The PDGF/VEGF Receptor Controls Blood Cell Survival in *Drosophila*

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Supplemental Experimental Procedures

Constructs and Fly Stocks

For pUAST-PVRΔC, the signal peptide and HA tag from HA-ephrinB1 (Brückner et al., 1999) was fused in frame with a partial coding sequence of *Pvr* (aa 23–845+stop), amplified from EST SD03187. Expression of the correct protein product was confirmed by transfection of SL2 cells and Western blot (Brückner et al., 2000). pCaSpeR4-*srp*HemoGAL4 was constructed from selected regions upstream of the *srp* gene, and the GAL4 cDNA and polyA from pGatB (Brand and Perrimon, 1993). The pCaSpeR-*tubulin*-PVR transgene contains a *Pvr* cDNA (LD04172) downstream of a 2.4 kb *tubulin* promoter fragment in pCaSpeR4. Transgenic fly strains were generated by standard methods. Additional fly lines used were: *lz*GAL4 (J. Pollock), *twi*GAL4 (Greig and Akam, 1993), UAS-lPVR (Duchek et al., 2001), UAS-p35 (Hay et al., 1994), UAS-DERdn (O'Keefe et al., 1997), UAS-RasN17 (Lee et al., 1996), UAS-RasV12 (X. Lin), UAS-spry (Casci et al., 1999), UAS-PTEN (Goberdhan et al., 1999), UAS-p110dn and UAS-p110CAAX (Leevers et al., 1996), UAS-Socs (Callus and Mathey-Prevot, 2002), UAS-Cactus (Qiu et al., 1998), UAS-p53 (Brodsky et al., 2000), UAS-HIDAla5 (Bergmann et al., 1998), UAS-srcEGFP and UAS-*lacZ*nls (E. Spana). To isolate mutations in *Pvr* (*Pvr1-7*), EMS mutagenesis was done by standard techniques, mutagenizing isogenized FRT40/FRT40 males, recovering single chromosomes and testing for lethality and sterility over the small deficiency *Df(2L)TE29Aa-14* which uncovers *Pvr*. Expression of a ubiquitous wild-type *Pvr* transgene rescued the recessive lethality associated with two of the alleles (*Pvr1* and *Pvr4*) to adulthood, demonstrating absence of additional mutations causing zygotic lethality (not shown). After complementation tests, lethals from each group were tested in clones for border cell migration defects and protein expression by anti-PVR antibody (Duchek et al., 2001). Intracellular regions of *Pvr1* and *Pvr4* were sequenced. *Pvr1* displayed a premature stop codon at Trp1087 (TGG® TGA); no missense mutation was found in the *Pvr4* cytoplasmic domain. For *Pvr* mutants, all trans-heterozygous combinations were rescued to adulthood by ubiquitously expressed PVR (pCaSpeR-*tubulin*-PVR).

To generate the construct srpHemoGAL4, the GAL4 and hsp70-polyA region from pGATB (Brand and Perrimon, 1993) was released and cloned into pCasSpeR4 (Thummel and Pirrotta, 1991), using a Kpn1-Not1 digest. The resulting vector was used to clone two upstream regions of the srp gene that were amplified by PCR from genomic DNA, using primers AGGGTACCCTACTGCTTCCCACTCTAAGACTTCCAGTTTTAGGCTACG (sense) and GGAATTCGGCAATGCCCCACCCCTTGGCTGGACGG (antisense) (product digested by EcoR1), as well as CGCGGTACCCAGCGGGAGCAACAGGATCAAATGCAGCAGCG (sense) and CGCGGTACCTATGGGATCCGTGCTGGGGTAGTGCTCGTAGAGC (antisense) (product digested by Kpn1), respectively.

Supplemental Legend to Figure 5

Average hemocyte numbers (in brackets) were as follows: wild type stage 11/12 (572), stage 15/16 (565); PVRΔC (*srp*HemoGAL4, UAS-srcEGFP; UAS-PVRΔC/UAS-*lacZ*nls) stage 11/12 (558), stage 15/16 (268); *Pvr1*/*Pvr1* stage 11/12 (330), stage 15/16 (176); p35 rescue of *Pvr1*/*Pvr1* stage 11/12 (506), stage 15/16 (394); lPVR rescue of *Pvr1*/*Pvr1* stage 11/12 (484), stage 15/16 (462); RasV12 rescue of *Pvr1*/*Pvr1* stage 11/12 (469), stage 15/16 (451); p110CAAX rescue of *Pvr1*/*Pvr1* stage 11/12 (452), stage 15/16 (247); p35 plus p110CAAX rescue of *Pvr1*/*Pvr1* stage 11/12 (510), stage 15/16 (418); wtPVR rescue of *Pvr1*/*Pvr1* stage 11/12 (520), stage 15/16 (524); heteroallelic combination *Pvr4*/*Pvr1* stage 11/12 (518), stage 15/16 (363); p35 rescue of *Pvr4*/*Pvr1* stage 11/12 (491), stage 15/16 (482).

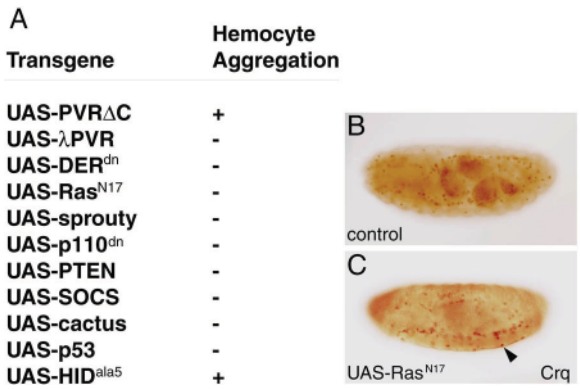
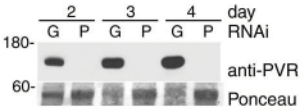


Figure S1.

It was tested whether inhibition of other signaling pathways would lead to a hemocyte aggregation phenotype. Several transgenes were expressed under control of the hemocyte specific *srp*HemoGAL4 (A). Dominant-negative *Drosophila* EGF receptor (DER), in particular in combination with dominant-negative PVR, was shown to have an inhibitory effect on border cell migration (Duchek et al., 2001). When expressed in embryonic hemocytes, no blood cell aggregation was induced. We further tested the effect of dominant negative Ras (RasN17) and the RTK signaling inhibitor Sprouty (Spry) (Casci et al., 1999; Reich et al., 1999). Neither RasN17 nor Spry induced large hemocyte aggregates to form. RasN17 expression resulted in mild enlargement of hemocytes at a low penetrance (one third of the embryos) (C) Arrowhead marks an example enlarged hemocyte. (B) Wild-type embryo shown as a reference. No large blood cell aggregates were seen with PTEN, a negative regulator of the PI3K/Akt pathway (Stocker et al., 2002), and a dominant-negative form of the p110 regulatory subunit of PI3K (Leevers et al., 1996). Negative results were also obtained with Socs (Callus and Mathey-Prevot, 2002) and Cactus (Qiu et al., 1998), negative regulators of the Jak/Stat and Toll/Cactus pathways, respectively (Govind, 1999; Mathey-Prevot and Perrimon, 1998). Likewise, *Drosophila* p53 (Brodsky et al., 2000; Ollmann et al., 2000) did not cause hemocyte aggregation in the embryo.

A



B

RNAi	sub-G0	G0/G1	S	G2/M
Pvr	33.86%	61.52%	2.18%	2.35%
GFP	22.99%	74.52%	2.20%	2.42%

C

RNAi	TUNEL
Pvr, day2	35.42%
GFP, day2	12.98%
Pvr, day4, no enzyme	1%
Pvr, day4	45.28%
GFP, day4, no enzyme	2.72%
GFP, day4	6.72%

Figure S2.
(A) Kc cells were treated with dsRNAs directed against *Pvr* (P) and GFP (G) (negative control). Western Blot analysis of equal amounts of protein extract shows absence of PVR protein in *Pvr* RNAi samples at 2–4 days after treatment. Ponceau stained membrane as loading control.
(B) Histogram statistics of propidium iodide cell cycle analysis shown in Figure 6B.
(C) Histogram statistics of TUNEL analysis shown in Figure 6C. Marker for TUNEL positive cells was set with reference to "Pvr no enzyme" control (1% TUNEL positive).

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