

Brief report

ORP-3, a human oxysterol-binding protein gene differentially expressed in hematopoietic cells

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Using differential display polymerase chain reaction, a gene was identified in CD34+ enriched populations that had with low or absent expression in CD34− populations. The full coding sequence of this transcript was obtained, and the predicted protein has a high degree of homology to oxysterol-binding protein. This gene has been designated OSBP-related protein 3 (ORP-3). Expression of ORP-3 was found to be 3- to 4-fold higher in CD34+ cells than in CD34− cells. Additionally, expression of this gene was 2-fold higher in the more primitive subfraction of hematopoietic cells defined by the CD34+38− phenotype and was down-regulated with the proliferation and differentiation of CD34+ cells. The ORP-3 predicted protein contains an oxysterol-binding domain. Well-characterized proteins expressing this domain bind oxysterols in a dose-dependent fashion. Biologic activities of oxysterols include inhibition of cholesterol biosynthesis and cell proliferation in a variety of cell types, among them hematopoietic cells. Characterization and differential expression of ORP-3 implicates a possible role in the mediation of oxysterol effects on hematopoiesis. (Blood. 2001;98:2279-2281)

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Introduction

Molecular processes that maintain the stem cell pool and govern the proliferation and differentiation of hematopoietic stem cells are widely investigated but incompletely understood. Identification of these genes will facilitate our understanding of hematopoiesis and may be used to improve clinical outcomes.

Using differential display-polymerase chain reaction (dd-PCR), we identified a differentially expressed transcript in CD34-enriched populations, with low or absent expression in CD34-depleted populations. Sequencing of this transcript revealed significant homology to oxysterol-binding protein (OSBP). On the basis of ESTs in the GenBank database, a family of up to 8 human genes that code for OSBP-related proteins is postulated to exist.1 These have been designated OSBP-related proteins (ORPs). A partial sequence for our OSBP-related gene was thus designated ORP-3.1

Study design

CD34+ purification

Umbilical cord blood (UCB) samples were obtained after uncomplicated vaginal or cesarean delivery. Adult bone marrow (ABM) cells were obtained from cell scrapings of discarded rib and femur heads after cardiothoracic or hip-replacement surgery. All samples were donated by volunteers according to approved institutional guidelines. Mononuclear cells were prepared by density-gradient separation through Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden). CD34+ cells were isolated using a MiniMacs bead separation kit (Miltenyi Biotec, Becton Dickinson, Sunnyvale, CA).

Flow cytometric analysis of CD34+ populations and fluorescence-activated cell sorting of CD34+ subsets

CD34+ cells were labeled with anti-CD38-fluorescein isothiocyanate, anti-CD45-phycocerythrin, and anti-CD45-PECYS (Coulter Immunotech, Fullerton, CA). CD34+ purity was determined by flow cytometric analysis (FacsCalibur, Becton Dickinson) following modified ISHAGE guidelines2 using CELLQuest software (Becton Dickinson). When required, CD34+38− cells were sorted using a FacsCalibur Cell Concentrator Sorting Module.

Culture of umbilical cord blood CD34+ cells

In selected experiments, isolated CD34+ cells were cultured for 1 week at 37°C in a humidified atmosphere flushed with 5% CO2 in air, at a concentration of 0.5 × 106 cells/mL, in α minimum essential medium (Trace, Noble Park, Victoria, Australia) with 20% fetal bovine serum (CSL Biosciences, Parkville, Victoria, Australia), 2 mM L-glutamine, 200 U/mL penicillin-streptomycin (Sigma, St Louis, MO), 20 ng/mL recombinant human stem cell factor (Amgen, Thousand Oaks, CA), 10 ng/mL IL-1B (Endogen, Woburn, MA), 10 ng/mL IL-3, 10 ng/mL IL-6, and 10 ng/mL G-CSF (Amrad, Boronia, Victoria, Australia).

dd-PCR

RNA was extracted by RNeasy total RNA isolation kit (Qiagen, Clifton Hill, Victoria, Australia) and was DNase treated (Invitrogen, Carlsbad, CA). Reverse transcription and dd-PCR were carried out as described in the RNAimage Differential Display System (GenHunter, Nashville, TN). We used ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) for all sequencing reactions, and sequences were determined using an ABI PRISM 373 DNA Sequencer (PE Applied Biosystems).

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mRNA extraction and rapid amplification of cDNA ends

mRNA was extracted from UCB mononuclear cells using Oligotex Direct mRNA Kit (Qiagen). 5' and 3' Rapid amplification of cDNA ends (RACE) was carried out using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA). All primers used in this study are presented in Table 1.

Northern hybridization

A PCR product spanning the ORP-3 coding region was amplified from UCB mononuclear cell cDNA in a standard PCR reaction and subcloned. The ORP-3 insert was isolated and labeled using [α-32P]dATP and an ORP-3 probe was hybridized to a human multiple tissue Northern blot (Clontech) with ULTRAhyb hybridization buffer (Ambion) and was visualized by autoradiography.

Taqman real-time polymerase chain reaction

Reverse transcription was carried out using the reverse transcription system (Promega, Madison, WI). Real-time PCR amplification of ORP-3 and β-actin was carried out using Taqman Universal PCR Mastermix on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems).

Bioinformatics

Nucleic acid and protein sequences were analyzed using software available from the National Center for Biotechnology Information and Swiss Institute of Bioinformatics databases.

Results and discussion

Full ORP-3 mRNA of 6.631 kb was obtained using EST database searching and RACE technology (GenBank accession no. AY008372). The size of the complete ORP-3 cDNA has been confirmed by Northern hybridization with the presence of a transcript at approximately 7.0 kb (results not included). Our results also revealed the presence of 2 other transcripts at approximately 4.4 and 3.6 kb. These splice variants were expressed in a variety of normal human tissues and might have been produced as a result of posttranscriptional modification. Further investigation is required to determine their specific functions.

We used Taqman real-time PCR to confirm the differential expression of ORP-3. Results indicate that the expression in CD34+ cells from UCB and ABM is 3- to 4-fold higher (respectively) than corresponding CD34- populations (Figure 1A). ORP-3 gene expression (normalized for the expression of β-actin24) was also significantly higher in CD34+ cells from ABM than in equivalent cell populations from UCB.

The CD34+ population of cells is heterogeneous and contains...
multopotential and lineage-restricted cells. Therefore, we investigated ORP-3 gene expression in this population before and after 7-day culture in media containing growth factors that promote the differentiation and proliferation of hematopoietic progenitors. 

Our results indicate that under these culture conditions, ORP-3 gene expression in freshly isolated, uncultured CD34+ cells from UCB and ABM was 2- to 3-fold higher (respectively) than in these same cells after 7 days’ culture (Figure 1B). Investigation of ORP-3 in the more primitive subset of cells defined by the CD34+38 -immunophenotype indicates higher expression than in the more mature CD34+38+ subset (Figure 1C). Taken together, ORP-3 expression data indicate higher expression in a less differentiated subset of hematopoietic cells. The physiological significance of this differential expression and the function of ORP-3 in these cell populations remain to be elucidated.

Translation of the ORP-3 open-reading frame yields an 887-amino acid protein that has a high degree of homology to OSBP. OSBP is a well-characterized and highly conserved protein that has been demonstrated to bind oxysterols in a dose-dependent fashion. ORP-3 and OSBP harbor the same modular domains—a C-terminal OSBP domain that is required for OS binding and an N-terminal pleckstrin homology domain that is required for translocation of the ligand protein to the Golgi apparatus. As do some OSBP, ORP-3 contains a putative leucine zipper region. It must be noted however, that though leucine zipper regions may be associated with DNA binding, this has not been demonstrated in any OSBPs.

To date, the complete coding sequence of only one human OSBP (OSBP-Hm) has been published; however, at least 8 other human ORP partial and complete gene sequences (designated ORP 1-8) exist on the GenBank database. The partial sequence for the human OSBP homologue, ORP-4, was identified by Fournier et al using dd-PCR as a screen to identify genes associated with metastatic potential. They designated the sequence HeLa metastatic gene (HLM). Northern blot analysis of ORP-4/HLM expression demonstrated that ORP-4/HLM expression is significantly associated with metastatic potential.

OSBPs have also been identified in lower species, and their expression has been strongly associated with cell cycle progression. Given our observations of differential expression of ORP-3 in cells with different cell cycle profiles (such as CD34+CD38+ and CD34+CD38- cells), we are investigating the possibility that ORP-3 may be involved in cell cycle regulation. Interestingly, it has been hypothesized that levels of OSBP-Hm vary as a function of cell cycle. This finding has not been confirmed, nor has its significance been reported.

Given the homology between ORP-3 and OSBP, it is likely that ORP-3 also plays a role in mediating oxysterol effects on cells. Oxysterols are hydroxylated derivatives of cholesterol that have been demonstrated to inhibit the transcription of many genes involved in cholesterol biosynthesis and cell replication and to be apoptotic to a variety of cell types. Additionally, investigations in our laboratory have demonstrated that oxysterols are potent inhibitors of HL60 and granulocyte macrophage–colony-forming unit cell growth and that they induce apoptosis in CD34+ cells (manuscript in preparation). Given the importance of these processes in hematopoiesis and the differential expression of ORP-3, we believe that characterization and investigation of the function of ORP-3 may provide useful insights into a possible regulatory role for oxysterols and their binding proteins in hematopoietic stem cell proliferation, differentiation, and self-renewal.

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We thank the medical and nursing staff of The Geelong Hospital and St John of God (Geelong, Victoria, Australia) for the collection of UCB and ABM samples.

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