Chromosomal abnormalities clustering in multiple myeloma reveals cytogenetic subgroups with nonrandom acquisition of chromosomal changes

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TO THE EDITOR

Since the introduction of cytogenetic analyses into the study of plasma cell neoplasias, it has been repeatedly shown that multiple myeloma (MM) is a heterogeneous disease with regard to the underlying chromosomal abnormalities. In a very recent issue of *Leukemia*, Debes-Marun *et al*[1] applied statistical cluster analyses towards the abnormal karyotypes of 254 myelomas from the Mayo Clinic (Rochester, MN, USA). By this novel approach, they were able to identify recurrent patterns of chromosomal changes and to define cytogenetic subgroups of the disease.

We recently analyzed the karyotypes of 276 cases of MM from European institutions with statistical methods similar to those applied by Debes-Marun *et al*.[1] In all, 138 karyotypes (including 72 published in Calasanz *et al*[2]) were retrieved from the cytogenetic database of the Department of Genetics in Pamplona (Spain). Moreover, 138 karyotypes from another published European series[3] were included. The population studied by us is, thus, completely independent of the series studied by Debes-Marun *et al*,[1] and therefore constitutes a proper statistical validation set. Only MM with abnormal karyotypes were considered and those patients with a diagnosis of a secondary neoplasia were excluded. The presence or absence of chromosomal aberrations was recorded in all cases. A total of 30 recurrent cytogenetic variables present in more than 5% of the cases entered the statistical analyses, from which at least one was present in 250 cases.

Due to the nature of the variables, average linkage hierarchical clustering with three methods for computing distances, that is, Jaccard's, Dice's and Sokal's coefficients, were performed using the Stata, Clustan and SPSS software packages. The cluster trees derived from the three approximations were closely related (data not shown). Thus, from the statistical point of view, the methods applied in the present study and by Debes-Marun *et al*,[1] who used Jaccard's an Kendall's tau coefficients, are completely comparable.

Our results (Figure 1a) are in excellent concordance with those published by Debes-Marun *et al*.[1] One cluster was characterized by hypodiploidy and included complete or partial losses of chromosomes X, 1, 2, 4, 5, 6, 8, 11, 13, 14, 16, 17, 20, 21 and 22. This cytogenetic cluster, besides the losses of chromosome 13 (51/76 cases in this cluster), also contained structural abnormalities in 14q32 (19/76 cases in this cluster) and 22q (29/76 cases in this cluster), suggestive for translocations involving the immunoglobulin genes, *IGH* and *IGL*, respectively. Translocations involving *IGH* might also be hidden in the cases with monosomy 14 (32/76 cases in this cluster), which may indicate the loss of the der(14) from a cryptic *IGH* translocation as has been reported to be recurrent in t(4;14)(p16;q32).[4] A second major cytogenetic branch was characterized by a hyperdiploid karyotype due to multiple trisomies (gains or partial gains of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21). The 95 cases assigned to this cytogenetic cluster only rarely contained breakpoints in 14q32 (9/95 cases). The incidence of cytogenetic 14q32 breakpoints and monosomy 13 in the hyperdiploid subgroup was significantly lower than in the hypodiploid cluster (9/95 *vs* 19/76, $P=0.006$ and 19/95 *vs* 51/76, $P<0.001$, respectively, by Fisher's exact test). Moreover, the frequency of appearance of monosomy 14 and 22q abnormalities, suggesting the presence of *IG* rearrangements, was also significantly lower in the hyperdiploid branch (8/95 *vs* 32/76, $P<0.001$ and 7/95 *vs* 29/76, $P<0.001$, respectively). A third major cytogenetic branch was characterized predominantly by structural aberrations in 14q32, but wide lack of a considerable number of chromosomal losses. Out of 49 MM assigned to this cluster, 37 carried 14q32 aberrations.

Thus, clustering of cytogenetic abnormalities in MM reveals two clusters with frequent *IG* translocations with and without marked hypodiploidy as well as a distinct hyperdiploid cluster. In addition, minor cytogenetic clusters might exist, such as MM with duplications in 1q or loss of the Y chromosome. Nevertheless, the biological meaning of these minor clusters warrants further investigations.

Whereas it can be assumed that *IG*-driven oncogene activation is the major underlying genetic change in the nonhyperdiploid clusters, the genetic basis of the hyperdiploid MM group is widely unknown. Besides the rarity of recurrent translocations, this group is characterized by trisomies of mostly structurally intact chromosomes as well as a comparably favorable prognosis.[3–5] Remarkably, another lymphocyte-

Figure 1 Bidimensional hierarchical cluster analyses (using Jaccard's distance measurement) of chromosome abnormalities. Alterations are plotted along the top horizontal axis and tumor samples along the vertical axis. Chromosome aberrations are arranged in a dendrogram, which groups alterations by their frequency of coappearance. Tumor samples are arranged in the same way along the vertical axis, and those cases with the most similar patterns of chromosome aberrations are adjacent to each other. Black cells indicate the absence of alteration, whereas colored cells indicate the presence of a given chromosomal abnormality: red cells indicate the presence of chromosomal gains; green cells, chromosomal losses; dark blue cells, 14q32 aberrations; light blue cells, deletion 22q and monosomy 14 (suggestive of *IG* rearrangements); white cells, nonresolved aberrations. (a) The analysis of 250 MM cases carrying at least one of the cytogenetic variables under study. Variables defined as partial chromosome gains or losses include also trisomies or monosomies of the complete chromosome, for example, trisomy 3 is included in the variable DUP3Q. The branch marked in red contains partial or complete chromosomal gains characteristic of hyperdiploid MM. Partial or complete chromosomal losses, typical of hypodiploid MM, are colored in green. Along the vertical axis, cases colored in red and green are the hyperdiploid and hypodiploid respectively, whereas cases colored in blue indicate the cluster defined by 14q32 aberrations and wide lack of numerical changes. (b) Different patterns of complete chromosome gains (defined by the presence of the centromere) between hyperdiploid MM and ALL variants. Along the vertical axis, cluster marked in red is characterized by hyperdiploid MM, whereas the branch colored in yellow groups ALL cases by their trisomies pattern.

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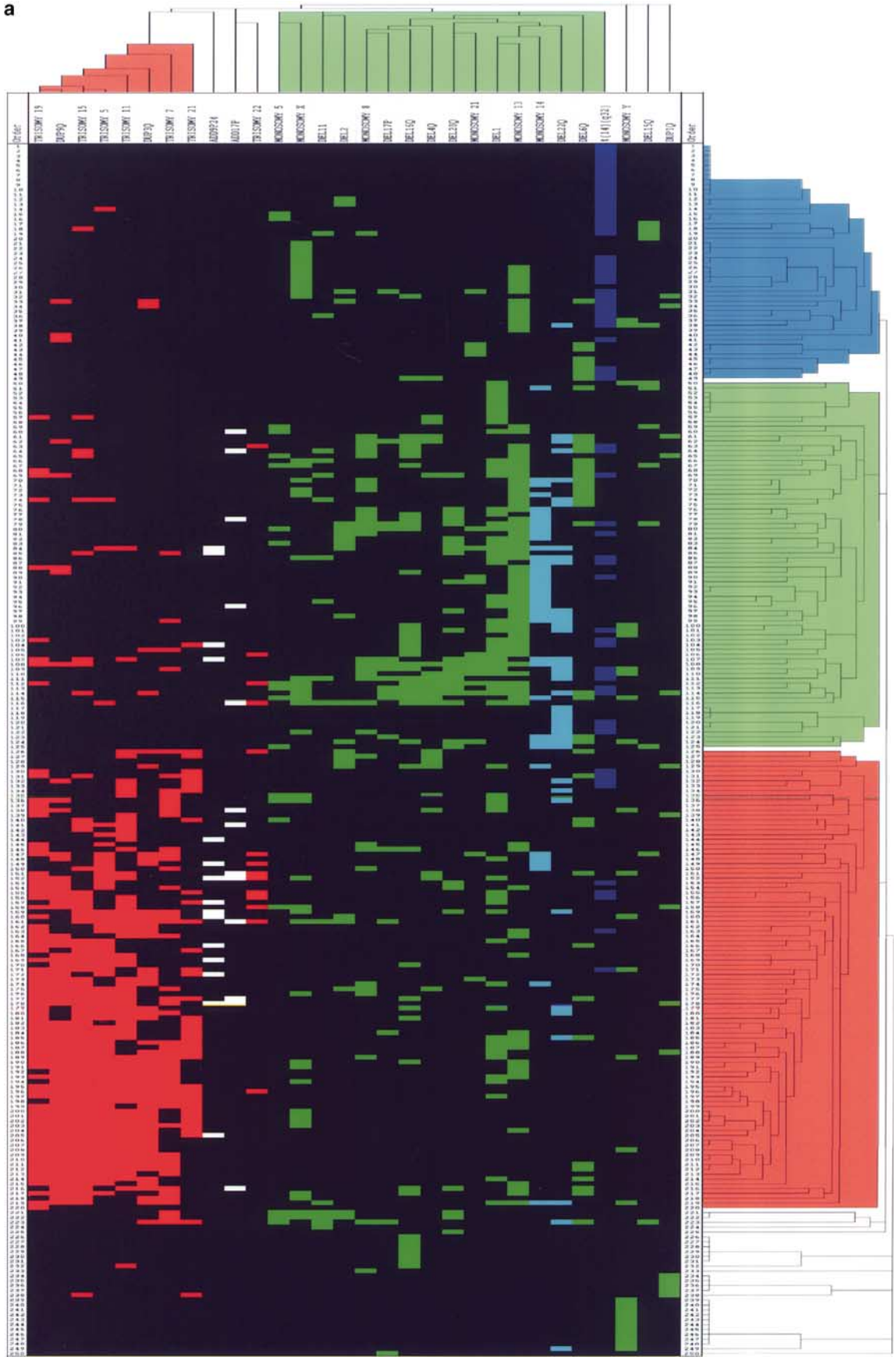
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Figure 1 Continued

derived neoplasia is characterized by the very same features, namely, hyperdiploid acute lymphoblastic leukemia (ALL) with more than 50 chromosomes.[6] This subtype of ALL, also recognized as a distinct entity by the WHO classification of hematologic neoplasia,[7] comprises approximately 20–25% of ALL and displays a characteristic gene expression profile.[8] It is intriguing to speculate that hyperdiploid MM and ALL may have in common a similar pathogenic mechanisms leading to the multiple chromosomal trisomies.

In order to compare the pattern of chromosomal gains between the hyperdiploid variants of MM and ALL, we performed clustering of karyotypes from these neoplasms. Based on the definition of hyperdiploid ALL by the WHO classification,[7] only those tumors entered this analysis, which contained more than 50 chromosomes (chromosomal gains defined by the presence of the respective centromere) and lacked any typical structural aberration like breakpoints in *IG* loci in MM or t(9;22)(q34;q11.2), 11q23 (*MLL*) rearrangements, t(1;19)(q23;p13.3) or t(12;21)(p13;q22) in ALL. Karyotypes from 23 hyperdiploid ALL with more than 50 chromosomes retrieved from the databases of the Department of Genetics in Pamplona and the Institute of Human Genetics in Kiel were compared to the 51 MM cases from the above-described hyperdiploid myeloma cluster fulfilling the inclusion criteria. Average linkage hierarchical clustering of the trisomies using Jaccard's coefficient resulted in a perfect differentiation between MM and ALL, (Figure 1b). Hyperdiploid MM were characterized by gains of chromosomes 3, 5, 7, 9, 11, 15 and 19, whereas hyperdiploid ALL showed predominately trisomies of chromosomes X, 4, 6, 8, 10, 14, 17, 18, 21 and 22 (Figure 1b). The different patterns of chromosomal aberrations in hyperdiploid MM and ALL suggest different but conserved mechanisms to underlie the occurrence of the trisomies in these disorders. Remarkably, the vast majority of chromosomes with frequent trisomies in ALL were recurrently lost in the hypodiploid chromosome cluster in MM. This might somehow suggest the existence of distinct sets of chromosomes with regard to chromosomal segregation.

In conclusion, the results presented here confirm in an independent series the presence of distinct cytogenetic clusters of MM described by Debes-Marun *et al*. The highly comparable results of the present and the published study,[1] which were obtained from independent MM series of more than 250 patients each from different continents, show the results to be valid. In the nonhyperdiploid subgroups of MM, the activation of oncogenes by *IG* translocations seems to be a major pathogenic mechanisms. Unfavorable prognostic factors like rearrangements in 14q32, monosomy 13, and 17p deletions and hypodiploidy[3–5] cluster together in the same subset of MM making it difficult to discern whether the unfavorable outcome is caused by one or by the conjunction of all these factors at the same time. The pathogenic mechanisms underlying hyperdiploid MM are hitherto unknown. Nevertheless, the distinct and widely nonoverlapping patterns of trisomies observed between MM and ALL patients suggest hyperdiploidy in both diseases to be caused by different but obviously nonrandom events, which might influence proper chromosome segregation in B-lymphocytes. The delineation of karyotypic patterns using statistical approaches provides a new perspective for studying cytogenetic variability and complexity in malignancies. Based on the statistical descriptions of cytogenetic evolution patterns, the hypothesis on the underlying cellular defects can be generated, which may warrant future experimental investigations.

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References

1 Debes-Marun CS, Dewald GW, Bryant S, Picken E, Santana-Davila R, Gonzalez-Paz N *et al*. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. *Leukemia* 2003; **17**: 427–436.
2 Calasanz MJ, Cigudosa JC, Odero MD, Ferreira C, Ardanaz MT, Fraile A *et al*. Cytogenetic analysis of 280 patients with multiple myeloma and related disorders: primary breakpoints and clinical correlations. *Genes Chromosomes Cancer* 1997; **18**: 84–93.
3 Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart CC, Groupe Francais de Cytogenetique Hematologique. Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood* 2001; **98**: 2229–2238.
4 Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA *et al*. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003; **101**: 4569–4575.
5 Calasanz MJ, Cigudosa JC, Odero MD, Garcia-Foncillas J, Marin J, Ardanaz MT *et al*. Hypodiploidy and 22q11 rearrangements at diagnosis are associated with poor prognosis in patients with multiple myeloma. *Br J Haematol* 1997; **98**: 418–425.
6 Forestier E, Johansson B, Gustafsson G, Borgstrom G, Kerndrup G, Johannsson J *et al*. Prognostic impact of karyotypic findings in childhood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. *Br J Haematol* 2000; **110**: 147–153.
7 Brunning RD, Borowitz M, Matutes E, Head D, Flandrin G, Swerdlow SH *et al*. Precursor B lymphoblastic leukaemia/lymphoblastic lymphoma. In: Jaffe ES, Harris NL, Stein H and Vardigan JV (eds). *World Health Organization Classification of Tumors. Pathology and Genetics of Tumor of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press, 2001, pp 111–114.
8 Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R *et al*. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 2002; **1**: 133–143.