Characterization of the Retention Motif in the C-terminal Part of the Long Splice Form of Platelet-derived Growth Factor A-chain*

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Platelet-derived growth factor (PDGF) A-chain is found in two different splice variants; the long A-chain variant differs from the short one in that it contains a stretch of basic amino acid residues in the C-terminal part that mediates retention of the growth factor inside the producer cell and to the cell matrix. By analyzing mutants in which different amino acid residues in the retention motif had been changed to alanine residues, we found that the total positive charge of the sequence is of importance for the function of the retention motif. Moreover, we showed that retention also occurs if only one of the polypeptides in the PDGF dimer carries the retention motif. Surface iodination and competition with a peptide having the sequence of the retention motif revealed that the long A-chain variant, in contrast to the short A-chain variant, is localized on the outside of the cells and is also associated to the cell matrix. The association is likely to be mediated partially through heparan sulfate proteoglycans since treatment of matrix with heparitinase released the long A-chain variant.

Platelet-derived growth factor (PDGF) is a 30-kDa dimeric protein composed of disulfide-linked A- and B-polypeptide chains that are 60% identical in their mature parts, with a perfect conservation of the 8 cysteine residues (reviewed in Refs. 1 and 2). The PDGF A-chain appears as long and short variants, as a result of differential splicing (3, 4). All three variants, as a result of differential splicing (3, 4). The corresponding expression vectors are named pSVA, and pSVAa, respectively. Mutations of codons in the long PDGF A-chain variant corresponding to amino acid residues (numbering as in Ref. 14) Gly-200, Lys-201, Lys-202, Arg-203, Arg-204, Arg-205, Lys-206, and Arg-207 to alanine codons were made using the method of Kunkel et al. (31) on a uracil-containing template encoding the long variant of PDGF A-chain. Two double mutants and one triple mutant in which amino acid residues Lys-201/Lys-202, Lys-206/Arg-207, or Arg-203/Lys-204/Arg-205 were replaced with alanine residues were also made. The expression vectors pSVA201, pSVA202, pSVA203, pSVA204, pSVA205, pSVA206, pSVA207, pSVA201/A202, pSVA206/A207, and pSVA203/A204/A205 were generated by cloning of the mutated fragments into the EcoRI sites of the expression vector pSV7d. Two other mutants, in which the 2nd cysteine residue from the N terminus (Cys-125) in the long A-chain variant and the 4th cysteine residue (Cys-132) in the short A-chain variant were changed to serine residues, were made using the same method; the expression vectors pSVA,2 and pSVA4 were generated by cloning into the EcoRI sites of the expression vector pSV7d. All the plasmids were sequenced over the region encoding the mature parts of the proteins.

MATERIALS AND METHODS

Construction of cDNAs Encoding PDGF Mutants—cDNAs encoding the long and the short variants of PDGF A-chain have been described (14). The corresponding expression vectors are named pSVA, and pSVAa, respectively. Mutations of codons in the long PDGF A-chain variant corresponding to amino acid residues (numbering as in Ref. 14) Gly-200, Lys-201, Lys-202, Arg-203, Lys-204, Arg-205, Lys-206, and Arg-207 to alanine codons were made using the method of Kunkel et al. (31) on a uracil-containing template encoding the long variant of PDGF A-chain. Two double mutants and one triple mutant in which amino acid residues Lys-201/Lys-202, Lys-206/Arg-207, or Arg-203/Lys-204/Arg-205 were replaced with alanine residues were also made. The expression vectors pSVA201, pSVA202, pSVA203, pSVA204, pSVA205, pSVA206, pSVA207, pSVA201/A202, pSVA206/A207, and pSVA203/A204/A205 were generated by cloning of the mutated fragments into the EcoRI sites of the expression vector pSV7d (32). Two other mutants, in which the 2nd cysteine residue from the N terminus (Cys-125) in the long A-chain variant and the 4th cysteine residue (Cys-132) in the short A-chain variant were changed to serine residues, were made using the same method; the expression vectors pSVA,2 and pSVA4 were generated by cloning into the EcoRI sites of the expression vector pSV7d. All the plasmids were sequenced over the region encoding the mature parts of the proteins.

Expression and Immunoprecipitation of Recombinant Proteins—The pSV constructions encoding the mutant PDGF A-chains as well as pSVAa and pSVA, were transfected into COS cells as described (24), using 15 µg of plasmid DNA and 0.5–1 x 10⁶ cells in 60-mm culture dishes. In case of double transfections, the 7.5 µg of each plasmid DNA was used. Two days after transfection, metabolic labeling was performed by growing the cells overnight in 1.5 ml of cysteine-free MCDB 104 medium supplemented with 0.1 mCi of [³⁵S]cysteine/ml, 10% dialyzed fetal calf serum, and antibiotics. After labeling, the media were collected and cleared of cell debris by centrifugation. The cells were...
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RESULTS

Mutational Analysis of the Retention Motif of the Long PDGF A-chain—In order to determine which of the amino acid residues in the C-terminal basic motif of the long version of the PDGF A-chain are important for retention, different residues in the motif were changed to alanine residues (Fig. 1B). The mutants were transfected into COS cells; analysis of immunoprecipitated samples was performed by SDS-gel electrophoresis under nonreducing conditions followed by fluorography.

Retention than the other charged amino acids.

Retention of a PDGF Heterodimer—In view of the fact that PDGF occurs as homo- and heterodimeric isoforms, we wanted to determine whether retention requires targeting motifs in both chains of the PDGF dimer or whether one motif is sufficient. As we have shown elsewhere (35), the interchain disulfide bonds in PDGF are located between cysteine residue 2 in one chain and cysteine residue 4 in the other chain, and vice versa. In order to specifically assemble a heterodimer with only one retention motif, we mutated cysteine residue 2 in the long A-chain variant. The amino acid residue sequences of the retention motifs are given in one-letter code. Arrowsheads indicate processing sites. Panel B illustrates the different single, double, and triple alanine residue mutants in the retention motif of the long A-chain variant that were used in the present study. Arrows and brackets indicate the amino acid residues that were substituted to alanine residues. Panel C shows the two serine residue mutants made. The cysteine residues in the A-chain are shown (C); S indicates that the corresponding cysteine residue was exchanged to a serine residue.

Localization of the Retained Material—Evidence has been presented that the retention motif of the long PDGF A-chain mediates interactions with components intracellularly (17, 33) as well as extracellularly (20–22). In order to characterize further the localization of the retained PDGF, we transfected COS cells with pSV_A and pSV_AL as described above. On the second day after transfection, the surface proteins were labeled with

washed once in PBS, collected by scraping, and lysed in 0.5 ml of 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5, 0.5% Triton X-100, 1% Trasylol (Sigma), and 1 mM phenylmethylsulfonyl fluoride. The cell lysates were centrifuged for 15 min at 10,000 × g, and the supernatants, as well as the cell culture supernatants, were subjected to immunoprecipitations. Samples were precleared by incubation with 15 μl of normal rabbit serum at 4 °C for 1 h, followed by addition of 60 μl of a 50% Protein A-Sepharose slurry in PBS. After incubation at 4 °C for 30 min, the beads were removed by centrifugation. The media and cell lysates then received 15 μl of rabbit antisera raised against PDGF-AA (33) and were then incubated at 4 °C for 2 h. After incubation with Protein A-Sepharose as above, the beads were washed 5 times with 0.5 M NaCl, 20 mM Tris, pH 7.5, 5 mg/ml bovine serum albumin, 1% Triton X-100, and 0.1% SDS, and once with 20 mM Tris-HCl, pH 7.5. The immunocomplexes were eluted by addition of 200 μl of nonreducing SDS sample buffer (34) and incubation at 95 °C for 3 min. Half of the eluted material was reduced by addition of dithiothreitol to a final concentration of 10 mM, followed by incubation at 95 °C for 2 min, and was then alkylated by addition of iodoacetamide to a final concentration of 50 mM. The samples were analyzed by SDS-gel electrophoresis (34), using 12–18% polyacrylamide gels, followed by fluorography.

Identification of Surface Proteins—Transfections of COS cells were performed with pSV_A and pSV_AL as described above. A mock transfection was also made. Two days after transfections, the surface proteins were labeled with 125I using the lactoperoxidase method. The cells were washed 3 times with PBS-G (PBS supplemented with 0.9 mg/ml glucose) at 37 °C. To each dish was added 1 ml of PBS-G with 0.1 unit/ml lactoperoxidase, 6 units/ml lactoperoxidase, and 0.2 mCi of 125I, followed by incubation at room temperature for 20 min. The cells were then washed 5 times with PBS, solubilized, immunoprecipitated, and analyzed by SDS-gel electrophoresis, as described above.

Release of the Long A-chain Variant from Labeled COS Cells after Incubation with Peptide—COS cells were transfected with pSV_A and pSV_AL as described above. Two days after transfection, metabolic labeling was performed by growing the cells for 16 h in 15 ml of cysteine-free MCDB 104 medium supplemented with 0.1 μCi of [35S]cysteine/ml, 10% dialyzed fetal calf serum, and antibiotics. After 12 h, a peptide derivative was added with the amino acid sequence RPRESGKKRKRKRLKRTAKGSTAG, thus resembling the C terminus of the long A-chain, was added at a concentration of 40 μM. The conditioned media and cell lysates were then immunoprecipitated and analyzed, as described above.

Release of the Long A-chain Variant from Extracellular Matrix—COS cells were transfected and labeled as described above. The cells were then lysed using 0.5% deoxycholate, 0.15 μM NaCl, 0.05 mM Tris-HCl, pH 8.0, and the remaining matrix washed once with PBS. Each dish was then incubated for 4 h at 37 °C in 1 ml of PBS containing 40 μM RPR peptide or 0.2 unit/ml hepatopirine (Heparinase III, Sigma). The eluate was then immunoprecipitated and analyzed, as described above.

Retention than the other charged amino acids.

Retention of a PDGF Heterodimer—In view of the fact that PDGF occurs as homo- and heterodimeric isoforms, we wanted to determine whether retention requires targeting motifs in both chains of the PDGF dimer or whether one motif is sufficient. As we have shown elsewhere (35), the interchain disulfide bonds in PDGF are located between cysteine residue 2 in one chain and cysteine residue 4 in the other chain, and vice versa. In order to specifically assemble a heterodimer with only one retention motif, we mutated cysteine residue 2 in the long A-chain variant to a serine residue, and cysteine residue 4 in the short A-chain variant to a serine residue (Fig. 1C). When these two mutants were transfected individually into COS cells, the long chain variant was retained whereas the short chain was secreted. Both proteins occurred as monomers, which is expected since in these mutants, interchain disulfide bonds cannot form. Co-transfection of the two plasmids yielded a dimeric molecule, presumably a heterodimer, which was only detected in the cell lysate (Fig. 3). Thus, we conclude that one retention motif in a PDGF dimer is sufficient for retention.

Localization of the Retained Material—Evidence has been presented that the retention motif of the long PDGF A-chain mediates interactions with components intracellularly (17, 33) as well as extracellularly (20–22). In order to characterize further the localization of the retained PDGF, we transfected COS cells with pSV_A and pSV_AL as described above. On the second day after transfection, the surface proteins were labeled with
Retention Motif in PDGF A-chain

**Fig. 2. Expression of the retention motif mutants in COS cells.** Fifteen μg of the pSV expression vectors containing the different long A-chain mutants, in which one or more amino acid residues in the retention motif were exchanged for alanine residues, was transfected into COS cells. A mock transfection (−) as well as transfections with pSVA_1_ and pSVA_2_ were also carried out. After metabolic labeling with [35S]cysteine, the conditioned media and cell lysates were immunoprecipitated with antisera against PDGF-AA. The immunoprecipitates were then analyzed by SDS-gel electrophoresis under nonreducing conditions, followed by fluorography. The mature form of PDGF-AA is represented by the 30-kDa band, and the bands of 35 and 40 kDa represent precursor forms of PDGF-AA. In order to quantify the amount of released material, each lane was subjected to scanning with an UltraScan XL enhanced laser densitometer; the amount of material in the region where the mature and precursor forms of PDGF are found (30-40 kDa) was determined. The amount of PDGF in the medium fraction in relation to that of the corresponding cell lysate fraction (average of two or three determinations for each mutant) is given at the bottom of the figure.

125I using the lactoperoxidase method. A metabolic labeling with [35S]cysteine was also performed on parallel transfected COS cells. Lysates from both the 125I- and [35S]cysteine-labeled cells were then immunoprecipitated with an antiserum against PDGF-AA and subjected to SDS-gel electrophoresis followed by autoradiography and fluorography, respectively. Analysis of 125I-labeled cells revealed that the long A-chain was present at the surface of transfected COS cells as three different species of 30, 35, and 40 kDa; no 125I-labeled PDGF A-chain was immunoprecipitated from cells transfected with the short form of the A-chain (Fig. 4A). Interestingly, comparison of immunoprecipitated 125I-labeled exterior A-chain with the A-chain from [35S]cysteine-labeled cells revealed that the latter contained two additional species of 25 and 33 kDa (Fig. 4B). We conclude that part of the long A-chain occurs at the cell surface or in the extracellular matrix where it is accessible to lactoperoxidase labeling, whereas additional species of 25 and 33 kDa of the long form of the A-chain occur inside the cell only.

In order to determine whether the long variant of PDGF-AA could be displaced from its extracellular binding sites, we transfected COS cells with pSVA_1_ labeled the cells with [35S]cysteine, and incubated them in the absence or the presence of 40 μg of a 17-amino acid peptide (RPR), with a sequence corresponding to the C terminus of the long A-chain encompassing the retention motif during the last 4 h of labeling. The presence of the RPR peptide led to the release of components of 30 and 40 kDa into the medium, representing the mature and precursor form of PDGF-AA_1_, respectively; in the absence of peptide none of these components was seen (Fig. 5). This is likely to be due to a specific competition between the long A-chain and the peptide for binding sites on the cell surface or in the matrix. As expected, the amount of short A-chain in medium from COS cells transfected with pSVA_2_ was similar in the absence or presence of the peptide (Fig. 5). In the presence of the RPR peptide relatively more of the precursor form of PDGF-AA_2_ (40 kDa) compared with the mature form (30 kDa) was seen. This may be due to an inhibition by the basic peptide of extracellular N-terminal processing of the A-chain precursor at the -Arg-Arg-Lys-Arg- site (14), where cleavage normally occurs.

To more specifically investigate the possibility that the long A-chain was associated with the cell matrix, COS cells transfected with pSVA_1_ or pSVA_2_ and labeled with [35S]cysteine were removed by incubation in a deoxycholate-containing buffer; the remaining matrix was then incubated with or without 40 μg RPR peptide for 4 h. Analysis by immunoprecipitation with a PDGF-AA antiserum revealed that the long A-chain was released from the matrix after incubation with the peptide (Fig. 6).

Many growth factors have been shown to bind to heparin or heparan sulfate. In order to determine whether the retention of the long version of PDGF-AA involved binding to heparin or heparan sulfate, we incubated matrix from COS cells prepared in the same way as described above with 0.2 unit/ml heparitinase for 4 h. The material released from the matrix after enzyme treatment was then immunoprecipitated with an antiserum against PDGF-AA. Incubation with heparitinase led to release of the long A-chain variant, whereas no release of the short variant was seen. Thus, part of the long A-chain is retained in the matrix bound to heparin/heparan sulfate proteoglycans (Fig. 6).

**DISCUSSION**

The object of the present study was to explore the structure/function relationship of the basic sequence in the C-terminal part of the longer splice variant of the PDGF A-chain that has been shown to serve as a retention motif. Our results suggest that the total negative charge rather than single amino acid residues in the retention motif is of importance for retention. Moreover, we demonstrate that the retention motif targets the protein to an intracellular destination as well as to the cell matrix, the latter in part through binding to heparin/heparan sulfate proteoglycans.

A heterodimer of one long and one short PDGF A-chain was
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Specifically assembled by coexpression in COS cells of PDGF A-chain mutants with the 2nd or 4th cysteine residues, respectively, substituted by serine residues. The heterodimer was found to remain associated with the producer cells; thus, in COS cells one retention motif per dimer is sufficient to mediate retention. In contrast, a PDGF-AB heterodimer produced in CHO cells stably transfected with the B-chain and the short

A-chain and thus having one retention motif per dimer was found to be secreted into the medium (16). It is thus possible that the retention motif in the long A-chain is more efficient than that in the B-chain. Alternatively, it is possible that the amounts of the components that bind the retention motif vary in different cell types. This notion is strengthened by the previous finding that PDGF-BB is efficiently secreted by human melanoma cells (36) but retained by several other cell types. COS cells may have a relative abundance of the components that associate with the retention motif, whereas CHO cells may have less, leading to saturation of the binding sites and thereby secretion of PDGF-AB heterodimers. Another possibility is that specific proteases cleave off the retention motif and release
PDGF into the medium. CHO cells, in comparison to COS cells, may have relatively much of such proteases.

Analysis by immunoprecipitation of the different forms of the long A-chain in lysates of metabolically labeled cells compared with [2,3H]-labeled A-chains in the matrix revealed two additional forms of 25 and 33 kDa in the cell lysate. In an analogous manner, the major part of PDGF-BB, which also contains the retention motif in the C-terminal prosequence, appears as a 24-kDa cell-associated form (16, 17, 37, 38). Previous studies have shown that the 24-kDa form of PDGF-BB has undergone processing, the major part of PDGF-BB, which also contains the long A-chain in lysates of metabolically labeled cells compared to the 24-kDa form of PDGF-BB.

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The data presented in this study support the notion that the positive charge of the retention motif in PDGF-AA correlates to the efficiency for retention. Future studies will be aimed at exploring whether the retention motif shows specificity for interaction with heparan sulfate proteoglycans or whether it also interacts with other proteoglycans and other negatively charged molecules.

REFERENCES

1. Heldin, C.-H., and Westermark, B. (1990) Cell Regul. 1, 555-566
2. Raines, E. W., Bowers-Pope, D. F., and Ross, R. (1990) in Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors (Stern, M. H., and Roberts, A. B., eds) Vol. 95, Part I, pp. 173-262, Springer-Verlag, Heidelberg
3. Rorsman, F., Bywater, M., Knott, T. J., Scott, J., and Betsholtz, C. (1988) Mol. Cell. Biol. 8, 571-577
4. Bonthron, D. T., Morton, C. C., Orkin, S. H., and Collins, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1492-1496
5. Heldin, C.-H., and Westermark, B. (1990) Transplant. Proc. 22, 2072-2074
6. Claesson-Welsh, L., Eriksson, A., Westermark, B., and Heldin, C.-H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4197-4201
7. Matsui, T., Hoideman, M., Kii, T., Mor, F., Fosse, N., La Rocch, W., Kraus, M., Pierie, J., and Aarson, S. A. (1989) Science 243, 800-803
8. Vardeny, Y., Escobedo, J. A., Kuan, W.-J., Yang-Feng, T. L., Daniel, T. O., Trembley, P. M., Chen, Y. E., Ando, W. M., Hanks, N., Kii, T., Friend, V. A., Ulrich, A., and Williams, L. T. (1989) Nature 332, 226-232
9. Heldin, C.-H., Erlund, S., Rorsman, C., and Rinnastrand, L. (1989) J. Biol. Chem. 264, 8905-8912
10. Eriksson, A., Siegh, B., Westermark, B., Heldin, C.-H., and Claesson-Welsh, L. (1992) EMBO J. 11, 543-550
11. Bishayey, S., Jeskum, S., Khire, J., and Des, M. (1989) J. Biol. Chem. 264, 11699-11705
12. Sefters, R. A., Hart, C. E., Philips, E. P., FreForest, J. W., Ross, R., Murray, M., and Bower-Pope, D. F. (1989) J. Biol. Chem. 264, 8771-8778
13. Kanakaraj, P. R., Raj, S., Khan, S. A., and Bishayey, S. (1991) Biochemistry 30, 1761-1767
14. Bonaldo, C., Johnson, A., Heldin, C.-H., Westermark, B., Lind, P., Ureda, M., Eddy, R. Shows, T. B., Philkoff, R., Moro, M., Oss, T. J., and Scott, J. (1996) Nature 330, 695-699
15. Joseph, S. F., Guo, C., Ratten, L., and Wong-Saa, F. (1984) Science 223, 487-491
16. Ostman, A., Rall, L., Hammersch, M., Wormstead, M. A., Colt, D., Vanzunel, P., Betsholtz, C., Westermark, B., and Heldin, C.-H. (1986) J. Biol. Chem. 261, 16200-16206
17. Ostman, A., Thijberg, T., Westermark, B., and Heldin, C.-H. (1992) J. Cell Biol. 118, 509-519
18. Robbins, K. C., Antoniadis, H. N., Devare, S. G., Hunkapiller, M. W., and Aarson, S. A. (1983) Nature 306, 605-609
19. Robbins, K. C., Leal, F., Pierie, J. H., and Aarson, S. A. (1984) EMBO J. 4, 1783-1792
20. Kelly, J. L., Sanchez, A., Brown, G. S., Chesterman, C. N., and Sleij, M. J. (1993) J. Cell Biol. 121, 1153-1163
21. Raines, K. W., and Ross, R. (1989) J. Cell Biol. 116, 533-543
22. Pollock, R. A., and Richardson, W. D. (1992) Growth Factors 7, 267-277
23. LaRocch, W. J., May-Siroff, M., Robbins, K. C., and Aarson, S. A. (1991) Genes & Dev. 5, 1191-1199
24. Ostman, A., Anderson, R. M., Betsholtz, C., Westermark, B., and Heldin, C.-H. (1991) Cell Regul. 2, 503-512
25. Khachigian, L. M., Owensby, D. A., and Chesterman, C. N. (1992) J. Biol. Chem. 267, 1660-1666
26. Betsholtz, C., Rorsman, F., Westermark, B., Ostman, A., and Heldin, C.-H. (1990) Nature 344, 299
27. Keck, P., Hansen, S. D., Krivi, G., Saro, K., Warren, T., Foder, J., and Connolly, D. T. (1989) Science 246, 1309-1315
28. Leung, D. W., Cachianes, G., Kuan, W.-J., Goeddel, D. V., and Ferrare, N. (1989) Science 246, 1306-1309
29. Magdione, D., Guerriero, V., Vignetti, G., Delli-Bovi, P., and Persico, M. G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9267-9271
30. Magdione, D., Guerriero, V., Vignetti, G., Ferrero, M. G., Apelzikona, O., Alitalo, K., Del Vecchio, S., Le, K.-J., Choo, J. V., and Persico, M. G. (1993) Oncogene 8, 925-931
31. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 267-268
32. Truet, M., Blacher, R., Burke, L. R., Capat, D., Choo, C., Dina, D., Hartog, K., Koo, C. H., Masiarz, F. R., Merryweather, P. J., Najarian, R., Pachl, C., Potter, S. J., Puma, J., Guarino, M., Rall, L. B., Randolph, A., Ureda, M. S., Valenzuela, M. F., Dali, H. H., Filavaro, J., Hansen, J., Nordfang, O., and Eason, M. (1985) DNA 4, 333-349
33. Thijberg, J., Ostman, A., Backström, G., Westermark, B., and Heldin, C.-H. (1990) J. Cell Sci. 97, 219-229
34. Blobel, G., and Dobberstein, B. (1975) J. Cell Biol. 87, 835-851
35. Anderson, M., Ostman, A., Backström, G., Hellman, U., George-Nacimento, C., Westermark, B., and Heldin, C.-H. (1992) J. Biol. Chem. 267, 11290-11296
36. Forsberg, K., Valyi-Nagy, I., Heldin, C.-H., Herlyn, M., and Westermark, B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 395-397
37. Beckmann, M. Z., Betsholtz, C., Heldin, C.-H., Westermark, B., Di Marco, E., Di Fiore, P. P., Robbins, K. C., and Aarson, S. A. (1988) Science 241, 1341-1349
38. Bywater, M., Rorsman, F., Bongcam-Rudloff, E., Mark, G., Hammersch, A., Heldin, C.-H., Westermark, B., and Betsholtz, C. (1988) Mol. Cell. Biol. 8, 2753-2762
39. Flanagan, J. G., Chan, D. C., and Leder, P. (1991) Cell 64, 1025-1035
40. Pandis, A., and Massague, J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1736-1730
41. Stein, J., and Bettezone, C. W. (1991) Oncogene 6, 601-605