

Increased expression of the pro-protein convertase furin predicts decreased survival in ovarian cancer¹

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Abstract. *Background:* Proprotein convertases (PCs) are serine proteases that after restricted proteolysis activate many proteins that play a crucial role in cancer such as metalloproteinases, growth factors and growth factor receptors, adhesion molecules, and angiogenic factors. Although the expression of several PCs is increased in many tumors, their expression in primary ovarian tumors has not been studied in detail. We sought to determine if there was an association between the expression of the ubiquitously expressed PCs, furin, PACE-4, PC-5 and PC-7, and ovarian tumor progression. *Methods:* We assessed their expression by RT-PCR, Real-time PCR, Western blot, and immunohistochemistry using cells derived from normal human ovarian surface epithelium (HOSE) and cancer cell lines as well as ovarian epithelial cancer specimens (45 RT-PCR/Real-time PCR, and 120 archival specimens for Immunohistochemistry). *Results:* We found that furin expression was restricted to the cancer cell lines. In contrast, PACE-4 and PC-7 showed expression only in normal HOSE cells lines. Furthermore, furin was predominantly expressed in primary tumors from patients who survived for less than five years. The other PCs are either expressed in the group of survivors (PC-7 and PACE4) or expressed in low amounts (PC-5). *Conclusions:* Our studies point to a clear relationship between furin and ovarian cancer. In addition, these results show that furin exhibits the closest association with ovarian cancer among the ubiquitously expressed PCs, arguing against the redundancy of these proteases. In summary, furin may constitute a marker for ovarian tumor progression and could contribute to predict the outcome of this disease.

Keywords: Proprotein convertases, metalloproteinases, survival, ovarian cancer, tumor progression

1. Introduction

Many proteins expressed during tumor progression need to be activated by specific proteolysis before exerting their biological role. A family of serine-proteases, the proprotein convertases (PCs) that are capable of recognizing and cleaving at the C-terminus

of the motif RXR/KR [18], have been implicated in this specific proteolysis. Several proteases, growth factors, growth factor receptors and pro-angiogenic proteins are known to be PCs substrates [13,31]. This PC family comprises seven members, PC-1/3, PC-2, PC-4, PC-5, PC-7, furin and PACE-4 and the recently identified SKI-1, and NARC-1. The first three show a restrictive pattern of expression to neuroendocrine tissues whereas PACE-4, furin, PC-5, and PC-7 are ubiquitously expressed [19]. SKI-1 and NARC-1 are expressed mainly in neural tissue and liver and unlike the other PCs cleave at non-basic residues [24,25].

Furin, the prototype of the PC family, is overexpressed in colon [14], head and neck [5] and breast [6] cancer. Interestingly, inhibition of furin activity leads

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to decreased proliferation and invasive ability [4,14]. PACE-4, another member of the group is overexpressed in chemically induced skin tumors [11], and PACE-4 transgenic animals showed increased susceptibility to carcinogens and marked degradation of the collagen IV component of the basement membrane [3].

Surprisingly, PACE-4 expression is clearly down-regulated in ovarian cancer cell lines such as Hey and Hey-C and a series of primary ovarian tumors [8,17]. Moreover, the analysis of the expression of other PC's such as PC-7 and furin does not show clear differences in cultures from normal and neoplastic HOSE (Human Ovarian Surface Epithelial) cells [8]. Thus, the possible role of furin in ovarian tumor progression remains elusive.

In order to determine if there is any association between PC expression and ovarian tumor progression, we evaluated the expression of the ubiquitously PC's-furin, PACE-4, PC-7 and PC-5- in normal and cancer ovarian cell lines and primary ovarian tumors showing different survival patterns.

2. Materials and methods

2.1. Culture of primary ovarian surface epithelial cells

Ovaries were obtained following laparoscopic procedures for nonmalignant gynecologic conditions. Informed consent was obtained in accordance with institutional regulations. The ovaries were collected under aseptic conditions and placed in 1 × PBS. The ovaries were then gently scraped with a cell scraper to obtain fragments of ovarian surface epithelium (OSE). The ovaries were then rinsed in 1 × PBS. Rinses and scrapes were pooled and placed in centrifuge tubes. The cells were collected by centrifugation. The resulting pellet was suspended in fresh media (a 1 : 1 mixture of medium 199 and MDCB 105 (Sigma, St. Louis, MO) supplemented with 15% FBS, penicillin, streptomycin, glutamine, bovine pituitary extract, and insulin. Bovine Pituitary extract was used only during the first 3–4 passages to increase cell proliferation. Cells were plated in T25 tissue culture flasks precoated with swine skin gelatin and incubated at 37°C. This precoating regimen appeared to increase the proportion of OSE fragments that attached to the plastic and, consequently, the survival and eventual yield of OSE. After 48 hours the flasks were washed twice with 1 × PBS to remove the residual red blood cells. Cells were fed twice weekly

with complete tissue culture media. Primary cultures were maintained in complete medium until the cells reached confluency (2 to 4 weeks). At that time, the cells were subcultured with 0.06% trypsin (1 : 250) and 0.01% ethylenediaminetetraacetic acid in calcium and magnesium-free balanced salt solution.

Under these conditions, primary HOSE cells can be passaged 10 to 15 times at a split ratio of 1 : 1.

The epithelial nature of these primary cultures was assessed by western blot to test keratin expression, using a monoclonal antibody directed against keratins (Abcam, mouse monoclonal antibody clone #50). In addition, the primary cultures were tested for vimentin expression, to discard any fibroblast contamination (Sigma, mouse monoclonal, clone VIM-13.2).

2.2. Cancer cell lines

The ovarian cancer cell lines OV-3 and SKOV-3 were purchased from ATCC and OV-4, OV-5, and OV-10 cell lines are described elsewhere [9]. Cancer cells were maintained in DMEM-10% FBS with the addition of 2 mM L-glutamine and 100 U/ml penicillin and 100 µg/ml streptomycin.

Tumors

Forty five ovarian tumors were obtained from the Tumor Bank at Fox Chase Cancer Center. The age of patients at the time of surgical resection was 28 to 88. The clinical stage, pathological grade, and survival time after the diagnosis of the tumors are summarized in Table 1.

2.3. RNA extraction and RT-PCR

RNA was extracted using the RNAqueous kit from Ambion (Ambion, Inc, Austin, TX). RNA s were freed from DNA by treatment with DNase. One hundred nanograms from this DNA-ase treated RNA was reverse transcribed and PCR-amplified using Superscript One-Step RT-PCR (Invitrogen, Carlsbad, CA). The primers used for PCs amplification are described elsewhere [6].

2.4. Real time-PCR

Contaminating DNA from RNA preparations was removed using TURBO DNA-free™ (Ambion, Austin, TX) and quantified with an Agilent 2100 BioAnalyzer in combination with RNA 6000 Nano LabChip. One hundred and 20 ng of RNA were reverse-transcribed using the M-MLV reverse transcriptase (Ambion) and

Table 1
Clinico-pathological characteristics of the tumor analyzed by RT-PCR

Parameter	Percentage of cases	
Diagnosis	Serous AdenoCa	69 (31/45)
	Mucinous AdenoCa	4 (2/45)
	Clear Cell	7 (3/45)
	Endometrioid	9 (4/45)
	Mixed mesodermal	2 (1/45)
	Others	9 (4/45)
Pathological grade	I	4.4
	II	13
	III	58
	IV	20
	Undetermined	4.4
Clinical stage	1 (A and C)	4.4 (2/45)
	2B	6.7 (3/45)
	3 (A and C)	55.5 (25/45)
	4	22 (10/45)
	Undetermined	11 (5/45)
Survival	>5 years	24.4 (11/45)
	<5 years	75.5 (34/45)

Table 2
Primers used to amplify the human furin and PACE-4 by real-time PCR and the expected size of the amplicon

Gene	
Furin	F: 5'GAGATTGAAAACACCAGCGAA3' R: 5'GCGGTGCCATAGAGTACGAG3' P: 5'AACAACACTATGGGACGCTGACCAAGTTCAC3'
PACE4	Assay on demand
BHD (folliculin)	F: 5'CAAGGCGCTCAAGGTGTTT3' R: 5'AATGGCGTGAAGGCTGTGT3' P: 5'AGTTTGGATGCCACAGCGTGCT3'

a mixture of anchored oligodT and random decamers. Real-time PCR assays were performed using an ABI 7900 HT instrument. For the furin and BHD (folliculin) transcripts, the primers and probes were designed using Primer Express™ version 1.5 software from Applied Biosystems (see Table 2). PACE4 primers and probes were purchased from Applied Biosystems (Assay on Demand). The 5'- and 3' ends of the probes were labeled with the reporter dye FAM (6-carboxy-fluorescein) (Glenn Research, Sterling, VA) and the quencher dye BHQ1 (Black Hole Quencher) (Biosearch Technologies, Novato, CA) respectively. Cycling conditions were 95°C, 15 min followed by 40 (2-steps) cycles (95°C, 15 sec; 60°C, 60 sec).

The primers and probes used are outlined in Table 2.

2.5. Protein extraction and Western blot

Cells were trypsinized, washed twice with phosphate-buffered saline, and resuspended in RIPA buffer (1× phosphate-buffered saline solution, 0.1% sodium dodecyl sulfate, 0.5% Na deoxycholate, and 1% Nonidet P40) with a cocktail of proteases inhibitors (Roche, Mannheim, Germany) and digested at 4°C for 30 min. The digests were centrifuged, and the supernatants were used as cell lysates. For furin analysis, 100 µg of cell lysates were fractionated on 8% sodium dode-

cyl sulfate/polyacrylamide gels. Furin expression was evaluated by Western blotting using a furin monoclonal antibody (MON-152; Axxora, San Diego, CA). For the detection of the pro- and activated form of MT1-MMP, we used a rabbit monoclonal antibody directed against the hinge region (AB815, Chemicon, Temecula, CA). To determine the proportion of unprocessed (proform) and mature form of MT-1MMP, the radiographic plates were scanned using the WIA-hp scan jet 8200n series scanner and the bands were measured using the GelPlot 2 software. Background readings were subtracted from the intensities corresponding to the bands corresponding to either the unprocessed or mature protein. The results are expressed as a ratio between the intensity unprocessed to total (processed and mature) MT1-MMP.

2.6. Immunohistochemistry

Furin immunohistochemistry of 120 (45 specimens used for RT-PCR plus 75 archival specimens) ovarian tumors was performed using paraffin-embedded specimens. All paraffin sections were subjected to antigen retrieval for 10 min, in 10 mM sodium citrate buffer, pH = 6. We used three antibodies: MON-152, MON-148 or a rabbit furin antiserum as primary antibody at a 1 : 100 dilution. An avidin-biotin-peroxidase kit (Vectastain Elite form Vector, Burlingame, CA) was employed, followed by the chromagen 3',3'-diaminobenzidine to develop the immunostain. Negative controls, not incubated with furin antibodies, were incubated with normal mouse immunoglobulin G. All sections were counterstained with hematoxylin and mounted. Grading of the immunostaining was based on a semi quantitative arbitrary scale. No staining or marginal staining was called 0, moderate 1, and intense staining 2.

2.7. Statistical analysis

Western blots and RT-PCR experiments were performed three times. Data from RT-PCR were analyzed with the Fisher exact two-tailed test. For the evaluation of furin immunostaining in tumors, individual tumors were scored in four to five different regions of the specimen. The scores were analyzed with the Wilcoxon two sample test. The results of these tests were considered significant when $P < 0.05$.

3. Results

3.1. PCs expression in normal and ovarian cancer cell lines

Furin is overexpressed in many epithelial cancers as diverse as squamous cell carcinomas from stratified epithelia, adenocarcinomas of the colon, and breast, as well as astrocytomas. Hence, we sought to determine if furin is also expressed in tumors derived from the ovarian surface epithelium. In order to determine furin expression in ovarian cancer, we evaluated its mRNA levels in five normal and five cancer cells by RT-PCR. Furin was expressed in all the cancer cell lines tested. In contrast, all the normal cells, except one, did not show expression of this PC.

As already stated, the ubiquitously expressed PCs furin, PACE-4, PC-7 and PC-5 can cleave substrates containing the PC consensus motif, pointing to a certain redundancy in their functions. To evaluate if the elevation of mRNA expression was unique to furin or if this was a shared behavior with the other ubiquitously expressed PCs, we studied PACE-4, PC-5 and PC-7 mRNA levels by RT-PCR. PACE-4 and PC-7 showed higher expression in normal than in cancer cells, suggesting that reduced rather than increased expression might be associated with ovarian carcinogenesis. PC-5 was expressed at marginal levels in normal and tumor cells, pointing to a less prominent role of this PC in ovarian cancer (Fig. 1).

This differential expression between furin and the other ubiquitously expressed PCs points to a unique role of furin in this disease, suggesting that the different PC activities are not fully redundant. In order to confirm the pattern of expression of furin at the protein level, cell lysates from normal and cancer cell lines were analyzed by Western blotting. Normal cell lines showed no or marginal levels of furin whereas cancer cell lines had higher expression of this PC (Fig. 2, panel A). This increased expression correlated with furin's biological activity. MT1-MMP, a protease implicated in ovarian tumorigenesis [12,33], was completely processed in cancer cells, whereas in normal cells, only a partial processing was achieved. This enhanced ability to process MT1-MMP in furin expressing cell lines supports the role of furin as facilitating ovarian tumor progression (Fig. 2, panel B). Calculation of the ratio between unprocessed and mature form of MT1-MMP indicated that HOSE cells, which expressed low amounts of furin, showed a reduction of 30% ($P < 0.001$) in their ability to process this metalloproteinase (Fig. 2E) compared to cancer cell lines.

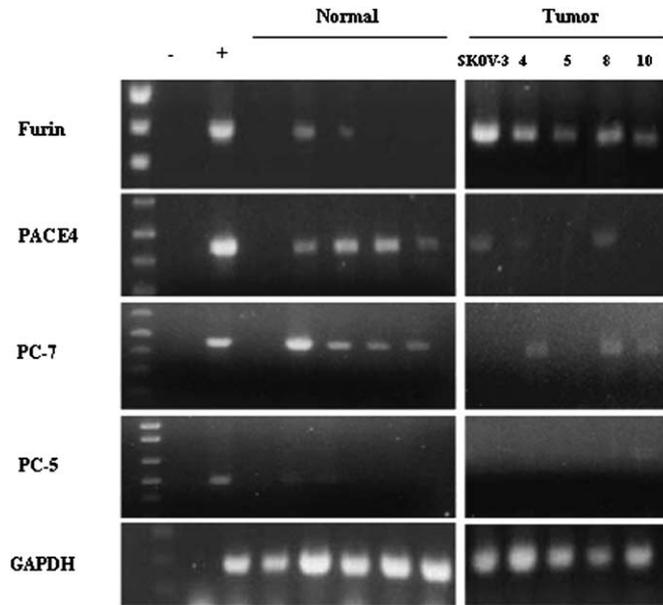


Fig. 1. PC's expression in normal or cancer cell lines. DNAase-treated RNA (100 ng) from five normal and five cancer cell lines were amplified by RT-PCR using specific primers for the ubiquitously expressed PCs, furin, PACE-4, PC-5, and PC-7. GAPDH amplification was used as loading controls. Note that PACE-4 and PC-5 are strongly expressed in the normal cells. In contrast furin expression is higher in cancer cells.

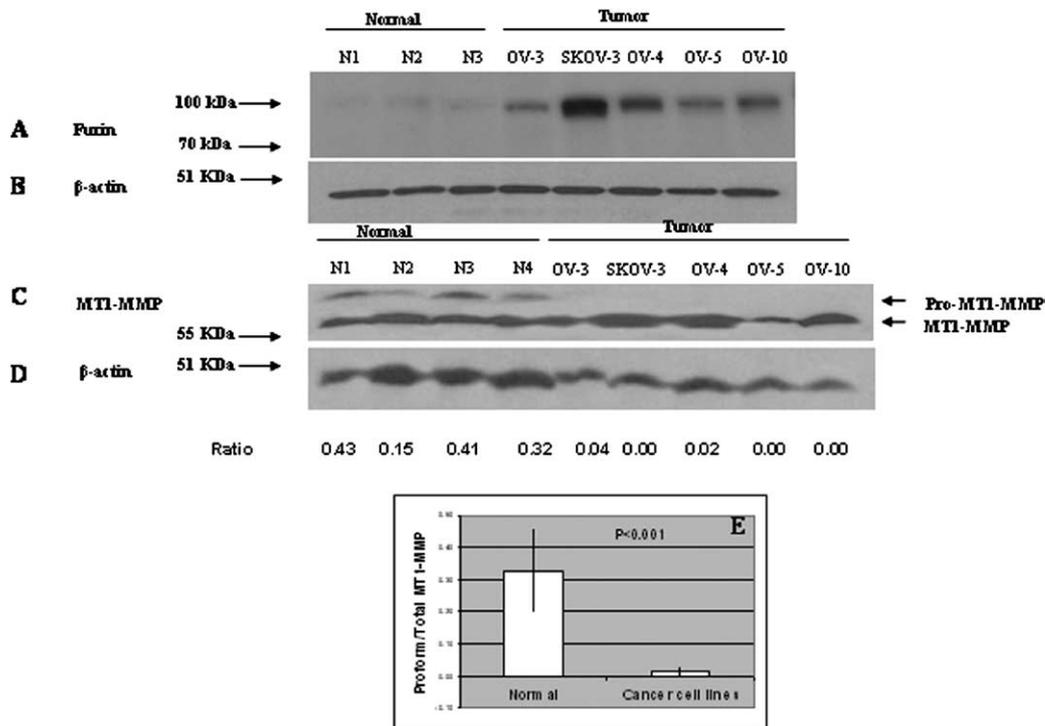


Fig. 2. Furin expression in normal or cancer cell lines. Cell lysates (100 μ g) were fractionated with a 10% PAGE-SDS gel. (A,B): Furin expression and its corresponding loading control (β -actin), respectively. Note furin expression in cancer cell lines but not in cells derived from normal surface epithelium. (C,D): MT1-MMP processing in normal or cancer cell lines (C) and its loading control (β -actin) (D). Note complete processing in cancer cell lines. (E): Increased MT1-MMP processing in furin-expressing cancer cell lines. Average of the ratio proform/total MT1-MMP in normal cells and cancer cell lines.

3.2. PC expression in primary ovarian tumors

Following a similar approach, we wanted to assess the physiological relevance of these differences in PC expression in surgical specimens. We analyzed furin mRNA expression from 45 ovarian tumors by RT-PCR. Thirty-three percent (15/45) of the tumors showed furin expression. To correlate these expression data with clinico-pathological data, we divided the tumors in two groups according to the five-year survival (less than five-year or more than five-year survival after diagnosis). We observed that furin was expressed only in tumors from patients surviving less than five years. In this subset of tumors, 44% (15/34) of the specimens strongly expressed furin. Remarkably, we found no furin expression in tumors from the five-year or longer survivor group (Fig. 3). These results indicated that furin was expressed preferentially in a subset of tumors from patients with shorter survival.

The expression of the other PCs in ovarian tumors showed a distinctive behavior as already observed in cell lines. Remarkably, 90% (10/11) of the five-year survivor group expressed PC-7, in sharp contrast with the non-survivor group, with only 23% (8/34) of the tumors expressing this PC.

Few tumors (15%, 7/45) exhibited PC-5 expression with no correlation between its expression and survival. Although we and others observed that PACE-4

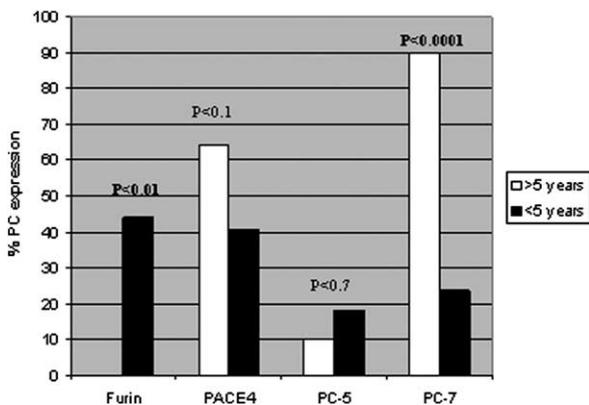


Fig. 3. PC's expression in tumors and survival time. DNAase-treated RNA from surgical specimens was amplified by RT-PCR. The expression of the ubiquitous expressed PCs (furin, PC4, PC-5 and PC-7) was analyzed in tumors from patients that survived longer ($n = 11$) and less ($n = 37$) than 5 years. The results are expressed as percentage of cases that showed the corresponding band. Significance was studied applying the Fisher exact tests. Note that furin is exclusively expressed in the group that survived less than five years. Conversely, PC-7 expression was significantly higher in the group that survived more than five years.

was preferentially expressed in HOSE cells [8], a high proportion of tumors (>70%) from both groups expressed PACE-4 and showed no correlation between PACE-4 expression and survival (Fig. 3).

In order to assess the relative contribution of furin and PACE-4 in ovarian tumors we evaluated the levels of these two PCs in 32 of the 45 epithelial cancers previously analyzed by quantitative real-time PCR. The tumors were selected according to their RNA quality or availability at the time of analysis. Furin is overexpressed (two-fold or more) in 18% of the cases (6/32) with respect to a reference gene, BDH. An additional ten percent showed a slight increased in furin expression (>1.5 folds than the reference) (Fig. 4, panel A). PACE-4 expression was significantly lower than the normal controls (Fig. 4, panel B). Only one case showed a marginally elevated expression.

3.3. Furin protein expression in ovarian tumors

In order to confirm the expression of furin at the protein level, we analyzed the pattern of furin expression in 18 normal ovarian surface epithelium and the previously analyzed 45 primary and metastatic tumors by immunohistochemistry. Fifty percent of the tumors showed furin staining. Its expression was predominantly cytoplasmic, but in some cases we observed a distinct staining at the tumor-stroma interface, suggesting that furin may localize near the invasion front (Fig. 5, left panels).

Although the normal ovarian epithelium showed minimal staining, it was mainly associated with the apical membrane and with the extracellular secretions at the epithelial-apical surface rather than in the cytoplasm, where furin is usually located. This pattern of staining contrasts with that obtained in the tumors and may represent non-specific staining associated with extracellular material rather than specific furin staining (Fig. 5, right panels).

In order to confirm the positive relationship between furin and poor prognosis, we immunostained 75 additional archival specimens of human ovarian epithelial tumors. Tumors were divided in two groups according to the survival time (more or less than five years). We observed that the amount of furin correlated significantly with survival ($P = 0.04$, Wilcoxon two sample test), confirming the correlation observed with the RNA expression levels (Fig. 6). No significant association between clinical stage and furin staining was observed.

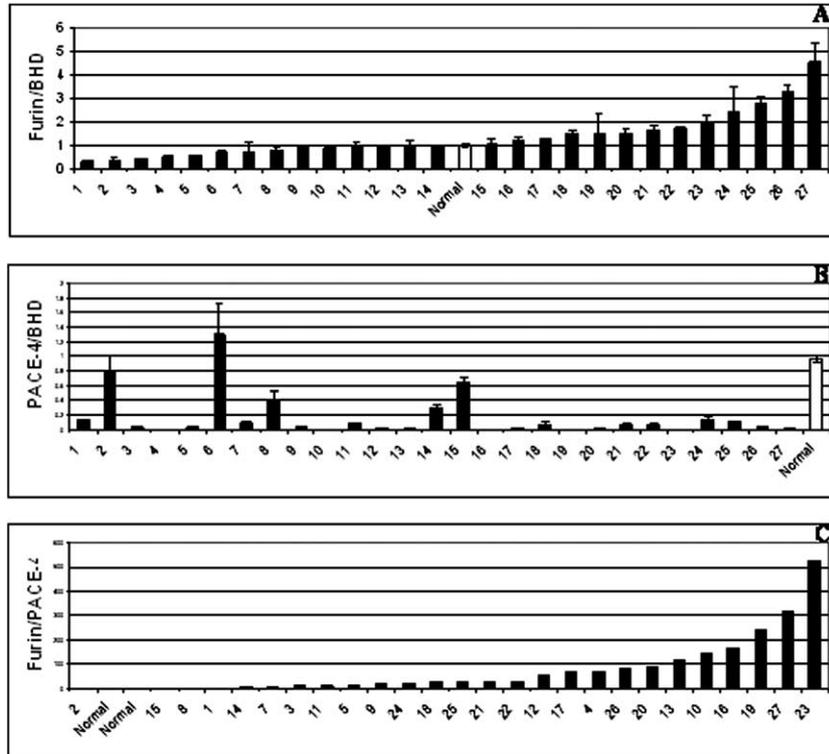


Fig. 4. Real-time PCR for furin (A) and PACE4 (B). Thirty percent of the samples had increased levels of furin. Most of the tumors expressed significant lower levels of PACE4 than the normal controls. (C): Ratio of furin to PACE4 expression. Note that furin is expressed in higher proportion than PACE4.

4. Discussion

Many proteins that are over-expressed during tumor progression require an activation step usually catalyzed by PCs. The acquisition of a malignant phenotype is associated with over-expression of proteins directly involved in invasion, proliferation, and survival. However, in some cases the de-regulation of the activating proteins, such as PCs, may contribute to tumor progression [16,23,30]. Therefore, the expression of PCs can be useful in diagnosis and/or prognosis of this disease.

In this study we provided evidence that furin is over-expressed in human ovarian cancer cell lines at the RNA and protein levels. In addition, this PC is also expressed preferentially in ovarian tumors with poor prognosis. Patients surviving less than 5 years, showed elevated levels of RNA expression and increased immunohistochemical staining.

HOSE cells showed only a marginal expression of furin. This lack of expression correlated with a diminished ability to process one of furin substrates, MT1-MMP [34], a key player in collagen IV degra-

dation [26], and, hence in tumor invasion and metastasis. MT1-MMP is also a metalloproteinase strongly associated with ovarian malignant behavior [7] and ovarian cancer cell migration through endothelial extracellular matrices [15]. This metalloproteinase contains two sites for PCs recognition and activation in the prodomain region, R⁸⁹-R-P-R-↓C⁹³ and R¹⁰⁸-R-K-R-↓Y¹¹² [20]. Active PCs display different ability to cleave these sites. For instance, furin has the ability to cleave both sites with high efficiency. Also, it has been reported that PC-7 cleaves MT1-MMP albeit with less efficiency than furin, and PACE4 is unable to cleave the first of these sites (R⁸⁹-R-P-R-↓C⁹³). These results highlight furin as the most efficient PC regarding its ability to cleave MT1-MMP [21].

Furin is synthesized as an inactive zymogen (~100 kDa), that is subsequently activated to the mature protein (~90 kDa) [1]. The apparent molecular weight observed in the western blot of the cancer cell lines, is consistent with the mature protein's molecular weight, suggesting that furin, although synthesized in higher amounts, is completely activated. Consequently, increased expression of furin correlated with increased

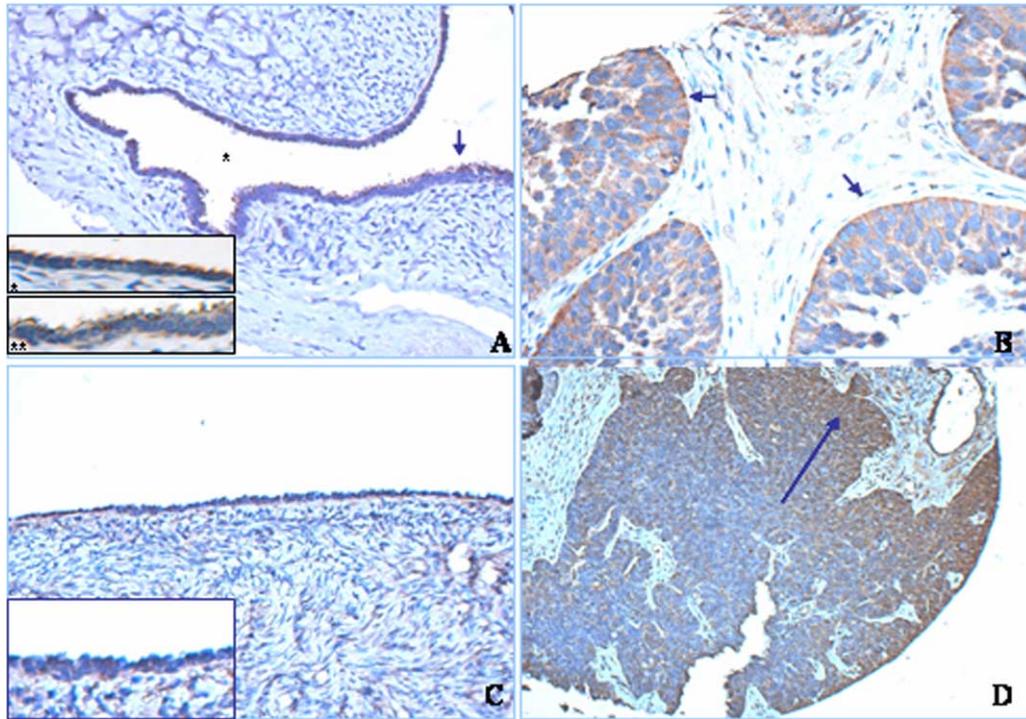


Fig. 5. Furin expression in tumors. Immunohistochemistry showing absent or low expression of furin in normal surface epithelium ((A) and (C)) and higher expression in tumors ((B) and (D)). The upper and lower panels show immunostaining using the monoclonal antifurin antibody MON-148 ((A)–(B)) and rabbit antifurin antiserum ((C)–(D)), respectively. Insets in (A): *negative control (without first antibody), **furin immunostaining. Arrow: Increased furin expression in the tumor-stroma boundary.

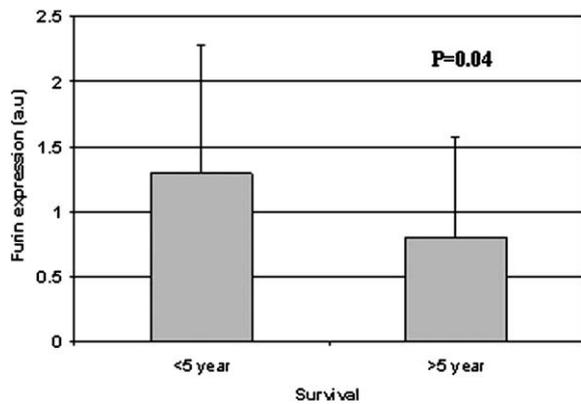


Fig. 6. Immunohistochemistry quantitation. Furin expression was scored 0 to 2 according to the immunostaining intensity. Note higher furin expression in the group that survived less than five years.

furin biological activity. MT1-MMP was completely processed in these samples, supporting a role of furin in ovarian tumor progression. Since the ubiquitously expressed PCs, PC-7 and PACE-4, which also activates MT1-MMP are expressed in HOSE cells (see below), the enhanced MT1-MMP processing in cancer

cell lines indicates that furin may play a distinctive role in ovarian tumor progression different from the other PCs. On the other hand, Fu et al. reported that furin RNA levels in ovarian cancer cell lines, like Hey and its derivative, Hey-C, did not differ significantly from the expression of normal cell lines. In agreement with these investigators, we found that furin was expressed at very low levels in Hey cell lines. However, in our experiments we found that most cancer cell lines tested expressed this PC, suggesting that furin may be over-expressed only in a subset of ovarian tumors.

In order to assess the physiological relevance of furin expression in ovarian tumor progression, we examined the expression of furin in 45 primary ovarian tumors. We found that 36% of the tumors expressed furin. Next we sought to determine if this expression was associated with a clinical parameter in this subset of ovarian tumors. Our data showed that there is a strong correlation between 5-year survival and furin expression. Forty four percent of the non-survivors expressed furin, whereas none of the survivors showed significant expression of this PC ($p < 0.01$). These results point to a preferential expression in tumors with poorer prognosis.

We also evaluated the expression of the other ubiquitously expressed PCs, PACE-4, PC-7 and PC-5. In agreement with the paper by Fu et al. [8], we found that PACE-4 expression was decreased in human ovarian cancer cell lines. Real-time PCR quantitation of the PACE4 transcript confirms this PCs downregulation in ovarian cancer. On the other hand, furin expression ranges from two to more than five hundred fold higher than PACE-4 in ovarian tumors, strongly suggesting that furin may have stronger functional significance than PACE-4 in ovarian tumor progression. This result is in agreement with published papers and supports the idea that PACE-4 is downregulated in ovarian tumors. Interestingly, furin expression is consistently higher than PACE4's. Most of the tumors (71%) expressed 10 times as much furin as PACE-4. Only 10% of these samples expressed less or the same levels of furin (Fig. 4, panel C). Overall furin is overexpressed in ovarian cancer, and its expression is decisively higher than PACE-4 highlighting the role of furin in tumor progression.

These results suggest that furin may have a unique role among the PCs in ovarian tumor progression and may be used as a prognostic marker. Interestingly, it has been demonstrated by cDNA microarrays, and Q-PCR, that furin is overexpressed in Paclitaxel-resistant breast (MCF-7) cells with respect to the sensitive wild type cell line [32]. These data indicate that furin is involved in drug resistance, and may be useful as a diagnostic and prognostic marker, helping decide therapeutic strategies. These studies are relevant to ovarian cancer, since paclitaxel is a key component of frontline chemotherapy for epithelial ovarian cancer [2]. In addition, PC-7, emerges as a PC whose activity seems to follow an opposite trend to furin, thus arguing against PCs redundancy.

PC-7 was also expressed at higher levels in normal OSE cells. Moreover, PC-7 expression correlated positively with survival, suggesting an opposite function to that of furin. Only one (9%) of the patients, who survived more than 5 years, did not express this PC. This percentage reached 23% in the case of the non-survivors ($p < 0.0001$).

Finally, PC-5 which was expressed in a limited number of cases does not seem to play a prominent role in ovarian tumor progression.

These results indicate that the role of the ubiquitously expressed PC to ovarian tumor progression may differ markedly. For instance, MT1-MMP, is not fully processed in HOSE cells that clearly expressed PC-7 and PACE-4, whereas, cancer cells that do not express

these PCs, but do express furin, achieved a complete processing (and activation) of this metalloproteinase. This suggests that furin is the PC totally or partially responsible for MT1-MMP activation, and consequently for the invasive phenotype. Also, furin seems to be preferentially expressed in tumors with poor prognosis. Marked increase in the expression of furin relative to PACE-4 in ovarian tumors points to a prominent role of furin in this disease. Therefore, furin may be considered as a candidate as a prognostic marker.

There are several lines of evidence suggesting that different PCs have different physiological roles. Furin shows the highest efficiency in PDGF-A processing [29], and metabolic labeling experiments using PDGF-A transfected cells demonstrated that furin was the only PC capable of cleaving this growth factor [29]. On the other hand, expression of PDGF receptor is a marker of poor prognosis in ovarian cancer, highlighting the role of activated PDGF and hence the contribution of furin to this disease [10]. Inhibition of each one of the PCs also points to a non-redundant role of these proteases. For instance, ppPC-7, the PC-7 prosegment, that strongly inhibits PC-7, but not furin or PC-5, is not able to reduce the PC mediated cleavage of PDGF-A [29] and B [28], and is less proficient in reverting VEGF-C processing [27], arguing against PC-7 proficiency in cleaving these substrates. Finally, an inducible furin knock-out animal model suggested that, ablation of furin expression in liver can be only partially rescued by the other ubiquitously expressed PCs [22], suggesting that there are some physiological activities performed by furin, and not by the other PCs.

Our data show that PC-7 and furin have opposite expression, suggesting a different physiological role. It may be speculated that PC-7 processes substrates that suppress tumor progression, and after loss of its expression, together with increased furin activity, tumor progression ensues.

These results also imply that the combination of furin and PC-7 may be used as a prognostic marker. Moreover, these observations point to a lack of redundancy and possible multitasking by PCs during ovarian tumor progression.

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