Identification in several human myeloid leukemias or cell lines of a DNA rearrangement next to the c-*mos* 3′-end

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A characteristic DNA rearrangement, the loss of an *Eco*RI cleavage site next to the 3′-end of the human c-*mos* gene, has been found to be frequently present in DNA from transformed hematopoietic cells of the myeloid lineage but not in DNA from either normal or transformed cells of different tissue types. Three established cell lines, respectively a pro-monocytic line (CM-S) and two precursor granulocytic lines (My/K1 and My/K5), carry the same genome rearrangement, but not fibroblasts obtained from the marrow of the same patients. This DNA rearrangement is maintained in three different hybridomas derived by fusion of CM-S cells with normal human embryo hepatocytes.

Proto-oncogene c-mos *DNA rearrangement* *Myeloid leukemia*

1. INTRODUCTION

The association between certain human neoplasias and characteristic chromosomal abnormalities or genome rearrangements [1,2] with cellular oncogenes has suggested the possibility of the involvement of oncogenes in these recombinations and a possible role that they may play in producing various types of derangements in the structure and function of other cellular gene sequences during the multistep evolution of fully malignant tumors. c-*mos* is a cellular oncogene which possesses the potential to become malignant through incorporation in the genome of retroviruses [3] or undergoing alteration within the cell [4]. The coding region of c-*mos* and v-*mos* [5,6] has been identified. The human c-*mos* has been mapped in the q22 region of chromosome 8 [7].

In the course of experiments aimed at examining the arrangements of c-*mos* sequences in the genome of human cells from different tissue origin or types, we have identified a characteristic DNA rearrangement next to the 3′-end of the c-*mos* gene. This genome alteration has been found to be frequently present in DNA from transformed hematopoietic cells of the myeloid lineage.

2. MATERIALS AND METHODS

DNA samples were obtained from peripheral blood leukocytes of 19 normal volunteers, 15 patients with chronic lymphoid leukemia (CLL), 12 with acute lymphoblastic leukemia (ALL), 9 with acute myelo-monocytic leukemia (M4), 8 with acute monocytic leukemia (M5), and 12 with acute myeloblastic leukemia (M1) [8]. DNA was also obtained from 4 lymphoma tumors, 6 hepatomas, 5 lung carcinomas, 6 melanomas, 4 gliomas and normal human placenta. We also examined DNAs obtained from a number of established human cell lines, including 2 melanoma, 1 teratocarcinoma, 5 different EBV-infected lymphoblastoma cell lines, the 2 myeloid K-562 and HL-60 cell lines, the pro-monocytic CM-S line and the fibroblast CM/FB line, both isolated from the bone marrow of a patient with congenital hypoplastic anemia (syndrome of Diamond-Blackfan) [9,10], and 2 myeloid cell lines (My/K1 and My/K5) and 2 fibroblastoid-like cell lines (FB-K1 and FB/K5),

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recently isolated in this laboratory from the marrow of 2 patients with M4. DNAs were cleaved with specific endonucleases, electrophoresed through a 1% agarose gel, blot hybridized by the Southern procedure [11] with a ^{32}P-labelled probe, as specified in the text, and radioautographs of the filters were made.

3. RESULTS AND DISCUSSION

Souther transfers containing *Eco*RI-digested DNAs from 2 patients with M5 and 3 with M4 when hybridized with an *Ava*I-*Sma*I specific fragment probe from human c-*mos* revealed that in addition to the expected *mos* specific *Eco*RI fragment of 2.4 kb, there was an additional *Eco*RI fragment of 5.5 kb. The results of a representative experiment are shown in fig.1. The same results (see example in fig.2, lower right) were obtained with DNAs from the CM-S cell line and with 2 other myeloid lines, My/K1 and My/K5, but not from marrow fibroblasts of the same patients. None of the other DNAs revealed any evidence of a similar *mos* hybridization pattern. The same radiolabelled *mos* probe hybridized with either the expected *mos* specific restriction fragments in *Hin*dIII, *Kpn*I or *Bgl*II-digested DNAs from all DNA preparations (not shown). This suggested that no gross rearrangement had occurred within the *mos* gene. We therefore examined the possibility that a small gene alteration had resulted in the loss of the *Eco*RI site either 5′ or 3′ to the *mos* locus, generating an *Eco*RI-restriction fragment length polymorphism (RLFP) which is detected by this probe. To determine whether this RFLP was generated by loss of the *Eco*RI site immediately 5′ to the *mos* coding region (see fig.2, upper) a *Kpn*I-*Xba*I fragment (fragment II) which maps 5′ to this *Eco*RI site was used to rehybridize the same blot after removal of the previous probe. With loss of this 5′-*Eco*RI site one would expect both fragments I and II to hybridize to the same *Eco*RI restriction fragment. However, fragment II hybridizes only in the expected *Eco*RI restriction fragment of 3.0 kb, suggesting that the *Eco*RI site 3′ to the *mos* gene must be altered (fig.2, lower left).

The availability of the 2 CM-S and CM/FB lines, which both derive from the same CHA patient but only one (CM-S) carries the gene rearrangement next to the c-*mos* 3′-end, offered a convenient system to examine the persistence of this rearrangement. Although the CM-S line derived from a non-neoplastic patient, these cells underwent spontaneous malignant transformation in vitro, with successive passage of the cells in liquid culture [12]. CM-S cells retained an euploid karyotype for over 100 continuous passages and during this period they consistently failed to form colonies in agar and to give rise to tumors when inoculated into athymic mice. On prolonged culture, however, CM-S cells gradually acquired nonrandom chromosome abnormalities and became tumorigenic in these hosts. Conversely, CM/FB cells remained euploid and consistently failed to form colonies in agar or tumors in mice [10,13].

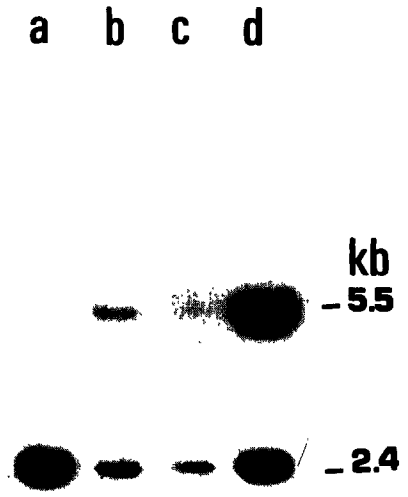

Fig.1. Southern blot analysis of *mos* sequence in *Eco*RI-digested DNA from normal human placenta (a), peripheral blood leukocytes of a patient with M4 (b) and 2 patients with M5 (c,d). High-M_r DNA (10 μg) was digested with the restriction enzyme, size fractionated on 0.7% agarose gel, and transferred to a nitrocellulose filter. Hybridization with the ^{32}P-labelled c-*mos* (an *Ava*I-*Sma*I fragment contained in the plasmid BR322) was in 10% dextran sulfate containing 50% formamide. Size markers indicate that bands were 2.4 and 5.5 kb, respectively.

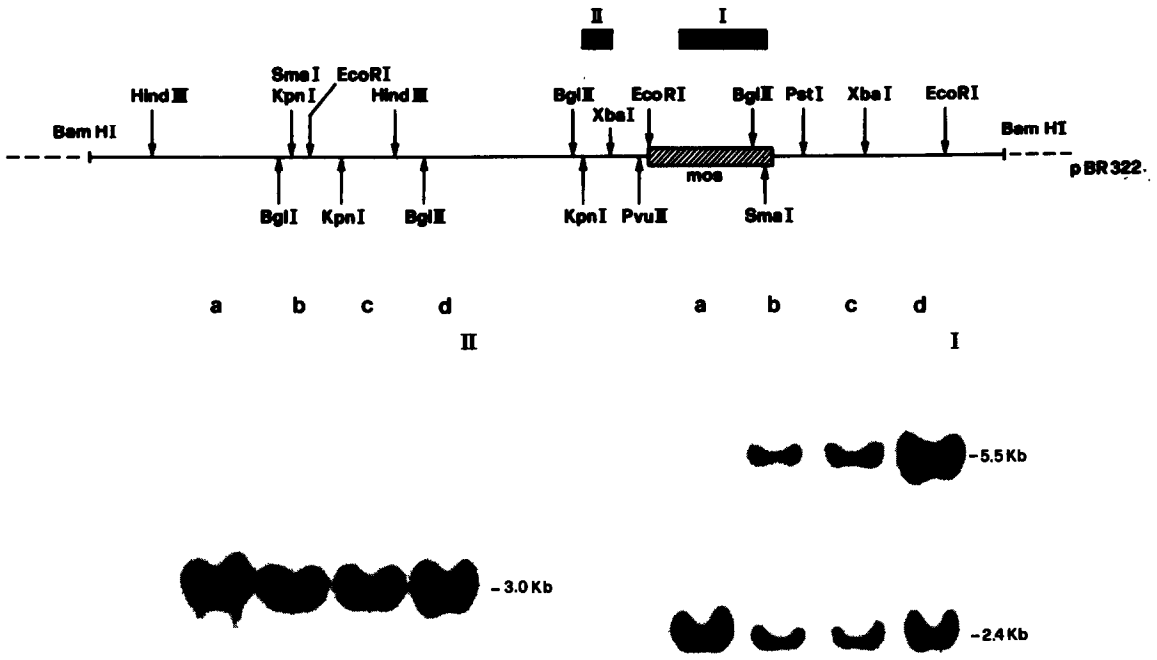

Fig.2. Physical map of the human c-*mos* locus coding region (upper), represented by the dashed bar. The strategy used to determine the structure and hybridization pattern of c-*mos* in the DNA preparations tested is shown. Black bar I delineates fragment I, an *Aval*-*Sma*I fragment in the c-*mos* locus and black bar II a *Kpn*I-*Xba*I fragment near the 5′-end of the c-*mos* gene. After *Eco*RI cleavage, upon hybridization with the ^{32}P-labelled probe (lower right) CM/FB DNA (a) reveals a single fragment of 2.4 kb, while respective DNAs from the pro-monocytic cell line CM-S (b), 2 myeloid lines My/K1 (c) and My/K5 (d) show an additional, identical fragment of 5.5 kb. After removal of the previous probe, the same DNA transfers were re-hybridized with the ^{32}P-labelled fragment II: all DNAs hybridized at the same expected 3.0 kb fragment (lower left).

Fig.3 shows that after *Eco*RI cleavage, DNA from euploid CM-S cells of the early passages or aneuploid, malignant cells of the late passages, showed 2 bands of hybridization with fragment I. Interestingly, the additional 5.5 kb band appeared more intense than the normal 2.4 kb band with DNA from cells of the late passages, as compared to DNA from cells of the early passages. With DNA obtained from different cell preparations, we have consistently observed this difference in intensity between the normal and additional band, suggesting that this rearrangement sequence may have undergone amplification. It is worthy of note that this increased intensity of hybridization shown with DNA from CM-S of the late passages correlated with the prevailing in culture of a clone of cells carrying trisomy of chromosome 8 [10,13]. Hybrids were also made by fusion (with PEG) [14] between a hypoxanthine phosphoribosyltransferase (HPRT) deficient clone of the late passage CM-S cells and human embryo hepatocytes in primary culture. Since the parental cells carried different histocompatibility (HLA.DR) antigens, enzyme and chromosome markers, hybrid clones isolated from agar in HAT-selecting medium could be easily identified (not shown). The results of a representative experiment of hybridization by the Southern procedure between *Eco*RI-cleaved DNA with the radiolabelled fragment I are shown in fig.4. We demonstrate here that the same DNA rearrangement downstream from the c-*mos* 3′-end, which is present in CM-S cells, was maintained in 3 out of 12 hybridomas tested in this experiment. As expected, DNA from the parental hepatocytes hybridized the fragment I probe with a single 2.4 kb band.

Poly(A)$^+$ RNA preparations were obtained from CM-S cells of an early (56th) or late (314th)

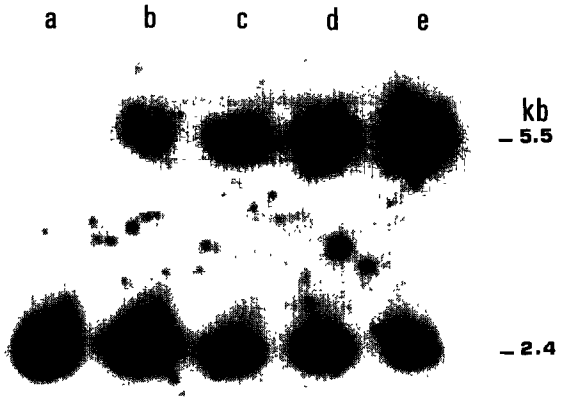

Fig.3. Southern blot analysis of c-*mos* sequence in *Eco*RI-digested DNAs from the CM/FB line (a) or the pro-monocytic CM-S line (passage 30, 156 and 290 at lane b–d, respectively). The 2.4 kb band represents the normal allele, while the 5.5 kb band represents the rearranged allele. The 5.5 kb band appears more intense than the normal 2.4 kb band with DNAs from CM-S cells of the later passage compared to DNAs from the earlier passages.

passage as well as from CM/FB cells. 15 μg poly(A)$^+$ RNA were analyzed by Northern hybridization [15], but no *mos* specific mRNA could be demonstrated. Whether the above gene rearrangement detected in CM-S cells, but not in fibroblasts of the same Diamond-Blackfan patient, had any relevance in the generation and development of the transformed phenotype of CM-S cells remains unknown.

In conclusion, we provide here evidence of a genome rearrangement resulting in the loss of an *Eco*RI cleavage site next to the 3′-end of the c-*mos* locus. This rearrangement appears to be not detected in a variety of normal tissues or cells of different types, nor to occur frequently in human disorders, except in certain myeloid leukemias. The same rearrangement has also been found in 3 established cell lines, all committed along the monocytic-granulocytic pathway. Altered c-*mos* sequences have been reported infrequently. A rearranged c-*mos* locus has been recently demonstrated [16–19] in murine plasmacytoma cell lines as well as in hybridomas. Activation of the c-*mos* gene was demonstrated in these cells and the altered gene transformed mouse fibroblasts in transfection experiments [16]. Multiple c-*mos* rearrangements were demonstrated in these myeloma cells, but all probably arose by the insertion within or next to the c-*mos* coding region [17–19] of a portion of the LTR sequence of an endogenous intracisternal-A particle genome (IAP) [17]. The latter are non-infectious retrovirus-like structures found regular-

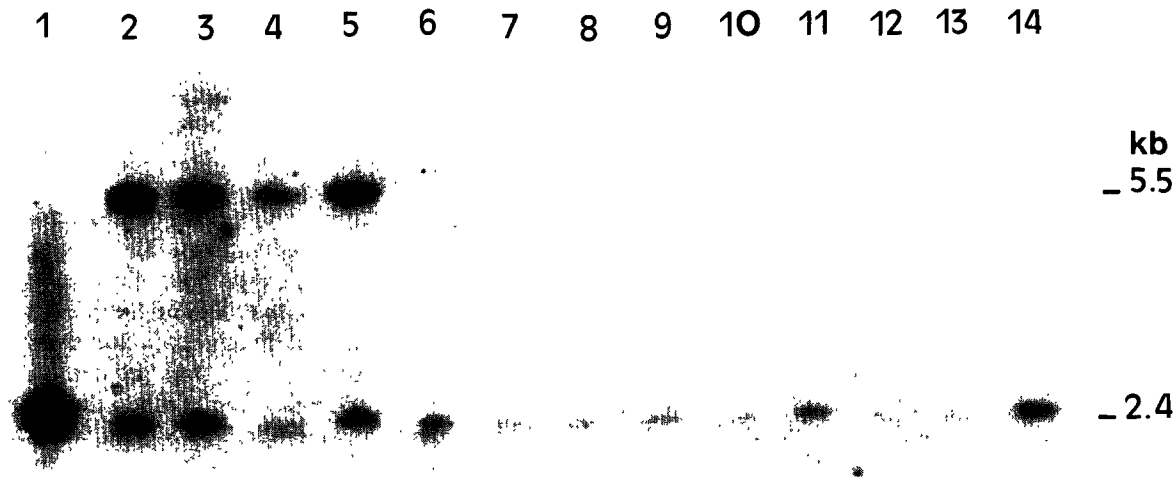

Fig.4. Southern blot analysis of c-*mos* sequences in DNA from primary cultures of normal human hepatocytes (1), an (HPRT-)clone of CM-S cells (2), and hybrids (3–12). High-M_r DNAs were digested with *Eco*RI endonuclease, size fractionated on 0.7% agarose gel (10 μg/lane) and transferred to a nitrocellulose filter. Hybridization with the radiolabelled c-*mos* fragment II probe was performed in 10% dextran sulfate containing 50% formamide.

ly in numerous copies in early mouse embryo [20–22], in plasmacytoma [23] or other mouse tumors [24]. IAPs seem to behave as movable elements with potential to alter profoundly gene expression either because they contain a transcriptional enhancer element [25] or because they change the structure of the chromatin and increase accessibility to enzymes participating in transcription [17]. More recently, Dr C.J. Larsen and his collaborators from the Hôpital St. Louis in Paris have found an *Eco*RI-RFLP downstream from the c-*mos* 3′-end in DNA of one patient with breast carcinoma (personal communication). Since suitable probes 3′ to the human c-*mos* coding region are not yet available, we are unable to confirm whether the common DNA rearrangement that we have described above in human myeloid cells is due to a small deletion, base alteration or gene insertion. It could be associated with the organization and assembly of a cellular gene coding for a stage-differentiation protein of precursor myeloid cells.

The cloning from CM-S and CM/FB cells of the two 2.4 and 5.5 kb sequences both hybridizing the c-*mos* specific probe, should allow the identification of this DNA rearrangement and clarify the significance of this event.

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