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cDNA cloning and chromosomal localization of the human β-adrenergic receptor kinase

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The β-adrenergic receptor kinase (βARK) mediates agonist-dependent phosphorylation of the β_2-adrenergic and related G protein-coupled receptors. A cDNA encoding bovine βARK has recently been isolated. In this work we have isolated a cDNA encoding human βARK from a retinal cDNA library. The cDNA encodes a protein of 689 amino acids with an overall 98.0% amino acid and 92.5% nucleotide identity with bovine βARK. Chromosomal location of the human βARK gene was determined by correlating the presence of the βARK locus with retention of a specific human chromosome in a rodent-human hybrid panel. This analysis revealed that the human βARK locus segregated with the long arm of chromosome 11, centromeric to 11q13.

β-Adrenergic receptor kinase; Human; Chromosome; Desensitization; Phosphorylation; cDNA

1. INTRODUCTION

The mechanisms which regulate G protein-receptor function are diverse and include rapid events which lead to receptor/G protein uncoupling as well as more long term events which eventually result in receptor degradation [1,2]. One of the major mechanisms implicated in the initial rapid uncoupling phase of desensitization appears to involve receptor phosphorylation. Utilizing the β_2-adrenergic receptor-coupled adenylyl cyclase system as a model it has been shown that receptor phosphorylation is mediated by at least two distinct protein kinases. The cAMP dependent protein kinase phosphorylates the β_2-adrenergic receptor (β_2AR) at two distinct sites and may be largely responsible for mediating desensitization observed at low agonist concentrations [3–5]. In contrast, the β-adrenergic receptor kinase (βARK) phosphorylates the β_2AR to a stoichiometry of ~8 mol phosphate/mol receptor and appears to mediate agonist-specific desensitization observed at high agonist concentrations [5–7].

βARK is a ubiquitous cytosolic enzyme which specifically phosphorylates the activated form of the β-adrenergic and related G protein-coupled receptors [7–9]. βARK, purified from bovine brain, consists of a single subunit of 80 000 Da which is not activated by a wide variety of kinase stimulatory agents [7]. Recently, we have isolated a cDNA encoding bovine βARK [10]. This cDNA encodes a protein of 689 amino acids (79.6 kDa) with a centrally localized catalytic domain which has highest homology with S6 kinase, the cAMP dependent protein kinase and protein kinase C. When inserted into a mammalian expression vector and expressed in COS-7 cells the βARK cDNA encoded a protein which specifically phosphorylated the agonist-occupied form of the β_2-adrenergic receptor and more weakly the light activated form of rhodopsin [10]. While significant work has been performed on bovine βARK little is known about the human form of this enzyme. In this work, we have used the bovine βARK cDNA to screen a human retinal library and isolate the cDNA encoding human βARK. The human βARK gene was then localized to the long arm of chromosome 11 by Southern hybridization analysis of a rodent-human hybrid panel.

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2. MATERIALS AND METHODS

2.1. *Isolation and sequencing of the human βARK cDNA*

A human retinal cDNA library (10^6 total clones) was screened with two ^{32}P labeled oligonucleotide probes which had previously been used to isolate the bovine βARK cDNA ([10], oligos no. 1, 2). The probes were hybridized for 48 h at 42°C in a solution containing 6 × saline sodium citrate (SSC), 5 × Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS) and 100 μg/ml denatured salmon sperm DNA. The filters were washed initially with 2 × SSC, 0.1% SDS at 60°C and the stringency was then increased by decreasing the salt concentration. A total of 5 clones which hybridized with both oligos at high stringency (0.2 × SSC, 60°C) were isolated. The isolated cDNAs were excised with *Eco*RI and inserted into the vector pSP 65 for sequencing.

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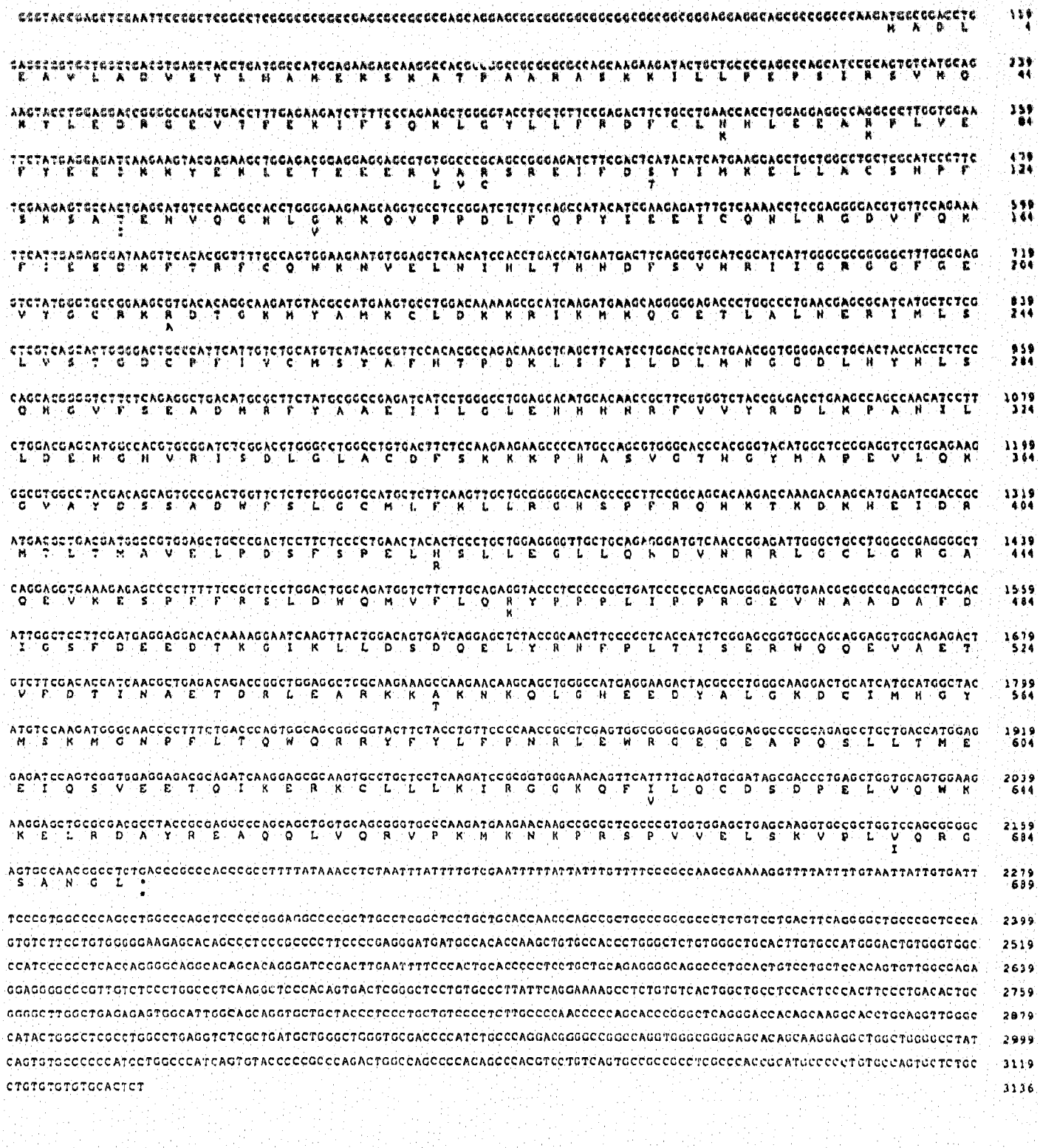

Fig. 1. Nucleotide and deduced amino acid sequence of human βARK. The nucleotides and amino acids are numbered on the right hand side. Shown also are the amino acids which differ in the bovine βARK sequence, in total there are 14 differences, six of which are conservative substitutions.

DNA sequencing was performed using the dideoxynucleotide chain termination method of Sanger [11].

2.2. *Cells*

Isolation, propagation and characterization of parental cells and somatic cell hybrids used in this study have been described [12]. The presence of specific human chromosomes or regions of chromosomes has been confirmed by DNA hybridization using probes for genes assigned to specific human chromosome regions.

2.3. *Southern blot analysis*

DNAs from human peripheral blood lymphocytes (PBL) or human cell lines, mouse cell lines, and rodent-human hybrid cell lines were extracted by cell lysis, proteinase K digestion, phenol extraction and ethanol precipitation. Cellular DNAs were digested with an excess of the restriction enzyme *Eco*RI, sized in 0.8% agarose gels and transferred to nylon filters and hybridized as described previously [13]. After hybridization, the filters were washed (0.1 × SSC, 0.1% SDS, 65°C) and exposed to Kodak XAR-5 film with intensifying screens.

3. RESULTS AND DISCUSSION

10^6 independent clones from a human retinal cDNA library were initially screened with two labeled bovine βARK oligonucleotides. A total of 5 clones which hybridized with both oligonucleotide probes at high stringency (0.2 × SSC, 60°C) were isolated. The clones were purified and when excised with *Eco*RI four of the clones yielded a single insert band ranging in size from 1.3 to 3.5 kb. The fifth clone when excised yielded two DNA bands of 0.35 and 0.8 kb due to the presence of an *Eco*RI site within the insert. The 0.35, 0.8 and ~2.8 kb of the 3.5 kb clone were sequenced on both strands and compared to the sequence of bovine βARK (Fig. 1). The open reading frame of the human βARK cDNA encodes a protein of 689 amino acids which has 98.0% amino acid and 92.5% nucleotide identity to bovine βARK. With conservative amino acid substitutions the homology increases to 98.8%. The sequenced portion of the human βARK clone contains 107 bp of 5′ untranslated sequence which has ~50% homology with the 84 bp of 5′ untranslated sequence from the bovine βARK cDNA [10]. This homology is largely due to the GC richness of both 5′ untranslated sequences (82% GC for human, 86% for bovine). In addition, the 3′ untranslated regions of the human and bovine clones contain a potentially interesting stretch of ~125 bp which are 95% identical at the nucleotide level. This stretch starts immediately after the stop codon in the human clone (bp 2178–2304) and 24 bases after the stop codon in the bovine clone (2180–2307). The 3′ localization and high degree of conservation of this region suggests that it may play some regulatory role in translation of the βARK message [14].

More than 20 rodent-human hybrids were then exam-

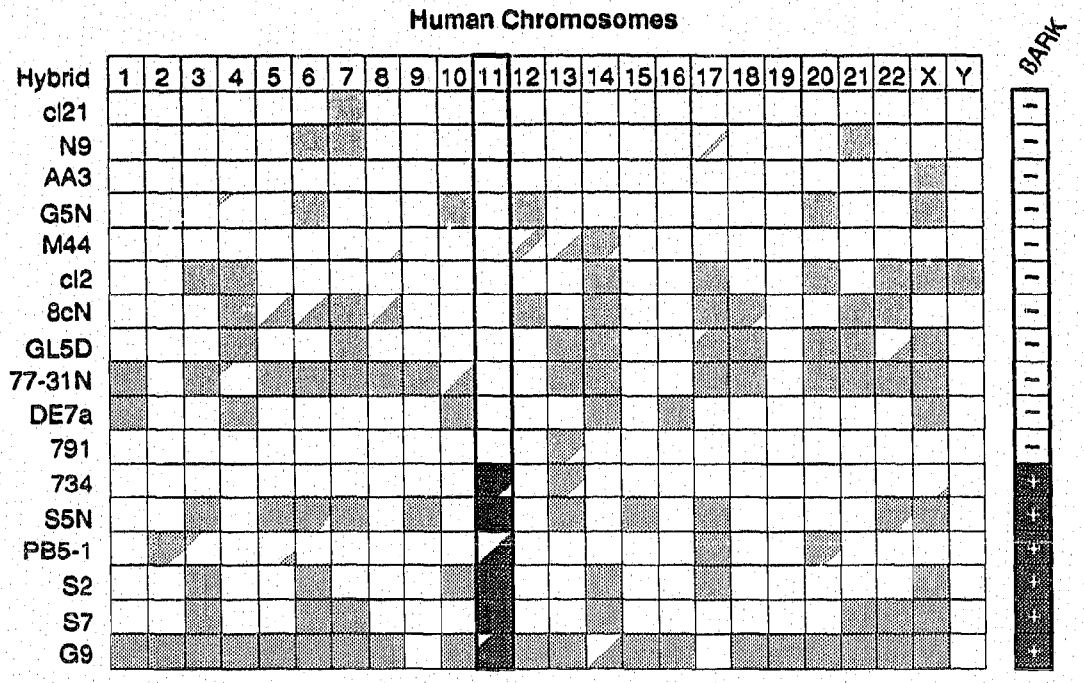

Fig. 2. Presence of the βARK gene in 17 rodent-human hybrids. A completely stippled box indicates that the hybrid named in the left column contains the chromosome indicated in the upper row; lower right stippling indicates presence of the long arm (or part of the long arm, indicated by a smaller fraction of stippling) of the chromosome shown above the column; upper left stippling indicates presence of the short arm (or partial short arm) of the chromosome listed above the column; no stippling indicates the absence of the chromosome listed above the column. The column for chromosome 11 is boldly outlined and stippled to highlight correlation of presence of this chromosome (or region of chromosome) with presence of the probe. The pattern of retention of the βARK gene in the panel is shown in the column to the right of the figure where presence of the probe in the hybrid is indicated by a stippled box with a plus sign and absence of the probe is indicated by an open box enclosing a minus sign.

Fig. 3. The human βARK gene maps to 11cen→11q23. *Eco*RI cleaved DNA (~10 μg/lane) from mouse (lane 1), human (lane 2), hybrid S2 retaining human chromosomes 3, 6, 10, 11, 14, 17 and X (lane 3), hybrid S7 retaining 3, 6, 7, 11, 14, 21, 22 and X (lane 4), hybrid PB5 retaining partial chromosome 2, partial 5, 8, 11q, 17, and partial 20 (lane 5), hybrid G9 retaining chromosomes 1-8, 10, 11p13→11qter, 12, 13, 14q11→14qter, 15, 16, 18-22 and X (lane 6), hybrid 734 retaining a der11 (11pter→11q23::Xq25→Xqter) and partial 13 (14, 15) (lane 7), hybrid PB5-1 retaining partial 2, partial 5, 11q, 17, partial 20 (lane 8) was fractionated, blotted to nylon filter and hybridized to a radiolabelled βARK probe.

ined for the presence of the βARK locus by hybridization of a radiolabeled human βARK probe to *Eco*RI cleaved hybrid and control DNAs immobilized on nylon filters. Results of testing of the entire panel demonstrated that the human βARK locus was present only in hybrids retaining chromosome 11 and was absent in all hybrids which did not contain chromosome 11 as summarized in Fig. 2. An example of results of such hybridization studies are shown in Fig. 3. The human βARK specific 15 kbp *Eco*RI fragment is present in the human DNA (lane 2) and each of the hybrids shown (lanes 3-8); a mouse specific *Eco*RI fragment (>23 kbp) is detected in the mouse DNA (lane 1) and is also present in each of the hybrid DNA containing lanes. Each of the hybrids in lanes 3-8 retains an entire or partial chromosome 11 as detailed in the legend to Fig. 3.

Fig. 3 also illustrates regional localization of the βARK gene since hybrids in lanes 5 and 8 retain 11q [12] and the hybrid (734) in lane 7 retains a der11 (11pter→11q23::Xq25-Xqter) [15]. A daughter clone derived by selection of the hybrid 734 in 6-thioguanine (to eliminate the der11 chromosome by selective killing of cells retaining the active HPRT gene on the X chromosome derived portion of the der11) [16] was negative for the βARK gene (not shown). Thus the βARK gene must localize to the portion of chromosome 11 (11cen→11q23) which is common to the hybrids in lanes 5-8. The final regional localization of the βARK gene is illustrated in Fig. 4 which provides a sketch of partial chromosome 11s retained in βARK positive hybrids. A βARK negative hybrid, CE4 (Southern data not shown), retained chromosome region 11q13-11qter with a break in the *BCL-1* locus [12], eliminating the region 11q13-11qter. Therefore, the βARK gene is located in the narrow chromosomal region between the centromere of chromosome 11 and the portion of 11q13 carrying the *BCL-1* locus, as indicated by the bracketed overlap region in Fig. 4.

Amplification of a cluster of loci at 11q13 usually in-

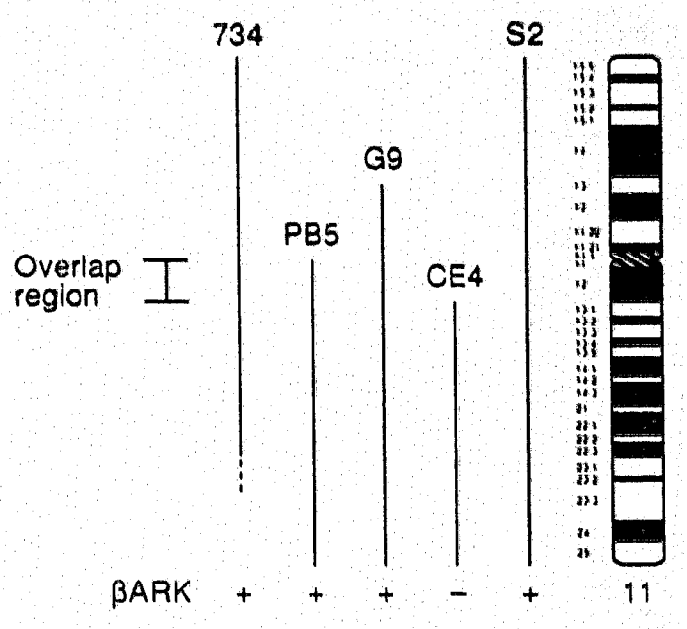

Fig. 4. Regional localization of the *β-ARK* locus. Diagram of chromosome 11 with portions of chromosome 11 retained by various hybrids sketched on the left. The dotted portion of the line representing hybrid 734 indicates uncertainty of the distal endpoint which cannot be determined exactly cytogenetically. The presence of the *βARK* locus in each of the hybrids is indicated below by a plus sign; on the far left the overlap region indicates the region of chromosome 11 common to the βARK positive hybrids. Thus the βARK gene resides within this region, 11cen→11q13.

cluding the *BCL-1*, *HSTF1*, and *INT2* loci has been observed in some human tumors [17-21]. Since the location of the mouse *Bark* gene (Benovic et al., in preparation) suggests that the human βARK gene most likely also maps near 11q13, mammary carcinoma derived lines carrying amplified *BCL-1/HSTF1/INT2* loci were tested for amplification of the *βARK* locus. The *βARK* locus was not amplified in any of the five lines tested (not shown).

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REFERENCES

[1] Benovic, J.L., Bouvier, M., Caron, M.G. and Lefkowitz, R.J. (1988) Annu. Rev. Cell Biol. 4, 405-428.
[2] Hausdorff, W.P., Caron, M.G. and Lefkowitz, R.J. (1990) FASEB J. 4, 2881-2889.
[3] Benovic, J.L., Pike, L.J., Cerione, R.A., Staniszewski, C., Yoshimasa, T., Codina, J., Birnbaumer, L., Caron, M.G. and Lefkowitz, R.J. (1985) J. Biol. Chem. 260, 7094-7101.
[4] Clark, R.B., Kunkel, M.W., Friedman, J., Goka, T.J. and Johnson, J.A. (1988) Proc. Natl. Acad. Sci. USA 85, 1442-1446.
[5] Lohse, M.J., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J. (1990) J. Biol. Chem. 265, 3202-3209.

[6] Lohse, M.J., Lefkowitz, R.J., Caron, M.G. and Benovic, J.L. (1989) Proc. Natl. Acad. Sci. USA 86, 3011–3015.
[7] Benovic, J.L., Mayor, F., Jr., Staniszewski, C., Lefkowitz, R.J. and Caron, M.G. (1987) J. Biol. Chem. 262, 9026–9032.
[8] Benovic, J.L., Regan, J.R., Matsui, H., Mayor, F., Jr., Cotecchia, S., Leeb-Lundberg, L.M.F., Caron, M.G. and Lefkowitz, R.J. (1987) J. Biol. Chem. 262, 17251–17253.
[9] Kwatra, M.M., Benovic, J.L., Caron, M.G., Lefkowitz, R.J. and Hosey, M.M. (1989) Biochemistry 28, 4543–4547.
[10] Benovic, J.L., DeBlasi, A., Stone, W.C., Caron, M.G. and Lefkowitz, R.J. (1989) Science 246, 235–240.
[11] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–
[12] Huebner, K., Ferrari, A.C., Delli, Bove, P., Croce, C.M. and Basilico, C. (1988) Oncogene Res. 3, 263–270.
[13] Bauer, S.K., Huebner, K., Budarf, M., Finan, J., Erikson, J., Emanuel, B., Nowell, P.C., Croce, C.M. and Melchers, F. (1988) Immunogenetics 28, 328–333.
[14] Jackson, R.J. and Standart, N. (1990) Cell 62, 15–24.
[15] Scott, A.F., Phillips III, J.A. and Migeon, B.R. (1979) Proc. Natl. Acad. Sci. USA 76, 4563–4565.
[16] Durst, M., Croce, C.M., Gissman, L., Schwarz, E. and Huebner, K. (1987) Proc. Natl. Acad. Sci. USA 84, 1070–1074.
[17] Adelaide, J., Mattei, M.-G., Maries, I, Raybaud, F., Planche, J., de Lapeyriere, O. and Birnbaum, D. (1988) Oncogene 2, 413–416.
[18] Lidereau, R., Callahan, R., Dickson, C., Peters, G., Escot, C. and Ali, I.U. (1988) Oncogene Res. 2, 285–291.
[19] Yoshida, M.C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T. and Terad, M. (1988) Proc. Natl. Acad. Sci. USA 85, 4861–4864.
[20] Gaudray, P., Theillet, C., Escot, C., Nguyen, C., Mattei, M.-G., Jordan, B., de Lapeyriere, O. and Birnbaum, D. (1989) Cancer Genet. Cytogenet. 38, 192.
[21] Theillet, C., Adnane, J., Szepetowski, P., Simon, M.P., Jeanteur, P., Birnbaum, D. and Gaudray, P. (1990) Oncogene 5, 147–149.