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.

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 cCI11-383, 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 cosm id 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 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 phosphatidylinosito-

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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 (Sagatagene). 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 β1 and β3 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 human PLCB3 cDNA as a probe. Lanes 1–5, chromosome 11-only hybrid, hamster, mouse, human, and EGCT, respectively.

FIG. 2. (A) Northern blot analysis of 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 11–363 cosmids used in this study. E. Schoenmakers is acknowledged for screening the CEPH-YAC library. This work was supported by the Dutch Cancer Society (Koningin Wilhelmina Fonds).

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