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Expression and clinical role of the dipeptidyl peptidases DPP8 and DPP9 in ovarian carcinoma

Running title: DPP8 and DPP9 in ovarian carcinoma

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Abstract

Dipeptidyl peptidase 9 (*DPP9*) was recently identified as fusion gene in ovarian high-grade serous carcinoma (HGSC). The aim of this study was to analyze the expression and clinical relevance of DPP8 and DPP9 in ovarian carcinoma, with focus on HGSC. mRNA expression by qRT-PCR of *DPP8* and *DPP9* was analyzed in 232 carcinomas, including 114 effusions and 108 surgical specimens (89 ovarian, 29 solid metastases). DPP8 and DPP9 protein expression was analyzed in 92 effusions. *DPP8* and *DPP9* mRNA was overexpressed in effusions compared to solid lesions in analysis of all histotypes ($p<0.001$ both), as well as in analysis limited to HGSC ($p<0.001$ for *DPP9*, $p=0.002$ for *DPP8*). *DPP9* mRNA was additionally overexpressed in HGSC compared to other histotypes ($p=0.021$). DPP8 and DPP9 protein was expressed in carcinoma cells in 31/92 (37%) and 81/92 (88%) effusions, respectively. DPP8 protein expression in HGSC effusions was significantly related to better (complete) chemoresponse at diagnosis ($p=0.005$). DPP8 and DPP9 mRNA and protein expression was unrelated to survival in analysis of the entire effusion cohort. However, higher *DPP9* mRNA levels were significantly related to longer overall survival in pre-chemotherapy effusions ($p=0.049$). In conclusion, *DPP8* and *DPP9* mRNA is frequently expressed in ovarian carcinoma, whereas DPP9 is more frequently expressed at the protein level. DPP8 and DPP9 may be related to less aggressive disease in advanced-stage HGSC.

Keywords: Ovarian carcinoma; Dipeptidyl peptidases; Disease progression; Survival; Immunohistochemistry; Quantitative PCR

Introduction

Ovarian cancer, consisting predominantly of ovarian carcinoma (OC), is the seventh most commonly diagnosed cancer among women in the world [1]. In 2018, it is estimated that 22,240 new cases will be diagnosed and 14,070 deaths will occur among women in the U.S. [2]. In Norway there are 450 new cases each year and ovarian cancer is the fourth most common killer among cancers in women [3]. The most common histological type of OC is high-grade serous carcinoma (HGSC), aggressive tumor that remains the leading cause of cancer-related deaths among all gynecological cancers and commonly metastasizes within the serosal cavities in the form of solid metastases and malignant effusions [4]. HGSC accounts for 70-80% of ovarian cancer deaths, and although overall survival (OS) has improved in recent years, it is still below 50% at 5 years [5]. The emergence of drug-resistant disease is a major problem in the clinical management of OC at advanced stage, and OC cells in effusions constitute a chemoresistant population [6]. In the context of the still unsatisfactory treatment outcomes, understanding the molecular and genetic mechanisms of HGSC cells in effusions is an important challenge.

Dipeptidyl peptidase-8 and -9 (DPP8, DPP9) are serine proteases that are members of the DPPIV family, together with the prototype member PPIV (a.k.a. CD26), fibroblast activation protein (FAP, a.k.a. Seprase), and the non-enzymes DPP6 and DPP10. Enzymes members of the DPPIV family cleave dipeptides from the N-terminus of substrates, with preference to proline in the penultimate position. Unlike DPPIV and FAP, which are cell surface and intracellular proteins, DPP8 and DPP9 are intracellular proteins. Both the latter have splice variants. DPP8 and DPP9 have been postulated to have a role in the regulation of apoptosis, proliferation, and interaction with the extracellular matrix (ECM) immune response, the latter through affecting adhesion and migration. Disease states in which these enzymes appear to have a role include inflammatory conditions, liver disease and cancer. Their substrates include multiple proteins, many of which have been implicated in these diseases, e.g. the chemokine CXCL10, collagen 7, and the metastasis promoter S100A10 [7-9].

DPP9-PPP6R3 fusion transcript was recently reported in a serous OC showing a matching 11;19 translocation, an additional tumor had a *DPP9-PLIN3* rearrangement [10]. A third fusion was reported with *PAX2* [11]. This prompted us to investigate the expression and clinical relevance of DPP8 and DPP9 in OC. In the present study, we analyzed the mRNA and protein expression of these proteases, with focus on HGSC effusions.

Material and Methods

Patients and specimens

OC specimens (n=232) and clinical data were obtained from patients treated at the Department of Gynecologic Oncology, Norwegian Radium Hospital and the Department of Gynecology, Ullevål University Hospital during the period of 1998 to 2006. As the fallopian tubes have not been adequately assessed in this cohort, tumors in the ovary are specified as such without reference to primary site. All tumors were reviewed by a surgical pathologist with experience in gynecologic pathology and cytopathology (BD) and diagnosed based on the combination of morphology and immunohistochemistry (IHC) according to the WHO 2014 guidelines [5]. The material studied using quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) is listed in **Table 1**. The 114 effusions analyzed for *DPP8* and *DPP9* mRNA expression consisted of 88 peritoneal and 26 pleural specimens from 114 patients. Clinicopathologic data for 107 patients with HGSC effusions are presented in **Table 2-A**. A validation series of 49 HGSC effusions tapped between 2002 and 2015 was independently studied for clinical relevance. Clinicopathologic data for these patients are detailed in **Table 2-B**. An overview of the studied material is shown in **Figure 1-A**.

Effusions were centrifuged immediately after tapping, and cell pellets were frozen at -70°C in equal amounts of RPMI 1640 medium (GIBCO-Invitrogen, Carlsbad, CA) containing 50% fetal calf serum (PAA Laboratories GmbH, Pasching, Austria) and 20% dimethylsulfoxide (Merck KGaA, Darmstadt, Germany). Cell blocks prepared using the Thrombin clot method. Sections from surgical specimens were frozen at -70°C without any treatment. Frozen sections from all solid tumors were reviewed by one of the authors (BD), and only specimens with tumor cell population >50% and minimal or no necrosis were included in this study.

Informed consent was obtained according to national and institutional guidelines. Study approval was given by the Regional Committee for Medical Research Ethics in Norway.

RNA extraction and cDNA synthesis

Total RNA was extracted using RNeasy kit (Qiagen, Hilden, Germany) and QIAcube (Qiagen). RNA concentration and quality was measured by the QIAexpert system (Qiagen) and 2100 Bioanalyzer (Agilent, Santa Clara, CA) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed in a 20µL reaction volume using iScript Advanced cDNA synthesis Kit for RT-PCR according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA).

qRT-PCR

DPP8 and *DPP9* expression was assessed using the CFX96 Touch Real-Time PCR detection system (Bio-Rad Laboratories). Reactions were carried out in quadruplicate using TaqMan Assays and the TaqMan Universal Master Mix II with UNG (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The primers used were for exons 12 and 13 for *DPP8* (Hs_00214745_m1); exons 8 and 9 (Hs_00373593_g1) and exons 19 and 20 (Hs01042066_m1) for *DPP9* (Applied Biosystems). The *DPP9* exon 8 and 9 assay detected the 5' end of the molecule, whereas the exon 19 and 20 assay was directed against the 3' end. *RPL4* (Hs_01939407_gH) was used as a reference gene as it has been reported to be stably expressed in ovarian cells [12]. Human Universal Reference Total RNA (Clontech, Mountain View, CA) was used as internal reaction control. The commercial Total RNA from the ovary, (Human Ovary Total RNA, Clontech), was used as reference for relative expression normalization. Expression data were analyzed using Bio-Rad CFX manager 3.1 (Bio-Rad). The normalized expression was calculated using the $2^{-\Delta\Delta Ct}$ (Livak) method [13].

Immunohistochemistry (IHC)

Formalin-fixed, paraffin-embedded sections from 92/107 HGSC effusions analyzed using qRT-PCR were analyzed for DPP8 and DPP9 protein expression using the Dako EnVision Flex + System

(K8012; Dako, Glostrup, Denmark). The DPP8 antibody was a mouse monoclonal antibody purchased from Novus Biologicals (cat # NBP2-01830, clone OTI1D2; Littleton, CO), applied at a 1:200 dilution. The DPP9 antibody was a rabbit polyclonal antibody purchased from Novus Biologicals (cat # NB100-59025), applied at a 1:100 dilution.

Following deparaffinization, sections were treated with EnVision™ Flex + mouse linker (15 min) and EnVision™ Flex/HRP enzyme (30 min) and stained for 10 min with 3'3-diaminobenzidine tetrahydrochloride (DAB), counterstained with hematoxylin, dehydrated and mounted in Richard-Allan Scientific Cyto seal XYL (Thermo Fisher Scientific, Waltham, MA). Positive and negative controls consisted of normal testis.

IHC scoring: Cytoplasmic staining was considered positive. Staining extent was scored by an experienced cytopathologist (BD), with a subset of the effusion specimens additionally scored by another author (MB), using a 0-4 scale as follows: 0=no staining, 1=1-5%, 2=6-25%, 3=26-75%, 4=76-100% of tumor cells.

Statistical analysis

Statistical analysis was performed applying the SPSS-PC package (Version 25). Probability of <0.05 was considered statistically significant. The association between DPP8 and DPP9 mRNA and protein expression and tumor type was performed using the Mann-Whitney U test (2-tier analyses) or the Kruskal Wallis H test (3-tier analyses). The same tests were applied to analysis of the association between DPP expression in HGSC effusions and clinicopathologic parameters. For this analysis, clinicopathologic parameters were grouped as follows: age: ≤60 vs. >60 years; effusion site: peritoneal vs. pleural; FIGO stage: III vs. IV; chemotherapy status: pre- vs. post-chemotherapy specimens; residual disease (RD): 0 cm vs. ≤1 cm vs. >1 cm, or 0 cm vs. any residual macroscopic disease; response to chemotherapy: complete response vs. partial response/stable disease/progressive disease. The association with CA 125 levels at diagnosis was analyzed using a

two-sided T-test. The Mann-Whitney U test was applied to analyses of the association between DPP expression and expression of AKT.

Progression-free survival (PFS) and OS were calculated from the date of the last chemotherapy treatment/diagnosis to the date of recurrence/death or last follow-up, respectively. Univariate survival analyses of PFS and OS were executed using the Kaplan-Meier method and log-rank test. Platinum resistance was defined as PFS≤6 months according to guidelines published by the Gynecologic Oncology Group (GOG) and progressive disease or recurrence was evaluated by the Response Evaluation Criteria In Solid Tumors (RECIST) criteria. For survival analyses, staining was grouped as high vs. low (extent: 0-2 vs. 3-4; combined score: low vs. high).

Results

DPP8 and DPP9 are differentially expressed as function of histological type and anatomic site

Comparative analysis of *DPP8* and *DPP9* mRNA levels in OC of different histology, analyzed in the entire material (n=232) showed overexpression of *DPP9* 5' in HGSC and carcinosarcoma compared to other histotypes (p=0.021), with comparable expression of *DPP9* 3' and *DPP8* (**Figure 1-B**). Comparative analysis of expression in effusion specimens, the ovarian tumors and solid metastases analyzing all tumors showed overexpression of *DPP9* 5' in effusions compared to the 2 other anatomic sites (p<0.001; **Figure 1-C**). *DPP9* 3' and *DPP8* were similarly overexpressed in effusions, but solid metastases had higher levels than the ovarian tumors (p<0.001 for both; **Figures 1-D, 1-E**, respectively). Limiting the analysis to HGSC alone, results were comparable (p<0.001 for *DPP9* 5' and *DPP9* 3'; p=0.002 for *DPP8*).

Two cases showed higher expression of *DPP9* 5'compared to *DPP9* 3', suggesting the presence of possible fusion genes. PCR analysis was performed with specific primers combinations for the already known fusion genes, but none of these transcripts was identified.

Based on these results, we chose to focus on analysis of DPP8 and DPP9 protein expression in HGSC effusions. DPP8 and DPP9 expression was predominantly localized to carcinoma cells, but expression in reactive mesothelial cells and leukocytes was found in some specimens, particularly of DPP9. In tumor cells, DPP8 was expressed in 31/92 (37%) HGSC effusions, with staining score =1 in 14 effusions, score =2 in 6, score=3 in 3, and score=4 in 8 specimens. DPP9 was expressed in 81/92 (88%) HGSC effusions, with staining score =1 in 10 effusions, score =2 in 10, score=3 in 21, and score=4 in 40 specimens (**Figure 2**). Inter-observer agreement was good (>80%).

In order to assess the cellular distribution of DPP proteins in solid specimens, we stained a small series of solid HGSC localized to the ovary (n=17) and peritoneum/omentum (n=11). As in effusions, DPP8 and DPP9 were predominantly expressed in carcinoma cells, with host cell expression in some specimens (**Figure 3**).Expression in carcinoma cells was as follows:

DPP8 ovary (n=17): score=0: 2; score =1: 1; score =2: 1; score=3: 2; score=4: 11 specimens.

DPP9 ovary (n=17): score=0: 0; score =1: 4; score =2: 7; score=3: 5; score=4: 1 specimens.
DPP8 omentum/peritoneum (n=11): score=0: 2; score =1: 2; score =2: 0; score=3: 4; score=4: 3 specimens.
DPP9 omentum/peritoneum (n=11): score=0: 0; score =1: 3; score =2: 3; score=3: 2; score=4: 3 specimens.
In the validation series of 49 HGSC effusions, DPP8 was expressed in carcinoma cells in 22/49 (45%) specimens, with a staining score =1 in 10 effusions, score =2 in 4, score=3 in 6, and score=4 in 2 specimens. DPP9 was expressed in 48/49 (98%) effusions, with staining score =1 in 9 effusions, score =2 in 5, score=3 in 13, and score=4 in 21 specimens

DPP8 and DPP9 are associated with chemotherapy response and survival

Original series: *DPP9* 5' mRNA levels were higher in HGSC effusions from older (>60 years) patients (p=0.039). DPP8 protein expression was higher in specimens from patients who had complete response to first-line chemotherapy compared to patients with unfavorable response (p=0.005). No associations were observed with other clinicopathologic parameters, including effusion site, FIGO stage, RD volume and intrinsic chemoresistance (p>0.05).
The follow-up period for the 107 patients with HGSC effusions studied for mRNA expression ranged from 1 to 179 months (mean = 37 months, median = 26 months). PFS ranged from 0 to 148 months (mean = 10 months, median = 6 months). At the last follow-up, 101 patients were dead of disease, 3 were alive with disease and 2 were with no evidence of disease. One patient was lost to follow-up.
In univariate survival analysis of all cases, DPP8 and DPP9 mRNA and protein expression was unrelated to survival (p>0.05; data not shown). However, in analysis limited to patients with pre-chemotherapy effusions tapped at diagnosis, higher *DPP9* 3' levels were significantly related to longer OS (p=0.049; **Figure 4**). Multivariate analysis was not performed since all the clinical variables were unrelated to OS (p>0.05; data not shown).

Validation series: In this series of 49 patients, DPP9 protein expression was higher in post-chemotherapy compared to pre-chemotherapy effusions (p=0.003). *DPP8* mRNA levels were higher in HGSC effusions from older (>60 years) patients (p=0.024), whereas DPP9 protein expression was higher in specimens from younger patients (p=0.027). *DPP9* 3' mRNA levels were higher in HGSC effusions from patients diagnosed at FIGO stage III compared to stage IV disease (p=0.049). No associations were observed with other clinicopathologic parameters, including effusion site, RD volume, response to first-line chemotherapy and intrinsic chemoresistance (p>0.05).

The follow-up period for this patient group ranged from 1 to 169 months (mean = 38 months, median = 31 months). PFS ranged from 0 to 116 months (mean = 14 months, median = 8 months). At the last follow-up, 36 patients were dead of disease, 11 were alive with disease and 2 died of complications.

In univariate survival analysis, DPP8 and DPP9 mRNA and protein expression was unrelated to survival (p>0.05; data not shown). The number of cases was deemed too small for separate analysis of pre- and post-chemotherapy specimens. Among the clinical variables, larger RD volume was associated with shorter OS (p=0.028) and PFS (p=0.001) for the 27 patients with upfront surgery who had data regarding this parameter, with no prognostic role for patient age and FIGO stage (data not shown).

Discussion

DPP8 and DPP9 are ubiquitously expressed in normal tissues, cancer specimens and cell lines, including in OC cell lines [8,14]. However, whether these molecules are tumor-promoting or -suppressing remains equivocal. DPP9 overexpression induced apoptosis via the intrinsic pathway and suppressed proliferation in HepG2 human hepatoma cells. The effect of DPP in these cells was via epidermal growth factor-specific signaling through phospoinositide 3-kinase (PI3K)/Akt, with no effect on ERK1/2 [15]. Conversely, silencing of DPP9 using short hairpin RNA in non-small cell lung cancer (NSCLC) cells resulted in suppression of proliferation, migration and invasion, with upregulation of epithelial markers and downregulation of mesenchymal ones. DPP9 silencing further induced the expression of the pro-apoptotic proteins p53, BAX and Apaf-1 *in vitro* and reduced tumorigenicity *in vivo* in a mouse model [16]. Analysis of the biological role of DPPIV, another member of this enzyme family, in OC cell lines showed that its overexpression reduced matrix metalloproteinase-2 (MMP2) and membrane-type 1 MMP (MT1-MMP) levels and inhibited ERK signaling, while upregulating tissue inhibitors of MMP (TIMP1 and TIMP2), E-cadherin and β-catenin [17].

Our group recently identified involvement of the *DPP9* gene in two different fusion transcripts in serous OC, suggesting this gene may have a role in tumorigenesis or progression of this tumor. The fusions lead to disruption and deregulation of *DPP9* gene expression at the 3'end, with potential loss of its tumor suppressor function [10]. In view of this finding, we wished to analyze *DPP9* mRNA expression in OC using specific primers for the 5' and 3' ends. We additionally assessed the mRNA expression of *DPP8,* a DPP9 homolog, and the protein expression of both molecules.

The role of DPP8 and DPP9 in OC progression has not been studied to date to the best of our knowledge. In the present study, *DPP8* and *DPP9* mRNA was overexpressed in effusion specimens compared to other anatomic sites was observed, with lowest levels in the ovary, in analysis of all histotypes, as well as in analysis limited to HGSC. We further observed overexpression of these

genes in HGSC and CS compared to other histotypes. The presence of DPP8 and DPP9 proteins in HGSC effusions and solid specimens was confirmed by IHC. The observation that *DPP8* and *DPP9* mRNA is more highly expressed in the clinically aggressive OC histotypes compared to less aggressive histotypes, and in extra-adnexal metastases compared to the adnexal lesions suggests they may be involved in disease progression in this cancer. However, in view of the small number of tumors with non-HGSC histology, this difference must be seen as a preliminary observation requiring validation in larger series of tumors of histological type other than HGSC.
By IHC, DPP8 and DPP9 protein expression was predominantly seen in carcinoma cells, but was observed in host cells in some specimens, particularly in the case of the latter. The possibility that this may have affected the anatomic site-related differences in the present study cannot be entirely ruled out. However, as we applied the same inclusion criterion, i.e. minimum 50% tumor cell content, to all specimens, this contribution is likely to be balanced.

Two effusion specimens showed expression difference between the *DPP9* 5' and *DPP9* 3'. However, none of the already known fusion genes was identified. The two possible explanations for this finding are either that *DPP9* is rearranged with a yet unknown partner in these tumors or that the gene is truncated. Unfortunately, we did not have remaining material from these cases for further analysis.

The potential effect of DPP8 and DPP9 expression on chemoresponse and patient survival in OC in general and HGSC in particular has not been studied to date, and current data regarding other members of the DPPIV family are contradictory. Transfection of OC cells with DPPIV increased the sensitivity of OC cells to paclitaxel *in vitro* and *in vivo* [18]. Conversely, FAP expression in the stroma of clinical OC specimens was significantly associated with chemoresistance and shorter time to recurrence, and its silencing in OC cells *in vitro* led to reduced proliferation [19].

In the present study, DPP8 protein expression was significantly associated with complete chemoresponse at diagnosis, whereas higher *DPP9* 3' level expression was related to longer OS in patients with pre-chemotherapy effusions. These data suggest a tumor suppressor role for DPP8 and DPP9 and appear to be in discordance with the above-discussed observation that these molecules are upregulated along tumor progression. It should nevertheless be commented that the finding in survival analysis, at p=0.049, was of marginal significance. Additionally, the association with chemoresponse and survival was not reproduced in a validation cohort, though its smaller size may have contributed to this failure.

In conclusion, DPP8 and DPP9 are frequently expressed in OC, particularly in HGSC. Despite their overexpression in metastatic disease and in aggressive OC histotypes, these molecules appear to be associated with better chemoresponse and longer OS. The reason for this discrepancy is unclear and merits additional research, including analysis of other tumor series. Validation of the performance of the antibodies used, with emphasis on and their role as predictive or prognosis markers, should similarly be undertaken in other cohorts. Analysis of other DPPIV family members in HGSC effusions may also be of interest, as would be further research directed at identifying the molecular partners of these molecules in OC.

Compliance with Ethical Standards: The study was approved by the Regional Committee for Medical Research Ethics in Norway.

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Conflict of interest: None declared

Author contributions:

MB: Performed the PCR experiments and wrote the manuscript.

AH: Performed the IHC experiments

IP: Participated in performing the PCR experiments, critically read the manuscript.

ACS: Provided clinical data and specimens, critically read the manuscript.

FM: Designed the study, supervised the PCR experiments, critically read the manuscript.

BD: Designed the study, performed the statistical analysis, scored the immunostains, and supervised the writing of the manuscript.

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Table 1: Specimens studied (n=232)

Histology	Anatomic site			Total
	Effusion	Ovary	Solid metastasis	
HGSC	107	68	25	200
LGSC	7	5	0	12
CCC	0	5	0	5
EC	0	6	4	10
Mixed type	0	2	0	2
CS	0	3	0	3
Total	**114**	**89**	**29**	**232**

Abbreviations: HGSC = High-grade serous carcinoma; LGSC = Low-grade serous carcinoma; CCC = Clear cell carcinoma; EC = Endometrioid carcinoma; CS = Carcinosarcoma

Table 2-A: Clinicopathologic parameters of the original HGSC effusion cohort (107 patients)

Parameter	Distribution
Age (mean)	35-85 years (61)
FIGO stage	
III	60
IV	47
Residual disease [a]	
≤1 cm	24
>1 cm	38
NA	5
CA 125 at diagnosis (range; median)	11-24290 (1156) [b]
Chemoresponse after primary treatment	
CR	54
PR	23
SD	8
PD	11
NA [c]	11

Abbreviations: NA = not available; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

[a] For 67 patients who received surgery as upfront treatment.

[b] Available for 72 patients

[c] Not available (missing data or disease response after chemotherapy could not be evaluated because of normalized CA 125 after primary surgery or missing CA 125 information and no residual tumor).

Table 2-B: Clinicopathologic parameters of the HGSC effusion validation cohort (49 patients)

Parameter	Distribution
Age (mean)	48-81 years (65)
FIGO stage	
II	1
III	30
IV	18
Residual disease [a]	
≤1 cm	19
>1 cm	8
NA	10
CA 125 at diagnosis (range; median)	128-28000 (1156) [b]
Chemoresponse after primary treatment	
CR	22
PR	19
SD	3
PD	0
NA [c]	5

Abbreviations: NA = not available; CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

[a] For 37 patients who received surgery as upfront treatment.

[b] Available for 43 patients

[c] Not available (missing data or disease response after chemotherapy could not be evaluated because of normalized CA 125 after primary surgery or missing CA 125 information and no residual tumor).

Figure legends

Figure 1: *DPP8* and *DPP9* mRNA expression in different ovarian carcinoma (OC) histotypes and at different anatomic sites

Figure 1-A: Overview of the tumors studied.

Figure 1-B: *DPP9 5'* mRNA is overexpressed in high-grade serous carcinoma and carcinosarcoma compared to other OC histotypes.

HGSC = high-grade serous carcinoma; LGSC = low-grade serous carcinoma; CCC = clear cell carcinoma; EC = endometrioid carcinoma; CS = carcinosarcoma

Figure 1-C: *DPP9 5'* mRNA is overexpressed in effusion specimens compared to the ovarian tumors and solid metastases.

Figure 1-D: *DPP9 3'* mRNA is overexpressed in effusion specimens compared to the ovarian tumors, with intermediate levels in solid metastases.

Figure 1-E: *DPP8* mRNA is overexpressed in effusion specimens compared to the ovarian tumors, with intermediate levels in solid metastases.

Figure 2: DPP8 and DPP9 protein expression in HGSC effusions

Cytoplasmic expression of DPP8 (A-B) and DPP9 (C-D) in tumor cells

Figure 3: DPP8 and DPP9 protein expression in surgical specimens from HGSC patients

(A-B) HGSC localized to the ovary expressing DPP8 (A) and DPP9. The majority of host cells are negative.

(C-D) Peritoneal metastasis with tumor cells diffusely positive for DPP8 (C) and focally positive for DPP9 (D). Stromal cells are weakly positive for DPP8 and strongly positive for DPP9.

Figure 4: *DPP9 3'* mRNA expression is associated with longer survival

Kaplan-Meier survival curve showing the association between *DPP9 3'* mRNA expression in pre-chemotherapy effusions (n=50) and overall survival (OS). Patients with effusions with high (above median) *DPP9 3'* mRNA expression levels (n=27; red line) had mean OS of 59 months compared to 31 months for patients with effusions having low *DPP9 3'* mRNA levels (n=23, blue line; p=0.049).