platelet count (116×10^9/L). In March 2006 she was urgently re-assessed and treated, due to bone pain in her left clavicle, subsequently shown to be due to a lytic lesion. The BM aspirate (sample 5) showed ~30% PC with binucleated forms present, while the biopsy revealed sheets of atypical PC in some areas. Hb was 106 g/L and platelets 97×10^9/L. The patient was entered into the MRC Myeloma IX Trial and treated with cyclophosphamide, thalidomide, and dexamethasone, followed by autologous stem cell transplantation in September 2006. She had an initial good response achieving PR, but relapsed with rising PP in September 2008.

Samples were received for genetic analysis from the same five BM samples as described above. All samples were tested by FISH on purified PC. Samples 2 and 5 were also adequate for cytogenetic analysis with sample 2 having sufficient material for aCGH analysis. Cytogenetic and interphase FISH (iFISH) methods, including the list of the probes used, have been previously published.[14,15] Additional probes used were for 1p12 (RP11-418J17), 1p32.3 (RP11-116M11), 1q12-q22 (RP11-373C9, *PDZK1* and RP11307C12, *CKS1B*), and 1q31.3 (RP11-32D17, *ASPM*).

For aCGH, genomic DNA was extracted from purified PC stored in Carnoy's fixative after washing with phospate buffered saline, using the Dneasy Blood and Tissue Kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. Pooled DNA from the peripheral blood of 10 healthy donors, sex-matched to the test sample, was used as a reference (Promega, Southampton, UK). Genomic DNA (1.9 μg) was hybridized to the 244k microarray (Agilent Technologies, Palo Alto, CA, USA) and processed according to the manufacturers' instructions. Slides were scanned using an Agilent Scanner and signal intensities from the generated TIF images were measured using Feature Extraction v9.5 and CGH Analytic v3.4.40, respectively (Agilent Technologies). Duplicated, amplified and deleted regions were defined using a 500 kb weighted moving-average window and the Aberration Detection Method-2 (ADM-2) algorithm of the CGH Analytics software with a threshold of 6.0.

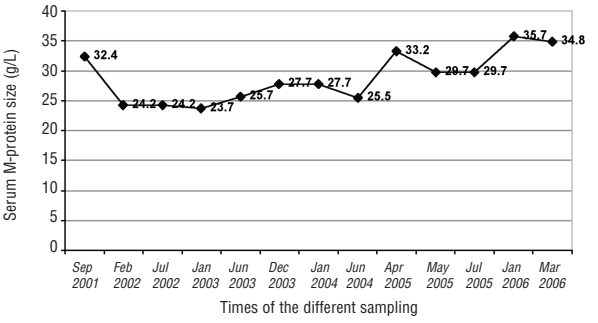

Figure 1. Chart showing the variation of the serum M-protein (IgA) from the diagnosis of asymptomatic smouldering multiple myeloma to diagnosis of symptomatic multiple myeloma.

Results and Discussion

This female patient was initially diagnosed with SMM at the unusually young age of 30 years; less than 3% of SMM patients are younger than 40 years of age.[4] Serial annual genetic analyses were carried out over a period of four and half years, at which time evolution to symptomatic MM had occurred. This gave a rare opportunity to compare the clinical course of the disease at the same time points as a detailed characterization of the genetic profile of her clonal PC at the different stages of her follow-up.

Clinical data

A number of diagnostic clinical parameters have been defined as predictive for disease progression to MM in patients with SMM: PP level, percentage of PC in BM, and IgA PP. Despite having evidence of IgA PP, the patient had <10% PC in the BM, and PP >30g/L. These levels have been associated with the lowest median rate and longest median time to progression in a large study of SMM patients.[4] Another study recognized two forms of SMM: *evolving* and *non-evolving* variants. Evolving SMM was characterized by a constant and progressive increase in PP, and by a previous MGUS phase in most cases, consistent with an early MM from the time of its appearance. The non-evolving variant was defined by a long-term stable PP level and usually no prior MGUS phase, consistent with a *de novo* SMM, stable until the occurrence of a postulated second hit leading to progression.[16] This patient did not show a progressive increase in PP (Figure 1). The

pattern of BM infiltration by PC, except for a slight increase, did not change significantly until the clinical diagnosis of MM. At this time, sheets of atypical plasma cells were present in the marrow. Overall, the patient conforms to the *non-evolving* type of SMM.

Genetic data

The main iFISH results are shown in Table 1. FISH indicated a hypodiploid karyotype for all samples (confirmed in sample 2 by aCGH and in sample 5 by cytogenetic analysis). At presentation all PC had a t(4;14) with loss of the derived chromosome 14 (der(14)). It is not clear whether loss of der(14) occurred at the time of t(4;14) formation or was a secondary change, although no signal patterns indicative of the balanced form were observed. No other abnormalities were detected apart from deletion of chromosome 13 in 18% PC. This is below the European Myeloma Network–agreed cut-off of 20%, but well above our laboratory false positive rate, indicating the presence of a low level population with this abnormality. The t(4;14) in both forms is associated with a poor outcome in MM.[13,17] In this patient at diagnosis it was not associated with an aggressive phenotype in agreement with previous reports of stable SMM with t(4;14).[18] Loss/deletion of chromosome 13 has also been reported as an early event in the pathogenesis of MM, but the relative timing of *IgH*

Table 1. Summary of the critical iFISH results for the five samples.

Samples (number, date, diagnosis)	Test	D13 (%PC)	IgHr (%PC)	t(4;14) Form (%PC)	CCND1	16q status	Dp53	1q status	1p32.3 status	t(8;14) & MYC split	CC
1 (09/2001) *SMM*	iFISH	(18%)	(98%)	Unbalanced (100%)	N	NT	N	N	N	N	Not set up
2 (06/2003) *SMM*	Metaphase analysis; iFISH, aCGH	(<20%)	(100%)	Unbalanced [1](100%)	N	16q23 deletion[1] (69%)	N	N[1]	N[1]	N	Normal: 46,XX[26]
3 (06/2004) *SMM*	iFISH	(40%)	(100%)	Unbalanced (100%)	N	16q23 deletion (89%)	N	NT	N	N	Not set up
4 (05/2005) *SMM*	iFISH	(74%)	(100%)	Unbalanced (100%)	N	16q23 deletion (97%)	N	N	deletion (42%)	MYC split (40%)†; t(8;14) negative	Not set up
5 (03/2006) *MM*	Metaphase analysis; iFISH	(100%)	(100%)	Unbalanced (100%)	N	NT	N	N	deletion (82%)	MYC split (100%)	Abnormal[2]

N indicates normal/negative result; IgHr, IgH rearrangement; unb, unbalanced; der(14), derivative(14) from an IgHt; NT, not tested. 16q status was deduced from loss of the c-MAF part of the Abbott IgH/MAF probe combination. [1]Result confirmed by array CGH. †The MYC split was detected with the Abbott MYC break apart probe combination; the t(8;14)(q24;32) was tested with the IgH/MYC, CEP 8 probe combination.
[2]Karyotype description: 40~41,X,X,del(1)(p13p3?2),add(3)(q2?6),der(8)t(8;13)(q24;q12),del(12)(q11),-13,-13,-14,-16,-20[cp2].

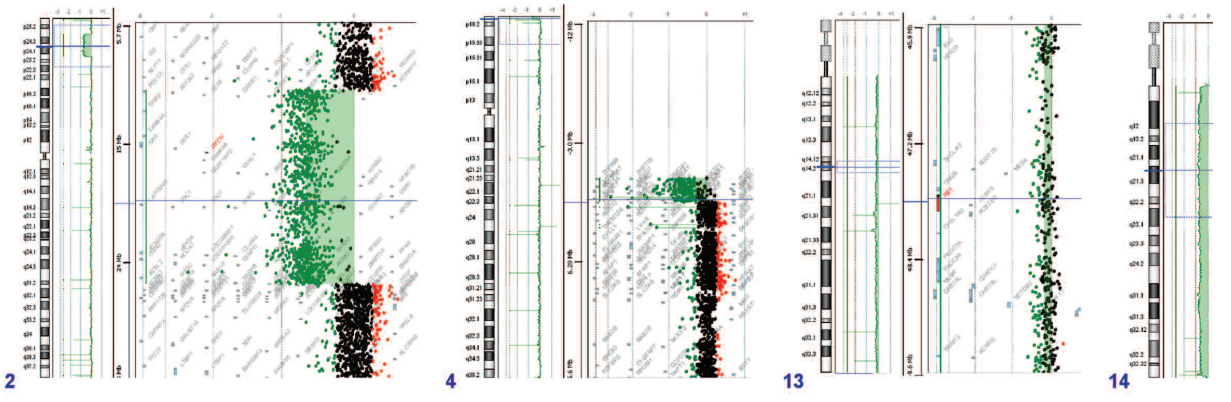

Figure 2. Array CGH analysis for chromosomes 2, 4, 13 and 14 with the G-banded ideograms (on the left). G-banded ideograms with the size of the abnormalities are shown for every chromosome; for the chromosomal regions 2p23-p25, 4p16 and 13q14 the plot of *calls* for every nucleotide is shown in detail. Gains are labeled in red, losses in green. ***Array results: Dim(1)(145.82[1q21.1/.2]-146.47[1q21.1/.2]); DimX2(1)(165.96[1q24.2]-165.99[1q24.2]); Dim (2)(11.19[2p25.1]-26.43[2p23.3]); Dim(2)(89.0[2p11.2]-89.31[2p11.2]); Dim (4)(0.04[4pter]-1.85[4p16.3]); Enh(5)(37.49[5p13.2]-37.5[5p13.2]); Dim (5)(172.59[5q35.1/.2]-172.6[5q35.1/.2]); Dim (6)(32.57[6p21.32]-32.63[6p21.32]); Dim (8)(6.93[8p23.1]-7.79[8p23.1]); Enh (8)(39.36[8p11.23]-39.51[8p11.23]); Dim (12)(34.42[12CEP]-132.39[12qter]); Dim(13)(18.07-114.12); Dim(14)(18.15-105.99); Enh(15)(18.68[15q11.2]-20.25[15q11.2]); Dim(16)(0.03[16pter]-10.97[16p13.13]); Dim(16)(34.06[16p11.2]-34.61[16p11.2]); Dim(16)(45.03[16q12.1]-88.69[16qter]); Dim(19)(47.99[19q13.13]-48.45[19q13.13]); Enh(22)(22.69[22q11.23]-22.73[22q11.23]); Dim(22)(37.68[22q13.1]-37.71[22q13.1]).*** (CNVs are written in italics). ***Dim*** and ***Enh*** describe loss and gain, respectively, with the genomic position of the abnormality shown in brackets after the chromosome. Trisomy and monosomy are described with ***Dim*** and ***Enh,*** respectively, with no additional breakpoint information.

translocations, deletion 13 and aneuploidy is not completely understood. In this case deletion 13 was clearly secondary to the *IgH* translocation. The increase in proportion of cells with deletion 13 was slow, particularly in the first three years (sample 1=18% to sample 3=40%, to 100% in sample 5 at diagnosis of MM). The array performed on sample 2 (2003) showed several copy number alterations (CNA). All were losses, involving chromosomal regions 2p, 4p, 12q, 16p, 16q, 19q and monosomies of chromosomes 13 and 14 (Figure 2); all chromosomal regions showed loss of one copy in all tumor cells, apart from chromosome 13 (Log_2 ratio=-0.18) consistent with the iFISH results exhibiting deletion 13 in only a minor population of cells. These data suggest that monosomy 13 was the latest numerical change to have been acquired by that time and that the abnormality might confer a proliferative advantage to the cell but not linked to the cause of malignant transformation.

Chromosome 1 abnormalities have previously been associated with the transition from MGUS/SMM to MM.[19] Particular emphasis has been given to the gain of 1q, which was found to be one of the most recurrent changes in a group of evolving SMM.[20] Based on these findings, we tested for 1q gains with three different probes spanning 1q21.1 to 1q31.3 which specifically mapped to the genes *PDZK1*, *CKS1B* and *ASPM*. No numerical 1q changes were detected by iFISH in any sample. As confirmation, no CNA of any region of chromosome 1 were found by array CGH in sample 2. The abnormal karyotype found at the time of diagnosis of MM showed an interstitial deletion on 1p (1p13 to 1p32). FISH analysis performed with two BAC clones located to 1p12 and 1p32.3, confirmed the involvement of 1p32.3 within the deletion. We then retrospectively tested the preceding samples for this abnormality. The first three were negative, but samples 4 and 5 showed 42 and 82% of PC, respectively, with the deletion. The deletion encompassed the *CDKN2C* locus. We have observed deletions of *CDKN2C* in 15% of newly diagnosed MM patients but rarely in MGUS or SMM; in MM the presence of the deletion was significantly associated with a poorer outcome.[21] These findings suggested a possible role of *CDKN2C* deletion in the mechanism of progression from MGUS/SMM to MM. The deletion observed in this patient was wider than the *CDKN2C* locus, therefore the possible importance of other genes within this chromosomal region cannot be ruled out. However smaller homozygous deletions only affecting *CDKN2C* have been found in three MM cell lines previously reported,[22] and one plasma cell leukemia analyzed by array CGH by our group (*data not shown*).

The karyotype showed a rearrangement involving 8q24, as an unbalanced translocation t(8;13). FISH confirmed that this rearrangement involved *MYC*. Similar to the 1p deletion, the *MYC* rearrangement was observed for the first time in sample 4 and at this time it involved only a sub-population of cells (40%). This rapidly increased to 100% in sample 5. Chromosomal rearrangements involving *MYC* are usually described as secondary events associated with tumor specific activation of one *MYC* allele, leading to enhanced proliferation. *MYC* translocations are absent or rare in MGUS or SMM, but occur in 15% of MM and 45% of advanced tumors.[7,23] Both *MYC* alterations and 1p deletions have been proposed to be late oncogenic events that occur at a time when MM is becoming more aggressive and eventually extramedullary.[24] Our findings, however, indicate that both abnormalities may be associated with the establishment of symptomatic MM.

Authorship and Disclosures

LC designed and performed research, analyzed data and wrote the first draft of the paper; GPD performed research and analyzed data; RKMP and DMS performed research; AGS, KHO, NCPC and CJH contributed to the analysis of the data; FMR designed and performed research, and analyzed data.

The authors reported no potential conflicts of interest.

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