Processing by Convertases Is Not Required for Glypican-3-
induced Stimulation of Hepatocellular Carcinoma Growth*

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Glypicans are a family of heparan sulfate proteoglycans that are bound to the cell surface by a lipid anchor. Six members of this family have been identified in mammals (GPC1–GPC6). Glypicans act as regulators of the activity of various cytokines, including Wnts, Hedgegogs, and bone morphogenetic proteins. It has been reported that processing by a convertase is required for GPC3 activity during convergent extension in zebrafish embryos, for GPC3-induced regulation of Wnt signaling, and for the binding of GPC3 to Wnt5a. In our laboratory, we have recently demonstrated that GPC3 promotes the growth of hepatocellular carcinomas (HCCs) by stimulating canonical Wnt signaling. Because there is increasing evidence indicating that the structural requirements for GPC3 activity are cell type specific, we decided to investigate whether GPC3 needs to be processed by convertases to stimulate cell proliferation and Wnt signaling in HCC cells. We report here that a mutant GPC3 that cannot be processed by convertases is still able to play its stimulatory role in Wnt activity and HCC growth.

Our laboratory has recently reported that GPC3 is up-regulated in many human hepatocellular carcinomas (HCCs)1 (34). In addition, we showed that GPC3 promotes the growth of HCC by stimulating canonical Wnt signaling (21). The growth-stimulating activity of GPC3 in HCC cells has also been reported by another laboratory (35). One interesting finding of our study was that the heparan sulfate chains were not essential for the GPC3-induced activation of Wnt signaling and for the GPC3-induced stimulation of HCC growth in vivo. Similarly, the heparan sulfate chains were shown to be dispensable for the regulatory activity of GPC4 in convergent extension during Xenopus gastrulation, a process that is also known to be driven by Wnt signaling (14). Contrarily, in the study by De Cat et al. (15) it was shown the heparan sulfate

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2 The abbreviations used are: HCC, hepatocellular carcinoma; GPC3, glypican-3; HA, hemagglutinin A; GPI, glycosyl-phosphatidylinositol; EF, elongation factor; FACS, fluorescence-activated cell sorting; wt, wild-type.
chains are essential for the regulatory activity of GPC3 on Wnt signaling in Chinese hamster ovary cells. Collectively, these results suggest that the structural requirements for GPC3-induced regulation on Wnt signaling are cell context specific. We decided therefore to investigate whether the processing of GPC3 by convertases is necessary for its regulatory activity of Wnt signaling in the context of HCC cells. We report here that a mutant GPC3 that cannot be cleaved by convertases is still able to stimulate the growth of HCC cells. Moreover, we also show that cleavage by convertases is not required for the interaction of GPC3 with Wnt7b and Wnt3a and for the potentiation of canonical Wnt signaling.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Plasmids**—PLC-PRF-5 and HLF cell lines were cultured in minimal essential medium with 10% fetal bovine serum (Hyclone) supplemented with minimal essential medium non-essential amino acids solution (Invitrogen), and sodium pyruvate (1 mM). The 293T cells were cultured in Dulbecco’s modified Eagle’s medium and 10% fetal bovine serum. The PLC-PRF-5 and 293T cell lines were obtained from the American Type Culture Collection (ATCC), and the HLF cell line was donated by Dr. Eiji Miyoshi (University of Osaka). L cells permanently transfected with Wnt3A-pLNCx or empty vector (pLNCx) as control were obtained from ATCC and cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. The PLC-PRF-5 and 293T cell lines were cultured in Dulbecco’s modified Eagle’s medium and 10% fetal bovine serum. The PLC-PRF-5 and 293T cell lines were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. To collect conditioned medium, the cells were grown at high density for 4 days. Rat1 cells permanently transfected with Wnt1-pLNCx or empty vector (pLNCx) were obtained from Dr. J. Kitajewsky (Columbia University).

Expression vectors containing N-terminal hemagglutinin A (HA)-tagged GPC3, the mutant GPC3 that cannot be glycated (GPC3ΔGAG), and the mutant GPC3 lacking the glycosyl-phosphatidylinositol (GPI)-anchoring domain (GPC3ΔGPI) were previously described (36).

**Generation of the Convertase-resistant GPC3 Mutant**—To eliminate the endoproteolytic cleavage site in the N-terminal HA-tagged GPC3 cDNA, arginine residues 355 and 358 were mutated to alanine by site-directed mutagenesis (GPC3 RR → AA), and mutations were confirmed by DNA sequencing. To verify that this mutant is convertase resistant, 293T cells were transiently transfected with the full-length wild-type GPC3 cDNA (GPC3 wt), the GPC3 RR → AA cDNA, or with vector alone (EF) using Lipofectamine 2000 (Invitrogen). Cells were lysed in radioimmune precipitation assay buffer and the lysates analyzed by Western blot with an anti-GPC3 (1G12) or anti-HA (12CA5; Roche Applied Science) monoclonal antibodies as previously described (34).

**Generation of Stable GPC3-expressing HCC Cell Lines**—PLC-PRF-5 and HLF cells were transfected with selectable expression vectors containing the full-length wild-type GPC3 cDNA (GPC3 wt), the GPC3 RR → AA cDNA, the GPC3ΔGAG cDNA, or with vector alone (EF) as a negative control using Lipofectin (Invitrogen). Transfected cells were selected and FACS-sorted as previously described (21). The expression levels of GPC3 in the different stably transfected cell populations was also analyzed by FACS as previously described (21).

**Cell Proliferation Assay**—Cells were plated in 24-well dishes (20,000 cells/well) in medium without antibiotics. At the indicated time points, cells were trypsinized and counted with a hemocytometer. Each experiment was done at least three times by quadruplicates.

**Tumorigenicity Assay**—5 × 10⁶ cells were injected subcutaneously into the left flank of 7-week-old CB-17 SCID mice (Charles River Canada) using a 26-gauge needle. At the indicated time points, the volumes of the tumors were determined by measuring the largest (a) and smallest (b) axis using a caliper. Volume was calculated according to the formula  V = 0.5 ab². Significance of differences between the groups of mice was determined by the Mann-Whitney test, and the level of significance was set as p < 0.05. Mice handling and experimental procedures were performed in accordance with institutional guidelines.

**Cytoplasmic β-Catenin Assessment**—Cells were grown in the absence of serum for 48 h, washed extensively with phosphate-buffered saline, and harvested with a cell scraper. Cytoplasmic fractions were prepared as previously described (21), and the level of cytoplasmic β-catenin was assessed by Western blot with the antibody clone 14 (BD Transduction Laboratories). Actin (AC-40 Ab; Sigma) was used as loading control.

**Luciferase Assay for TOPFLASH Reporter Activity**—293T cells were plated in 24-well plates at a density of 200,000 cells/well and co-transfected with a luciferase reporter vector driven by the TOPFLASH promoter (37), a β-galactosidase expression vector, and GPC3 wt, GPC3 RR → AA, or vector alone (EF) using Lipofectamine 2000 (Invitrogen). One day after transfection the cells were incubated for 6 h with conditioned media from Wnt3A-transfected L cells or L cells transfected with empty vector as control. Cells were then lysed, and luciferase activity (Luciferase Assay System; Promega) and β-galactosidase activity were determined. Each luciferase value was normalized for transfection efficiency using β-galactosidase activity. Each experiment was performed three
times by triplicates. To perform the luciferase assay with PLC-PRF-5 cells we followed a procedure similar to that for the 293T cells, except that we used Lipofectin (Invitrogen) for transfection, and for stimulating Wnt signaling the cells were co-cultured with 300,000 Wnt1-expressing Rat1 cells or Rat1 cells transfected with empty vector as control for 16 h.

**Wnt-GPC3 Co-immunoprecipitation**—293T cells were transfected with GPC3 wt or GPC3 RR \(^{335}AA\) mutant and HA-tagged Wnt3A or Wnt7B expression vectors using Lipofectamine 2000 (Invitrogen). Two days after transfection, cell lysates were prepared in 1% Triton X-100, 0.5% deoxycholate in phosphate-buffered saline. Lysates containing 400 μg of protein were precleared with protein G-Sepharose (fast flow; Sigma) during 1 h at 4 °C. After centrifugation, GPC3 was immunoprecipitated from the precleared lysates adding anti-GPC3 1G12 monoclonal antibody (2.5 μg/ml) overnight at 4 °C. After washing, the beads were collected and the presence of Wnt in the immunoprecipitated material analyzed by Western blot using an anti-HA antibody (3E10; Roche Applied Science). To control for efficiency of transfection, GPC3 and Wnt levels were assessed by Western blot analysis of total lysates.

**RESULTS AND DISCUSSION**

**Generation of the Convertase-resistant GPC3 Mutant**—A mutant GPC3 (GPC3 RR \(^{335}AA\)) that cannot be processed by convertases was generated by replacing Arg \(^{355}\) and Arg \(^{358}\) in the intraproteolytic processing site RQYR with alanine residues. The resistance of this mutant GPC3 to convertase-mediated processing was verified by Western blot analysis of transiently transfected 293T cells. As shown in Fig. 1B, when the anti-GPC3 antibody 1G12 (which detects an epitope located in the C terminus) is used for Western blot analysis in reducing conditions, wild-type GPC3 generates a smear that extends from 30 to 200 kDa, which corresponds mostly to the products of the glycanation of the C terminus 30-kDa fragment (Fig. 1A). It should also be noted that a portion of the smear corresponds to the glycanation products of the full-length GPC3, because analysis with the anti-HA antibody shows that not all the GPC3 that is on the cell surface is processed by convertases in transiently transfected 293T cells. In addition, the Western blot analysis revealed the presence of a ~70-kDa band. This band most likely represents the GPC3 core protein that has not been glycanated yet. As expected, Western blot analysis of the GPC3 RR \(^{335}AA\) mutant with the 1G12 antibody shows the disappearance of the portion of the smear with a range of molecular masses that is lower than the full-length GPC3 core protein, confirming that GPC3 cleavage and the generation of the 30-kDa C terminus does not occur when the site recognized by the convertases is mutated.

When the cell lysate containing wild-type GPC3 was analyzed with an anti-HA antibody (12CA5) that recognizes the HA tag located at the N terminus, the intensity of the smear is dramatically reduced. This is expected because the anti-HA antibody cannot recognize the 30-kDa...
subunit and its glycanated products. On the other hand the anti-HA antibody recognizes a 40-kDa fragment that corresponds to the N terminus generated as a result of convertase-driven cleavage of GPC3. In addition, a limited amount of smear corresponding to non-cleaved glycanated GPC3 is observed. Analysis of the GPC3 RR → AA mutant with the anti-HA antibody therefore confirms that this mutant cannot be cleaved by convertases.

**GPC3 Cleavage by Convertases Is Not Required for the Stimulation of HCC Cell Proliferation**—We have previously shown that ectopic GPC3 stimulates the *in vitro* and *in vivo* growth of PLC-PRF-5 and HLF, two HCC cell lines that express very little and no endogenous GPC3, respectively (21). Both cell lines are able to process GPC3, as indicated by the fact that ectopic wild-type GPC3 generates in these cells smears with a smaller molecular mass range (Fig. 2). In addition, the ability of the HCC cells to cleave GPC3 can be recognized by the presence of a 40-kDa band in Western blots probed with the anti-HA antibody (Fig. 2). To provide a better estimation of the proportion of GPC3 that is cleaved by convertases, we transfected the two HCC cell lines with a mutant GPC3 that cannot be glycanated (22). Based on the intensity of the ~30-kDa band that corresponds to the non-glycanated C terminus, we estimate that ~50% of the GPC3 expressed by the PLC-PRF-5 and HLF cell lines is cleaved by convertases (Fig. 2F). To determine whether the processing of GPC3 by convertases is necessary for its growth-stimulatory activity, we also transfected PLC-PRF-5 and HLF cells with the GPC3 RR → AA mutant. Populations of transfected cells expressing high levels of mutant GPC3 were then isolated by FACS, and the expression of similar GPC3 levels in the various cell populations was confirmed by Western blot and FACS analysis (Fig. 2, C–E). It should be noted that the HLF cells seem to be able to synthesize much longer heparan sulfate chains, because the range of molecular masses of the smear detected in these cells is significantly larger than that observed for PLC-PRF-5 cells.

Next, the effect of ectopic GPC3 on the proliferation rate of the transfected HCC cells was investigated. As shown in Fig. 2, we found that both the wild-type GPC3 and the RR → AA mutant GPC3 were able to stimulate the proliferation rate of the two HCC cell lines to the same extent. Moreover, wild-type or RR → AA mutant GPC3 similarly stimulated the tumorigenicity of PLC-PRF-5 cells in mice (Fig. 3). We conclude, therefore, that the processing of GPC3 by convertases is not necessary for its *in vitro* and *in vivo* growth-stimulatory activity in HCC cells. These findings are consistent with the hypothesis that the structural requirements for glypican activity are cell context specific.

In principle, it could be argued that processing of GPC3 in the transfected HCC cells is not required because the ectopic levels of GPC3 are very high. It should be noted, however, that we have already shown that the levels of GPC3 expressed by transfected PLC-PRF-5 and HLF cells are within the physiological range, because they are lower than those found in HepG2, a GPC3-positive HCC cell line (21).

**The GPC3-induced Stimulation of Canonical Wnt Signaling in HCC Cells Is Independent of Cleavage by Convertases**—We recently reported that GPC3 promotes HCC growth by stimulating the canonical Wnt pathway (21). Because De Cat et al. (15) showed that convertase activity is required for GPC3-induced regulation of canonical Wnt activity and it is well established that glypicans can also potentiate the activity of other heparin binding growth factors, we decided to investigate whether non-cleaved GPC3 also stimulates HCC cell proliferation by activating Wnt activity. To this end we studied the effect of wild-type or RR → AA mutant GPC3 on the levels of cytoplasmic β-catenin in the PLC-PRF-5 and HLF cells. As shown in Fig. 4A, the mutant GPC3 is able to induce the accumulation of levels of cytoplasmic β-catenin similar to wild-type GPC3. We conclude, therefore, that the processing of GPC3 by convertases is not required for the stimulation of canonical Wnt signaling in HCC cells.

De Cat et al. (15) showed that processing by convertases is required for the GPC3-induced regulation of canonical Wnt1 in Chinese hamster ovary and MCF-7 cells. Because PLC-PRF-5 and HLF cells do not express Wnt1 (21), it could be proposed that the lack of requirement of convertase processing for GPC3-induced activation of autocrine Wnt signaling in HCC cells that we observed is due to the fact that this
requirement is restricted for the regulation of Wnt1 activity. To test this possibility we compared the effect of GPC3 and the RR → AA mutant in the response of PLC-PRF-5 cells to exogenous Wnt1. Because soluble Wnt1 is mostly inactive, we decided to co-culture the GPC3-transfected PLC-PRF-5 cells with Rat1 cells permanently transfected with a Wnt1 expression vector. To measure the response of the PLC-PRF-5 cells to Wnt1 we transiently transfected these cells with the canonical Wnt-responsive TOPFLASH luciferase reporter plasmid. The experiments were performed three times by triplicates, and a representative experiment is shown. A, levels of GPC3 expression in the transfected cells. Arrowhead indicates GPC3 core protein; arrow indicates the N terminus 40-kDa fragment; and the asterisks mark the position of two nonspecific bands recognized by the 12CA5 antibody. Molecular mass markers are indicated on the right.

**FIGURE 5.** GPC3 processing by convertases is not required for stimulation of canonical Wnt signaling in 293T cells. A, luciferase activity of transiently transfected 293T cells (EF, GPC3 wt; RR → AA mutant, or GPC3ΔGPI) in response to Wnt3A-transfected L cell-conditioned medium. Luciferase activity was normalized for transfection efficiency by β-galactosidase activity. The bars represent the fold stimulation induced by Wnt3a on the normalized luciferase activity. The experiments were performed three times by triplicates, and a representative experiment is shown. B, levels of GPC3 expression in the transfected cells. Arrowhead indicates GPC3 core protein; arrow indicates the N terminus 40-kDa fragment; and the asterisks mark the position of two nonspecific bands recognized by the 12CA5 antibody. Molecular mass markers are indicated on the right.

GPC3 Processing by Convertases Is Not Necessary to Stimulate Canonical Wnt Signaling in 293T Cells—To determine whether the lack of requirement of convertase processing for GPC3-induced stimulation of Wnt activity is a phenomenon restricted to HCC cells, we decided to study the effect of the RR → AA mutant GPC3 on the response to exogenous canonical Wnt in 293T cell lines. To this end, we measured Wnt1-induced transcriptional activity in 293T cells transiently transfected with the TOPFLASH-luciferase reporter plasmid and various GPC3 expression vectors. Conditioned medium from Wnt3A-transfected L cells was used as the source of exogenous Wnt. Fig. 5, upper panel, shows that wild-type GPC3 significantly increases the response of 293T cells to Wnt3A and that the RR → AA mutant GPC3 induces a similar increase. As a negative control we also tested the effect of a mutant GPC3 lacking the GPI-anchoring domain (GPC3ΔGPI). This mutant cannot be attached to the cell membrane and did not stimulate Wnt signaling in HCC cells (21). As expected, GPC3ΔGPI was unable to stimulate luciferase activity in 293T cells (Fig. 5). Western blot analysis of GPC3 in the transiently transfected 293T cells showed that the wild-type GPC3 and the RR → AA mutant constructs generated similar expression levels (Fig. 5, lower panel).

**FIGURE 6.** Convertase processing is not required for co-immunoprecipitation of GPC3 and Wnt. 293T cells were transfected with the indicated expression vectors, and GPC3 was immunoprecipitated with the anti-GPC3 antibody (1G12). In the upper panel, the expression levels of ectopic GPC3 and Wnt in whole lysates were assessed by Western blot using anti-GPC3 (1G12) and anti-HA (3F10) monoclonal antibodies, respectively. In the lower panel, the presence of Wnt in the immunoprecipitates was probed with an anti-HA antibody (JF16). The positions of Wnt (open arrow), GPC3 core protein (arrowhead), and the IgG heavy chain (HC) and IgG light chain (LC) from the anti-GPC3 monoclonal antibody are indicated on the right. Molecular mass marker positions are shown on the left.

**Convertase Processing Is Not Required for the Co-immunoprecipitation of GPC3 and Wnts—**It has been proposed that glypicans stimulate Wnt signaling by facilitating the interaction of Wnts and their signaling receptors (8). Consistent with this, we have demonstrated that in 293T cells GPC3 co-immunoprecipitates with Wnt3a and Wnt7b (21). Interestingly, De Cat et al. (15) have shown that in MCF-7 breast cancer cells the non-cleavable GPC3 does not co-immunoprecipitate with Wnt5a. We decided to investigate, therefore, whether the RR → AA mutant GPC3 co-immunoprecipitates with Wnt3b and Wnt7b in 293T cells. To this end we transiently transfected wild-type or RR → AA mutant GPC3 and HA-tagged Wnt3a and Wnt7b into 293T cells. GPC3 was then immunoprecipitated from the cell lysates using the anti-GPC3 1G12 antibody, and the presence of Wnt in the precipitated material was analyzed by Western blot with an anti-HA antibody. Fig. 6 shows that both Wnt3A and Wnt7B co-immunoprecipitated indistinctly with wild-type or mutant GPC3.

We have shown here that in the context of HCC cells, GPC3 does not need to be processed by convertases to be able to potentiate canonical Wnt signaling and to exert its in vitro and in vivo growth-stimulatory activity. Moreover, we have shown that unprocessed GPC3 can co-immunoprecipitate with Wnts and stimulate their signaling activity in
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293T cells, indicating that the convertase-independent activity of GPC3 is not specific for HCC cells.

Currently we do not know why convertase processing is required for GPC3 activity in some contexts but not in others. The interaction of Wnts with their signaling receptors is highly complex. Co-receptors and other extracellular proteins that regulate Wnt-Frizzled interactions have been identified (38). We speculate that the requirement of convertase processing for GPC3 activity may be determined by the particular composition of the ligand-receptor complex in a given cell context and/or by the expression levels of the components of such complex.

REFERENCES
1. Lander, A. D., Stipp, C. S., and Ivins, J. K. (1998) Perspect. Dev. Neurobiol. 1, 1–7
2. Veugelers, M., and David, G. (1998) Trends Glycosc. Glycothec. 10, 145–152
3. Filmus, J., and Selleck, S. B. (2001) J. Clin. Investig. 108, 497–501
4. Filmus, J., and Song, H. H. (2000) in Proteoglycans (Iozzo, R. V., ed) pp. 161–176, Marcel Dekker, New York
5. Veugelers, M., De Cat, B., Ceulemans, H., Bruystens, A. M., Coomans, C., Durr, J., Vermeesch, J., Marynen, P., and David, G. (1999) J. Biol. Chem. 274, 26968–26977
6. Song, H. H., and Filmus, J. (2002) Biochim. Biophys. Acta 1573, 241–246
7. Jackson, S. M., Nakato, H., Sugiuira, M., Januszi, A., Oakes, R., Kaluzia, V., Golden, C., and Selleck, S. B. (1997) Development (Camb.) 124, 4113–4120
8. Baeg, G. H., and Perrimon, N. (2000) Curr. Opin. Cell Biol. 12, 575–580
9. Perrimon, N., and Bernfield, M. (2000) Nature 404, 725–728
10. Paine-Sauders, S., Viviano, B. L., Zupicich, J., Skarnes, W. C., and Saunders, S. (2000) Dev. Biol. 225, 179–187
11. Lums, L., Yao, M., Mozer, B., Rovescalli, A., Von Kessler, D., Nirenberg, M., and Beachy, P. A. (2003) Science 299, 2039–2045
12. Desbordes, S. C., and Sanson, B. (2003) Development (Camb.) 130, 6245–6255
13. Topczewsky, J., Sepich, D. S., Myers, D. C., Walker, C., Amores, A., Lele, Z., Hammerschmidt, M., Postlethwait, J., and Solnica-Krezel, L. (2001) Dev. Cell 1, 251–264
14. Ohkarawa, B., Yamamoto, T. S., Tada, M., and Ueno, N. (2003) Development (Camb.) 130, 2129–2138
15. De Cat, B., Muyldermans, S. Y., Coomans, C., Degeest, G., Vanderschueren, B., Creemers, J., Biemar, F., Peers, B., and David, G. (2003) J. Cell Biol. 163, 625–635
16. Kramer, K. L., and Yost, H. J. (2003) Annu. Rev. Genet. 37, 461–484
17. Baeg, G. H., Lin, X., Khare, N., Baumgartner, S., and Perrimon, N. (2001) Development (Camb.) 128, 87–94
18. Song, H. H., Shi, W., Xiang, Y., and Filmus, J. (2005) J. Biol. Chem. 280, 2116–2125
19. Cumberledge, S., and Reichman, F. (1997) Trends Genet. 13, 421–423
20. Ai, X., Do, A. T., Lozynska, O., Kusche-Gullberg, M., Lindhal, U., and Emerson, C. P. (2003) J. Cell Biol. 162, 341–351
21. Capurro, M., Xiang, Y. Y., Lobe, C., and Filmus, J. (2005) Cancer Res. 65, 6245–6254
22. Gonzalez, E. M., Mongiat, M., Slater, S. J., Baffa, R., and Iozzo, R. V. (2003) J. Biol. Chem. 278, 38113–38116
23. Fujise, M., Takeo, S., Kamimura, K., Matsuo, T., Aigaki, T., Izumi, S., and Nakato, H. (2003) Development (Camb.) 130, 1515–1522
24. Han, C., Belenkaya, T. Y., Wang, B., and Li, X. (2004) Development (Camb.) 131, 601–611
25. Blair, S. S. (2005) Curr. Biol. 15, R92–R93
26. Baeg, G. H., Selva, E. M., Goodman, R. M., Dasgupta, R., and Perrimon, N. (2004) Dev. Biol. 276, 89–100
27. Han, C., Yan, D., Belenkaya, T. Y., and Lin, X. (2005) Development (Camb.) 132, 667–679
28. Kirkpatrick, C. A., Dimitroff, B. D., Rawson, J. M., and Selleck, S. B. (2004) Dev. Cell 7, 513–523
29. David, G., Lories, V., Decock, B., Marynne, P., Cassiman, J., and Van Den Berghe, H. (1999) J. Cell Biol. 111, 3165–3176
30. Filmus, J., Shi, W., Wong, Z. M., and Wong, M. I. (1995) Biochem. J. 311, 561–565
31. Watanabe, K., Yamada, H., and Yamaguchi, Y. (1995) J. Cell Biol. 130, 1207–1218
32. Taylor, N. A., Van de Ven, W. J. M., and Creemers, J. W. M. (2003) FASEB J. 17, 1215–1227
33. Khatib, A. M., Siegfried, G., Chretien, M., Metrakos, P., and Seidah, N. G. (2002) Ann. J. Pathol. 160, 1921–1935
34. Capurro, M., Wallner, I. R., Sherman, M., Deboer, G., Shi, W., Miyoshi, E., and Filmus, J. (2003) Gastroenterology 125, 81–90
35. Huang, J. S., Chao, C. C., Su, T. L., Yeh, S. H., Chen, D. S., Chen, C. T., Chen, P. J., and Jou, Y. S. (2004) Biochem. Biophys. Res. Commun. 315, 950–958
36. Duenas Gonzales, A., Kaya, M., Shi, W., Song, H., Testa, J. R., Penn, L. Z., and Filmus, J. (1998) J. Cell Biol. 141, 1407–1414
37. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787
38. Logan, C. Y., and Nusse, R. (2004) Annu. Rev. Cell Dev. Biol. 20, 781–810