Localization of the cellular retinoic acid binding protein (CRABP) gene relative to the acute promyelocytic leukemia-associated breakpoint on human chromosome 15

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Summary. A human genomic fragment comprising the cellular retinoic acid binding protein (CRABP) gene was isolated. By using a panel of somatic cell hybrids, this gene could be assigned to human chromosome 15. Subsequently, a possible involvement of the CRABP gene in translocation (15;17) (q22;q11) positive acute promyelocytic leukemia (APL) was investigated. Although transposition of the CRABP gene could be demonstrated, we did not observe any gross CRABP rearrangement in a series of primary APL patients, nor in the acute myeloblastic leukemia cell line HL-60. Thus, the observed lack of CRABP expression in these leukemic cells may not be caused by disruption of its gene. CRABP maps to the region 15q22-qter.

Introduction

Retinoic acid (RA), a biologically active metabolite of retinol (vitamin A), plays an important role in various growth and differentiation processes during both embryonic development and adult life (Lotan 1980; Omori and Chytil 1982; Chytil 1984; Oikarinen et al. 1985). RA has also been reported to induce in vitro differentiation of cancerous cells, including human and murine embryonal carcinoma cells (Strickland and Mahdavi 1978; Andrews et al. 1986), the acute myeloblastic leukemia cell line HL-60, the monoblastic leukemia cell line U-937 (Breitman et al. 1980; Hozumi 1982; Dalton et al. 1988) and blasts from acute myeloid leukemia (AML) patients in primary culture (Breitman et al. 1981; Honma et al. 1983). Consequently, RA has on various occasions been

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suggested as a potent agent for the treatment of AML, in particular acute promyelocytic leukemia (APL) patients (Breitman et al. 1981; Meng-er et al. 1988; Hassan and Rees 1990).

The molecular mechanisms underlying RA action are currently the subject of intensive investigations. Recent work indicates that RA acts as an endogenous signal that regulates gene expression via nuclear receptors (RARs) in a dose-dependent way (Edwards and McBurney 1983; Evans 1988). The discovery of an RA gradient in the developing embryo by Thaller and Eichele (1987) is in full agreement with this notion. In addition to these nuclear receptors, cellular (cytoplasmic) RA-binding protein (CRABP) seems to play a role in RA-mediated signal transduction. During embryonic development, the spatial and temporal expression of the CRABP gene appears to be strictly regulated (Vaessen et al. 1989, 1990; Perez-Castro et al. 1989; Maden et al. 1988, 1989a). Hence, it has been suggested that CRABP could be involved in the formation of gradients of RA across various developing tissues (Maden et al. 1989b).

Here, we report the isolation of the human homolog of the CRABP gene and its chromosomal localization. Subsequently, a possible involvement of CRABP in the APL specific translocation (15;17) (q22;q11) (Larson et al. 1984) was evaluated. Interestingly, although both primary APL cells and the acute myeloblastic leukemia-derived cell line HL-60 are responsive to differentiation-induction by RA, they seem to be deficient in the cellular RA-binding protein (Douer and Koeffler 1982; Jetten et al. 1987; Allegretto et al. 1983; Largman et al. 1989; C. Chomienne, personal communication). By using our newly isolated CRABP probe, we have studied its expression at the RNA level in HL-60 cells prior to and after differentiation-induction.

Materials and methods

Cells and culture conditions

The panel of human-rodent hybrid cell lines used for the chromosomal localization of the CRABP gene has been described before (Geurts van Kessel et al. 1983), as have the t(15;17)-derived cell hybrids C73/PJT2A1 and P12.3B6 (Sheer et al. 1983; van Tuinen et al.1987). The hybrid (and parental) cells were grown in F10 or DMEM medium according to standard procedures. Chromosome analyses, using GTG-banding or R-banding after heat denaturation, and DNA extractions were carried out on the same batches of cells.

HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, glutamine $(2\,\mathrm{m}M)$, penicillin (100 U/ml) and streptomycin (100 µg/ml). After addition of $10^{-6}\,M$ all-trans RA (from a 3 mg/ml stock in dimethyl sulfoxide; DMSO) or 1% DMSO, the cells were allowed to differentiate for 6 days (Breitman et al. 1980). During this period, the medium was replenished twice. As estimated from May Grünwald-Giemsa stained cytospin preparations, over 70% of the cells had differentiated into granulocytes and monocytes, respectively.

Origin of the human CRABP probe

Screening of a human genomic phage library (CMLO, kindly provided by Dr. G. Grosveld) with mouse and chicken CRABP cDNA probes (Vaessen et al. 1989, 1990) yielded several CRABP-positive clones. A 700-bp repeat-free human XhoII fragment was subcloned into the BamHI site of pTZ18R and its nucleotide sequence was determined via the dideoxy chain termination method (Sanger et al. 1977).

Southern analysis

DNAs were extracted according to standard procedures (Jeffreys and Flavell 1977). Following cleavage with restriction enzymes, DNA fragments were separated by electrophoresis on agarose gels and transferred to Genescreen (NEN) filters (Southern 1975). The human CRABP probe used was labeled with ^{32}P using random priming as described by Feinberg and Vogelstein (1983). Hybridizations were carried out overnight at 65°C in the presence of 0.5 M phosphate, 7% SDS and 1 mM EDTA. Filters were washed at 65°C, 3 × 5 min, in 40 mM phosphate/0.1% SDS, followed by a 30-min wash in 10 mM phosphate 0.1% SDS. X-ray films were exposed using intensifier screens.

Northern analysis

Total RNA was isolated from cell lines using the LiCl/urea method described by Auffray and Rougeon (1980), electrophoresed on 1% agarose gels in the presence of formaldehyde, and transferred to Genescreen filters (Maniatis et al. 1982). Prior to electrophoresis, ethidium bromide was added to the RNA samples in order to allow visualization of the ribosomal RNAs in the gel (Fourney et al. 1989). Probe labeling, hybridization (in the presence of 10% dextran sulfate) and washing conditions were essentially as described above.

Results and discussion

A 700-bp repeat-free human XhoII CRABP fragment was subcloned into the Bam-Hl site of pTZ18R and its nucleotide sequence was determined (details will be published elsewhere). Comparison of this sequence with the murine cDNA sequence revealed a 92% homology in the coding region from nucleotide 380 to 464 (num-
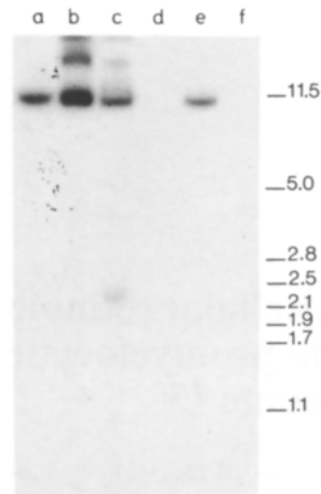

Fig.1. Southern blot analysis of EcoR1-digested normal human fibroblast (a), myeloblastic leukemia cell line HL-60 (b), chromosome-15-only somatic cell hybrid HORL-1 (c), 15/17 translocation-derivative positive hybrid C73/PJT2A1 (d), 17/15 translocation-derivative positive hybrid P12.3B6 (e) and control mouse (f) DNAs, after hybridization with the human CRABP probe. The slow migrating (> 10 kb) bands (b, c) are a result of incomplete digestions (not observed in other similarly obtained preparations). The indicated molecular weight markers are derived from co-electrophoresed PstI-digested bacteriophage lambda DNA (in kb)

bering according to Vaessen et al. 1989). Nucleotide 464 represents an exon/intron boundary, which is apparently conserved in the human and bovine genome (Shubeita et al. 1987). Comparison of the predicted amino acid sequence revealed that our human clone codes for a protein identical to CRABP from other species (bovine: Shubeita et al.1987; mouse: Vaessen et al. 1989). Hence, the 700-bp subclone was named HuCRABP and used as a human CRABP-specific probe in the present study.

For the chromosomal localization of the human CRABP gene, a panel of 19 well-defined (Geurts van Kessel et al. 1983) human-rodent somatic cell hybrids was used. High molecular weight DNA extracted from these cells and control rodent (hamster, mouse) and human cells was cleaved with EcoR1 and, after Southern blotting, analyzed for the presence of CRABP sequences using HuCRABP DNA as a probe. This probe hybridizes to a human EcoR1 fragment of approximately 10 kb (see Fig. 1) and does not hybridize to mouse or hamster DNA under the stringency conditions used. A high concordancy score (95%) was observed for chromosome 15 (Table 1), whereas for all other human chromosomes, concordancy scores were invariably lower. Therefore, we conclude that the CRABP gene must reside on human chromosome 15. The only discordant clone (see Table 1) had retained chromosome 15 in a low percentage of cells (10%). This assignment is not in agreement with a previous report in which a bovine cDNA was used as a probe under low stringency conditions (Nilsson et al. 1988). The most likely explanation for this descrepancy is that the previously observed hybridization signals were the result of cross-hybridization of the bovine probe with a related gene(s) on human chromosome 3.

Table 1. Correlation between the presence or absence of human chromosomes and the CRABP gene in 19 human-rodent somatic cell hybrids

Chromosome	Chromosome/CRABP; no. of clones				Discordancy (%)
	+/+	+/−	−/+	−/−	
1	4	5	6	4	58
2	2	2	8	7	53
3	5	4	5	5	47
4	6	3	4	6	37
5	5	5	5	4	43
6	5	4	5	5	47
7	5	2	5	7	37
8	7	6	3	3	47
9	6	6	4	3	53
10	3	4	7	5	58
11	4	5	6	4	58
12	7	2	3	7	26
13	7	5	3	4	42
14	8	5	2	4	37
15	10	1[a]	0	8	5
16	7	6	3	3	47
17	9	5	1	4	32
18	4	5	6	4	58
19	7	5	3	4	42
20	9	7	1	2	42
21	7	7	3	2	53
22	7	6	3	3	47
X	6	2	4	7	32

[a] This hybrid clone contains chromosome 15 in a low percentage of cells (10%)

In order to establish the localization of the CRABP gene relative to the acute promyeolocytic leukemia (APL)-associated t(15;17)(q22;q11) breakpoint on chromosome 15, hybrid cell lines derived from the fusion of cells from two t(15;17)-positive APL patients, having retained either the 15/17 or the 17/15 translocation derivative in the absence of a normal chromosome 15, were subjected to Southern blot analysis. A human-chromosome-15-only hybrid (HORL-1, N. Spurr unpublished result), normal human fibroblasts and the acute myeloblastic leukemia cell line HL-60 were also included in this analysis. Figure 1 shows that HORL-1 (lane c) is positive for CRABP; this confirms our localization of this gene on human chromosome 15. Similarly hybridizing EcoR1 fragments were found in normal human fibroblast (lane a) and HL-60 (lane b) DNAs, indicating that no gross rearrangements have occurred in this CRABP sequence in the leukemic cell line HL-60. Hybrid C73/PJT2A1 (lane d), which contains the 15/17 derivative of the t(15;17), is negative, whereas P12.3B6 (lane e) containing the 17/15 derivative of this translocation is positive for CRABP. From these results, we conclude that, as a result of the t(15;17), CRABP is transposed from chromosome 15 to chromosome 17 in APL. As in HL-60 DNA, the APL-derived hybrids show no aberrantly hybridizing fragments. Similar results were obtained with

BamHI-cleaved (hybrid) DNAs. In addition, DNAs from another eight t(15;17)-positive APL patients were tested for the presence of gross rearrangements in the CRABP gene via EcoR1 or BamH1 digestion and Southern blotting. Again, no aberrantly hybridizing fragments were observed (not shown). Apparently, the break in t(15;17) positive APL does not occur within restriction fragments hybridizing to our CRABP probe.

Previously, it was reported that, at the protein level, no CRABP expression could be detected in the myeloblastic leukemia-derived cell line HL-60 (Douer and Koeffler 1982; Jetten et al. 1987; Largman et al. 1989). In order to extend these studies to the RNA level, a Northern blot analysis was carried out using RNA extracted from exponentially growing HL-60 cells and HL-60 cells that were induced to differentiate for 6 days using 10^{-6} M all-trans RA (granulocytic differentiation) or 1% DMSO (monocytic differentiation) according to standard protocols (Breitman et al. 1980). By using HuCRABP as a probe, no CRABP transcripts could be detected in any of the HL-60 derived RNAs (not shown). DMSO induction of CRABP was previously observed in a murine embryonal carcinoma (EC) cell system (Vaessen et al. 1989). In HL-60 cells, however, DMSO exposure does not result in expression of detectable amounts of CRABP mRNA. A positive CRABP signal was observed in a co-electrophoresed RNA sample extracted from the human EC cell line N-Tera 2 (Andrews et al. 1986). Since the CRABP gene does not seem to be grossly disrupted in HL-60 cells (or primary APL samples), lack of CRABP expression in these cells may be caused by transcriptional repression.

In conclusion, we have isolated genomic fragments corresponding to the human CRABP gene. Subsequently, this gene could be localized on human chromosome 15, distal to the APL-associated breakpoint (region 15q22-ter). As a result of this translocation, the CRABP gene does not seem to be grossly rearranged. Further experiments are required to evaluate a possible role of CRABP in (aberrant) myeloid differentiation processes and the development of APL.

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Note added in proof. After submission of this manuscript, three independent groups reported that the APL breakpoint at chromosome 17 is located within the retinoic acid receptor-α gene.