Supporting Information

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SI Text

Methods. ***Chemical Crosslinking of Receptor Dimers.*** After overnight serum starvation, 3T3 cells expressing wild type VEGFR2/PDGFR chimeric receptor (WT) or the R720/ D725 double mutant (RD/2A) were incubated with indicated concentration of VEGF-A in DMEM containing 50mM Hepes (pH 7) at 4 °C. After 90 min the cells were extensively washed with PBS (pH 7.4) to remove unbound ligand molecules. The samples were transferred to room temperature and disuccinimidyl suberate (DSS) was added to a final concentration of 0.5 mM. Crosslinking reaction was quenched after 30 min by incubation with 10 mM Tris buffer. Cell lysates were immunoprecipitated with anti-PDGFR antibodies to pull-down the chimeric receptors. Immuno-pellets were resolved by SDS-PAGE followed by immunoblotting with antibodies against FLAG-tag to detect receptor level.

Amino Acid Sequence Alignment. Amino acid sequence alignment of D7 was performed using ClustalW (1) and then manually adjusted based on the I-set IgSF fold restrains for 20 key residues. Amino acid sequences of human VEGFRs were used as query to search the nonredundant database for homologous sequences, using PSI-BLAST (2). The alignment of amino acid sequences as well as D7 PDB file were submitted to the Consurf 3.0 server (3) to generate maximum-likelihood normalized evolutionary rates for each position of the alignment where low rates of divergence correspond to high sequence conservation. As with the Consurf output, the continuous nine conservation scores are partitioned into a discrete scale of nine bins for visualization, such that bin 9 contains the most conserved (maroon) positions and bin 1 contains the most variable (cyan) positions. Amino acid sequences used in the analysis include: VEGFR2_HUMAN (gi:11321597), VEGFR2_DOG (gi:114158632), VEGFR2_HORSE (gi:194209154), VEGFR2_CATTLE (gi:158508551), VEGFR2_RAT (gi:56269800), VEGFR2_MOUSE (gi:27777648), VEGFR2_CHICK (gi:52138639), VEGFR2_QUAIL (gi:1718188), VEGFR2_ZEBRAFISH (gi:46401444), VEGFR1_HUMAN (gi:143811474), VEGFR1_MOUSE (gi:148673892), VEFGR1_RAT (gi:149034835), VEFGR1_HORSE (gi:149730119), VEGFR1_CHICK (gi:82105132), VEGFR1_ZEBRAFISH (gi:72535148), VEGFR_SEAURCHIN (gi:144226988), VER1_C_ELEGANS (gi:6003694), VER3_C_ELEGANS (gi:3877967), VER4_C_ELEGANS (gi:3877968), PVR_DROSOPHILA (gi:45552252), and VEGFR_SEASQUIRT (gi:198434052).

Cell Lines and Expression Vectors. 3T3 cell lines stably expressing the VEGFR1/2-PDGFR chimeric receptor were generated by retroviral infection as previously described (4). Cells were selected with L-histidinol, and pools matched for similar expression level were used in the experiments. HEK293 cells were transiently transfected with 1 μg of DNA and serum-starved overnight prior to VEGF-A stimulation. Cells were treated with 50, 100, or 200 ng/ml VEGF-A, and cell lysates were immunoprecipitated with antibodies against VEGFR1 or VEGFR2 followed by immunoblotting with anti-pTyr antibodies (PY20, Santa Cruz). Total cell lysates were analyzed by SDS-PAGE and subjected to immunoblotting with anti-phosphoMAPK or anti-MAPK antibodies (Cell Signaling), respectively.

VEGF-A was produced in sf9 cells using baculovirus expression vector pFastBac1 as previously described (5). VEGF-A was purified using heparin sepharose beads to >80% purity by Commassie blue stained SDS-PAGE experiments.

Analytical Ultracentrifugation. Sedimentation velocity experiments were performed with a Beckman Optima XL-I at the Center for Analytical Ultracentrifugation of Macromolecular Assemblies (Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX). D7 protein at concentration of 4×10^{-5} M, 8×10^{-5} M, and 1.6×10^{-4} M in buffer containing 25 mM Tris, pH 8, and 100 mM NaCl were subjected to centrifugation at 50,000 rpm at 20 °C in 1.2 cm Epon/charcoal centerpieces in an AN-60–TI rotor. Velocity data were analyzed with two-dimensional spectrum analysis combine with Monte Carlo analysis.

1. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice *Nucleic Acids Res* 22 (22): 4673–4680.
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool *J Mol Biol* 215 (3): 403–410.
3. Landau M, et al. (2005) ConSurf 2005: The projection of evolutionary conservation scores of residues on protein structures *Nucleic Acids Res* 33 (Web server issue): W299–302.
4. Yang Y, Yuzawa S, Schlessinger J (2008) Contacts between membrane proximal regions of the PDGF receptor ectodomain are required for receptor activation but not for receptor dimerization *Proc Natl Acad Sci USA* 105 (22): 7681–7686.
5. Cohen T, Gitay-Goren H, Neufeld G, Levi BZ (1992) High levels of biologically active vascular endothelial growth factor (VEGF) are produced by the baculovirus expression system *Growth Factors* 7 (2): 131–138.

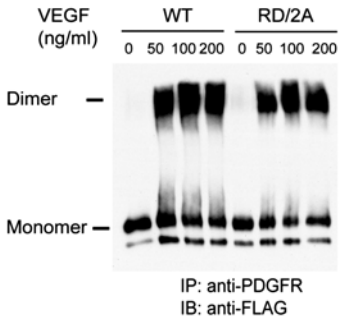

Fig. S1. VEGF-A stimulates dimerization of chimeric VEGFR2/PDGFR as well as chimeric receptor harboring D7 mutation. 3T3 cells expressing either VEGFR2/PDGFR chimeric receptor (WT) or cells expressing Arg720 and Asp725 to Ala (RD/2A) chimeric receptor mutants were serum starved overnight, followed by incubation with indicated amount of VEGF-A for 90 min at 4 °C. After extensive washing to remove unbound ligand, cells were incubated with 0.5mM DSS in PBS for 30min. Cell lysates were immunoprecipitated with anti-PDGFR antibody followed by SDS-PAGE and immunobloting with anti-FLAG antibody.