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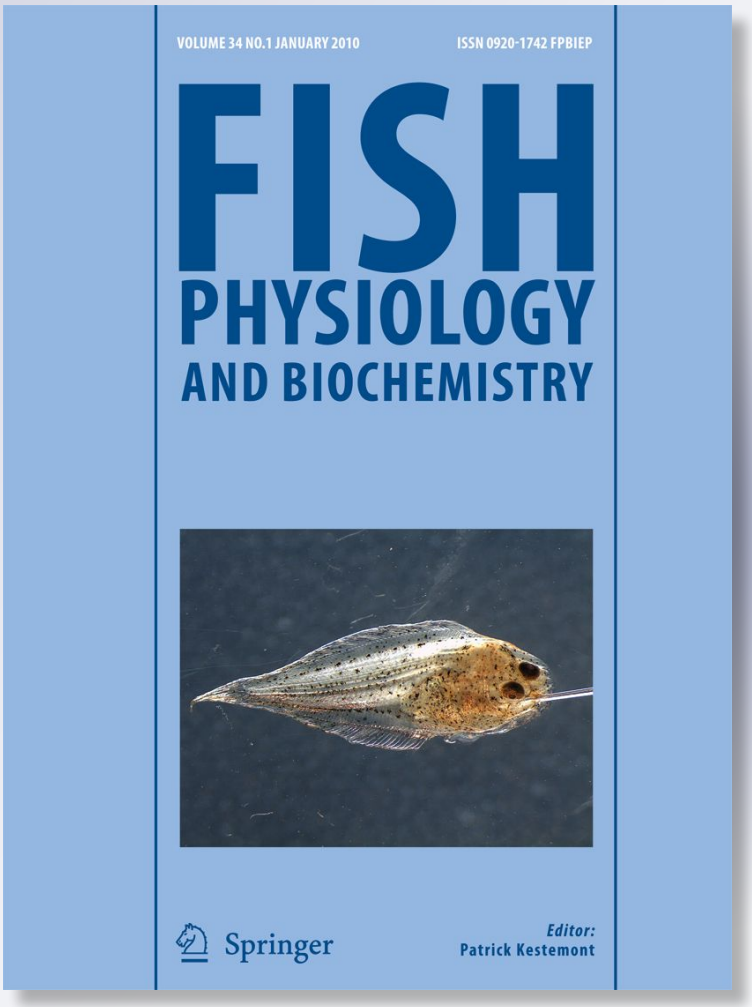

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Molecular cloning and expression analysis of P-selectin glycoprotein ligand-1 from zebrafish (*Danio rerio*)

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Abstract To date, the best characterized glycoprotein ligand for P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1). In this study, we cloned the full-length cDNA of PSGL-1 from zebrafish (*Danio rerio*). Zebrafish PSGl-1 cDNA is 1,594 bp and encodes a putative 284 amino acid protein with a theoretical molecular weight of 30.33 kDa and isoelectric point of 7.96. A signal peptide of 27 amino acids is predicted. The putative protein contains an extracellular mucin-like domain, a transmembrane domain and a cytoplasmic domain, with homology to mammalian PSGL-1. In the putative P-selectin binding region, there are 1 potential tyrosine sulfation site and 12 potential threonine O-glycosylation sites. A single extracellular cysteine, at the junction of the extracellular and transmembrane domains, suggests a disulfide-bonding pattern. The amino acid sequence of zebrafish PSGL-1 is 19–22% identical to that of mammalian PSGL-1. RT–PCR and whole-mount in situ hybridization analysis revealed that zebrafish PSGL-1 was expressed in early embryonic development, and the expression has an increased trend from 0.2 (1-cell stage) to 72 hpf. The results indicate that the general domain structure of PSGL-1 protein is conserved among species, and zebrafish PSGL-1 plays important roles in embryonic development and probably has similar biological function to that of mammalian PSGL-1.

Keywords PSGL-1 · Zebrafish · Cloning · Expression · cDNA

G. Sun · K. Liu (✉) · S. Wang · X. Wang · X. Wang
Biology Institute of Shandong Academy of Sciences,
No. 19, Keyuan Road, Jinan 250014,
People's Republic of China
e-mail: hliukch@keylab.net

G. Sun · J. Pan
College of Life Sciences, Shandong Normal University,
Jinan 250014, People's Republic of China

Introduction

P-selectin, L-selectin and E-selectin belong to the selectin family of adhesion molecules. P-selectin is normally located in the alpha granules of platelets and Weibel-Palade bodies of endothelial cells, with low or absent expression; with activation of endothelial cells and platelets by a variety of stimuli, P-selectin moves to cell surfaces, where the expression is markedly increased. E-selectin is expressed by activated endothelial cells. L-selectin is constitutively expressed on the surface membrane of all leukocytes (Kansas 1996). In the past few years, there has been rapid growth in understanding on the structures and functions of the selectins, but our understanding on the structure and function of the selectin ligand is still limited. To date, the best characterized glycoprotein ligand for P-selectin is P-selectin glycoprotein ligand-1 (PSGL-1) (Moore et al. 1992; Sako et al. 1993).

PSGL-1 is a homodimeric mucin-like glycoprotein that is rich in O-and N-glycans (Moore et al. 1992; Sako et al. 1993) and expressed on leukocyte microvilli (Spertini et al. 1996; Walcheck et al. 1996). At the same time, PSGL-also serves as a ligand for both E- and L-selectin (Spertini et al. 1996; Asa et al. 1995). PSGL-1 is required for the recognition of P-selectin by all classes of leukocytes. Both tyrosine sulfation and threonine O-glycosylation are required for human PSGL-1 and mouse PSGL-1 binding to P-selectin and L-selectin (Bernimoulin et al. 2003; Sako et al. 1995; Somers et al. 2000; Martinez et al. 2005). In addition to sulfation and O-glycosylation, PSGL-1 requires post-translation modifications to recognize P-selectin, including addition of fucose in an α1-3 linkage and modification of O-glycans (Baïsse et al. 2007).

The selectin family of adhesion molecules and PSGL-1 plays critical roles in regulating leukocyte rolling and recruitment in appropriate inflammatory responses (Ley 2003). Early an inflammatory response, interactions of P-selectin with PSGL-1 mediate leukocyte rolling (Norman et al. 1995); interactions of L-selectin with PSGL-1 strongly amplify leukocyte recruitment (Walcheck et al. 1996; Sperandio et al. 2003); moreover, interactions of E-selectin with PSGL-1 and CD44 and/or other potential ligands support leukocyte slow rolling along inflamed endothelium (Norman et al. 2000; Katayama et al. 2005; Xia et al. 2002). Leukocyte rolling, extravasation and hemostasis are defective in P-selectin-deficient mice (Mayadas et al. 1993; Subramaniam et al. (1996)). Rolling and migration of leukocytes are impaired in PSGL-1-deficient mice (Sperandio et al. 2003; Xia et al. 2002; Yang et al. 1999). PSGL-1 has been as attractive therapeutic targets for inflammation and cardiovascular diseases (Rijcken et al. 2004; Takada et al. 1997; Wang et al. 1996; Blann et al. 2003; Vandendries et al. 2004). So, a better understanding on PSGL-1 structure and function may be helpful to the treatments in inflammatory and cardiovascular diseases.

Zebrafish (*Danio rerio*) has become a prominent vertebrate animal model for investigating human development and diseases (Dooley and Li 2000), because of its advantages of high fecundity, external embryonic development, transparency of the embryos, advanced genomic resources and similar organ systems and gene sequences to humans. Zebrafish is especially appropriate for the study of cardiovascular disease because zebrafish embryos and larvae are transparent, and cardiac function and blood circulation are readily observed after 24 h post-fertilization (hpf) (Jagadeeswaran 2005; Jagadeeswaran et al. 2005).

To our knowledge, zebrafish PSGL-1 has not been characterized. In the present study, we cloned the full-length cDNA of zebrafish PSGL-1 and analyzed its temporal and spatial expression patterns.

Materials and methods

Cloning of zebrafish PSGL-1 cDNA

We performed a BLAST search of zebrafish PSGL-1 cDNA sequence in the NCBI database (http://www.ncbi.nlm.nih.gov) and obtained cDNA sequence of *Danio rerio* similar to P-selectin glycoprotein ligand (Accession No. XM_001334699). According to the predicted sequence, the primers (5′-CATCGGTGC CTATTAAGGTAACAG-3′ and 5′-AAGTCAGGTC ATCTCCATCAGG-3′) were designed to amplify zebrafish PSGl-1 cDNA by RT–PCR. RT–PCR involved use of total RNA extracted from zebrafish embryos at 72 hpf. PCR was performed using Ex Taq (TaKaRa), and amplification conditions were 3 min at 94°C; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. On the basis of the cloned cDNA fragment sequence, the primers (5′-GGTGTGGTGGTAATCTGTAACCGT TGAG-3′ and 5′-CATCAACCGTAGCCGAAGC-3′) for 5′-rapid amplification of cDNA ends (5′-RACE) and 3′-RACE were designed to amplifythe 5′- and 3′-untranslated regions (UTRs) with use of the SMART-RACE cDNA amplification Kit (Clontech). The primers (5′-GGGCGTCACAGTGACAGAAC-3′ and 5′-AAGAAAGTGTTTATTTTGGGGTGAA-3′) were used to amplify the full-length cDNA of zebrafish PSGL-1 for verification. PCR was performed using Ex Taq (TaKaRa) and the amplification program was 94°C for 5 min; 35 cycles of 94°C for 30 s, 68°C for 30 s, 72°C for 2 min and a final extension at 72°C for 10 min. All PCR products were cloned into pGEM-T easy vector (Promega) and sequenced.

Bioinformatics analysis of zebrafish PSGL-1

The nucleotide and amino acid sequences, exon–intron organization and chromosomal location, and

conserved domain were analyzed online with use of the NCBI Blast server 2.0, the zebrafish whole-genome sequence project database (http://www.ensembl.org/Danio_rerio/blastview) and the Conserved Domains Database (http://smart.embl-heidelberg.de). The deduced signal peptide was identified by use of SignalP (http://genome.cbs.dtu.dk/services/SignalP/). Homology between the amino acid sequences of zebrafish and mammalian PSGL-1 was analyzed by ClustalW (http://www.ebi.ac.uk/clustalw). The phylogenetic tree was constructed with use of Mega3 by the neighbor-joining method. The genetic distance was calculated by the ρ-distance method.

Real-time quantitative PCR

Total RNA was extracted from embryos at 0.2 (1-cell), 12, 24, 48 and 72 hpf using TRIzol reagent (Invitrogen). Approximately 2 μg of total RNA was used for reverse transcription for first-strand cDNA with Moloney murine leukemia virus reverse transcriptase (Promega). Real-time quantitative PCR, with the first-strand cDNA used as a template, involved use of primers: for β-actin, (5′-TGGCTTCTGCTCTGTATGGC-3′ and 5′-CCCTGTTAGACAACTACCTCCCT-3′) (Accession No. NM_131031); for normalization and PSGL-1 (5′-TCCAGTGCAGACCGTTAATG-3′ and 5′-GTTGGGTGTGCAAACTAATC-3′). The protocol was initial denaturation at 95°C for 5 min, then 95°C for 10 s, 59°C for 15 s, 72°C for 20 s and 79°C 5 s (fluorescent data were acquired), repeated for 30 cycles. For every assay, the negative control was without cDNA template. The 2-ΔΔCt method was used to analyze data, and the expression at 0.2 hpf was used to calibrate temporal expression.

Whole-mount in situ hybridization

Whole-mount in situ hybridization was performed as described (Thisse and Thisse 2008). Embryos were fixed in 4% paraformaldehyde (Sigma, UK). A 402 bp antisense RNA probe was generated from ebrafish PSGL-1 coding sequence (598–999 bp of the cDNA sequence, primers-5′- CATCGGTGCCTATTAAGGTAACAG-3′ and 5′-AAGTCAGGTCATCTCCATCAGG-3′). This probe was synthesized by linearizing the PSGL-1-T easy plasmid with NdeI and transcribing with T7 RNA polymerase. All probes were made using a Digoxigenin RNA labelling kit (Roche Diagnostics). The hybridization was performed with 0.5 ng/ml digoxigenin (DIG)-labeled RNA probes at 65°C for 16 h. The hybridized embryos were incubated with alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche) at 4°C overnight and the hybridization signals were visualized using NBT/BCIP.

Results

Nucleotide sequence analysis of zebrafish PSGL-1

We obtained a full-length cDNA sequence of zebrafish PSGL-1 by a combination of RT–PCR and RACE-PCR. Zebrafish PSGL-1 cDNA is 1,594 bp and contains a 182-bp 5′-UTR, an 855-bp coding sequence and a 557-bp 3′-UTR (Fig. 1). The sequence was deposited in GenBank (Accession No. HQ542297). The zebrafish PSGL-1 gene contains 2 exons and 1 intron and is located on chromosome 5. The first exon covers 5′UTR, and the second exon covers ORF and 3′UTR.

Amino acid sequence analysis of zebrafish PSGL-1

Zebrafish PSGL-1 cDNA encodes a putative 284 amino acid protein (Fig. 1) with a theoretical molecular weight of 30.33 kDa and isoelectric point of 7.96 (using DNAMAN). A signal peptide cleavage site is predicted between residues 27 and 28 in putative amino acid sequences. The sequence of the putative mature protein contains the following structural feature: a large extracellular domain of 160 residues, a transmembrane domain of 23 residues and a cytoplasmic tail of 73 residues, as observed in mammals (Baïsse et al. 2007).

In this region of zebrafish PSGL-1 (residues 29–93) corresponding to the putative P-selectin binding region of human PSGL-1 (Moore et al. 1992; Sako et al. 1993), there is 1 tyrosine (Tyr86) for potential sulfation site, 12 threonines for potential O-glycosylation sites and 5 consensus sequences (NXS/T) for potential N-glycosylation sites. The whole extracellular domain of zebrafish PSGL-1 has 31 threonine and 22 serine residues for potential O-glycosylation sites and 8 consensus sequences for

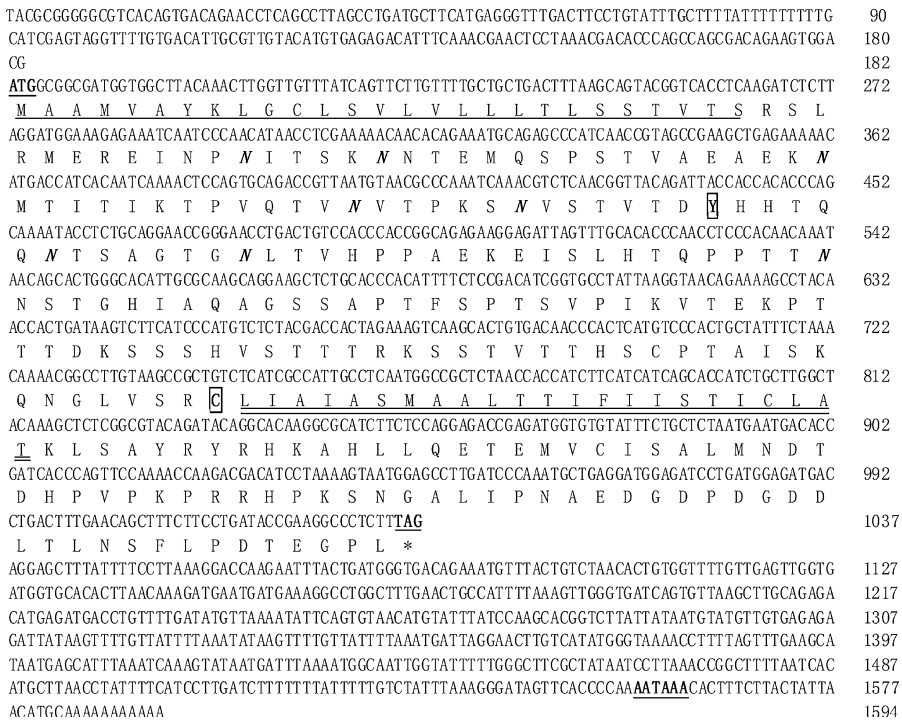

Fig. 1 Full-length nucleotide and deduced amino acid sequences for zebrafish PSGL-1. The start codon and stop codon are *bold* and *underlined*. The putative signal peptide is underlined. Asparagine residues for potential N-glycosylation sites in the extracellular domain are *bold* and *italic*. The conserved tyrosine residue and cysteine residue are *boxed*. The transmembrane domain is *double-underlined*

potential N-glycosylation sites. The160-residue extracellular domain is rich in serine, threonine and proline (22Ser/31Thr/13Pro), characteristic of mucin-like domains, but the striking repeat pattern of the decameric repeats is not observed in the extracellular domain. Zebrafish PSGL-1 has 5 cysteine residues. Among them, one (Cys11) is in the putative signal peptide region, two (Cys174 and Cys188) is in the extracellular region, one (Cys208) is in the transmembrane region and one (Cys232) is in the cytoplasmic tail region. Cys188 is at the proposed junction of the extracellular and transmembrane domains. The amino acid sequence of zebrafish PSGL-1 is 19–22% identical to that of mammalian PSGL-1 (Fig. 2). The signal peptide, extracellular, transmembrane and cytoplasmic tail regions of zebrafish PSGL-1 share 23–35%, 13–16%, 39–47% and 33–37% sequence homologies with those of human (Moore et al. 1992; Sako et al. 1993), mouse (Yang et al. 1996), horse (Xu et al. 2005), rat (Tsuchihashi et al. 2006), bovine (Xu et al. 2006) and pig (Accession No. CAO91826).

In order to study the evolutionary relationship of zebrafish PSGL-1 with mammalian PSGL-1, we constructed a phylogenetic tree by aligning the amino acid sequences of zebrafish PSGL-1 to that of mammalian PSGL-1. As shown in Fig. 3, the 7 species were divided into 2 different branches, the first of mammal and the other zebrafish.

Expression pattern of zebrafish PSGL-1 during embryonic development

The temporal expression pattern of zebrafish PSGL-1 during embryonic development was examined by real-time quantitative PCR. Results revealed the increased trend of PSGL-1 expression from 0.2 (1-cell stage) to 72 hpf (Fig. 4). As shown in Fig. 4, the weak expression was observed at 1-cell stage (0.2 hpf) and then the expression outstandingly increased from 0.2 to 48 hpf, and the expression was maintained at a relatively high level at 48 and 72 hpf.

The temporal and spatial expression patterns of zebrafish PSGL-1 during embryonic development were further examined by whole-mount in situ hybridization. PSGL-1 was expressed in the embryo anterior at 90% epiboly (Fig. 5A) and ubiquitously expressed at 12 hpf (Fig. 5C). At 26 hpf, the expression was strong in the head region, the whole myotome region of ventral trunk and intermediate

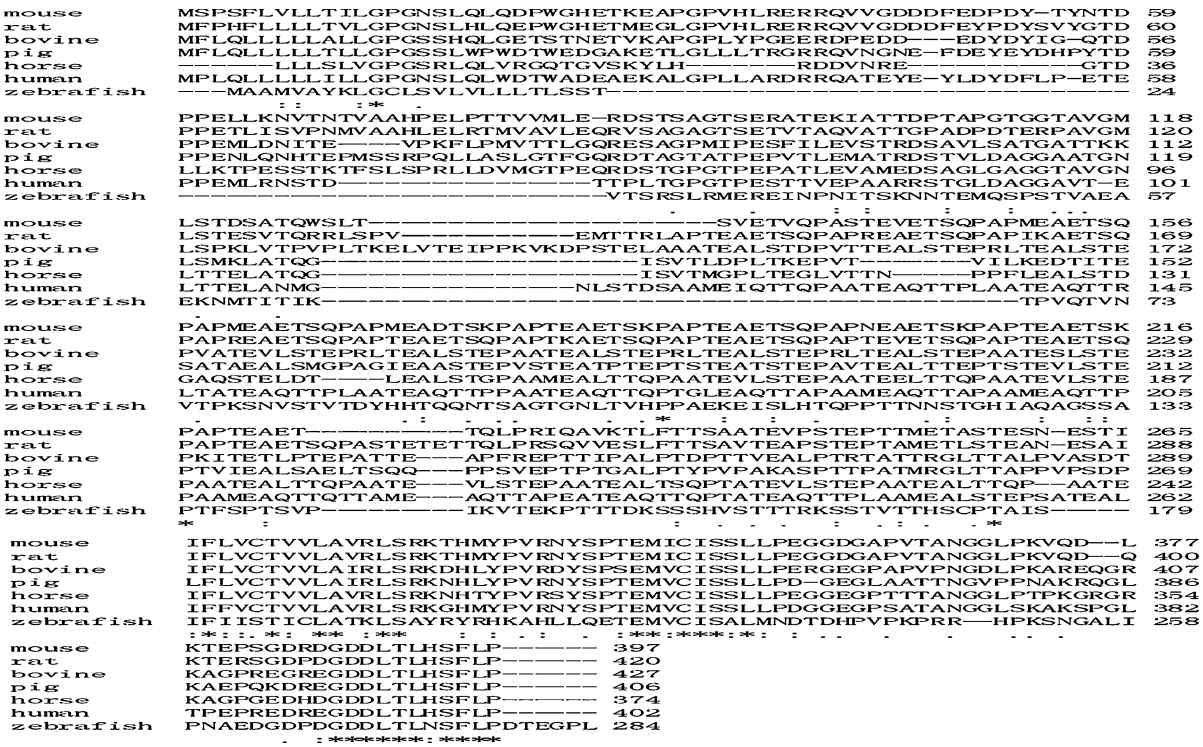

Fig. 2 Comparison analysis of the mouse, rat, bovine, pig, horse, human and zebrafish PSGL-1 amino acid sequence (CLUSTALW alignment). An *asterisk* indicates a residue that is invariant among species; a colon indicates a residue that is high conserved among species; a period indicates a residue that is moderately conserved among species

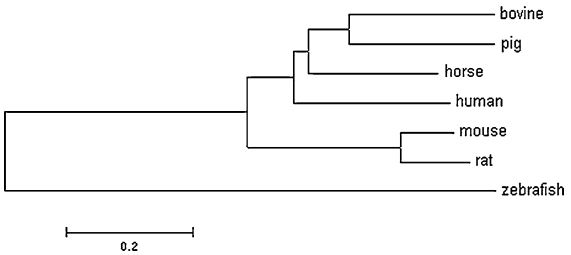

Fig. 3 Phylogenetic tree of PSGL-1 family members. The numbers indicate the bootstrap confidence values obtained for each node after 1,000 replications. Shows human (Accession No. NP_002997), horse (Accession No. AAQ63184), mouse (Accession No. CAA62583), rat (Accession No. CAO91829), bovine (Accession No. AAY21013) and pig proteins (Accession No. CAO91826)

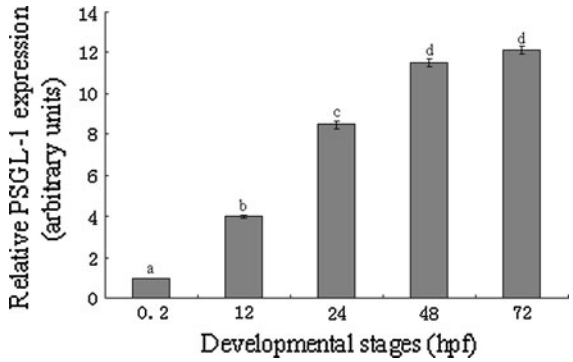

Fig. 4 Expression analysis of zebrafish PSGL-1 during embryo development by real-time quantitative PCR. *Different letters* indicate significant differences ($P < 0.05$)

cell mass region, and there was also a weak signal in the ventral vascular system (Fig. 5E). At 48 hpf, the expression was persisted in the head region, obvious in the whole ventral trunk, especially in the ventral vascular system, and there was still a strong signal in the intermediate cell mass region (Fig. 5G). At 72 hpf, the expression was confined to the cardiovascular system in the head region, the signal existed in the whole trunk and the signal was stronger in the ventral trunk than that of the dorsal trunk, the signal was persisted in the intermediate cell mass region and there was no signal in the caudal region (Fig. 5I).

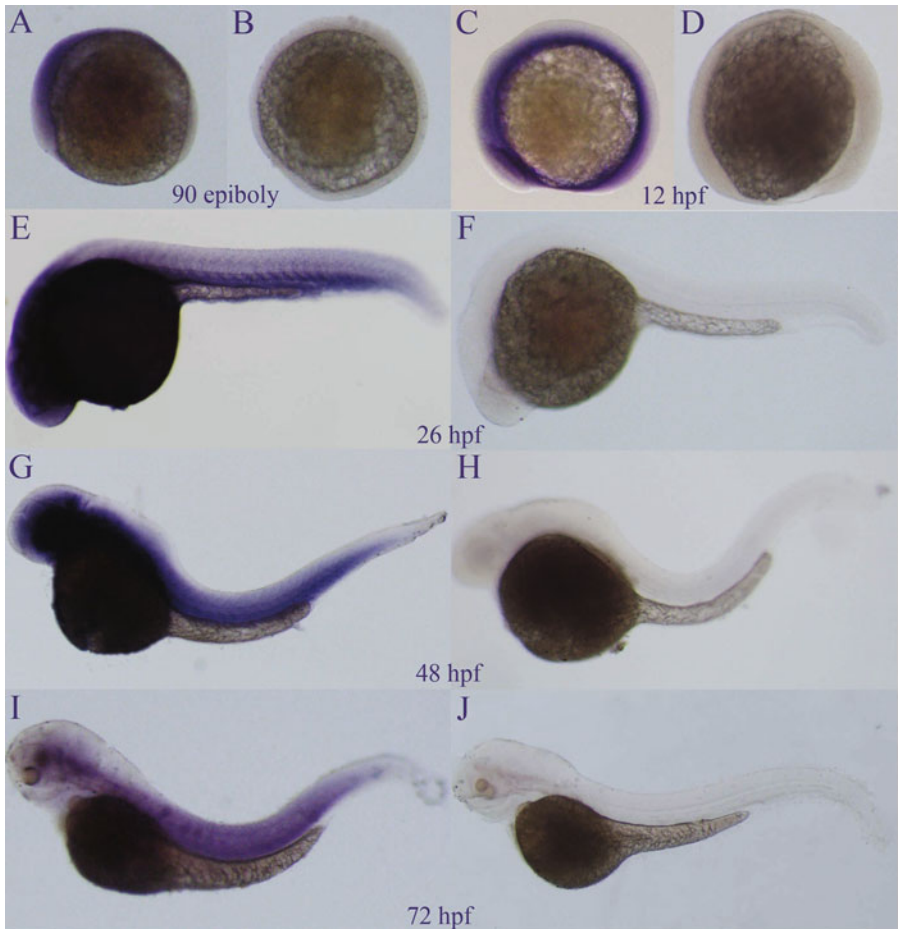

Fig. 5 Expression pattern of PSGL-1 in zebrafish embryos. **A–J** Whole-mount in situ hybridization of zebrafish embryos at 90% epiboly, 12 hpf, 26 hpf, 48 hpf, 72 hpf with antisenseprobes in the *left-hand panel* and control sense probes in the *right-hand panel*. Lateral view, anterior to *left*

Discussion

Human PSGL-1 was first identified from neutrophils and the myeloid cell line HL-60 (Moore et al. 1992), and human PSGL-1 cDNA was isolated from an HL-60 cell cDNA library (Sako et al. 1993). Subsequently, PSGL-1 gene was isolated and characterized in mouse, horse, rat, bovine (Yang et al. 1996; Xu et al. 2005; Tsuchihashi et al. 2006; Xu et al. 2006) and pig (Accession No. CAO91826). To further study the characteristic and expression of this gene, a full-length cDNA of PSGL-1 was cloned from zebrafish, a widely studied animal model for human diseases. To our knowledge, this represents the first in nonmammalian vertebrates. The cloned full-length cDNA sequence is 1,594 bp. The cDNA sequence of *Danio rerio* similar to P-selectin glycoprotein ligand (Accession No. XM_001334699) is 855 bp. By the gene structure analysis, we found that the sequence (Accession No. XM_001334699) was only the coding region of the cloned sequence.

Although the low amino acid sequence homology (19–22%) between zebrafish and mammalian PSGL-1, the general domain structure is conserved, including a signal peptide domain, a Ser/Thr/Pro-rich extracellular domain, a transmembrane domain and a cytoplasmic tail domain. This highlights that the primary structure of PSGL-1 protein is relatively conserved across species, and this also indicates the biological function of zebrafish PSGL-1 is probably similar to that of mammalian PSGL-1.

A signal peptide of 27 amino acids is predicted at the N-terminus of the putative protein, as observed in

mammals. The PACE (paired basic amino acid-converting enzyme) consensus sequence (Li et al. 1996), RX [R/K]R, is not observed in zebrafish PSGL-1. As a result, there is likely no propeptide following the signal peptide and consequently, the mature zebrafish PSGl-1 protein likely begins at residue 29.

Previous researches have shown that both tyrosine sulfation and threonine O-glycosylation are required for human and mouse PSGL-1 binding to P-selectin and L-selectin (Bernimoulin et al. 2003; Sako et al. 1995; Somers et al. 2000; Martinez et al. 2005). The putative P-selectin binding region of human and mouse PSGL-1 s has 3 (Tyr46, Tyr48 and Tyr51) and 2 tyrosine residues (Tyr54 and Tyr56) for potential sulfation sites (Moore et al. 1992; Sako et al. 1993; Yang et al. 1996), respectively, and the same region of zebrafish PSGL-1 (residues 29–93) contains only 1 tyrosine residue (Tyr 86). The acidic residue (Asp85) is at position-1 of Tyr 86 in zebrafish PSGL-1. According to Bundgaard et al. predictions (Bundgaard et al. 1997), a neutral or acidic charge of the residue in the amino terminal position (-1) of the tyrosine is critical for sulfation. The Asp85 presence of zebrafish PSGL-1 suggests that sulfation at Tyr86 is likely. The putative P-selectin binding region of human and mouse PSGL-1 s has 2 threonine residues (human: Thr44 and Thr57, mouse: Thr55 and Thr58) for potential O-glycosylation sites (Moore et al. 1992; Sako et al. 1993; Yang et al. 1996), respectively. Zebrafish PSGL-1 contains 12 threonine residues for potential O-glycosylation sites. Among them, there are 4 threonine residues (Thr82, Thr84, Thr89 and Thr93) corresponding to threonine residue (Thr57) in human PSGL-1 and threonine residues (Thr55 and Thr58) in mouse PSGL-1, which are located 2–9 residues from tyrosine residue (Tyr86).

In mouse, Tyr54 regulates PSGL-1 interactions with P-selectin (Xia et al. 2003), which differs significantly from human PSGL-1, where each of 3 tyrosine sulfates contributes to the affinity of binding to P-selectin. Because only one tyrosine is used, it is suggested that mouse PSGL-1 binding may rely more on O-glycosylation in the extracellular domain and on other peptide components than does human PSGL-1 (Xia et al. 2003). Because zebrafish PSGL-1 has also only 1 single tyrosine residue in the putative P-selectin binding region, it is possible that zebrafish PSGL-1 may also rely more on *O*-glycosylation and other peptide components to bind to P-selectin, similar to mouse PSGL-1. In the putative P-selectin binding region of horse PSGL-1, there is only 1 tyrosine (Tyr30) (Xu et al. 2005), and Tyr30 may not be sulfated according to Bundgaard et al. predictions (Bundgaard et al. 1997). So, it is also likely that other potential sites of sulfation in the putative P-selectin binding region of horse PSGL-1 are able to confer high-affinity binding, and horse PSGL-1 uses a different configuration of residues to bind to horse P-selectin (Xu et al. 2005). This also supports the concept that tyrosine sulfation enhances but is not required for PSGL-1 rolling adhesion on P-selectin (Rodgers et al. 2001). The difference in the putative P-selectin binding region of different species suggests some flexibility in the configuration of the actual P-selectin recognition region among species.

The O-glycosylations in other regions of the extracellular domain of human and bovine PSGL-1 may contribute to the interactions with P-selectin (Xu et al. 2006; Walcheck et al. 1996). In other regions of the extracellular domain of zebrafish PSGL-1, there are multi Thr and Ser residues for potential O-glycosylations (20Thrs and 16Sers), which also may contribute to the interactions with zebrafish P-selectin. The percentage of Thr/Ser residues for potential O-glycosylation sites in the extracellular domain of zebrafish PSGL-1 is greater than that in human, mouse, rat, bovine, pig and horse PSGL-1 (32.9% vs. 24.21–27.37%). This may further support the above premise that zebrafish PSGL-1 may also rely more on *O*-glycosylation and other peptide components to bind to P-selectin. Additionally, the actual molecular weight (120 kDa) of human PSGL-1 is larger than the theoretical molecular weight of 42 kDa, and multi O-glycosylations in human PSGL-1 are the possible reason. So, it is possible that zebrafish PSGL-1 should actually be heavier than its theoretical molecular weight of 30.3 kDa, too.

Eight consensus sequences for potential N-glycosylation sites exist within the extracellular domain of zebrafish PSGL-1. Among them, five exists in the putative P-selectin binding region. The potential N-glycosylation sites of zebrafish PSGL-1 are more than that of human (3) (Moore et al. 1992; Sako et al. 1993) and mouse (3) (Yang et al. 1996). So, it is possible that N-glycosylations on zebrafish PSGL-1 play a role in the interaction with the selectins. This also supports the viewpoint that the N-linked

oligosaccharides on PSGL-1 may be involved in the interaction with the selectins (Aeed et al. 2001). However, previous work has revealed that the N-linked glycans on PSGL-1 are not required for P-selectin recognition (Somers et al. 2000; Moore et al. 1994; Eppanen et al. 2000).

The160-residue extracellular domain of zebrafish PSGL-1 is rich in serine, threonine and proline (22Ser/31Thr/13Pro), which is similar to that of human and mouse PSGL-1. But the notable repeat pattern is not observed, this is different from that of human and mouse PSGl-1, which have repeat sequences.

The putative zebrafish PSGL-1 protein is 90–143 amino acid shorter than human (Moore et al. 1992; Sako et al. 1993), mouse (Yang et al. 1996), horse (Xu et al. 2005), rat (Tsuchihashi et al. 2006), bovine (Xu et al. 2006) and pig proteins (Accession No. CAO91826). The differences mainly lies in the length variance of extracellular domains between zebrafish and mammalian PSGL-1, and there are 160, 269, 265, 289, 266, 318 and 274 amino acids in zebrafish, human, horse, rat, mouse, bovine and pig PSGL-1 extracellular domains, respectively. This is also in agreement with the low homology between zebrafish and mammalian PSGL-1.

Previous researches indicate that the conserved cysteine residue at the proposed junction of the extracellular and transmembrane domains is responsible for dimerization of the surface expressed molecule and PSGL-1 dimerization is essential for functional recognition of P-selectin (Fujimoto et al. 1996; Snapp et al. 1998). At the proposed junction of the extracellular and transmembrane domains of zebrafish PSGL-1, there is also a single extracellular cysteine residue (Cys189), which suggests zebrafish PSGL-1 dimerization also contributes to functional recognition of P-selectin. But, Epperson et al. results demonstrated that Cys^{320}-dependent dimerization of PSGL-1 was not required for binding to P-selectin and that a small monomeric fragment of PSGL-1 was sufficient for P-selectin recognition (Epperson et al. 2000).

So, our results in the present study, together with the previous reports in different vertebrate models, suggest that some questions are worth further exploring in the future. For example, are the tyrosine sulfation and N-glycosylation of PSGL-1 required for P-selectin recognition? What is the difference in the configuration of the actual P-selectin recognition site among species? What are the role of dimerization of PSGL-1 and the repeat sequences of the extracellular domain?

Phylogenetic analysis indicates that mammalian and zebrafish PSGL-1 s form distinct clades. These results indicate a relatively distant evolutionary relationship between mammals and zebrafish, which is consistent with the different evolutionary ranks between fish and mammals.

PSGL-1 expression pattern has also been documented in human and mouse (Yang et al. 1996; Laszik et al. 1996). Human PSGL-1 is broadly expressed on differentiating and mature cells in the myeloid, lymphoid, and dendritic lineage and detected in some nonhematopoietic cells (Laszik et al. 1996). Mouse PSGL-1 is expressed in most tissues including heart, kidney, liver, muscle, ovary, stomach, blood, bone marrow, brain, adipose tissue, spleen and thymus (Yang et al. 1996). This indicates that the expression of human and mouse PSGL-1 is broad. To clarify the role of zebrafish PSGL-1, we analyzed its temporal and spatial expression patterns during embryonic development using RT–PCR and whole-mount in situ hybridization. The results showed that zebrafish PSGL-1 was expressed in embryonic development, and the expression increased gradually from 0.2 (1-cell stage) to 72 hpf. At 72 hpf, the signal existed in the cardiovascular system in the head region, the whole trunk and intermediate cell mass region, and the expression was broad, which is similar to that of human and mouse. Our results suggest that zebrafish PSGL-1 is expressed maternally, plays important roles in embryonic development and may be involved in many aspects of embryonic development, especially the development of some organs in the head region, muscle segments and circulatory system. However, the exact organs and cell types that contain zebrafish PSGL-1 are unknown. To further understand the precise role of zebrafish PSGL-1 in embryonic development and define the organs and cell types, further researches are needed.

In summary, we successfully isolated a cDNA encoding the homolog of PSGL-1 from zebrafish. The general domain structure of PSGL-1 protein is conserved between fish and mammals, including an extracellular mucin-like domain, a transmembrane domain and a cytoplasmic domain, despite a poor conservation of the extracellular domain. The potential tyrosine sulfation and threonine O-glycosylation

sites in the putative P-selectin binding region of zebrafish PSGL-1, and a single extracellular cysteine at the transmembrane and extracellular domain junction suggesting a disulfide-bonding pattern are evolutionary conserved between fish and mammals. Our study may provide additional insights into the understanding of PSGL-1 structure and function.

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