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# Proteases and their inhibitors as prognostic factors for high-grade serous ovarian cancer

Dominique Trudel[a,b,c,d,*], Luminita-Mihaela Avarvarei[d], Michèle Orain[d], Stéphane Turcotte[d], Marie Plante[d,e], Jean Grégoire[d,e], Reinhild Kappelhoff[f,g], David P. Labbé[h], Dimcho Bachvarov[d], Bernard Têtu[d,i], Christopher M. Overall[f,g], Isabelle Bairati[d]

[a] Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM)/ Institut du cancer de Montréal, Montréal, QC, H2X 0A9, Canada
[b] Department of Pathology, Centre Hospitalier de l'Université de Montréal, Montréal, QC, H2X 3O4, Canada
[c] Department of Pathology and Cellular Biology, Faculty of Medicine, Université de Montréal, Montréal, QC, H3T 1J4, Canada
[d] Laval University Cancer Research Center and Research Center of the Centre Hospitalier Universitaire (CHU) de Québec, Ville de Québec, QC, G1R 3S3, Canada
[e] Gynecologic Oncology Division, Centre Hospitalier Universitaire (CHU) de Québec, Québec, QC, G1R 2J6, Canada
[f] Centre for Blood Research, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada
[g] Department of Oral Biological and Medical Sciences and Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada
[h] Department of Surgery, Division of Urology, Faculty of Medicine, McGill University, Montréal, QC, H3A 0G4, Canada
[i] Anatomic Pathology and Cytology Department, Hôpital du St-Sacrement, Centre Hospitalier Universitaire de Québec, Laval University, Québec, QC, G1S 4L8, Canada

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ABSTRACT

Ovarian carcinoma is one of the most lethal malignancies, but only very few prognostic biomarkers are known. The degradome, comprising proteases, protease non-proteolytic homologues and inhibitors, have been involved in the prognosis of many cancer types, including ovarian carcinoma. The prognostic significance of the whole degradome family has not been specifically studied in high-grade serous ovarian cancer. A targeted DNA microarray known as the CLIP-CHIP microarray was used to identify potential prognostic factors in ten high-grade serous ovarian cancer women who had early recurrence (< 1.6 years) or late/no recurrence after first line surgery and chemotherapy. In women with early recurrence, we identified seven upregulated genes (*TMPRSS4, MASP1/3, SPC18, PSMB1, IGFBP2, CFI* – encoding Complement Factor I – and *MMP9*) and one down-regulated gene (*ADAM-10*). Using immunohistochemistry, we evaluated the prognostic effect of these 8 candidate genes in an independent cohort of 112 high-grade serous ovarian cancer women. Outcomes were progression, defined according to CA-125 criteria, and death. Multivariate Cox proportional hazard regression models were done to estimate the associations between each protein and each outcome. High ADAM-10 expression (intensity of 2–3) was associated with a lower risk of progression (adjusted hazard ratio (HR): 0.51; 95% confidence interval (CI): 0.29-0.87). High complement factor I expression (intensity 2–3) was associated with a higher risk of progression (adjusted HR: 2.30, 95% CI: 1.17–4.53) and death (adjusted HR: 3.42; 95% CI: 1.72–6.79). Overall, we identified the prognostic value of two proteases, ADAM-10 and complement factor I, for high-grade serous ovarian cancer which could have clinical significance.

## 1. Introduction

In Canada, ovarian carcinoma is the second most frequent gynecologic cancer in women and represents the leading cause of death by gynecologic cancer [1]. In about 70% of cases, women are diagnosed with an advanced stage ovarian carcinoma [1], for which extensive oncologic debulking surgery followed by placlitaxel-carboplatin based chemotherapy is the current standard of care [2,3]. With this extensive

*Abbreviations:* ADAM-10, A Disintegrin and Metalloproteinase domain containing protein-10; aHR, adjusted hazard ratio; CI, confidence interval; FFPE, formalin-fixed/paraffin-embedded; HGSOC, high-grade serous ovarian cancer; HR, hazard ratio; IGFBP-2, insulin-like growth factor binding protein-2; LCM, laser-capture microdissection; MASP-1/3, mannose-associated serine protease 1/3; MMP-2, matrix metalloproteinase 2; PFS, progression-free survival; PSMB-1, proteasome beta-1 subunit; SEC11A, serine protease SEC11 Homolog A; SDS, sodium dodecylsulfate; SSC, saline sodium citrate; TIMP-3, tissue inhibitor of metalloproteinase-3; TMA, tissue microarray; TMPR, SS-4transmembrane protease, serine-4

* Corresponding author at: Hôpital Saint-Luc 1058, rue Saint-Denis, Montréal, Québec, H2X 3J4, Canada.
*E-mail address:* dominique.trudel.chum@ssss.gouv.qc.ca (D. Trudel).

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therapy, about 60% of ovarian carcinoma patients will respond completely to treatment [3,4]. Maintaining a sustained response to treatment is however an important problem, since 60% of women with complete response will relapse within 18 months [3,4]. There are no well-established prognostic biomarkers available that can be used to identify women who will have early progression [3]. Moreover, no targeted therapy has been clinically validated for ovarian carcinoma patients [3,5]. Molecular studies recently improved our histologic assessment of ovarian carcinoma [6,7]. Over the years, high-grade serous ovarian cancer (HGSOC), a specific molecular subtype of ovarian cancer, has emerged as the most significant disease in terms of incidence and in terms of poor prognosis [6,7].

It has been repeatedly shown that different members of the degradome, comprising proteases, protease non-proteolytic homologues and inhibitors, have been involved in progression, invasion and metastasis of many cancer types, including ovarian cancer [8–14]. We have previously demonstrated that: (a) Matrix Metalloproteinase 2 (MMP-2) expression by ovarian cancer peritoneal cells was associated with a decreased specific survival [15]; (b) MMP-1 overexpression tended to be associated with a higher risk of progression [16,17]; (c) Tissue Inhibitor of Metalloproteinase-3 (TIMP-3) expression was downregulated in chemoresistant ovarian cancer [18]; (d) MMP-14 expression was associated with a lower risk of complete response to treatment [19], yet MMPs are now recognized as more often than not being beneficial for survival by providing essential host defense functions by cleaving bioactive mediators [20–22]. However, the whole degradome has not been specifically investigated in a family wide screen to determine their relationship to HGSOC survival.

Our prior experience prompted us to use the CLIP-CHIP microarray, a DNA microarray comprising all 721 known gene members of the degradome [23,24] to contrast tumor samples from HGSOC women with early and late recurrence after complete response to treatment. Compared to other gene chips, the CLIP-CHIP is the only gene array covering the degradome [24]. For each promising biomarker identified in the CLIP-CHIP analysis, we validated in an independent cohort whether the individual biomarkers were significantly associated with the prognosis (recurrence and death) of women with HGSOC.

## 2. Materials and methods

### 2.1. Patients

Eligible women were all diagnosed with a primary HGSOC, and underwent debulking surgery followed by adjuvant chemotherapy when indicated [25] at the Centre Hospitalier Universitaire (CHU) de Québec, Canada, between 1993 and 2006. Those who received neoadjuvant chemotherapy were excluded. To identify candidate genes potentially associated with the prognosis of women with HGSOC, an exploratory study was conducted using snap-frozen primary HGSOC tissue from 10 women with FIGO stage III-IV tumor, selecting from patients with high quality frozen tissue, five patients with early recurrence and five patients with late recurrence (overall < 1.6 years vs late/no recurrence > 1.6 years) [26] (supplementary Table A.1). Candidate genes were further evaluated on a larger independent cohort of 243 women with a primary ovarian carcinoma, in which we studied 112 women with primary HGSOC [27] of any FIGO stage, with available formalin-fixed/paraffin-embedded (FFPE) material. All women signed an informed consent form to participate to our biobanking activities, approved annually by the local Research Ethics Board. This study was also independently approved by the local Research Ethics Board.

### 2.2. Baseline data and follow-up

Patients' medical records were reviewed to retrieve baseline and follow-up data. Age at diagnosis, pre-operative CA-125 levels, FIGO stage (according to FIGO stage 1988) and first line treatment description (residual tumor after debulking surgery, chemotherapy regimens) were collected. CA-125 levels and/or the Response Evaluation Criteria In Solid Tumors (RECIST) criteria [28] were used to identify progression after the first-line therapy, as proposed by the Gynecologic Cancer InterGroup (GCIG) [26]. When identified by two modalities, the earliest event was recorded as the date of progression. Death and cause of death were obtained by record linkage with the Québec mortality files using a unique identifier (Québec health insurance number). Clinical follow-up data was last updated in June 2012.

### 2.3. Exploratory study

#### 2.3.1. Laser-capture microdissection and RNA extraction

All tissue underwent histologic quality control performed by two pathologists (DT, BT). Ten µm sections of the snap frozen tissue from the primary tumors were cut on nuclease and human nucleic acid free PEN-membrane slides (Leica, Concord, ON, Canada, #11505189) and were stained with hematoxylin and eosin (H&E) in nuclease-free conditions. Briefly, slides were dehydrated in 75% ethanol, dipped 20 s in Mayer's hematoxylin, rinsed in molecular-grade water, dipped 20 s in eosin Y and finally rinsed in 100% ethanol. Mayer's hematoxylin and eosin Y prepared with molecular-grade water and filtered (0.20 µm) prior to use. For each case, within 3 h after the staining, tumor was extracted from the stroma with the Leica AS LMD laser-capture microdissection (LCM) system. Microdissected tissue was collected in 30 µL of RLT buffer (Qiagen, Toronto, ON, Canada, # 79216) with β-mercaptoethanol (1:100). RNA was extracted using the RNeasy Plus micro kit (Qiagen, #74034), following manufacturer's instruction. A bioanalyzer 2100 (Agilent, #G2940CA) was used to assess the extracted RNA concentration and purity before amplification with the MessageAmp II aRNA amplification kit (Ambion, Burlington, ON, Canada, # AM1751) as previously described [23].

#### 2.3.2. CLIP- CHIP microarray analysis

Using the ULS aRNA fluorescent labeling kit (Kreatech Biotechnology, Amsterdam, Netherlands), sample aRNA was labeled with ULS-Cy-3 while a control mix including a 1:1 ratio of human universal cDNA (Biochain, Newark, CA, USA # C4234565) with normal ovary cDNA (Ambion # AM3330) was labeled with ULS-Cy-5. For each sample, a 1:1 mix of sample aRNA and control aRNA was hybridized to the CLIP-CHIP microarray in duplicate [23]. Briefly, microarray slides were pre-hybridized 45 min in 5X saline sodium citrate (SSC) with 0.1% (w/v) sodium dodecylsulfate (SDS) and 0.2% (w/v) bovine serum albumin (BSA) at 48 °C, then rinsed 4x in nuclease-free water and dipped in isopropanol 15–30 s. aRNA samples were incubated 15 min in the fragmentation buffer from the amplification kit at 70 °C, then resuspended in 10 µL of nuclease-free water and 10 µL of KREAblock from the labeling kit and incubated 3 min at 95 °C. Twenty microliters of pre-heated (42 °C) hybridization buffer (formamide, 10 µL; 20XSSC, 10 µL, SDS 10% (p/v), 2 µL) were added to each sample before incubation with the CLIP-CHIP slide in a humid chamber at 42 °C for 18 h. Slides were then successively rinsed in 1XSSC with 0.2% (w/v) SDS, in 0.1XSSC with 0.2% (w/v) SDS and 0.1XSSC, all at 42 °C, then dipped into nuclease-free water and dried. A 428 arrayscanner (Affymetrix, Santa Clara, CA, USA) was used to scan the slides. Signals were measured with the Imagene 6.1 software (BioDiscovery, Hawthorne, CA, USA) then normalized using CarmaWEB (comprehensive R- and bioconductor-based web service, Medical University Innsbruck, [29]) and visualized with the MultiExperiment Viewer software (www.TM4.org [30]).

#### 2.3.3. Statistical Analysis

Data from the CLIP-CHIP microarray were analyzed using the LIMMA module [31] within the BioConductor Software (www.bioconductor.org). After background correction following the normexp method, data were further normalized following the *locally*

*weighted scatterplot smoothing* (*loess*) method. A t-statistic was generated using an empiric Bayesian method to compare the gene expression in tumors from women with early recurrence (median time to recurrence: 0.98 years, to the gene expression in tumors from women with late or no recurrence (median time to recurrence/last follow-up: 7.25 years). Only genes with sufficient expression ( > 6) and a > 2 fold-change were included in the statistical analysis. P-values were adjusted for multiple testing according to the Benjamini-Hochberg method [32].

### 2.4. Cohort study

#### 2.4.1. Tissue Microarray Construction

For each tumor of the validation cohort, the most representative FFPE block was selected for tissue microarray (TMA) construction after review of the diagnostic slides (BT, DT) to confirm the histological subtype. Tissue microarrays were constructed using a tissue arrayer (Beecher Instruments® Tissue Microarray Technology, Estigen, Sun Prairie, WI, USA), using randomly distributed triplicates of 0.6 mm cores for each tumor.

#### 2.4.2. Immunohistochemistry

Immunohistochemistry was performed on the TMAs with each of the identified candidate genes, except MMP-9 which was studied with other MMPs [33] (Table 1). Before antigen retrieval and incubation with the primary antibody (Table 2), 4μm-thick sections of the TMAs were deparaffinised in toluene and rehydrated in graded alcohols, then incubated with 10% hydrogen peroxide (10 min) and normal goat serum (20 min). The Super Stain HRP detection kit (IDLabs, London, ON, Canada, #IDMR-2001) and 3, 3′-diaminobenzidine (DAB) (IDLabs, #BP1110) were used to complete the immunohistochemistry reactions and the slides were counterstained with Harris hematoxylin. For each antibody, the full TMA series was stained in a single batch. Positive controls recommended by the manufacturers and negative controls with antibody diluent instead of primary antibodies were performed in parallel to each immunohistochemistry reaction.

#### 2.4.3. Slide Digitization and Immunohistochemistry I nterpretation

Immunohistochemistry-stained TMA slides were digitized at 20x using a slide scanner (NanoZoomer 2.0-HT, Hamamatsu, Bridgewater, NJ, USA) and visualized using a specialized web-based application (mScope, Aurora Interactive, Montréal, QC, Canada). Each core was first evaluated for adequacy ( > 30% of the surface covered by tumor) and then for the expression of the candidate protein according to percentage and intensity of the staining, without knowledge of the clinical data. On each core, percentage of positive cells was evaluated using a 20% increasing scale and intensity was evaluated as low or high, according to the average of expression for each marker as determined by the observed spectrum of intensities throughout the TMAs. For each potential biomarker, threshold cores were identified and served as references to classify each core (supplemental Fig. 1). Following the

**Table 1**
Expression levels of the differentially expressed genes in tumors from 5 women with early recurrence compared to tumors of 5 women with late/no recurrence.

| Gene | Description | Fold-Change | Adjusted p-value |
|---|---|---|---|
| ADAM10 | A disintegrin and metalloproteinase containing protein-10 | 0.23 | 0.02 |
| TMPRSS4 | Transmembrane serine-protease 4 | 2.05 | 0.02 |
| MASP1/3 | Mannan-binding lectin-associated serine protease-1/3 | 2.24 | 0.03 |
| SEC11A | Signalase 18 kDa component | 2.37 | 0.048 |
| PSMB1 | Proteasome beta 1 subunit | 2.54 | 0.02 |
| IGFBP2 | IGF binding protein 2 | 2.80 | 0.02 |
| CFI | Complement factor I | 2.81 | 0.03 |
| MMP9 | Matrix metalloproteinase 9 | 2.89 | 0.04 |

**Table 2**
Experimental conditions and evaluation categories for immunohistochemistry. Endogenous biotin was blocked using a biotin-blocking system (DAKO X0590) for the anti-proteasome beta-1 subunit antibody. Sodium citrate antigen retrieval was performed at pH 6.0 and EDTA antigen retrieval was performed at pH 9.0. MMP-9 results are to be published elsewhere.

| Gene | Protein | Abbreviation | Provider | Catalog Number | Type | Specie | Antigen Retrieval | Dilution | Incubation Time |
|---|---|---|---|---|---|---|---|---|---|
| ADAM10 | A disintegrin and metalloproteinase domain-containing protein 10 | ADAM-10 | Millipore | AB19031 | Polyclonal | Mouse | No | 1/100 | 1h |
| TMPRSS4 | Transmembrane serine protease 4 | TMPRSS-4 | Lifespan Biosciences | LS-A9910 | Polyclonal | Rabbit | Citrate | 1/200 | 2h |
| MASP1/3 | Mannan-binding lectin-associated serine protease-1/3 | MASP-1/3 | Santa Cruz | sc-166816 | Monoclonal | Mouse | Citrate | 1/50 | 1h |
| SPC18 | Signalase 18k component | SEC11A | Proteintech Group | 14753-1-AP | Polyclonal | Rabbit | No | 1/100 | O/N |
| PSMB1 | Proteasome beta 1 subunit | PSMB-1 | Sigma | HPA029635 | Polyclonal | Rabbit | Citrate | 1/3000 | 1h |
| IGFBP2 | Insulin growth factor binding protein 2 | IGFBP-2 | Santa Cruz | sc-6001 | Polyclonal | Goat | EDTA | 1/50 | O/N |
| CFI | Complement factor I | CFI | Abnova | MAB7963 | Monoclonal | Mouse | Citrate | 1/50 | O/N |

O/N: overnight.

variability in the intensity of SEC11 A positive cells, percentage of intensely positive cells was also evaluated using a 10% increasing scale. Tumors with ≤ 1 adequate core were excluded from the statistical analysis. For each tumor with > 1 adequate core, percentage of positive cells was averaged and the maximal intensity of staining was identified.

#### 2.4.4. Statistical Analysis

Reporting of tumor MARKer Studies (REMARK) guidelines were followed [34] to conduct the statistical analysis. Analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA). All tests were two-sided. When staining was evaluated according to an ordinal scale, the ordinal variable was used. Logistic regressions were performed to assess the associations between protein expression levels and the standard prognostic factors of ovarian carcinoma: age (≥ 60 vs < 60 years at diagnosis), preoperative CA-125 levels (median levels), FIGO stage (III-IV vs II-I) and residual tumor (presence vs none). Progression-free survival (PFS) was defined as the time between the debulking surgery and the earliest of the following: progression according to CA-125 levels [26], death, or the most recent follow-up visit. Overall survival was defined as the time between the debulking surgery and the date of death or most recent follow-up visit. Cox proportional hazard regression models were done to evaluate the relationship between each protein and each outcome. Crude and adjusted hazard ratios (HR), as well as their 95% confidence intervals (CI), were estimated. Using a backward selection, prognostic factors associated (p-values ≤ 0.10) with prognosis (progression and death) were identified. Final multivariate models included the age at diagnosis, the FIGO stage and the preoperative CA-125 levels. Because of the strong association between the FIGO stage and the residual tumor after debulking surgery (p < 0.0001), the latter was not included in the multivariate models.

## 3. Results

### 3.1. Identification of differentially expressed genes in women with early recurrence

The overall median concentration of extracted RNA from the tumors was 3.5 ng/μL. Of the 721 proteases, inhibitors and non-proteolytic homologs included on the CLIP-CHIP microarray, 7 genes were found to be significantly upregulated while one gene was down-regulated in tumors from women with early recurrence compared to tumors from women with late or no recurrence (Table 1, Supplemental Fig. 2).

### 3.2. Evaluation of the prognostic effect of the proteins encoded by the identified genes

#### 3.2.1. Cohort

Between 1996 and 2003, 243 women with a primary ovarian carcinoma were included in the CHU de Québec Biobank (Canada). Of these, 112 eligible women had a HGSOC and their clinico-pathological characteristics are shown in Table 3. The number of women available for the statistical analyses varied from protein to protein (minimum: 80, maximum: 93) according to the number of adequate tumor cores for immunohistochemistry evaluation (Fig. 1). For the 112 women with HGSOC, the median follow-up time until progression was 1.36 years (94 progressions) and the median follow-up time until death was 4.06 years (84 deaths). The five-year PFS was 0.19 (0.12-0.27) and the five-year overall survival was 0.44 (0.34-0.53).

#### 3.2.2. Immunohistochemistry

All proteins were detected in the cytoplasm indicating either proteins in the secretory pathway or intracellular proteases (Fig. 2). Overall, most of the variability was observed in the intensity of staining (Table 4) as the median percentage of positive cells was > 80% but for transmembrane protease, serine-4 (TMPRSS-4) and insulin-like growth factor binding protein-2 (IGFBP-2) (median percentage of positive cells: 60% and 80%, respectively). The serine protease SEC11 Homolog A (SEC11 A, encoded by *SPC18*) showed a high variability when the percentage of highly intense positive cell was evaluated (average number of intensely positive cells/per case: 0: 30.2% of the cases; [0–20]: 47.7%;]20-40]: 15.1%;]40-60]:4.6%;]60-80]:1.2%;]80-100]: 1.2%).

**Table 3**
Clinico-pathological characteristics of the 112 women with high-grade serous ovarian carcinoma included in the validation study.

| Characteristic | Patients (N = 112) |
|---|---|
| Age at diagnosis, years, mean (standard deviation) | 61.1 (10.8) |
| Preoperative CA-125 levels, U/L, median (interquartile ranges) | 681 (302,1759) |
| FIGO Stage*, n(%) | |
| I-II | 8 (7.1) |
| III-IV | 104 (92.9) |
| Residual tumor, n(%) | |
| None | 18 (16.1) |
| Presence | 94 (83.9) |
| Chemotherapy | |
| Paclitaxel and platine | 103 (92.0) |
| Other type of chemotherapy | 9 (8.0) |

* According to FIGO stage 1988.

#### 3.2.3. Associations with recognized prognostic factors

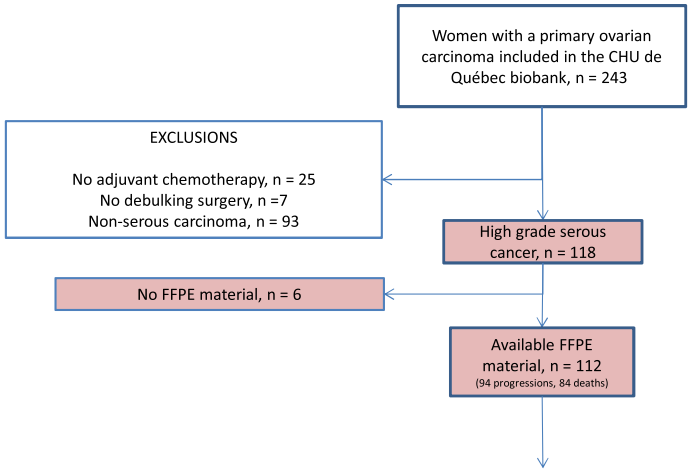
High average intensity of mannose-associated serine protease 1/3 (MASP-1/3) expression was significantly associated with higher age at diagnosis (odds ratio for high average intensity, women ≥ 60 year-old vs women < 60 year-old: 3.04; 95% CI: 1.38–6.69; p = 0.006). Intensity of the proteasome beta-1 subunit (PSMB-1) expression was inversely and significantly associated with the presence of residual tumor after debulking surgery (odds ratio: 0.32, 95% CI: 0.11-0.95; p = 0.02). Otherwise, no significant associations were found between protein expression and the standard prognostic factors of ovarian carcinoma (data not shown).

#### 3.2.4. Associations with progression and death

Percentage of positive cells was not associated with the outcomes (data not shown). All statistically significant associations identified in this cohort mirrored those identified using the CLIP-CHIP microarray. The expression levels of A Disintegrin and Metalloproteinase domain containing protein-10 (ADAM-10), of SEC11 A and of complement factor I were significantly associated with the outcomes of ovarian carcinoma after the first line therapy (Table 5). The intensity of ADAM-10 staining was associated with a lower risk of progression (adjusted HR: 0.51; 95% CI: 0.29-0.87). Each 10% increase of the maximal percentage of SEC11A-intensely positive cells was associated with an increased risk of progression of 12% (95% CI: 1.02–1.24) and with an increased risk of death of 12% (95% CI: 1.01–1.25). Although of similar magnitudes, adjusted HR did not reach statistical significance. Higher risks of progression and death were observed with high intensity of complement factor I staining (adjusted HR: 2.30, 95% CI: 1.17–4.53 and adjusted HR: 3.42, 95% CI: 1.72–6.79, respectively).

## 4. Discussion

A majority of women with ovarian cancer will experience an early relapse (< 18 months) following complete response to the current standard therapies. Therefore, discrimination of highly aggressive ovarian cancer from more indolent ovarian cancer is of paramount importance in order to provide targeted treatments to high-risk patients. Through our exploratory and validation studies, we here showed for the first time that high tissue expression of ADAM-10 was associated with a decreased risk of progression after a primary diagnosis of HGSOC and that high tissue expression of complement factor I was associated




| Protein | ADAM10 | Complement factor I | IGFBP2 | PSMB1 | SEC11A | TMPRSS4 | MASP1-3 |
|---|---|---|---|---|---|---|---|
| Evaluated women (≥ 2 available cores) | 83 | 80 | 90 | 93 | 86 | 90 | 93 |
| Progressions (%) | 70 (84.3) | 67 (83.8) | 77 (85.6) | 78 (83.9) | 73 (84.9) | 73 (81.1) | 78 (83.9) |
| Deaths (%) | 60 (72.3) | 59 (73.8) | 67 (74.4) | 68 (73.1) | 63 (73.3) | 66 (73.3) | 69 (74.2) |

**Fig. 1.** Validation study flow-chart.

with an increased risk of progression and of death.

Previous work underlined the prognostic potential for some members of the degradome, including the MMPs on HGSOC [8–19]. The CLIP-CHIP microarray allowed us to expand our investigations to all 721 known gene members of the degradome [23] in 5 women with early and in 5 women with late/no recurrence after a primary diagnostic of HGSOC. Using this approach, we narrowed down our focus to 8 candidates bearing potential prognostic impact in HGSOC. Among those, some were previously described in ovarian cancer such as ADAM-10, which was detected in exosomes released by OC cells [35] or IGFBP2, which was found to be upregulated in OC [36] and to promote cell invasion [37,38]. Notably because of its close association with MMP-2 [39], MMP-9 is the focus of a separate study [33]. Significantly, the CLIP-CHIP microarray allowed us to discover novel potential biomarkers such as complement factor I and SEC11 A for which the biological potential in cancer is only very partly known. As our study was designed to evaluate the association between each biomarker and the prognosis of chemotherapy-naïve HGSOC, further studies will be needed to estimate the interactions between ADAM-10, complement factor I and SEC11 A as well as the possibility to generate a prognostic signature using those biomarkers. Studies comparing pre- and post-chemotherapy specimens will also provide another view on the role of the degradome in HGSOC.

In order to evaluate the prognostic impact of this abbreviated list of the degradome, we systematically analyzed their protein expression by immunohistochemistry on a tissue microarray regrouping HGSOC tumors from up to 93 patients. Overall, we observed concordance between RNA and protein expression for 3 out of 8 candidates, which provides for a validation of the downstream effects of the RNA expression.

SEC11 A, which would be one of the subunits of the signal peptidase complex, has never been studied in ovarian cancer. In gastric carcinoma, high expression of SEC11 A was associated with advanced tumor

**Table 4**
Distribution of the intensity of the expression of the proteins encoded by the differentially expressed genes in women with high-grade serous carcinoma and early recurrence in a larger cohort of women with high-grade serous carcinoma and long-term follow-up. SEC11 A has been evaluated according to the percentage of highly intense positive cell.

| Protein | Categories | Max Intensity, n (%) |
|---|---|---|
| ADAM-10 | Low Intensity | 24 (28.9) |
| | High Intensity | 59 (71.1) |
| TMPRSS-4 | Low Intensity | 65 (72.2) |
| | High Intensity | 25 (27.8) |
| MASP-1/3 | Low Intensity | 30 (32.3) |
| | High Intensity | 63 (67.7) |
| PSMB-1 | Low Intensity | 68 (73.1) |
| | High Intensity | 25 (26.9) |
| IGFBP-2 | Low Intensity | 46 (51.1) |
| | High Intensity | 44 (48.9) |
| CFI | Low Intensity | 66 (82.5) |
| | High Intensity | 14 (17.5) |

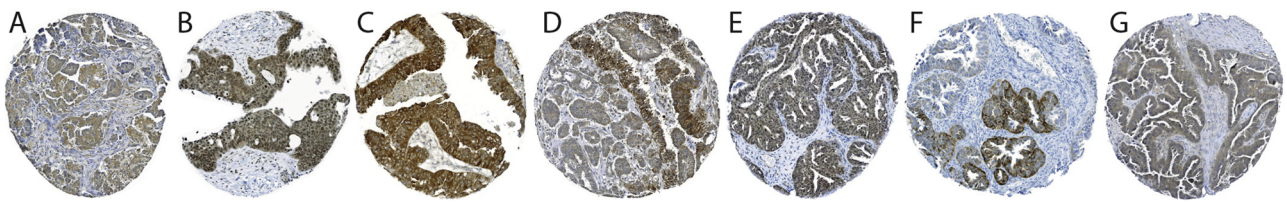



**Fig. 2.** Representative cores for the proteins as evaluated by immunohistochemistry. Intensity levels were determined according to the global distribution of the marker in the different TMAs. A. ADAM-10, high expression; B. TMPRSS-4, high expression; C. MASP1/3, high expression; D. SEC11 A, 30% of intensely positive cells; E. PSMB-1, high expression; F. IGFBP-2, high expression; G. Complement Factor I, high expression. Images were adjusted to harmonize hematoxylin counterstaining.

**Table 5**
Associations between maximal intensity of staining and outcomes. Bolded results are statistically significant.

| Marker | Progression | | Death | |
|---|---|---|---|---|
| | Crude HR (95% CI) | Adjusted HR (95% CI) | Crude HR (95% CI) | Adjusted HR (95% CI) |
| ADAM-10 | **0.58 (0.35-0.97); p = 0.04** | **0.51 (0.29-0.87); p = 0.01** | 0.86 (0.49-1.52); p = 0.61 | 0.77 (0.43-1.38); p = 0.38 |
| TMPRSS-4 | 1.13 (0.68-1.88); p = 0.63 | 1.12 (0.67-1.86); p = 0.68 | 1.39 (0.82-2.36); p = 0.22 | 1.33 (0.78-2.26); p = 0.29 |
| MASP-1/3 | 0.95 (0.59-1.54); p = 0.83 | 0.88 (0.54-1.44); p = 0.61 | 1.12 (0.67-1.88); p = 0.66 | 0.96 (0.56-1.64); p = 0.88 |
| SEC11A[1] | **1.12[1] (1.02-1.24); p = 0.02** | 1.10[1] (0.99-1.21); p = 0.07 | **1.12[1] (1.01-1.25); p = 0.03** | 1.07[1] (0.96-1.18); p = 0.24 |
| PSMB-1 | 1.03 (0.62-1.69); p = 0.92 | 1.14 (0.69-1.89); p = 0.61 | 0.79 (0.45-1.38); p = 0.41 | 0.79 (0.45-1.40); p = 0.42 |
| IGFBP-2 | 1.40 (0.89-2.21); p = 0.14 | 1.31 (0.83-2.08); p = 0.25 | 0.97 (0.60-1.57); p = 0.90 | 0.87 (0.54-1.41); p = 0.57 |
| CFI | 1.37 (0.73-2.57); p = 0.33 | **2.30 (1.17-4.53); p = 0.02** | 1.77 (0.93-3.35); p = 0.08 | **3.42 (1.72-6.79); p = 0.0004** |

HR, Hazard Ratio; CI, Confidence Intervals.
[1] Ordinal evaluation, hazard ratio for a 10% increase in the maximal percentage of positive cells.

stage [40]. To our knowledge, no other human studies have been reported. Our finding of an association between high SEC11 A expression and poor prognosis in HGSOC, even if only significant for crude analysis, is however in line with the limited available human literature on SEC11 A. It is likely that studies with higher statistical power would confirm the prognostic impact of SEC11 A in HGSOC.

ADAM-10 can degrade collagen IV and gelatin and it has been shown to cleave many cell adhesion molecules such as CD44 and E-cadherin, thus promoting cell growth and invasion [41–44]. However, although being identified in ascites in the form of exosomes [35], the prognostic impact of ADAM-10 in ovarian cancer has never been studied. In our study we showed consistently that ADAM-10 overexpression (either according to RNA levels or to maximal intensity of immunostaining) in the primary tumor is associated with a decreased risk of progression after standard treatment for a primary HGSOC. Studies on larger cohorts of women could allow to confirm the protective effect of ADAM-10 on HGSOC and to detect a potential effect on overall survival. Further studies are needed to elucidate the roles of ADAM-10 in ovarian cancer.

The serine protease complement factor I has a crucial role in the inhibition of the complement cascade through the degradation of the activated complement proteins C3b and C4b [45]. Overexpressed in non small cell lung carcinoma and by gliomas, complement factor I is thought to play a role in the tumour immune escape by avoiding complement attack [45]. To our knowledge, we here present the first assessment of the role of complement factor I in ovarian cancer, and our findings of an association between high expression and poor prognosis are in line with the expected role of complement factor I in other tumours.

## 5. Conclusions

In conclusion, we conducted a targeted study of potential protease and inhibitor prognostic factors of HGSOC, undercovering ADAM-10 and complement factor I as prognostic biomarkers. As the identified proteins were potentially expected to play a role in cancer progression, to our knowledge, this is the first assessment of their prognostic value in ovarian cancer. These results justify further investigations on the functional role of ADAM-10 and of complement factor I as potential tumor-suppressor or oncogene, respectively, in ovarian cancer. Finally, our study provides a much-needed screening method to identify highly aggressive ovarian cancer tumors in order to eventually be able to offer complementary and/or alternative treatments to those patients.

## Disclosure/Conflict of interest

There is no duality of interest for any authors.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.02.019.

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