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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 β3 gene (PLCB3). Since we considered PLCB3 a candidate gene for these EGCTs, we set out to clone the PLCB3 cDNA, from which the tol-specific phospholipase C β3 gene (PLCB3). Since we considered PLCB3 a candidate gene for these EGCTs, we set out to clone the PLCB3 cDNA, from which the tol-specific phospholipase C β3 gene (PLCB3).

To saturate this genomic region with new probes, sequence-tagged sites (STSs) were generated from single-copy subclones of the breakpoint-matching cosmids (cCI11-247 and cCI11-383, respectively; 10). These STSs, in turn, were used to screen a total human YAC library (CEPH) (1). This resulted in one positive clone, PLCB3H, with the STS primer set corresponding to cosmid cCI11-247 (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-matching cosmids (cCI11-247 and cCI11-383, respectively; 10).

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-matching cosmids (cCI11-247 and cCI11-383, 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 cosmid cCI11-247 (D11S457). FISH analysis revealed that this YAC gives only one specific hybridization signal on 11q13, indicating that this YAC is non-chimeric. Furthermore, the 255H9 YAC appeared to map proximal to the breakpoint region in EGCTs, 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).

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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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 \( \beta_1 \) and \( \beta_3 \) sequences were compared. By using the entire cDNA as a probe on 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 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