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SHORT COMMUNICATION

Localization of the Human Phosphatidylinositol-Specific Phospholipase C $\beta_3$ Gene (PLCB3) within Chromosome Band 11q13

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...In course of the molecular characterization of a human extragonadal germ cell tumor (EGCT)-associated chromosomal translocation, we identified YACs and cosmids from the 11q13 region. The endclone of one of these YACs appeared to contain a stretch of DNA homologous to part of the human phosphatidylinositol-specific phospholipase C $\beta_3$ gene (PLCB3). Since we considered PLCB3 a candidate gene for these EGCTs, we set out to clone the PLCB3 cDNA, from which the 5' end was still missing, and performed Northern and Southern blot analyses. The localization of PLCB3 to 11q13 was confirmed. In addition, we were able to exclude the gene from involvement in EGCT development. © 1995 Academic Press, Inc.

Recently, we started the molecular characterization of a recurring complex chromosomal translocation, involving breaks in 6p21, 6p22, 6q23, and 11q13, specific for a newly defined subgroup of human extragonadal germ cell tumors (EGCTs) (4). Band 11q13 was chosen as the starting point for our experiments. By using FISH, we were able to narrow the breakpoint region to an interval between loci D11S457 and D11S546 (8). To saturate this genomic region with new probes, sequence-tagged sites (STSs) were generated from single-copy subclones of the breakpoint-bracketing cosmids (cCI11-247 and cCI11-363, respectively; 10). These STSs, in turn, were used to screen a total human YAC library (CEPH) (1). This resulted in one positive clone, designated 255H9, with the STS primer set corresponding to an interval between loci D11S457 and D11S546 (8). To saturate this genomic region with new probes, sequence-tagged sites (STSs) were generated from single-copy subclones of the breakpoint-bracketing cosmids (cCI11-247 and cCI11-363, respectively; 10). These STSs, in turn, were used to screen a total human YAC library (CEPH) (1). This resulted in one positive clone, designated 255H9, with the STS primer set corresponding to cCI11-247 (D11S457). FISH analysis revealed that this YAC gives only one specific hybridization signal on 11q13, indicating that this YAC is nonchimeric. Furthermore, the 255H9 YAC appeared to map proximal to the breakpoint region in EGCTs, as did the cCI11-247 cosmid. For further genome walking experiments, we isolated the endclones of YAC 255H9 via Alu-vector PCR. These endclones were sequenced to obtain new STSs. Surprisingly, database sequence comparison revealed that one of these endclones (L73) contains a stretch of DNA completely homologous to positions 1000–1250 of the human phosphatidylinositol-specific phospholipase C $\beta_3$ (PLCB3) cDNA (3). In addition, this endclone was used to screen a flow-sorted chromosome 11 cosmid library (ICRF) (6). This resulted in four overlapping cosmids that, again, could be localized in the 11q13 region using FISH. Together, these observations unambiguously map the corresponding gene PLCB3 within chromosome 11q13.

A

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B

**FIG. 1.** (A) The 5' sequence of cDNA clone c7. The translation initiation site is shown in boldface. The position where the previously known PLCB3 sequence begins (3) is underlined. (B) Amino acid alignment of the N-terminal regions of the three phosphatidylinositol-specific phospholipases C: PLCB1; PLCB2; PLCB3. The asterisks below the sequences indicate identical amino acids; the dots indicate conservative changes. The position where the previously known PLCB3 sequence begins (3) is underlined.

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the chromosomal localization of the PLGB3 gene (Fig. 1). By using the entire cDNA as a probe on a poly(A)+ RNA from different human tissues using the human PLCB3 cDNA as a probe. Lanes 1–8, heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas, respectively. The major 5.5-kb transcript is indicated by an arrow. (B) Southern blot analysis of EcoRI-digested DNAs using the human PLCB3 cDNA as a probe. Lanes 1–5, chromosome 11only hybrid, hamster, mouse, human, and EGCT, respectively.

gene within chromosome band 11q13. However, so far only part of the PLCB3 cDNA was cloned and sequenced. The 5’ end of the cDNA, encoding approximately 180 amino acids, was still missing (3). Since phospholipases are involved in cellular signaling and as such are thought to play a role in differentiation and proliferation processes (7), we considered the PLCB3 gene a candidate in the development of EGCTs. To test this possibility further, we used the L73 clone to screen a human fetal brain Lambda ZAP cDNA library (Stratagene). This resulted in five independent clones. Here, we report the remaining 5’-cDNA sequence of the PLCB3 cDNA (Fig. 1A). In addition, a comparison of the putative protein sequence with known sequences of two other members of the beta family of phospholipase C is provided (Fig. 1B). Based on this comparison, three of our clones probably contain the full-length cDNA. The degree of homology turned out to be high, especially when /?1 and /?2 sequences were compared. By using the entire cDNA as a probe for a poly(A)+ Northern blot (Clontech), a major transcript of approximately 5.5 kb was detected in all tissues tested, with additional transcripts (of unknown origin) in heart and skeletal muscle (Fig. 2A). The 5.5-kb mRNA was also detected on Northern blots containing RNA isolated from several germ cell tumor-derived cell lines, including EGCTs (not shown). Southern blot analysis using, again, the entire cDNA as a probe once more confirmed the chromosomal localization of the PLCB3 gene (Fig. 2B, lane 1). No aberrantly hybridizing fragments were observed in EGCT DNAs (Fig. 2B, lanes 4 and 5). Together with the mRNA studies, this result indicates that the PLCB3 gene can be excluded as a candidate in the development of this group of germ cell tumors. However, since the 11q13 region is involved in a number of other neoplastic disorders (2, 5, 8–10), the gene must be considered a candidate for either one of them.

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REFERENCES