Functional Sialylated O-Glycan to Platelet Aggregation on Aggrus (T1α/Podoplanin) Molecules Expressed in Chinese Hamster Ovary Cells*

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Aggrus, also called T1α and podoplanin, is a novel platelet aggregation-inducing factor that is expressed in various carcinoma cells. Aggrus/T1α/podoplanin is known to be expressed in lung type I alveolar cells or lymphatic endothelial cells. However, its physiological role has not been clarified. To assess the attribution of glycosylation to Aggrus platelet aggregation activity, recombinant molecules were stably expressed in a series of Chinese hamster ovary (CHO) cell mutants, N-glycan-deficient Lec1, CMP-sialic acid transporter-deficient Lec2, and UDP-galactose transporter-deficient Lec8. A new anti-human Aggrus monoclonal antibody, YM-1, was established to detect the expression of human Aggrus on these CHO cell mutants. Aggrus on Lec1 cells induced platelet aggregation, but those on Lec2 and Lec8 cells did not. Further, the glycans on Aggrus were analyzed by lectin blotting. Aggrus expressed in CHO and Lec1 cells showed Wheat-germ agglutinin, Jacalin, and Vicia villosa lectin bindings. Lectin blotting results indicated that sialylated core 1 structures, sialic acid plus Galβ1,3GalNAc-Ser/Thr, were critical for the platelet aggregation activity. This oligosaccharide structure is known as tumor-associated antigen, which is potentially related to the metastasis process of cancer cells.

The concept has been accepted that tumor metastasis is associated with platelet-aggregation activity possessed by human and other mammalian cancer cells (1, 2). A previous study has clarified that membranous 44- and 36-kDa sialoglycoproteins in the cancer cells of mice and humans, respectively, aggregated platelets with no relation to plasma components (3–5). Ectopic expression of these molecules was observed in a variety of cancer cells including colorectal and testicular tumors, in which up-regulated expression was evident (3, 6). The molecules, designated as Aggrus, belong to a type-I transmembrane sialomucin-like glycoprotein that consisted of an extra-cellular domain with abundant Ser and Thr residues as potentially O-glycosylation sites, a single transmembrane portion, and a short cytoplasmic tail with putative sites for protein kinase C and cAMP phosphorylation (3, 7).

Homologous molecules to Aggrus have been identified independently in several mammalians; T1α (7, 8), podoplanin (9), PA2.26 (10), gp40 (11), RANDAM-2 (12) are isolated from rat alveolar type-I cells in lung, rat glomerular epithelial cell (podocyte), mouse keratinocytes, Madin-Darby canine kidney cells (type I), and glutamatergic neuronal cells in mouse cerebrum, respectively. Podoplanin (gp36) is utilized as a specific marker for lymphatic endothelium in histopathology (13, 14). Ramirez and co-workers (15, 16) showed that T1α null mice, generated by a targeted dysfunction of the gene, died at birth from lethal respiratory failure accompanied by immature lymphatic vessel formation (15, 16). T1α/podoplanin conceivably plays an important role in regulating peripheral lung cell proliferation and lymphatic vascular development.

The present study examined the association of glycosylation with the platelet-aggregation activity; recombinant Aggrus molecules were expressed in a set of three defective cell lines from Pro-5 Chinese hamster ovary (CHO)1 cells. Those mutant cells have defects in distinct steps of glycosylation. Lec1 CHO cells lack the glycosyltransferase termed GlcNAc-TI, which produces incomplete intermediates of N-linked carbohydrates (18–20). Lec2 cells are CMP-sialic acid transporter-deficient. In addition, both glycoproteins and glycolipids lack 90% of sialic acids (SA). Lec8 cells exhibit a remarkable reduction of the ability to transport UDP-galactose (Gal) into the Golgi compartment (22, 23). Platelet aggregation-inducing activity was examined in those stably transfected cells. Moreover, the structure of active glycans was examined by the lectin-blot analysis. In addition, a monoclonal antibody to human Aggrus (hAggrus) was obtained by immunizing rat with the synthetic hAggrus peptide.

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EXPERIMENTAL PROCEDURES

Materials—Lymphatic endothelial cell was purchased from AngioBio (Del Mar, CA). Lung carcinoma cells, NCI-H226, and NCI-H520 were obtained from the American Type Culture Collection (ATCC). The anti-mouse Aggrus rat monoclonal antibody (mAb), 8F11, was prepared previously (24). Mouse anti-human podoplanin (gp36) mAb was obtained from Research Diagnostics Inc. (Flanders, NJ). Anti-FLAG antibody (M2) was purchased from Sigma-Aldrich. Horseradish peroxidase-conjugated secondary antibodies were obtained from DakoCytomation Co., Ltd. (Glostrup, Denmark). Fluorescein-isothiocyanate-conjugated anti-rat IgG was obtained from Cappel (Cochranville, PA). Fluorescein-isothiocyanate-conjugated anti-mouse IgG was from MC Biomedicals (Irvine, CA). Horseradish peroxidase-conjugated streptavidin was obtained from Amersham Biosciences. The following biotinylated lectins were purchased from Vector Laboratories Inc. (Peterborough, UK), concanavalin A, Dolichos biflorus agglutinin, peanuts agglutinin, Ricinus

Fig. 1. Characterization of YM-1 and platelet aggregation-inducing activity of cell lines. A, immunohistochemical staining with YM-1 on human specimens derived from prostate. The scale bar represents 10 μm. B, the cell lysate of lymphatic endothelial cell, NCI-H226, CHO-hAggrus, and CHO were electrophoresed and immunoblotted with YM-1 and anti-podoplanin mAb. C, flow cytometric analysis of YM-1 and anti-podoplanin reactivity to CHO-hAggrus, lymphatic endothelial cell, and NCI-H226. D, CHO-hAggrus, lymphatic endothelial cell, and NCI-H226 were incubated with mouse platelet-rich plasma, and platelet aggregation activity was confirmed. Parental CHO served a control. WB, Western blot.
communis agglutinin I, soybean agglutinin, Wheat-germ agglutinin (WGA), succinylated WGA (s-WGA), Griffonia simplicifolia lectin I and II, Sophora japonica agglutinin, Datura stramonium lectin, Jacalin, Sambucus nigra lectin, and Maackia amurensis lectin I and II.

Hybridoma Production—Two Sprague-Dawley rats were immunized with the synthetic peptide, CEGGVAMPGAEDDVV, corresponding to amino acids 38–51 of hAggrus plus an N-terminal cysteine. The spleen cells were fused with mouse myeloma P3U1 cells. Then the culture supernatants of the hybridomas were screened by enzyme-linked immunosorbent assay for the binding activity to the Aggrus peptide. The established mAb was designated YM-1.

Immunohistochemistry—YM-1 was added to the deparaffinized and rehydrated specimens for 4 h at 23 °C. Biotin-conjugated secondary anti-rat IgG antibody was added, followed by incubation with peroxidase-conjugated avidin-streptavidin complex (Vectastain ABC Kit, Vector Laboratories Inc.). Color was developed with 3,3-diaminobenzidine tetrahydrochloride tablet sets (DakoCytomation Co. Ltd.) used for 10 min. The human specimens were derived from prostate cancer (25).

Production of Aggrus in CHO/Lec Cells—Lec1, Lec2, and Lec8 cells of CHO mutant cell lines were obtained from ATCC (18–23). Cells were cultured at 37 °C in 5% CO₂ with α-minimal essential medium (Sigma-Aldrich) containing 10% fetal calf serum (Moregate Biotech, Queensland, Australia), supplemented with l-glutamine (2 mM), L-proline (0.04 mg/ml), penicillin (100 units/ml), and streptomycin (100 µg/ml). Human and mouse aggrus cDNAs (AB127958, AJ297944), incorporated with FLAG tag sequence, were subcloned into a vector of pcDNA3 (Invitrogen), which were named as pcDNA-hAGR and pcDNA-mAGR, respectively. Lec1, Lec2, and Lec8 cells were transfected with pcDNA-hAGR and pcDNA-mAGR by a procedure using LipofectAMINE reagent (Invitrogen) as described previously (3). Stable production was obtained using a selective culture of the transfectants in a medium containing Geneticin (G418; Sigma-Aldrich) at a concentration of 1.0 mg/ml for a couple of weeks.

Flow Cytometry—The expression levels of hAggrus and mouse Aggrus (mAggrus) were confirmed comparatively using by flow cytometry, as described previously (26). Briefly, lymphatic endothelial cells, NCI-H226 cells, parental CHO cells, and Lec cells, collected by trypsin-EDTA treatment, were incubated with antibodies for 1 h at room temperature, YM-1 culture supernatant, and 8F11 in phosphate-buffered saline at a concentration of 1 µg/ml. Then the cells were incubated...
with fluorescein isothiocyanate-conjugated antibodies for 30 min. Flow cytometry was performed using FACS Caliber (BD Biosciences).

**Platelet Aggregation**—Platelet aggregation was examined by the procedures of Toyoshima et al. (5). Briefly, mouse platelet-rich plasma was prepared from fresh heparinized blood extracted from BALB/c mice. An aliquot of 200 μl of platelet-rich plasma was incubated in a cuvette at 37 °C with continuous stirring. After 5 min, 5 μl of phosphate-buffered saline-washed cells were added. Then light transmittance was monitored at 660 nm for 10 min. This assay was performed with a NIKK HEMA Tracer I (Niko Bioscientific Co., Tokyo, Japan). A photograph was taken using a phase contrast microscope (100×, microphoto Fxα; Nikon Corp.) at the same time.

**Western Blot Analyses and Lectin Blotting**—Cultured cell pellets were lysed with a buffer for 30 min on ice. The buffer consisted of 25 mM Tris, 50 mM sodium chloride, 0.2% SDS, 0.5% sodium deoxycholate, 2% Nonidet P-40, 50 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride, pH 7.4. The lysate supernatants were centrifuged for 15 min at 15,000 rpm to remove cellular debris and nuclei. Cell lysates containing 5 μg of total protein were prepared for Western blot analysis by boiling in SDS sample buffer (50 mM Tris, 2% SDS, 5% glycerol, 10% 2-mercaptoethanol, pH 6.8).

For lectin blotting analysis, cell lysates were immunoprecipitated with anti-FLAG antibody conjugated agarose for 1 h at 4 °C. After the complexes were washed, elution was performed with a SDS sample buffer by boiling for 5 min, followed by polyacrylamide gel electrophoresis in 10–20% gradient gels. After proteins were transferred onto an immobilon PVDF membrane (Millipore Corp., Billerica, MA), the strips were incubated with YM-1 antibody or biotinylated lectins (20 μg/ml) for 1 h at room temperature followed by incubation with horseradish peroxidase-conjugated anti-rat IgG or streptavidin coupled to peroxidase. The enzyme activity was developed using an enhanced chemiluminescence kit (ECL, Amersham Biosciences).

**RESULTS**

Establishment of Anti-human Aggrus Monoclonal Antibody—We generated a mAb to hAggrus (YM-1) as described in the previous section. The reactivity of YM-1 was confirmed by immunohistochemical analysis, Western blot analysis, and flow cytomteric analysis (Fig. 1, A–C). YM-1 clearly recognized lymphatic vessel via immunostaining (Fig. 1A). Approximately 36 kDa of Aggrus expressed on human lymphatic endothelial cells and lung cancer cell line NCI-H226 were detected by YM-1 but not anti-podoplanin mAb (Fig. 1B). This result indicated that the YM-1 exhibit more intense immunoreactivity for Aggrus than that of the anti-podoplanin mAb. Furthermore, flow cytomteric analysis showed that the YM-1 could react more strongly than anti-podoplanin (Fig. 1C).

**Platelet Aggregation by Lymphatic Endothelial Cells and NCI-H226 Cells**—As shown in Fig. 1D, lymphatic endothelial and NCI-H226 cells also induced platelet aggregation as hAggrus on CHO cells did. However, another lung cancer cell line NCI-H520, which did not express hAggrus, did not induce platelet aggregation (data not shown). These results indicated that the spontaneous hAggrus also induced platelet aggregation.

**Platelet Aggregation by Lec Transfectants**—Recombinants were generated in the three mutant cells and parental CHO cells by transfection of pcDNA-hAGR and pcDNA-mAGR to assess the physiological importance of glycosylation on Aggrus molecules. The stable transfectants were established by the Genetin-selective culture. Expression of recombinant molecules was comparatively confirmed by flow cytometry using the specific antibodies of YM-1 and 8F11 (Fig. 2A). Judging from the shift of peaks, expression of hAggrus were high and equal among these cell transfectants.

When the platelet aggregation activity of the established transfectants was examined, both hAggrus and mAggrus in Lecl cells induced complete platelet aggregation as well as recombinants in parental CHO cells (Fig. 2B). The aggregation was confirmed using varying cell numbers in three independent experiments (data not shown). Real features of induced platelet aggregation are also demonstrated in Fig. 2C as a photograph. In contrast, aggregation was absent in Aggrus molecules expressed in Lecl2 and Lecl6 cells.

Lecl CHO cells are incapable of synthesizing N-glycan complexes (18–20). The amino acid sequence of mAggrus contained one consensus sites for N-glycosylation but did not in hAggrus. Because Lecl cells lacked N-glycosylation, platelet aggregation activity was not associated with this type of modification. Furthermore, CMP-SA transporter-deficient Lecl2 cells lack 90% of common SA decoration in both glycoproteins and glycolipids (21). Maturation of sialylated structures is inhibited in Lecl8 cells, because Gal is the major acceptor for SA in glycoconjugates (22, 23). Molecules that are modified by these two cell lines form insufficient SA decoration. Based on these results and interpretation, the presence of SA on O-glycans was conclusively critical for the induction of platelet aggregation.

**Glycans on Aggrus Expressed in CHO and Lec Cells**—To type the O-glycan structure on active hAggrus molecules in the CHO cells, lectin blotting using the 18 lectins, was further performed with immunoprecipitates of the cell lysates (Fig. 3). Table I summarizes this result. The hAggrus that was expressed in Lecl and parental CHO cells reacted with WGA but not s-WGA. The aggregation-inducing molecules carried SA at their termini because s-WGA recognizes naked GlcNAc in contrast to the interaction of WGA to SA-bound GlcNac. Jacalin, which recognizes core 1 structure (Galβ1,3GalNAc), reacted with hAggrus molecules expressed in parental, Lecl, and Lecl2 CHO cells. This result implies that active molecules possess a sialylated core 1 structure. Furthermore, hAggrus from Lecl8...
cells was detected by VVA (to GalNAc-Ser/Thr, called Tn-antigen), Griffonia simplicifolia lectin I, and soybean agglutinin (to GalNAc residue), indicating that the major glycan on hAggrus from Lec8 was Tn-antigen. In addition, hAggrus molecules from Lec2 cells showed slower movement in electrophoresis compared with others. This lower mobility might result from the reduction of negative charges or elongation of O-glycan chain because of the lack of SA. Fig. 4 schematically illustrates the theoretically possible glycosylation structures of Aggrus expressed in the parental CHO and Lec mutant cells.

**DISCUSSION**

We generated anti-hAggrus mAb by immunizing rats with the synthetic Aggrus peptide. Recently, Aggrus/podoplanin has been utilized as a lymphatic endothelium marker in histopathology. The results of YM-1 immunostaining indicated that this antibody has superior sensitivity to lymphatic endothelium (Fig. 1A). Western blot and flow cytometric analyses indicated that the hAggrus protein is expressed in the lymphatic endothelial cell line and lung cancer cell line, NCI-H226 (Fig. 1, B and C). Furthermore, these results showed that the YM-1 antibody reacted more strongly than the anti-podoplanin antibody.

The present study utilized the advantage of unique characteristics of CHO mutant cell lines to show the sialylated O-glycan was critical for the platelet aggregation-inducing activity of Aggrus.

**TABLE I**

Summary of lectin blot analysis to immunoprecipitated hAggrus

For concanavalin A, D. biflorus agglutinin, S. japonica agglutinin, L. esculentum lectin, S. tuberosum lectin, D. stramonium lectin, M. amurensis lectins I and II, and S. nigra lectin, lectin did not react toward recombinant hAggrus. −, negative; +, positive; w/o, without.

| Lectin                        | Specificity | CHO/hAggrus | Lec1/hAggrus | Lec2/hAggrus | Lec8/hAggrus |
|-------------------------------|-------------|-------------|--------------|--------------|--------------|
| WGA                           | (GlcNAc)_, SA | +           | +            | +            | −            |
| α-WGA                         | (GlcNAc)   | +           | −            | −            | −            |
| Jacalin                       | Galβ1,3GalNAc | +           | +            | +            | −            |
| Peanut agglutinin             | Galβ1,3GalNAc (w/o SA) | −           | −            | +            | −            |
| VVA                           | GalNAc, β   | −           | −            | −            | −            |
| R. communis agglutinin I      | Gal, GalNAc (w/o SA) | −           | −            | +            | −            |
| G. simplicifolia lectin       | GalNAc, Galα | −           | −            | +            | −            |
| Soybean agglutinin            | GalNAc, β   | −           | −            | −            | +            |

**Fig. 4. Scheme representing a possible pathway for O-glycans of Aggrus expressed in Lec mutant cells.** The initiating structure of O-glycan is the addition of the monosaccharide GalNAc to serine and threonine residues (Tn antigen). Subsequently, particular glycosyltransferases form T or sialyl Tn. Possible glycan structures formed by Lec mutant cells appear at intermediate products. Lectins that recognized the oligosaccharide were shown in parentheses.
Aggrus Activity Is Regulated by Glycosylation

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Aggrus in CHO cells, which provoked platelet aggregation, might be decorated as well as glycosylation in cancer cells. Although the physiological importance of the platelet-aggregating activity remains obscure, the fact that Aggrus is expressed in many tumor cells indicated that cancerous glycosylation of Aggrus is attributable to the tumor-induced activity of platelet aggregation during metastasis (3, 6).

Lectin blot analysis showed that active Aggrus molecules expressed in CHO cells bore a sialylated core 1 structure (SA-aggregation during metastasis (3, 6). In many tumor cells indicated that cancerous glycosylation of Aggrus in CHO cells, which provoked platelet aggregation, SA-binding Ig-like lectins (Siglec) of Siglec-3, Siglec-5, and Siglec-7, which are expressed in platelets (40). However, no positive evidence has been obtained by Western blotting and flow cytometry to date (data not shown). In the next step, it will be important to determine which acceptant component on platelets is attributable to the potent activity toward aggregation by Aggrus.

In conclusion, we generated a new anti-hAggrus antibody, YM-1. Then we showed that the decoration of SA on O-glycan is essential for platelet aggregation by membrane Aggrus molecules. Such data seem particularly important in light of the fact that the platelet aggregation activity is related to tumor metastasis.

REFERENCES

1. Honn, K. V., Tang, D. G., and Crissman, J. D. (1992) Cancer Metastasis Rev. 11, 325–351
2. Bastida, E., and Ordinas, A. (1988) Haemostasis 18, 29–36
3. Kato, Y., Fujita, N., Kunita, A., Sato, S., Kaneko, M., Osawa, M., and Tsuruo, T. (2003) J. Biol. Chem. 278, 51599–51605
4. Watanabe, M., Sugimoto, Y., and Tsuruo, T. (1999) Cancer Res. 59, 6657–6662
5. Toyoshima, M., Nakajima, Y., Yamori, T., and Tsuruo, T. (1995) Cancer Res. 55, 767–773
6. Kato, Y., Sasagawa, I., Kaneko, M., Osawa, M., Fujita, N., and Tsuruo, T. (2004) Oncogene in press
7. Dobbs, L. G., Williams, M. C., and Gonzalez, R. (1988) Biochim. Biophys. Acta 970, 146–156
8. 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, 294–306
9. Breiteneder-Geleff, S., Matsui, K., Soleiman, A., Meraner, P., Paczewski, H., Kalt, R., Schaffner, G., and Kerjaschki, D. (1997) Am. J. Pathol. 151, 1141–1152
10. Garcia-Diaz, A., Scholl, F. G., Benito, N., Gamallo, C., and Quintanilla, M. (1997) Mol. Carcinog. 20, 10–18
11. Zimmer, G., Klenk, H. D., and Herrer, G. (1995) J. Biol. Chem. 270, 17815–17822
12. Kato, Y., Tajima, Y., Otsani, T., Irie, A., Iwasuki, K., Kanai-Azuma, M., Imada, M., Kato, H., Shiitara, H., Kubo, H., and Sakuraba, H. (2003) J. Neurosci. Res. 73, 603–613
13. Maltesen, T., Veikko, T., Mustjoki, S., Karpanen, T., Catimel, B., Nice, E. C., Wise, L., Merer, A., Kowalski, H., Kerjaschki, D., Stacker, S. A., Achen, M. G., and Altola, K. (2001) EMBO J. 20, 4762–4773
14. Petrova, T. V., Makinen, T., Makela, T. P., Saarela, J., Virtanen, I., Ferrell, R. E., Finegold, D. N., Kerjaschki, D., Yla-Herttuala, S., and Altola, K. (2002) EMBO J. 21, 4593–4599
15. Ramirez, M. I., Millien, G., Hinds, A., Seldin, D. C., and Williams, M. C. (2005) Dev. Cell 8, 71–72
16. Schacht, V., Ramirez, M. I., Hong, Y. K., Hirakawa, S., Feng, D., Harvey, N., Williams, M., Dvorak, A. M., Dvorak, H. F., Oliver, G., and Detmar, M. (2003) EMBO J. 22, 3546–3556
17. Zimmer, G., Lottspeich, F., Maisner, A., Klenk, H. D., and Herrer, G. (1997) Biochem. J. 326, 99–108
18. Stanley, P., Califfit, V., and Siminovitch, L. (1975) Cell 6, 121–128
19. Stanley, P., Narasimhan, S., Siminovitch, L., and Schachter, H. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3323–3327
20. Stanley, P., Califfit, V., and Siminovitch, L. (1977) Somatic Cell Genet. 3, 391–405
21. Deutscher, S. L., Nuwayhid, N., Stanley, P., Briles, E. I., and Hirschberg, C. B. (1984) Cell 39, 295–299
22. Stanley, P. (1981) Mol. Cell. Biol. 1, 687–696
23. Deutscher, S. L., and Hirschberg, C. B. (1986) J. Biol. Chem. 261, 96–100
24. Watanabe, M., Okochi, E., Sugimoto, Y., and Tsuruo, T. (1988) Cancer Res. 48, 6411–6416
25. Tian, W., Osawa, M., Horuichi, H., and Tomita, Y. (2004) Cancer Sci. 95, 491–495
26. Kaneko, M., Kato, Y., Horuichi, H., and Osawa, M. (2003) Immunol. Lett. 86, 45–51
27. Backstrom, M., Link, T., Olson, F. J., Karlsson, H., Graham, R., Picco, G., Burchell, J., Taylor-Papadimitriou, J., Noll, T., and Hansson, G. C. (2003) Biochem. J. 376, 677–686
28. Scholl, F. G., Gamallas, C., Vilaro, S., and Quintanilla, M. (1999) J. Cell. Sci. 112, 4601–4613
29. Deblieker, R. E., Jay, J. P., and Kerjaschki, D. (1989) J. Biol. Chem. 264, 872–883
30. Lloyd, K. O., Burchell, J., Kudryavtsev, V., Yin, B. W., and Taylor-Papadimitriou, J. (1996) J. Biol. Chem. 271, 33325–33334
31. Brockhausen, I., Yang, J. M., Burchell, J., Whitehouse, C., and Taylor-Papadimitriou, J. (1995) Eur. J. Biochem. 233, 607–617
32. Hanisch, G., Fasdie, T. R., Deutzmann, F., and Peter-Katalinic, J. (1996) Eur. J. Biochem. 236, 318–327
33. Muller, S., and Hanisch, F. G. (2002) J. Biol. Chem. 277, 26103–26112
34. Moloney, D., Panin, V. M., Johnston, S. H., Chen, J., Shao, L., Wilson, R., Wang, Y., Stanley, P., Irvine, K. D., Haltiwanger, R. S., and Vogt, T. F. (2000) Nature 406, 369–375
35. Chen, J., Moloney, D. J., and Stanley, P. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13716–13721
36. Bruckner, K., Perez, L., Clausen, H., and Cohen, S. (2000) Nature 406, 411–415
37. Hicks, C., Johnston, S. H., diSilvio, G., Collazo, A., Vogt, T. F., and Weinmaster, G. (2000) Nat. Cell Biol. 2, 515–520
38. Breguet, P., Dugrillon, A., Garin, J. M., Eichler, H., and Kluter, H. (2003) Thromb. Haemostasis 90, 738–748