Platelets play an important role in hemostasis, thrombosis, and antimicrobial host defense and are also involved in the induction of inflammation, tissue repair, and tumor metastasis. We have previously characterized the platelet aggregation-inducing sialoglycoprotein (Aggrus/gp44) overexpressed on the surface of tumor cells. Because a platelet aggregation-neutralizing 8F11 monoclonal antibody that could specifically recognize Aggrus suppressed tumor-induced platelet aggregation, we have previously purified Aggrus by 8F11-affinity chromatography and found that purified Aggrus possessed the ability to induce aggregation of platelets. Here we show that Aggrus is identical to the T1α/gp38P/OTS-8 antigen, the function of which in tumors is unknown. Expression of mouse Aggrus and its homologue (also known as T1α-2/gp36) induced platelet aggregation without requiring plasma components. Using the 8F11 antibody, we identified the highly conserved platelet aggregation-stimulating domain with putative O-glycosylated threonine residues as the critical determinant for exhibiting platelet aggregation-inducing capabilities. We compared the expression level of human aggrus mRNAs using an array containing 160 cDNA pair samples derived from multiple human tumor-igenic and corresponding normal tissues from individual patients. We found that expression level of aggrus was enhanced in most colorectal tumor patients. To confirm the protein expression, we generated anti-human Aggrus polyclonal antibodies. Immunohistochemical analysis revealed that Aggrus expression was frequently up-regulated in colorectal tumors. These results suggest that Aggrus/T1α is a newly identified, platelet aggregation-inducing factor expressed in colorectal tumors.

Specific glycoproteins expressed on the surface of platelets enable the platelets to adhere to receptors exposed in areas of vascular damage (1). The process of adhesion activates platelet aggregation, leading to the formation of a platelet plug in the vessel wall. Activated platelets also induce the formation of a fibrin clot by carrying coagulation factors and providing a catalytic surface for the major interactions of the coagulation cascade. Because there exists a clear link between atherosclerotic vascular disease, inflammation, tumor metastasis, and thrombosis (1–3), it is important to identify the mechanisms of platelet aggregation that have pathobiologic, prognostic, and treatment-related relevance. Studies on cancer metastasis have shown that some human and animal tumor cells possess platelet aggregation-inducing abilities that correlate with their metastatic potential (2, 3). Interactions between tumor cells and platelets have been considered to facilitate the arrest of tumor cell cluster in the microcirculation with the subsequent formation of experimental metastasis. However, the molecules associated with the tumor-induced platelet aggregation have not yet been identified.

We previously established several clones possessing different platelet aggregation-inducing capabilities from a mouse colon adenocarcinoma 26 cell line (4). By generating monoclonal antibodies, we revealed that the established 8F11 monoclonal antibody inhibited platelet aggregation in vitro (5, 6) and the pulmonary metastasis of highly metastatic clones of mouse colon adenocarcinoma 26 cell line in vivo (7). The 8F11 antibody recognized a cell-surface 44- or 41-kDa sialoglycoprotein (Aggrus) (5, 6) Using 8F11 affinity-column chromatography, we purified the 44-kDa mouse Aggrus and found that purified Aggrus itself could induce platelet aggregation with no requirement for plasma components (8). We tried to identify the protein; however, we could not obtain the peptide sequence from the purified Aggrus protein because of the abundant carbohydrate chains.

In this study, we identified the 8F11 antibody-reactive mouse Aggrus as mouse T1α antigen (also known as mT1α/gp38P/OTS-8) (9–11). Using the platelet aggregation-neutralizing 8F11 antibody, we identified the residue in PLAG1 domain critical for platelet aggregation-inducing capability of mouse Aggrus. Although mouse and human Aggrus (also known as T1α-2/gp36) (9, 12) proteins have only 39% amino acid identity, the PLAG domains are highly conserved. Mutation of threonine residues in the PLAG domain abolished the platelet aggregation-inducing abilities of human and mouse Aggrus proteins. Using Cancer Profiling Array II, we found that the expression level of aggrus was enhanced in colorectal tumor patients. By generating polyclonal antibodies to human Aggrus/T1α-2/gp36, we confirmed the increased human Aggrus/T1α-2/gp36 protein expression in colorectal tumors. Therefore, Aggrus is a newly identified platelet aggregation-inducing factor that is overexpressed in colorectal tumors.
Aggrus Possesses Platelet Aggregation-inducing Capability

EXPERIMENTAL PROCEDURES

Plasmids, siRNA, and Antibodies—Mouse aggrus/mT1aggsP38 cDNA (GenBank accession no. AJ259794 or AJ250246) (9, 10) was obtained from Dr. Z. Aggrus plus N-terminal cysteine and purified using a column linked to 3° was purchased from QIAGEN (Valencia, CA). The oligonucleotides had CE(G)GV AMPG AEDDVV, corresponding to amino acids 38–51 of human Aggrus plus N-terminal cysteine and purified using a column linked to the peptide. Rat monoclonal 8F11 antibody was purified from ascites (Stratagene, La Jolla, CA) (13, 14). The mouse aggrus deleted cDNAs were accomplished using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) (13, 14). The mouse aggrus deleted cDNAs were also ligated into pET-21a vector (Novagen, Darmstadt, Germany), (13, 14). The mouse aggrus deleted cDNAs were ligated into pET-21a vector (Novagen, Darmstadt, Germany), and the recombinant mouse Aggrus deletion mutants proteins were expressed in Escherichia coli. Five small interfering RNA oligonucleotide duplexes (siRNAs) were designed from the mouse aggrus/mT1α sequence. In our experiments to suppress mouse Aggrus expression in NL-17 cells, we used the most effective siRNA from among them. The coding strands of the siRNA directed to residues 180–198 of mouse aggrus cDNA were 5’-UGAAUCAUCCGGAAGGCGC-3’ and 5’-UGCC-UUGCCAGUAGGAUUA-3’, respectively. Non-silencing control siRNA was purchased from (Qiagen, Venlo, The Netherlands). The oligonucleotides had 3’ ddT overhangs. The anti-human Aggrus polyclonal antibody (TT679) was obtained by immunizing rabbits with the synthetic peptide CEGVGAMPAGAEDDVV, corresponding to amino acids 38–51 of human Aggrus plus N-terminal cysteine and purified using a column linked to the peptide. Rat monoclonal 8F11 antibody was purified from ascites fluid (5). Control rat IgG, anti-FLAG-M2 antibody, and anti-β-actin antibody were purchased from Sigma.

Cell Culture Conditions—Chinese hamster ovary (CHO), mouse col adenocarcinoma NL-14 and NL-17 (4), mouse lymph node stromal cells expressing the mouse Aggrus antigen (also known as mT1α antigen (11, 12)), and macrophages expressed mouse Aggrus protein (Fig. 1A). Several reports suggested that mouse T1α antigen (also known as mT1aggsP38/OTS-8 (9–11)) and its human homologue (T1α-2/Aggrus) (9, 12) were expressed in MC3T3-E1 cells (11) and lymphoid tissues (19). Because of the expression pattern, we hypothesized that mT1α, which is required for normal lung cell proliferation and alveolus formation in vivo (20), is identical to an unidentified platelet aggregation-inducing factor (Aggrus). We generated pcDNA3 vector containing FLAG-tagged full-length mT1α cDNA. When CHO cells were stably transfected with mT1α cDNA (CHO/mAGR), transfected mT1α antigen was expressed on the cell surface and was recognized by the 8F11 antibody (Fig. 1B). Immunoprecipitation followed by immunoblot analysis revealed that 8F11 antibody recognized the antigen itself (Fig. 1C). We designed five siRNAs (21) and found that the siRNA directed to residues 180–198 of mT1α decreased the expression of endogenous mouse Aggrus in highly metastatic NL-17 and B16-F10 cells (Fig. 1D and data not shown). As we have reported previously (5), protein expression level of gp44/mouse Aggrus in highly metastatic NL-17 cells was higher than that in poorly metastatic NL-14 cells (Fig. 1E). Semiquantitative PCR analysis revealed the increased aggrus mRNA expression in NL-17 cells (Fig. 1E). These results indicate that the mT1α/gp38P/OTS-8 antigen is identical to our previously characterized mouse Aggrus/gp44 protein.

Aggrus Promotes Aggregation of Human and Mouse Platelets without Requiring Plasma Components—When CHO/mAGR cells were incubated with mouse PRP, CHO/mAGR cells, but not parental CHO cells, induced mouse and human platelet aggregation (Fig. 2A and B). The aggregation was suppressed by pretreatment of the cells with 8F11 antibody (Fig. 2C). Consistent with our previous report (8), plasma components were not required because washed platelets were also aggregated by incubation with CHO/mAGR (Fig. 2D). We cloned human aggrus cDNA (hAGR; also known as hT1α-2 or gp38) (9, 12) and transiently transfected it into CHO cells (CHO/hAGR). Expression of human Aggrus on CHO cell sur-
face induced mouse and human platelet aggregation (Fig. 2, A and B), regardless of poor homology (39% amino acid identity with mouse Aggrus) between mouse and human Aggrus proteins (9). These results indicate that human and mouse Aggrus proteins possessed the ability to induce platelet aggregation.

Identification of the 8F11 Recognition Domain in Mouse Aggrus—The mouse Aggrus-induced mouse platelet aggregation was perfectly suppressed by pretreatment of CHO/mAGR cells with 8F11 antibody (Fig. 2C). Thus, 8F11 may recognize the specific epitope of mouse Aggrus that interacts with platelets. To clarify the residues critical for platelet aggregation, we examined the reactivity of 8F11 antibody to recombinant mouse Aggrus protein expressed in E. coli (Fig. 3, A–C). The 39-DGMVP-44 residue was identified as essential for 8F11 binding to recombinant mouse Aggrus protein. To confirm the result, we transiently transfected the pcDNA3 vector containing mouse aggrus-M41A cDNA (CHO/M41A). CHO/M41A had almost the same platelet aggregation-inducing capability as parental CHO or CHO/mAGR cells (Fig. 5A). Therefore, 39-DGMVP-44 is the 8F11 recognition domain in mouse Aggrus.

These results suggest that the identified 8F11 recognition domain may not be involved in direct binding to platelets.

Identification of the Platelet Aggregation-stimulating Domain in Aggrus—As the Critical Determinant for Exhibiting Platelet Aggregation-inducing Capabilities—As we have characterized previously (8), carbohydrate chains of mouse Aggrus/gp44 are involved in the platelet aggregation-inducing capability. We hypothesized that the 8F11 antibody might neutralize platelet the aggregation-inducing capability of mouse Aggrus by conformationally interfering with the carbohydrate chains near the 8F11 binding domain. We then generated several mouse aggrus point mutants in which putative O-glycosylated threonine residues near the 8F11-binding domain were converted to alanine residues. Mutation of these threonine residues affected neither 8F11 binding (Fig. 4A) nor cell surface expression (data not shown). However, conversion of threonine-34 to alanine (T34A) in mouse Aggrus eliminated the platelet aggregation-inducing capability (Fig. 5A). Because threonine followed by proline is likely to be O-glycosylated (22) and Edman microsequencing of the equivalent residues in the dog homologue of Aggrus revealed gaps at the threonine residues (23), threonine-34 in mouse Aggrus might be O-glycosylated, and the carbohydrate chain was involved in the interaction of the platelets and subsequent platelet aggregation. Sequence comparison of the residues around threonine-34 in mouse Aggrus and residues around threonine-52 in human, threonine-34 in rat (24), and threonine-41 in threonine-50 in dog (23) homologues showed mouse Aggrus to be highly conserved (Fig. 5B). We therefore termed the residues as PLAG (platelet aggregation-stimulating) domains. As expected, conversion of threonine-52 to alanine in human Aggrus (T52A-hAGR) specifically abolished its platelet aggregation-inducing
capability (Fig. 5C). These results indicate that the PLAG domain (especially O-glycosylated threonine residues) is critical for platelet aggregation.

Expression of Aggrus in Colorectal Tumors—Mouse aggrus mRNA expression was observed in parental mouse colon adenocarcinoma 26 and its sublines, and the expression levels of aggrus mRNA in these cells were higher than that in normal mouse colon tissue (data not shown). Thus, we estimated the human aggrus expression in human tumors using Cancer Profiling Array II. In this array, normalized cDNA pairs from 160 primary tumor tissues and the corresponding normal tissues from individual patients were immobilized on nylon membrane (17, 18). Human aggrus expression increased in almost all intestinal tumors (colon, rectum, and small intestine) compared with corresponding normal tissues (Fig. 6A). After hybridization with 32P-labeled ubiquitin cDNA, human aggrus expression in each patient was normalized. The average expression ratio of human aggrus in colon (n = 10), rectum (n = 7), and small intestine (n = 10) tumor patients was about 2.8, 3.2, and 3.9, respectively (Fig. 6B). To confirm the result, we performed PCR with matched cDNA pairs from colon adenocarcinoma patient 5 and small intestine adenocarcinoma patient 2, whose cDNAs were immobilized in Cancer Profiling Array II (Fig. 6A, circled). Semiquantitative PCR analysis revealed that human aggrus expression was clearly enhanced in both tumor tissues (Fig. 6C). In contrast, human aggrus expression in lung, breast, and ovary varied from patient to patient (Fig. 6, A and B), and the average expression ratio in lung, breast, and ovary was about 1.6, 0.94, and 1.1, respectively. Human aggrus expression in adenocarcinoma of the intestine was higher than that in colon adenomas or small intestine carcinoid tumors (data not shown). Therefore, human aggrus expression might be specific for carcinoma. We obtained similar results using Cancer Profiling Array I (data not shown).

To confirm the increased human Aggrus protein expression in colorectal tumors, we generated polyclonal antibodies to human Aggrus (TT679) because the 8F11 antibody cannot recognize human Aggrus (Fig. 7A). The generated TT679 antibody recognized the 36-kDa human Aggrus protein and did not cross-react with other protein species (data not shown). Strong staining was seen in human specimens derived from colon adenocarcinoma tissues (Fig. 7, B and C) but not in samples from normal colon tissues (Fig. 7, D and E). These results indicate that expression level of Aggrus is enhanced in most colorectal tumor patients.

DISCUSSION

Several reports suggested that tumor-induced platelet aggregation is an early step in the development of a metastatic lesion (2, 3). Ultrastructural studies have also demonstrated that
platelets seem to enhance the development of arrested tumor emboli into a secondary metastatic colony (25, 26). Although several inhibitors of platelet aggregation have been reported to retard tumor metastasis in certain animal models (27–29), the tumor adhesive glycoproteins have not yet been identified. We previously established several clones possessing different metastatic abilities from a mouse colon adenocarcinoma 26 cell line (4). Among these clones, a highly metastatic clone, NL-17, was found to exhibit a high platelet aggregation-inducing activity, although a poorly metastatic clone, NL-14, had marginal platelet aggregation-inducing capability (28, 30). Therefore, the ability to induce platelet aggregation was related to the metastatic potential. To identify the platelet aggregation-inducing molecule(s) associated with tumor metastasis, we generated monoclonal antibodies by immunizing rats with NL-17 cells. One of the established antibodies, 8F11, exhibited the platelet aggregation-neutralizing activity in vitro and the inhibitory activity of lung colonization in vivo (5, 7). The 8F11 antibody recognized a 44-kDa membrane sialoglycoprotein (mouse Aggrus/gp44) on NL-17 cells (5). Consistent with the platelet aggregation-inducing capabilities of NL-17 and NL-14 cells (28, 30), the expression level of 8F11 antibody-reactive mouse Aggrus in NL-17 cells was higher than that in NL-14 cells (Fig. 1E). We previously succeeded in purifying the mouse Aggrus from NL-17 cells (8) and found that the purified mouse

![Image](image-url)
Aggrus itself possessed the ability to induce platelet aggregation. These data indicate that 8F11 antibody-reactive Aggrus is a platelet aggregation-inducing factor expressed on tumors.

We could not obtain the peptide sequence from the purified mouse Aggrus. Thus, we searched cells expressing mouse Aggrus. We found that mouse Aggrus was expressed on the surface of mouse osteoblastic MC3T3-E1 cells (Fig. 1A). Because the 38-kDa mouse T1α/gp38P/OTS-8 antigen was originally isolated from MC3T3-E1 cells (11), we hypothesized that mouse Aggrus was identical to mouse T1α/gp38P/OTS-8. Four lines of evidence support this assumption. First, the 8F11 antibody recognized CHO cells that had been transfected with mouse T1α/gp38P/OTS-8 cDNA (Fig. 1A); second, the immuno-precipitated FLAG-tagged mouse T1α/gp38P/OTS-8 protein was recognized by 8F11 antibody (Fig. 1C); third, siRNA directed to mouse T1α/gp38P/OTS-8 decreased the 8F11 antibody-reactive endogenous mouse Aggrus expression in NL-17 cells (data not shown). Therefore, we think these variations are caused by differences in post-translational modification.

The identified mouse Aggrus was first identified as an early-response protein (OTS-8) that was induced by phorbol ester in osteoblastic MC3T3-E1 cells (11). Mouse Aggrus has also been reported as a cell-surface antigen (gp38/gp38P) that is expressed in type-I thymus epithelial cells and in peripheral lymphoid tissues (10, 19). Mouse Aggrus is a high content of serine and threonine residues that might be O-glycosylated. In dog Aggrus/gp40, 14 serine and threonine residues were identified by Edman degradation to be modified by O-glycosylation (23). Because our data (Fig. 4) suggested that 8F11 antibody might neutralize mouse Aggrus-induced platelet aggregation by conformationally interfering the carbohydrate chains near the 8F11-binding domain, we generated several mouse aggrus point mutants in which putative O-glycosylated threonine residues around the 8F11-binding domain. We revealed that putative O-glycosylated threonine-34 of mouse Aggrus is a critical residue for its platelet aggregation-inducing capability (Fig. 5). This result is consistent with our previous report (8) that sialylated carbohydrate chains of Aggrus/gp44 were involved in their platelet aggregation-inducing capabilities. Although human and mouse Aggrus proteins have only 39% amino acid identity (9), the residues around threonine-34 of mouse Aggrus are highly conserved (Fig. 5B). Mutation of the equivalent threonine-52 in human Aggrus abolished its platelet aggregation-inducing activity (Fig. 5C).

We also cloned rat homologue of Aggrus/RT140/E11/podoplanin (33–35) and found that it could induce platelet aggregation (data not shown). Mutation of the threonine-34 to alanine in rat Aggrus abolished its platelet aggregation-inducing capability (data not shown). Thus, the PLAG domain is identified as critical residues for their platelet aggregation-inducing capability. Because heavily O-glycosylated proteins were identified as counter receptors for selectins (36, 37), it is possible that Aggrus binds to selectins for triggering platelet aggregation.

We also observed increased aggrus mRNA expression in most patients with intestinal tumors (Fig. 7, B–E). This result was consistent with the result that aggrus mRNA expression in colon adenocarcinoma 26 sublines was higher than that in normal mouse colon. Interestingly, the aggrus expression in adenocarcinoma of the intestine was higher than that in colon adenomas or small intestine carcinoid tumors (data not shown). Therefore, human aggrus expression might be specific for carcinoma and was strongly correlated with tumor progression.

Our results indicate that Aggrus is a newly identified platelet aggregation-inducing factor expressed on the surface of several tumors. Because platelet aggregation is associated with the development of arrested tumor emboli into a secondary
metastatic colony, therapies aimed at neutralizing Aggrus function could prove successful in inhibiting tumor metastasis.

REFERENCES
1. Klinger, M. H., and Jelkmann, W. (2002) J. Interferon Cytokine Res. 22, 913–922
2. Oleksowicz, L., and Dutcher, J. P. (1995) Med. Oncol. 12, 95–102
3. Honn, K. V., Tang, D. G., and Crissman, J. D. (1992) Cancer Metastasis Rev. 11, 325–351
4. Tsuruo, T., Yamori, T., Naganuma, K., Tsukagoshi, S., and Sakurai, Y. (1983) Cancer Res. 43, 5437–5442
5. Watanabe, M., Okochi, K., Sugimoto, Y., and Tsuruo, T. (1988) Cancer Res. 48, 6411–6416
6. Watanabe, M., Sugimoto, Y., and Tsuruo, T. (1990) Cancer Res. 50, 6657–6662
7. Sugimoto, Y., Watanabe, M., Oh-hara, T., Sato, S., Isoe, T., and Tsuruo, T. (1991) Cancer Res. 51, 921–925
8. Toyoshima, M., Nakajima, M., Yamori, T., and Tsuruo, T. (1995) Cancer Res. 55, 767–773
9. Ma, T., Yang, B., Matthay, M. A., and Verkman, A. S. (1996) Am. J. Respir. Cell Mol. Biol. 19, 143–149
10. Boucherot, A., Schreiber, R., Pavenstadt, H., and Kunzelmann, K. (2002) Nephrol. Dial Transplant. 17, 978–984
11. Nose, K., Saito, H., and Kuroki, T. (1990) Cancer Res. 50, 10832–10837
12. Zimmer, G., Oeffner, F., Von Messling, V., Tschernig, T., Groness, H. J., Klenk, H. D., and Herrler, G. (1999) Biochem. J. 341, 277–284
13. Fujita, N., Sato, S., Katayama, K., and Tsuruo, T. (2002) J. Biol. Chem. 277, 28796–28811
14. Sato, S., Fujita, N., and Tsuruo, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10832–10837
15. Fujita, N., Kataoka, S., Ito, M., Heike, Y., Boku, N., Nakajima, M., and Tsuruo, T. (1993) Cancer Res. 53, 5022–5027
16. Kato, Y., Fujita, N., Yano, H., and Tsuruo, T. (1997) Cancer Res. 57, 3049–3054
17. Wiechen, K., Diatchenko, L., Agoulnik, A., Scharff, K. M., Schober, H., Artl, K., Zhumbabayeva, B., Siebert, P. D., Dietel, M., Schäfer, R., and Sers, C. (2001) Am. J. Pathol. 159, 1635–1643
18. Zhumbabayeva, B., Diatchenko, L., Chenchik, A., and Siebert, P. D. (2001) Biotechniques 30, 158–163
19. Farr, A. G., Berry, M. L., Kim, A., Nelson, A. J., Welch, M. P., and Aruffo, A. (1992) J. Exp. Med. 176, 1477–1482
20. Ramirez, M. I., Millien, G., Hinds, A., Cao, Y., Seldin, D. C., and Williams, M. C. (2003) Dev. Biol. 256, 61–72
21. Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001) Nature 411, 494–498
22. Yoshida, A., Suzuki, M., Ikenaga, H., and Takeuchi, M. (1997) J. Biol. Chem. 272, 16884–16888
23. Zimmer, G., Lottepeich, F., Maisner, A., Klenk, H. D., and Herrler, G. (1997) Biochem. J. 326, 99–108
24. Rishi, A. K., Joyce-Brady, M., Fisher, J., Dobbs, L. G., Floros, J., VanderSpek, J., Brody, J. S., and Williams, M. C. (1995) Dev. Biol. 167, 284–306
25. Crissman, J. D., Hatfield, J. S., Menter, D. G., Sloane, B., and Honn, K. V. (1988) Cancer Res. 48, 4065–4072
26. Bradley, C. J., Dauer, R. J., Thurlow, P. J., and Connellan, J. M. (1997) Pathology 29, 189–195
27. Honn, K. V., Onoda, J. M., Diglio, C. A., Carufel, M. M., Taylor, J. D., and Sloane, B. F. (1984) Clin. Exp. Metastasis 2, 61–72
28. Tsuruo, T., Iida, H., Makishima, F., Yamori, T., Kawabata, H., Tsukagoshi, S., and Sakurai, Y. (1985) Cancer Chemother. Pharmacol. 14, 30–33
29. Honn, K. V., Onoda, J. M., Pampalona, K., Battaglia, M., Neagos, G., Taylor, J. D., Diglio, C. A., and Sloane, B. F. (1985) Biochem. Pharmacol. 34, 235–241
30. Tsuruo, T., Kawabata, H., Iida, H., and Yamori, T. (1986) Clin. Exp. Metastasis 4, 25–33
31. Gandarillas, A., Scholl, F. G., Benito, N., Gamallo, C., Quintanilla, M. (1997) Mol. Carcinog. 20, 10–18
32. Zimmer, G., Klenk, H. D., and Herrler, G. (1995) J. Biol. Chem. 270, 17815–17822
33. McElroy, M. C., Pittet, J. F., Allen, L., Wiener-Kronish, J. P., and Dobbs, L. G. (1997) Am. J. Physiol. 273, L1228–L1234
34. Metcalf, L. M., Flicker, W., Ceechini, M. G., Lanske, B., Wagner, C., Fleisch, H., and Atkinson, M. (1986) Bone 18, 125–132
35. Breiteneder-Geloff, S., Matsu, K., Soleiman, A., Merrer, P., Peczewski, H., Kalt, R., Schaffner, G., and Kerjaschki, D. (1997) Am. J. Pathol. 151, 1141–1152
36. Zamboni, S., Singer, M. S., Henzel, W., Hemmerich, S., Renz, M., Rosen, S. D., and Lasky, L. A. (1990) Science 262, 436–438
37. Sassetti, C., Tangemann, K., Singer, M. S., Kershaw, D. B., and Rosen, S. D. (1998) J. Exp. Med. 187, 1965–1975