Prodomain of the proprotein convertase subtilisin/kexin Furin (ppFurin) protects from tumor progression and metastasis

Nathalie Scamuffa1-2, Fatma Sfaxi1-2, Jia Ma1-2, Claude Lalou1-2, Nabil Seidah3, Fabien Calvo4 and Abdel-Majid Khatib1-2-6

1Université Bordeaux 1, Angiogenesis Laboratory, Talence, France, 2INSERM, UMR 1029, F-33400 Talence, France, Laboratory of Biochemical Neuropathocinthesis, Clinical Research Institute of Montreal (IRCM), University of Montreal, Montreal, Quebec, Canada and 3Institut de Génétique Moléculaire, INSERM, UMR 940, Université Paris 7, Paris, France.

*To whom correspondence should be addressed. Tel: +33 5 40 00 29 53; Fax: +33 5 40 00 87 05; E-mail: majid.khatib@inserm.fr

Proteolytic activity of various proprotein convertases by the pro-protein convertase Furin is now considered as a crucial step in tumor progression and metastasis. Here, we report the repression of the malignant and metastatic potential of carcinoma cells by the prodomain region of Furin (ppFurin), a naturally occurring inhibitor of this convertase. Overexpression of ppFurin in carcinoma cells in a stable manner significantly reduced their convertase activity and ability to mediate processing of the Furin cancer-related substrates platelet-derived growth factor (PDGF)-A and insulin-like growth factor-I receptor precursors. Unprocessed platelet-derived growth factor-A produced by ppFurin expressing cells failed to activate Akt in the platelet-derived growth factor receptor-expressing cells NIH BALB/c-3T3 and treatment of ppFurin expressing cells with insulin-like growth factor-I failed to induce Akt phosphorylation, compared with controls. The malignant potential of ppFurin expressing cells was significantly reduced as revealed by the loss of anchorage-independent growth and survival that associated their increased chemosensitivity. In vivo, comparative studies revealed that expression of ppFurin in the carcinoma cells MDA-MB-231 and CT-26 cells inhibited tumor growth when subcutaneously inoculated in nude mice. An experiment of an experimental liver colorectal metastasis model revealed the reduced ability of metastatic carcinoma CT-26 cells to colonize the liver in response to intrasplenic portal inoculation. Further analyses revealed reduced Furin activity in tumors derived from intrasplenic inoculated mice with ppFurin expressing CT-26 cells. This finding highlights the role of Furin in the malignant and metastatic potential of tumor cells and suggests the possible consideration of using its naturally occurring inhibitor ppFurin in anticancer therapy.

Introduction

Tumor progression is characterized by the gradual acquisition of various cellular aberrations resulting in the malignant behavior including lack of control in cell proliferation and survival. Similarly, cancer metastasis is a complex process involving sequential interactions between disseminating tumor cells and a continuously changing host microenvironment. To date, it is well established that the proprotein convertases (PCs) are involved in all these processes (1–10). These proteases process precursor proteins at basic residues within the general motif (K/R)-(X)n-(K/R)n, where n = 2, 2, 4 or 6 and X any amino acid. They include PC1, PC2, Furin, PC4, PACE4, PC5 and PC7 (1–10). These multidomain serine proteases are consisting of a signal peptide followed by prodomain, catalytic, middle and carboxy-terminal domains. Following signal sequence removal, Furin precursor was found to undergo an autoproteolytic cleavage of its prodomain region (ppFurin). The latter remains associated with the enzyme and functions as a potent autoinhibitor (11,12). Similar mechanisms were also suggested for the prodomains of various PCs that indicate an inhibitory action overlapping toward various PCs (11,12). Indeed, in vitro experiments demonstrated that the prosegment of Furin (ppFurin) is 10-fold more potent toward PC5A, whereas the prosegment of PC7 is a relatively selective inhibitor of its cognate enzyme (11). On the other hand, cell transfection studies revealed that overexpression of ppFurin and ppPC7 in cells resulted in potent but moderately selective inhibition of their parent enzyme (11,12).

The implication of PCs, particularly Furin, in tumor progression and metastasis is due to their ability to generate following proteolysis active proteins involved in the acquisition of the malignant and metastatic phenotype of tumor cells (1–10,13,14). Among these substrates, various growth factors and their receptors occupy the primordial place (1–10,13,14). A wide range of these molecules were found to mediate a mitogenic and/or antiapoptotic function through Akt activation, including the growth factors insulin-like growth factor (IGF)-1 and platelet-derived growth factor (PDGF) (7,8,15). Previously, Akt activation was found to induce tumor cell proliferation and survival and found to be upregulated in a number of tumor types and cancer cell lines (8,16,17). In parallel, higher levels of Akt expression contribute to the acquisition of malignancy and antiapoptotic activity within the tumors (16–18). Thereby, directly targeting its activity was suggested as a potential effective therapy (16–18). In this study, we assessed the effect of the Furin prodomain (ppFurin) on Furin substrates maturation and activity in carcinoma cells and on their malignant potential in vitro and during tumor progression and metastasis.

Materials and methods

Cell transfection and culture

The breast carcinoma MDA-MB-231 cells and the colon carcinoma CT-26 cells derived from American Type Culture Collection were stably transfected with empty pIRE2-enhanced green fluorescent protein (EGFP) vector (Control) or with the same vector containing whole Furin prodomain complementary DNA (ppFurin cDNA). To generate single-mixture cells expressing stably ppFurin, MDA/ppFurin and CT-26/ppFurin expressing cells were cultured in the presence of 1 μg/ml Pseudomonas exotoxin A, as described previously (7,8). This toxin mediates cells death only after its cleavage by the PCs. Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 200 μg/ml G418. In some experiments, cells were transiently transfected with pIRE2-EGFP-V5 empty vector or the same vector containing PDGF-A cDNA to assess the activity of the PCs. All transfections were carried out using Lipofectamine reagent (Invitrogen, Cergy Pontoise, France), as recommended by the manufacturer. For experiments dealing with Akt activation by PDGF-A, the fibroblast NIH BALB/c-3T3 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin.

Real-time PCR analysis

Using Trizol reagent (Invitrogen), total RNA was extracted from tumor cells as reported by the manufacturer’s instructions. One microgram of total RNA was subjected to cDNA synthesis using the Superscript II first strand cDNA synthesis system (Invitrogen) and oligo DT12-20 as primers. The relative quantification of specific messenger RNAs (mRNAs) was performed by real-time PCR using the StepOnePlus™ Real-Time PCR System and PCR Master Mix (Applied Biosystems, Courtaboeuf, France) according to the manufacturer’s instructions. The reaction mixture of the reaction (20 μl) contained 2 μl of cDNA resulting of 5-fold dilution of the reverse transcriptase mixture product, 2x TaqMan Universal PCR Master Mix, 0.3 μM of the probe and 0.9 μM of the forward and reverse primers (8). PCR reaction was performed at 94°C for 15 s and at 60°C for 1 min during 40 cycles. The transcription of β2-microglobulin evaluated in each sample was used as endogenous control.

Western blotting

Following cell lysis in phosphate-buffered saline containing 2% Nonidet P-40 and protease inhibitors (Roche), media or lysates were subjected to...
SDS–polyacrylamide gel electrophoresis in 8% gels. The primary antibodies used were anti-IGF-1 Receptor (IGF-1R; Santa Cruz Biotechnology, Heidelberg, Germany) and anti-V5 for PDGF-A-V5 detection (Invitrogen).

horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL+Plus, Amersham Pharmacia Biotech, GE Healthcare, Vélizy Villacoublay, France) were used for primary antibodies revelation according to the manufacturers' instructions.

Akt tyrosine phosphorylation

For the analysis of Akt phosphorylation, tumor cells were maintained in serum-free media condition for 24–48 h and incubated with or without IGF-1 (50 ng/ml) for 15–30 min at 37°C. In other experiments, fibroblast NIH BALB/c-3T3 cells were maintained in serum-free Dulbecco’s modified Eagle’s medium for 24 h and incubated for 15–30 min with medium derived from control or ppFurin expressing cells transfected with pIRE2-EGFP-V5 empty vector or the same vector containing PDGF-A cDNA. Cells were lysed with lysis buffer (50 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 150 mM NaCl, 1% Triton X-100, 2 mM vanadate, 100 mM sodium fluoride and 0.40 mg/ml phenylmethylsulfonyl fluoride) and cell lysates were analyzed by western blotting for Akt phosphorylation using anti-phospho-Akt (Cell Signaling). The blots were stripped and reprobed with anti-Akt (Cell Signaling Saint Quentin en Yvelines, France) and antiaclinin (Sigma-Aldrich Saint Quentin Fallavier, France) for data normalization.

Measurement of PCs activity

PC activity in tumor cells and tissues was assessed by evaluating their ability to digest the universal PCs substrate the fluorogenic peptide pERTKR-MCA, as described previously (8,9). In brief, cell extracts were incubated with pERTKR-MCA (100 μM) during various time periods in the presence of 25 mM Tris–HCl (pH 7.4), 25 mM methyl-ethane-sulfonic acid and 2.5 mM CaCl2, at 37°C and the fluorometric measurements were performed using a spectrofluorometer (FLUOSstar OPTIMA; BMG Labtech Champigny sur Marne, France).

Soft agar assay

Tumor cells (4 x 10⁴ cells) were suspended in complete medium containing 0.8% agar and seeded in triplicate in six-well plates onto a basal layer of complete medium containing 3% agar. Medium was added every 3 days. After 2 weeks of cell growth, colonies were analyzed using inverted microscopy and the results were represented as percent of formed colonies, as described previously (7).

Cell death assay

Control tumor cells CT-26 and CT-26/ppFurin were incubated for 6h without or with 1 μM staurosporin or 5 mM H2O2. Following incubation, cells were stained with phycocerythrin-labeled annexin V (AN) and 7-aminocoumarin D (7AAD) using the Phycocerythrin Annexin V Apoptosis Detection Kit I (BD Pharmingen™, Le Pont de Claire, France), as described by the manufacturer. Cell analysis by flow cytometry (fluorescence-activated cell sorting CaptoDil) detected the populations AN⁺/7AAD⁺, AN⁺/7AAD⁻, AN⁻/7AAD⁺ and AN⁻/7AAD⁻ that correspond to live cells, early apoptotic cells, necrotic cells and late apoptotic cells, respectively.

Tumorigenicity assay

All the performed experiments were done according to protocols approved by the health science animal policy and welfare committee at the University of Bordeaux-1, in accordance with French animal care guidelines. Female 4- to 6-week-old female nu/nu mice from Charles River Laboratories were used for all of the experiments. To assess the effect of ppFurin on tumor growth, 1 x 10⁶ control cells (MDA/Control, CT-26/Control) and ppFurin expressing cells (MDA/ppFurin, CT-26/ppFurin) were injected subcutaneously into nude mice and tumor formation was monitored during various periods of the experiments. Tumor volume was calculated, as described previously (7,8).

Experimental liver metastases

To evaluate the effect of PCs inhibition by ppFurin on metastasis, we focused on rapid formation of metastases in the liver as a primary metastasis site by the use of an experimental colorectal liver metastasis. Nu/nu mice (4- to 6-week-old female) were anesthetized by methoxyflurane and the spleens were exposed through a small abdominal incision. The colon CT-26 tumor cells (1 x 10⁶) in saline were inoculated into the hepatic circulation, as described previously (8). Livers were removed and the formed metastases were enumerated, without prior fixation.

Results

PCs activity in ppFurin expressing tumor cells

To investigate the effect of ppFurin on the malignant and/or metastatic phenotypes of carcinoma cells, MDA-MB231 and CT-26 cells were stably transfected with pIRE2-EGFP empty vector (MDA/Control; CT-26/Control) or containing Furin prodomain (MDA/ppFurin; CT-26/ppFurin). We first assessed the effect of ppFurin expression on two PC substrates PDGF-A (19) and IGF-IR (7,8). As shown in Figure 1A, MDA/Control cells transfected with PDGF-A expressed mostly the mature form of PDGF-A. In contrast, MDA/ppFurin cells showed a predominant unprocessed form of PDGF-A. Similarly, expression of ppFurin in MDA/Control cells blocked the processing of IGF-1R, as revealed by the accumulation of the precursor form and reduction of the mature form of IGF-1R (Figure 1B). Analysis of CT-26/Control and CT-26/ppFurin revealed that ppFurin expression in these cells inhibited also the processing of these substrates (data not shown). Using an in vitro enzymatic digestion assay (8,9) and the universal PC substrate, the fluorogenic peptide pERTKR-MCA, we confirmed the presence of high PC activity in MDA/Control cells that were inhibited following ppFurin expression (Figure 1C–D). Previously, MDA/Control cells were found to express high level of Furin compared with PACE4 and PC7 that were found to be expressed at relatively similar amounts. PC5 was detected at lower amount than these PCs (20). Using specific primers for PCs found in the secretory pathway, real-time PCR confirmed the presence of all these PCs in MDA/Control cells. In MDA/ppFurin cells, the mRNA level of Furin and PACE4 was reduced. PC5 mRNA was increased and PC7 remained unchanged (Figure 1E–H).

Inhibition of Akt phosphorylation by ppFurin

To examine the effect of ppFurin on Akt activation, we assessed the ability of processed and unprocessed IGF-1R and PDGF-A to mediate Akt phosphorylation. As illustrated in Figure 2A, the presence of IGF-1 (50 ng/ml) induced Akt phosphorylation in MDA/Control cells after 15 or 30 min of incubation. In contrast, under the same conditions, MDA/ppFurin cells were insensitive to IGF-1-mediated Akt phosphorylation. Previously, interfering with PDGF receptor activation was found to inhibit Akt phosphorylation and causes a reversion of the transformed phenotype (19). Thereby, to examine whether pro-PDGF-A processing inhibition by ppFurin will affect PDGF-A-induced Akt phosphorylation, conditioned media derived from MDA/Control and MDA/ppFurin cells were tested on Akt phosphorylation using NIH/BALB-c 3T3 cells that express PDGF receptor (19). Incubation of these cells with media derived from MDA/Control cells transfected with PDGF-A cDNA induced Akt phosphorylation significantly. In contrast, media derived from MDA/ppFurin cells transfected with PDGF-A cDNA were not effective (Figure 2B).

Inhibition of anchorage-independent growth by ppFurin

Akt activation was found to be critical in anchorage-independent colony formation and tumorigenesis (18) and Akt activation is mediated by various PCs substrates including IGF-1 and PDGFs (7,8,19). Consequently, we tested the effect of ppFurin expression on colony formation in soft agar. Compared with MDA/Control cells, MDA/ppFurin cells exhibited a complete inhibition of their anchorage-independent growth (Figure 2C).

Tumorigenicity of ppFurin expressing cancer cells

To assess the effect of ppFurin expression on tumorigenesis, nude mice were subcutaneously inoculated with MDA/Control or MDA/ppFurin. To highlight the effect of ppFurin on tumor growth, we also inoculated mice with the colon cancer cells, CT-26/Control or CT-26/ppFurin. Tumors size was measured at various intervals, and data are summarized in Figure 3A and B. Subcutaneous inoculation of 1 x 10⁶ cells revealed that all mice receiving injections of MDA/Control developed local tumors by day 3 postinoculation. In contrast to controls, only 50% of the mice inoculated with MDA/ppFurin cells developed tumors.
under the same conditions. After this time period, the growth of developed tumors induced by MDA/Control cells was reduced dramatically. In animals injected with CT-26/ppFurin cells exhibited a significant delay in tumor growth compared with CT-26/Control cells (Figure 3B).

**Reduced PCs activity in cancer cells expressing ppFurin-derived tumors**

To evaluate the effect of ppFurin on PC activity in tumors derived from control and ppFurin expressing tumor cells, CT-26/Control or CT-26/ppFurin cells were subcutaneously injected in mice. Formed tumors were removed and PC activity was analyzed by assessing the ability of tumor-derived protein extracts to cleave the universal PC substrate, the fluorogenic peptide pERTKR-MCA during the indicated time periods. Note the reduced digestion of pERTKR-MCA by media derived from ppFurin expressing cells. In (D) bars denote the corresponding percentages of PCs activity measured following pERTKR-MCA digestion. Values are mean ± SEM (n = 3 per group). ***P < 0.001. (E–H) PCs expression in MDA/Control and MDA/ppFurin cells. Following total RNA extraction from 10^6 cells, real-time PCR was performed using specific primers for Furin (E), PC5 (F), PACE4 (G), PC7 (H) or β2-microglobulin as described in Materials and methods. During PCR, the transcription of β2-microglobulin that was evaluated in each sample was used as endogenous control. Results are expressed as the percentage of the indicated transcripts relative to control transcript (100%). Data are shown as means ± SE of three experiments performed in duplicate. ***P < 0.001.
Expression of ppFurin prevents experimental colorectal liver metastasis

To evaluate the effect of ppFurin on the metastatic ability of tumor cells, we focused on rapid metastases formation (in one organ) by using an experimental liver colorectal metastasis assay, as described previously (8). Indeed, injection of the colon cancer cells, CT-26 (but not the injection of breast cancer MDA-MB cells), in the intrasplenic route directly induces metastasis in the liver. CT-26/Control and CT-26/ppFurin cells were injected in mice through the intrasplenic/portal route and their ability to colonize the liver was evaluated 2 weeks after the injection. After this period, livers were removed and the number of metastases was determined (Figure 4A and 4B). The number of hepatic metastases was reduced by up to 67% (P < 0.001) in CT-26/ppFurin-injected mice relative to animals injected with control cells (Mann–Whitney test).

ppFurin alters survival and increased chemosensitivity of tumor cells

To further investigate the mechanism responsible for the antiproliferative effect of ppFurin observed during tumor progression and/or metastases formation, flow-cytometric analysis of cell death was performed on CT-26/ppFurin cells. Using annexin V and 7AAD as markers, flow cytometric analysis revealed that ppFurin expression in CT-26 cells induced tumor cell death (Figure 5A and B). To assess the effect of ppFurin expression on cell survival in the presence of apoptotic agents, CT-26 cells were treated for 6 h with H2O2 (5 mM) or staurosporin (1 μM). As illustrated, in CT-26/Control cells, H2O2 (5 mM) induced cell death. In contrast, under the same conditions, CT-26/ppFurin cells survived and the proportion of viable cells was increased (Figure 5C).
conditions, expression of ppFurin in CT-26 cells increased their chemosensitivity to staurosporin, as revealed by the increased number of apoptotic cells.

Discussion

Based on various in vitro and in vivo studies, Furin is now considered as a potential target for several cancers treatment (1–10). Indeed, like other PCs, Furin is overexpressed in many human cancer cell lines and tumors and its increased expression was found to predict decreased survival in several human cancers (4,21). Furin overexpression resulted in increased invasiveness in vitro and in vivo (2,4) and its in vivo deficiency resulted in delayed tumorigenesis induced in mice (22). Recently, in a phase I autologous tumor cell vaccine (FANG) vaccine trial, an autologous tumor-based product incorporating a plasmid encoding GM-CSF and a bifunctional short hairpin RNAi targeting Furin was found to be beneficial with high success rate in patients with advanced cancer (23). This promising clinical trial results reinforce the need for development and identification of specific and potent inhibitors of PCs that could be applied in anticancer therapy. In this study, we investigated the effect of PC inhibition by the prodomain of Furin on the malignant and metastatic potential of

Fig. 3. Inhibition of tumor growth by ppFurin. MDA/Control or MDA/ppFurin (A) and (B) CT-26/Control or CT-26/ppFurin (1 x 10⁶) were injected subcutaneously into 4- to 6-week-old nude mice. The animals were monitored for tumor formation during indicated periods. Note the smaller size of tumors induced by tumor cells expressing ppFurin. Results are representative of three experiments. Values are mean ± SEM (n = 6 per group). ***P < 0.001. (C–D) PC activity in CT-26/ppFurin cells-derived tumors. Subcutaneously developed tumors were removed and lysed in lysis buffer. Protein extracts were incubated with the fluorogenic peptide PC substrate pERTKR-MCA. Substrate cleavage was evaluated as raw fluorescence intensity at indicated time periods (C). Results shown in the bar graph represent PCs activity after 2h of incubation (D). Results are representative of two experiments performed in triplicate and data are mean ± SE ***P < 0.001. (E–F) Following total RNA extraction from CT-26/Control and CT-26/ppFurin tumor cells, real-time PCR analysis was performed using specific primers for PACE4, PC5, PC7 or β2-microglobulin as described in Materials and methods. During PCR, the transcription of β2-microglobulin that was evaluated in each sample was used as endogenous control. Results are shown in the bar graph and are expressed as the percentage of the indicated transcripts relative to control transcript (100%). Data are shown as means ± SE of three experiments performed in duplicate. **P < 0.005.
tumor cells. The observed effect of ppFurin on tumor cell phenotype is probably due to the inhibition of the proteolytic processing of various proteins, including growth factors and/or their corresponding receptors by Furin and probably by other PCs previously reported to be involved in the malignant phenotype of tumor cells, such as PACE4 and PC7 (1–10) (Figure 6). Indeed, ppFurin was reported to inhibit not only Furin but also these PCs (1,2,11,12). Interestingly, expression of ppFurin in tumor cells seemed to reduce also when present the expression of Furin and PACE4 (Figures 1 and 3). We found that expression of ppFurin in tumor cells blocks the processing of PDGF-A and IGF-1R (Figures 1), leading to their inability to mediate Akt activation (Figure 2A and B). Expression of ppFurin in tumor cells significantly alters their ability to proliferate and form colonies in soft agar (Figure 2C). Further analysis revealed that ppFurin expression induced tumor cell apoptosis and treatment with H$_2$O$_2$ or staurosporin increased the number of apoptotic cells, compared with controls, revealing their increased chemosensitivity (Figure 5). The capacity of ppFurin to abrogate Akt pathway activation in tumor cells seems to be responsible for these effects. Indeed, the mitogenic and survival effect of many growth factors including IGF-1 and PDGF is apparently dependent on their ability to induce a cascade of events leading to Akt phosphorylation (16). Active IGF-1 and PDGF receptors are known to be required for cell growth of various transformed cells (24–26) (Figure 6). Overexpression and/or constitutive activation of these receptors in a variety of cell types induced their ability to form colonies in soft agar and tumors in mice (26,27). In addition, activation of Akt by IGF-1 or PDGF was reported to play a crucial role in cell growth, tumorigenesis and metastasis (8,15–18). Taking advantage of MDA-MB-231 and CT-26 carcinoma cells that induce tumor formation in nude mice when inoculated subcutaneously and CT-126 that induce liver metastasis following their intrasplenic injection (8), we found that expression of ppFurin in CT-26 and MDA-MB-231 cells delayed tumor development that was associated with reduced PC activity in ppFurin expressing tumor cell-derived tumors (Figure 3C). Similarly, injection of CT-26/ppFurin into hepatic circulation reduced liver metastases formation (Figure 4). Although Akt was found to be overexpressed in several cancers and its phosphorylation correlates with cell proliferation and inhibition of apoptosis (17,18), the inhibition of growth factor and growth factor receptor activation leading to Akt phosphorylation blockade is not the only mechanism responsible for ppFurin effects on the metastatic phenotype of tumor cells. Definitely, other PC substrates processing inhibition contribute to the final biological effect of ppFurin such as matrix metalloproteinases. The processing inhibition of these substrates by ppFurin was previously linked to reduced invasiveness of tumor cells (19,28).

Previously, inhibition of PCs by the general bioengineered PC inhibitors α1-PDX resulted in variable outcomes regarding malignant phenotypes of tumor cells and tumorigenesis. Indeed, expression of α1-PDX in different tumor cell systems resulted in a reduced invasiveness and in vivo tumorigenicity in nude mice (1–5,7,8,10,14). In other studies, this inhibitor was found to increase cell migration and accelerate the process of metastasis (29), suggesting that PC expression and/or function in cancer cells varies in a tumor-specific fashion.

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**Fig. 4.** Inhibition of colorectal liver metastasis by ppFurin. (A) Experimental colorectal liver metastases were generated by intrasplenic/portal injection of CT-26/ control and CT-26/ppFurin colon tumor cells (1 × 10$^6$). Liver metastases were enumerated 2 weeks after injection. (B) Data summary of metastasis incidence and prevalence weight. Shown are representative results of 3 experiments (n = 6 per group, Mann–Whitney test).
and raises the possibility that alteration in PCs expression/activity may positively or negatively regulate human tumor biology. Overall, in view of its antitrypsin backbone, α1-PDX may have effects other than inhibition of PCs, which need further investigation. In addition to Furin, ppFurin was found also to inhibit various PCs such as PACE4 and found to affect their levels (Figure 1), this inhibitor has thus far consistently resulted in reduced malignant phenotypes of all tested cancer cells including those used in this study (19,28). These findings suggest the potential use of this naturally occurring inhibitor or derivate(s) in anticancer therapy. Advanced molecular therapies aimed at down modulating the level or expressing of a given genes in model organisms have been successfully established (30). Likewise, small interfering RNA technologies allow effective introduction of artificial RNA guide strands in the RNA-induced signaling complex in primates (30). We thus envision that similar strategies may be exploited to express ppFurin for cancer therapy.

Fig. 5. Inhibition of PCs by ppFurin alters CT-26 tumor cells survival and increased their chemosensitivity. (A) Fluorescence-activated cell sorter scatter plots of CT-26/Control and CT-26/ppFurin cells incubated for 6h without or with H2O2 (5 mM) or staurosporin (1 μM). After incubation, cells were double stained with annexin V and 7AAD. The use of fluorescence-activated cell sorter detected viable (negative for both dyes; lower left), early apoptotic (Annexin+/7AAD−, lower right), necrotic cells (Annexin−/7AAD+, upper left) and late apoptotic (Annexin+/7AAD+, upper right) cells. (B) Summary of data obtained in (A). Note that ppFurin increased the number of death in tumor cells and exaggerated this affected in the presence of staurosporin and H2O2. Data are mean ± SEM (n = 3 per group). **P < 0.005. ***P < 0.001.
Fig. 6. Schematic representation of ppFurin effects on tumor progression and metastasis. The generation of active Furin following an autocatalytic cleavage of its precursor pFurin at the endoplasmic reticulum (ER) (1) favors the accumulation of active growth factors (A) and growth factor receptors (B). Furin and these precursors interaction may occur intracellularly or on the cell surface (2). The active growth factors and growth factor receptors that interact with their specific initiators induce signaling pathways involved in tumor cells proliferation, survival and invasion and thereby tumor progression and metastasis (3). These processes are inhibited following inhibition of Furin activity by the accumulation of ppFurin (4) and unprocessed inactive pGF and pGFr (5 and 6).

Funding

Institut National du Cancer (INCA2009-1-PL. BIO-09); la Ligue National Contre le Cancer Aquitaine Charente.

Conflict of Interest Statement: None declared.

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Received June 5, 2013; revised August 2, 2013; accepted September 14, 2013.