Cyclin D1 expression in patients with multiple myeloma

X Troussard*,1,2, H Avet-Loiseau3, M Macro4, MP Mellerin3, M Malet1, M Roussel1 and B Sola2

1Laboratoire d’Hématologie, CHU Caen, France; 2UPRES-EA 2128, UFR de Médecine, Caen, France; 3Laboratoire de Cytogénétique Hématologique, Nantes, France; 4Service d’Hématologie Clinique, CHU Caen, France

Introduction: Chromosomal abnormalities are detected in 50 to 70% of patients with multiple myeloma (MM). By conventional cytogenetic analysis, a t(11;14)(q13;q32) is observed at a frequency of 3 to 14%.

Materials and methods: To demonstrate a cyclin D1 expression in MM patients or MM cell lines, 14 patients with multiple myeloma (MM) and nine human multiple myeloma cell lines (HMCL) were screened by a competitive RT–PCR and/or Northern blot analysis for cyclin D1 expression. Furthermore, we screened 10 MM patients with FISH to demonstrate a relationship between the cyclin D1 expression and the presence of the t(11;14).

Results: Five HMCL had a cyclin D1 overexpression: three of them had a t(11;14)(q13;q32) and two had extra copies of chromosome 11. A cyclin D1 expression was found at diagnosis in seven out of 14 untreated MM patients (50%). Out of 14 MM patients, FISH studies were performed in 10 patients. A t(11;14) was detected in three out of 10 patients and extra copies of chromosome 11 were found in two additional patients.

Conclusion: Cyclin D1 expression is a common event in MM patients (50%) and is associated either with a t(11;14)(q13;q32) or extra copies of chromosome 11. The prognostic role of the cyclin D1 expression and the level of this expression, as compared to other B-cell chronic lymphoproliferative disorders such as mantle cell lymphoma or hairy cell leukemia, remains to be determined in the pathogenesis of multiple myeloma.

Keywords: multiple myeloma; cyclin D1 overexpression; RT–PCR; t(11;14)(q13;q32)

Introduction

When using conventional cytogenetic analysis, the incidence of numerical and structural chromosomal abnormalities in patients with multiple myeloma (MM) is about 40% (ranging between 20 to 60%), depending on the series and the status of the disease. Chromosomal translocations involving band 14q32.33 occur in approximately 20% of cytogenetically abnormal cases. Among translocations to the IgH locus at 14q32, three loci are frequently involved, cyclin D1 on 11q13, FGFR3 or MMSET on 4p16 and c-maf on 16q23. The expression of these genes is thought to be dysregulated by juxtaposition of endogenous promoters to powerful regulatory regions of the IgH locus. Using conventional cytogenetics the translocation t(11;14)(q13;q32), involving the BCL1/cyclin D1 region at 11q13, is detected in mantle cell lymphoma and has been reported in other chronic B-lymphoproliferative disorders including splenic lymphoma with villous lymphocytes, rare cases of classic CD5 chronic lymphocytic leukemia, hairy cell leukemia and, at a frequency of 3 to 14%, in MM.1–5 In contrast, when using fluorescence in situ hybridization (FISH), tumor-specific rearrangements of the IgH gene are detected in 50 to 70% of MM cases, the partner sites being 11q13 in approximately 15 to 20% of cases.6,7 The cyclin-D1 gene product is one of the key proteins regulating progression through the G1 phase of the cell cycle and has been implicated in the molecular pathogenesis of human cancers. Progression through the cell cycle is controlled by regulatory proteins such as cyclins and their catalytic partners, ie, cyclin-dependent kinases. Cyclins D (D1,D2,D3) are not functionally redundant and appear strictly tissue-specific: lymphocytes express cyclin D3, cyclin D2 to a lesser extent, but no cyclin D1. Amplification of the CCND1 gene or overexpression of the cyclin D1 protein releases a cell from its normal control and could cause a transformation to a malignant phenotype. The t(11;14)(q13;q32) chromosomal translocation in MM cells is possibly associated with the overexpression of cyclin D1 gene.8 By immunohistochem-

*Correspondence: X Troussard, Laboratoire d’Hématologie, CHU de Caen, Côte de Nacre, 14033 Caen, France; Tel: +33 02 31 06 50 14; Fax: +33 02 31 06 50 15; E-mail: troussard-x@chu-caen.fr
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Human myeloma cell lines

Nine human MM cell lines (HMCL) were studied: LP1, NCI H929, RPMI 8226, L363, XG6, JJN3, MDN, U266 and Karpas 620. All these human HMCL have been previously reported, except MDN, which has been recently established in the laboratory from peripheral blood of a patient with a secondary plasma cell leukemia with t(11;14).12 With this expression was detected in the peripheral blood or polymerase chain reaction (RT–PCR), a cyclin D1 expression is correlated to the presence of a t(11;14)(q13;q32).

Competitive RT–PCR for analyzing the expression of cyclins D1, D2 and D3

The expression of cyclins D1, D2 and D3 was assayed by RT–PCR as described previously,12 with slight modifications. Total RNA was purified from peripheral lymphocytes with the TRIzol reagent as recommended by the supplier (Life Technologies, USA) and 2 µg of total RNA were reverse-transcribed with M-MuLV reverse transcriptase and random hexanucleotide primers (Life Technologies). One-tenth of the RT reaction product was incubated in the PCR reaction mixture which contained 2 mM MgCl₂, 0.2 mM dNTPs, 0.25 µM of each primer, and 1 U Taq Polymerase (Life Technologies). After 35 amplifications cycles (denaturation step at 94°C for 30 s; annealing step at 51°C for 45 s; elongation step at 72°C for 1 min), PCR products (20 µl) were analyzed onto 1.5% agarose gels. A single upstream primer DIS (5'-CTGGGCCATGAATCACTCTGGA-3') was common to the three cyclin D sequences (D1 nucleotides (nt) 385–404, D2 nt 256–275, D3 nt 403–422) when the three downstream primers were specific to their respective cyclin D: D1AS (5'-GTCACACTTGATCACTCTGG-3'), nt 403–422, 12 and D3AS (5'-CATGGCAAACCTTACCTGG-3', nt 590–609), and D3AS (5'-CCAGGAAATCATGTGCACAATC-3', nt 630–649). Because the upstream primer DIS is shared by all 3 cyclins, each PCR product serves as an internal competitor and the intensity of PCR product fragments is representative of each cyclin D gene transcription level.

Materials and methods

Table 1  Cyclin D1 expression in multiple myeloma

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Sex</th>
<th>Stage</th>
<th>Hb (g/dl)</th>
<th>Ig</th>
<th>Value (g/l)</th>
<th>K/L</th>
<th>Sample</th>
<th>% of plasma cells in bone marrow</th>
<th>RT–PCR</th>
<th>FISH</th>
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<tr>
<td>1</td>
<td>69</td>
<td>M</td>
<td>I</td>
<td>12.2</td>
<td>PBJs</td>
<td>NE</td>
<td>K</td>
<td>BM/PB</td>
<td>6</td>
<td>/–</td>
<td>+/–</td>
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<tr>
<td>2</td>
<td>74</td>
<td>F</td>
<td>PCL</td>
<td>7.7</td>
<td>Ig G</td>
<td>65</td>
<td>K</td>
<td>PB</td>
<td>90</td>
<td>+</td>
<td>+/–</td>
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<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>II</td>
<td>14.3</td>
<td>PBJs</td>
<td>NE</td>
<td>K</td>
<td>BM</td>
<td>11</td>
<td>–/–</td>
<td>+/–</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>M</td>
<td>II</td>
<td>10.6</td>
<td>NE</td>
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<td>K</td>
<td>BM</td>
<td>50</td>
<td>+/–</td>
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<tr>
<td>5</td>
<td>73</td>
<td>M</td>
<td>I</td>
<td>9.5</td>
<td>IgG</td>
<td>6</td>
<td>K</td>
<td>BM</td>
<td>12</td>
<td>+/–</td>
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<tr>
<td>6</td>
<td>73</td>
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<td>II</td>
<td>8.5</td>
<td>IgG</td>
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<td>K</td>
<td>BM</td>
<td>7</td>
<td>–/–</td>
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<tr>
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<td>K</td>
<td>PB</td>
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<tr>
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<tr>
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<td>K</td>
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<td>+/–</td>
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<tr>
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<td>IgG</td>
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<td>K</td>
<td>BM/PB</td>
<td>32</td>
<td>/–</td>
<td>+/–</td>
</tr>
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</table>

BM = bone marrow, K = kappa, L = lambda, PB = peripheral blood, PCL = plasma cell leukemia; ND = not determined, NE = not evaluated, NE* = patient without M protein.

The main characteristics of these patients are detailed in Table 1. Two patients presented a plasma cell leukemia (PCL), and 12 patients presented a MM (five patients with stage I, five with stage II and two patients with stage III). The median bone marrow plasmocytosis was 27% (range: 3 to 90%).
Fluorescence in situ hybridization (FISH)

Cytospin slides were fixed in methanol/acetic acid (3 vol/1 vol) for 30 min, air-dried and dehydrated in ethanol series. FISH experiments were performed as previously reported, using the following probes: CCND1 (Vysis, Downers Grove, IL, USA), labeled in red, Ig10 and 158A2, labeled in green. After overnight hybridization, slides were washed in 2xSSC for 4 min, air-dried, and counterstained with DAPI in antifade. Slides were then examined using an epifluorescence microscope (Axioskop 2, Zeiss, Iena, Germany), equipped with appropriate filters, as previously described.14

Results

Human myeloma cell lines (HMCL)

All three HMCL (MDN, U266, Karpas 620) with a t(11;14)(q13;q32) and a IgH-CCND1 fusion also had a cyclin D1 expression, using RT–PCR and/or Northern blot. Two additional HMCL (NCI H929 and RPMI 8226) had extra copies of CCND1 corresponding to extra chromosomes 11, as shown by hybridization experiments with a chromosome 11 centromeric probe (Vysis). Thus, a perfect correlation was found between abnormalities of CCND1 copy numbers or rearrangement with IgH, and the overexpression of cyclin D1 as detected by the RT–PCR assay. However, Northern blot analysis clearly showed that the level of cyclin D1 expression was incomparable in both situations, with a much higher level in HMCL harboring t(11;14) (Figure 1).

Patients

Cyclin D expression was tested by RT–PCR in bone marrow (n = 14) and peripheral blood (n = 7). The test was simultaneously performed in bone marrow and peripheral blood in four cases. RT–PCR analysis was not performed in two patients because RNA was insufficient. Cyclin D1 expression was found in seven out of 14 patients (50%) (Figure 2). In two patients, cyclin D1 overexpression was exclusively detected in bone marrow cells, with peripheral lymphoid cells only expressing cyclin D2 and cyclin D3. The intensity of cyclin D1 PCR fragment was similar to that observed in mantle cell lymphoma (not shown) and independent of the percentage of plasma cells in the bone marrow. To demonstrate the dependence of the cyclin D1 expression and the t(11;14), FISH studies were conducted on samples from 10 out of 14 MM patients, in which five samples showed cyclin D1 expression. A t(11;14) was detected by FISH in three out of 10 (30%). All three patients showed cyclin D1 expression by RT–PCR. Furthermore, extra copies of chromosome 11 were found in two additional MM patients with cyclin D1 expression. Two MM patients (UPN 14 and 15) had cyclin D1 expression: unfortu-
nately FISH studies were not performed. One other patient (UPN 1) was RT–PCR-negative and also had an extra copy of chromosome 11. These results suggest the presence of heterogeneity in 11q13 rearrangements. However, we were unable to specify the level of this expression in patients with either a t(11;14) or chromosome 11 abnormalities.

Discussion

A cyclin D1 expression was detected in HMCL (five out of nine) and MM patients (seven out of 14). The high incidence (50%) of cyclin D1 expression by RT–PCR and Northern blot analysis suggests that, in MM patients, the chromosome 11q13 expression were found. Taken together these results support the presence of heterogeneity in 11q13 rearrangements. An extra copy of chromosome 11 was detected. These results suggest that, in MM patients, the chromosome 11q13 breakpoints could be more heterogeneous, extending or not into the CCND1/cyclin D1 gene.

In patients with mantle cell lymphoma, the t(11;14)(q13;q32) is associated with cyclin D1 expression. A t(11;14) has also been reported in chronic B-lymphoproliferative disorders, including splenic lymphoma with villous lymphocytes, B-prolymphocytic leukemia, and apparently even in rare cases of classic CD5+ chronic lymphocytic leukemia. In MM, a t(11;14) has been identified by standard cytogenetic studies in 5% of cases. The t(11;14) is regarded as a very poor prognostic factor in MM patients and could be associated with an aggressive clinical course: short survival, high bone marrow labeling index and a high number of circulating plasma cells. The differences in the survival in cyclin D1-negative MM patients (one year survival: 81%) have to be compared to those who were cyclin D1-positive MM patients (63%). However, recent research suggests that the impact of the t(11;14) on the prognosis in patients with multiple myeloma is debatable. The median follow-up in our study was insufficient to analyze the survival in patients with cyclin D1 expression.

In conclusion, a cyclin D1 expression is frequently observed in patients with MM (40%). Competitive RT–PCR could be a rapid, reliable and routine procedure as compared to Northern blot analysis and/or immunohistochemical studies. In the majority of cases, cyclin D1 expression is associated with a t(11;14) or extra copies of chromosome 11. However the role of the cyclin D1 expression in the pathogenesis of multiple myeloma remains to be determined.

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References


