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ORIGINAL ARTICLE

Histotype-specific analysis of acid ceramidase expression in ovarian cancer

Ahmed El-Balat[1] · Thomas Karn[1] · Uwe Holtrich[1] · Sven Becker[1] · Stefan Kommoss[2] · Balázs Győrffy[3] · Michael S. Anglesio[4] · David G. Huntsman[4,5] · Zacharias Drosos[6] · Achim Rody[6] · Heidrun Gevensleben[7] · Lars C. Hanker[6]

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Abstract

Acid ceramidase (ASAH1) is a key player in sphingolipid metabolism and signaling. It has prognostic value for several cancers, but histotype-specific analyses of ovarian cancer are not yet available. We used three retrospective TMA cohorts encompassing a total of 1106 ovarian cancers with follow-up data for immunohistochemical analysis of acid ceramidase (ASAH1) expression. Patients with sub-optimal debulking and persistent residual tumor after surgery introduced bias in the prognostic analysis and were excluded from further studies. Overall, we detected an association of ASAH1 expression with better prognosis in ovarian cancer patients. ASAH1 expression differed between histological ovarian cancer histotypes with most frequent expression in endometrioid and clear cell ovarian cancer, which are both associated with good prognosis. Stratified subgroup analyses within these histotypes did not reveal significant survival differences, but the power of the analysis may be limited by smaller sample sizes. In contrast to breast cancer, we found only a modest concordance between estrogen receptor status and ASAH1 expression within the endometrioid ovarian cancer histotype. In an exploratory analysis of estrogen receptor negative endometrioid ovarian cancer, ASAH1 expression was associated with significantly better overall survival ($P = 0.007$). Acid ceramidase is most frequently expressed in endometrioid and clear cell histotypes and could add independent prognostic value to estrogen receptor in endometrioid ovarian cancer. Modulating sphingolipid metabolism may lead to novel therapeutic intervention strategies for this disease.

Keywords Ovarian cancer histotypes · Sphingolipid signaling · Prognosis

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✉ Ahmed El-Balat
ahmed.el-balat@kgu.de

[1] Goethe University Frankfurt, Department of Obstetrics and Gynecology, Frankfurt, Germany

[2] Department of Woman's Health, Tuebingen University Hospital, Tuebingen, Germany

[3] MTA TTK Lendület Cancer Biomarker Research Group & Semmelweis, University Second Department of Pediatrics, Budapest, Hungary

[4] Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, Canada

[5] Department of Molecular Oncology, BC Cancer Research Centre, Vancouver, Canada

[6] Gynecology and Obstetrics, University Hospital Lübeck, Lübeck, Germany

[7] Institute of Pathology, University Hospital Bonn, Bonn, Germany

Introduction

Ovarian cancer (OC) is among the most frequent cancers of women and accounts for an estimated 22,240 new cases and 14,070 deaths in the USA (2018) [1]. There are five main histotypes of epithelial ovarian carcinoma [2]. These histotypes comprise high-grade serous, endometrioid, clear cell, mucinous carcinoma, and low-grade serous, of which high-grade serous carcinoma is the most common [3]. Findings support the assumption that besides the cells covering the ovaries [2, 3], the fallopian tubes could also be a site of origin of serous ovarian cancer [4–6], whereas endometrioid and clear cell tumors may originate from endometriotic lesions [4, 7]. The differences between ovarian tumor histotypes suggest that they describe biologically distinct malignancies, which has also important implications for biomarker analyses. Studies have shown that combining all histopathological subtypes in corresponding analyses obscured the prognostic value of the biomarkers

[8]. Thus, it is crucial to perform separate analyses by histotype to decipher the prognostic value [9, 10].

Acid ceramidase (ASAH1) is a key player in sphingolipid metabolism and signaling [11]. ASAH1 converts ceramide into sphingosine thereby altering the "sphingolipid rheostat" which governs apoptosis and proliferation of cancer cells [11]. ASAH1 expression has a positive prognostic value in several cancer types including ovarian cancer [12–15], but distinct subtypes within these cancer types differ in their ASAH1 expression which can confound analyses.

Therefore, in the current study we used a large cohort of 1106 ovarian cancer cases of different histotypes to validate and study in more detail the potential prognostic value of ASAH1 in ovarian cancer.

Materials and methods

All analyses in this study were performed according to the "REporting recommendations for tumour MARKer prognostic studies" (REMARK) (Supplementary Fig. S1) [16]. Three large ovarian cancer cohorts (OOU n = 540, OOUE $n = 250$, VOA $n = 316$) were applied to validate the prognostic effect of ASAH1 expression by immunohistochemistry. Details of these population-based ovarian cancer cohorts from British Columbia with long-term follow-up data have been described before [8, 17–19]. OOU and OOUE cohorts were treated at the British Columbia Cancer Agency (BCCA) with tumor samples derived from > 20 hospitals. The VOA cohort consists of samples from the Vancouver General Hospital Tissue Bank. In this study, the eligibility was a diagnosis of chemotherapy naïve ovarian carcinoma with either optimal surgical debulking (OOU and VOA) or macroscopic residual tumor after primary surgery (OOUE). Clinical follow-up data was collected through the Cheryl Brown Ovarian Cancer Outcomes Unit as an ovarian cancer database of the BCCA for all three patient cohorts, and approval for the study was obtained from the Research Ethics Board of the University of British Columbia. All samples underwent contemporary gynecopathological review including predictions of an IHC-based Calculator of Ovarian Carcinoma Subtype (COSP) [19] and a tissue microarray (TMA) was available through earlier studies. Paraffin sections of TMAs were dewaxed in xylene and rehydrated to water through a graduated ethanol series. For antigen retrieval, sections were incubated for 20 min in a microwave oven (800 W) using EDTA buffer (10 mmol/L; pH 8.0). Sections were incubated with a monoclonal anti-ASAH1 antibody (Biozol Diagnostica, Germany; cat. no. H00000427-M01, Clone2C9) at a 1:100 dilution for 1 h at room temperature. For secondary antibody incubation, the Dako REAL Detection System Alkaline Phosphatase (Dako, Denmark) was applied, following the instructions of the vendor. Sections were counterstained with hematoxylin. Staining intensity was assigned semiquantitatively as negative/weak or moderate/strong dichotomizing the sample cohort into low or high ASAH1 expression (Fig. 1). Estrogen receptor (ER) expression was obtained from a previous study [8]. All assessments were made blinded with respect to clinical patient data. RNA expression of ASAH1 through Affymetrix U133A microarray data were analyzed as previously described [14, 20]. Progression-free survival (PFS) and overall survival (OS) were used as outcome variables ($n = 1106$). Follow-up data for patients in whom the envisaged end point was not reached were censored as of the last follow-up date or at 120 months. Kaplan Meier analysis and log-rank test were performed overall and separately in subcohorts, stratified by efficiency of debulking, and histological subtypes. Chi-square test was used for comparing cohort characteristics. All P values are two-sided and $P = 0.05$ was considered as significant. All analyses were performed in SPSS Statistics Version 25 (IBM Corp.).

Results

ASAH1 expression as prognostic factor among 1106 ovarian cancer samples

We used three large ovarian cancer cohorts with long-term follow-up data that have been described before to validate the prognostic effect of ASAH1 expression by immunohistochemistry (Table 1): The "Ovarian outcome unit" (OOU) cohort consisting of 540 ovarian cancers with optimal debulking ("BC-NoRes") [8, 17], the "Ovarian outcome unit extreme risk "(OOUE) cohort of 250 cases all with residual tumor after surgery ("BC-Res") [17], and the VOA cohort of 316 cases with both optimal and suboptimal debulking. The clinical parameters of these three cohorts differed significantly. One important reason for this fact is the selection of cases according to debulking in the OOU and OOUE cohorts. A further effect of this sampling strategy is the lower frequency of cases with higher stages in the OOU cohort as compared to OOUE and VOA. Also the relative frequency of non-HGSC histotypes is increased in OOU as has been reported before [8]. Strong ASAH1 expression was seen overall in 55.5% of the samples with a higher frequency in the OOU cohort (60.4%) compared to the VOA (56.6%) and OOUE (41.2%) cohorts (Table 1). As shown in Fig. 2, overall ASAH1 expression is associated with a better prognosis

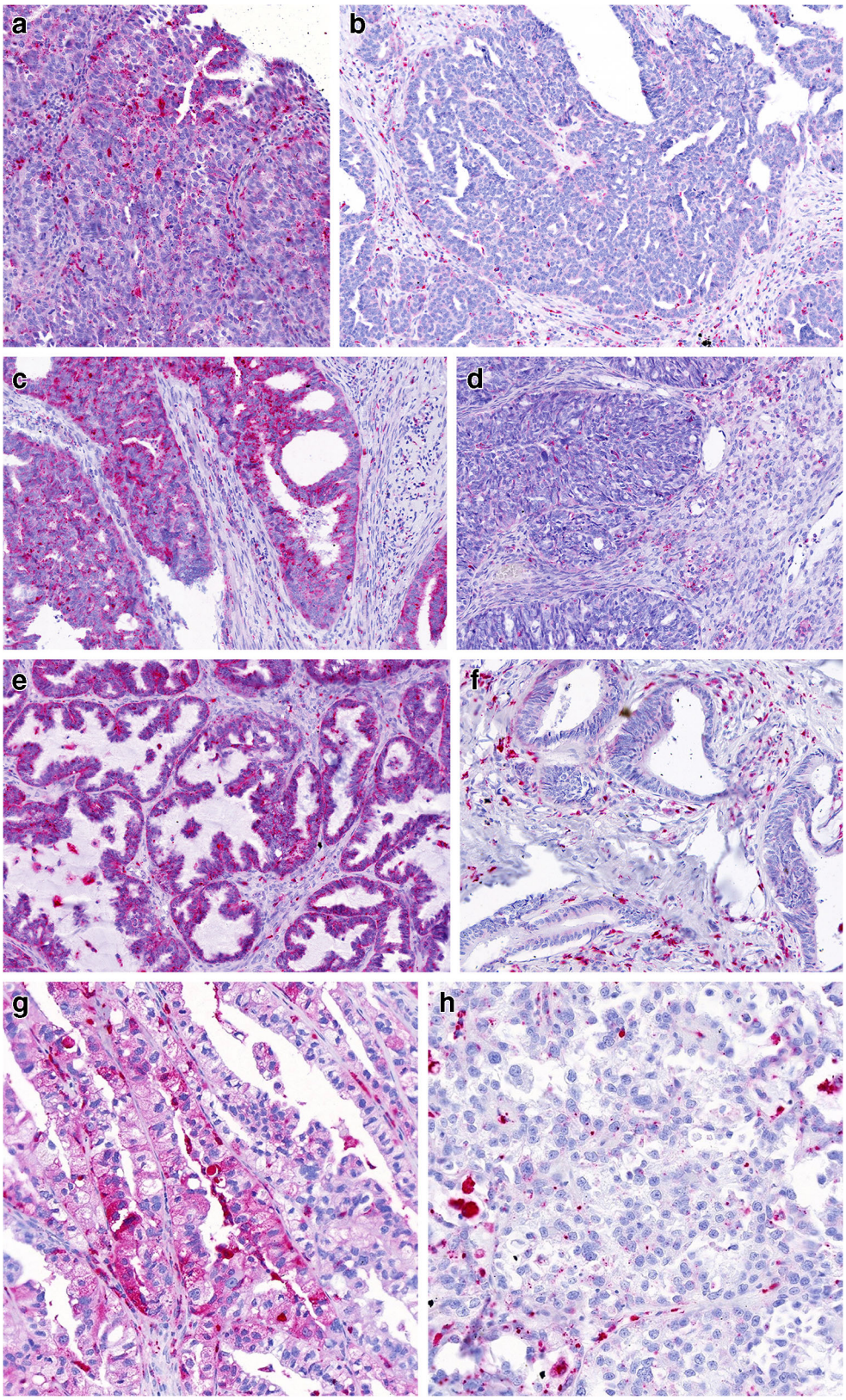

Fig. 1 Acid ceramidase (ASAH1) staining of ovarian cancer tissue. Representative examples ovarian cancer tissues with high (**a**, **c**, **e**, **g**) and low (**b**, **d**, **f**, **h**) ASAH1 expression are shown. Counterstain: Mayer hematoxylin (blue) A + B: serous OC, C + D: endometrioid OC, E + F: mucinous OC, G + H: clear cell OC

in ovarian cancer patients. This effect was detected both in the OOU (Fig. 2a, b) and in the VOA cohort (Fig. 2c, d), but not in the OOUE cohort (Fig. 2e, f), which consists solely of patients with sub-optimal debulking and persistent residual tumor after surgery. In addition, further study of the VOA cohort, which contains samples

Table 1 Clinical parameters of the analyzed cohorts

		TMA cohort				
Parameter		OOU/BC-No Res ($n = 540$)	OOUE/BC-Res ($n = 250$)	VOA ($n = 316$)	Total ($n = 1106$)	P value
Debulking	Optimal	540 (100%)	0 (0%)	119 (37.7%)	659 (59.6%)	
	Sub-optimal	0 (0%)	250 (100%)	197 (62.3%)	447 (40.4%)	$P < 0.001$
Age	< 50 years	157 29.1%)	41 (16.4%)	82 (25.9%)	280 (25.3%)	
	≥ 50 years	383 (70.9%)	209 (83.6%)	234 (74.1%)	826 (74.7%)	$P = 0.001$
Stage	I	221 (40.9%)	1 (0.4%)	65 (21.7%)	287 (26.4%)	
	II	232 (43.0%)	16 (6.5%)	21 (7.0%)	269 (24.7%)	
	III	87 (16.1%)	207 (83.5%)	184 (61.3%)	478 (43.9%)	
	IV	0 (0%)	24 (9.7%)	30 (10.0%)	54 (5.0%)	$P < 0.001$
Histotype	Endometrioid	192 (23.9%)	n.a.	25 (8.5%)	154 (18.5%)	
	Mucinous	35 (6.5%)	n.a.	8 (2.7%)	43 (5.2%)	
	Clear cell	130 (24.1%)	n.a.	29 (9.8%)	159 (19.1%)	
	HGSC	209 (38.8%)	n.a.	201 (68.1%)	410 (49.2%)	
	Other	36 (6.7%)	n.a.	32 (10.8%)	86 (8.2%)	$P < 0.001$
ASAH1 expression	Low	214 (39.6%)	147 (58.8%)	137 (43.4%)	498 (45.0%)	
	High	326 (60.4%)	103 (41.2%)	179 (56.6%)	608 (55.5%)	$P < 0.001$

n.a. not available

with both optimal as well as sub-optimal debulking, demonstrated a prognostic effect of ASAH1 only in those patients with optimal debulking and no residual tumor after surgery (PFS, $P = 0.062$, Supplementary Fig. S2). Thus, a strong bias is introduced in the analysis if patients with and without residual tumor after surgery are analyzed together, and we decided to restrict all further analyses to patients with optimal debulking. Moreover, we have previously shown a strong relationship between ASAH1 and estrogen receptor (ER) status in breast cancer [12]. Only for the samples from the OOU cohort the ER status was available from a previous study [8]. Therefore, we focused on these 540 samples with optimal debulking in all subsequent analyses (Supplementary Fig. S1).

Analysis of ASAH1 in tumors with optimal debulking according to histotype

We next studied potential differences in ASAH1 expression between histotypes of ovarian cancer in the OOU cohort (Table 2). We found that strong ASAH1 expression was most frequent in endometrioid subtype (79.8%) and clear cell ovarian cancer (83.1%), and less frequent in mucinous subtype (62.9%), high grade serous ovarian cancer (34.9%), and other (52.8%) histological subtypes ($P < 0.001$). We verified these differences in ASAH1 expression between histotypes on the level of mRNA expression by using Affymetrix microarray data, where the strongest expression of ASAH1 was in clear cell and endometrioid histotypes (Supplementary Fig. S3).

The observed differences of ASAH1 expression between the histological types could have a confounding effect, for example histotypes with higher ASAH1 expression also tend to be enriched for lower stage cancers and it is well recognized that each histotype has distinct biology, risk factors, response to therapy, and overall outcomes (Supplementary Fig. S4). Accordingly, while we observed a better outcome across our cohort associated with ASAH1 expression (Fig. 2), this was confounded by the distribution of expression across histotypes. We therefore analyzed ASAH1 expression and prognosis in a histotype-specific analysis (Supplementary Fig. S5).

Across histotypes we found that ASAH1 was associated with considerably better overall survival in the mucinous histotype. However, we were unable to detect a similar trend for progression-free survival with our limited sample size. In contrast, we detected an opposite effect in HGSC with reduced overall survival correlated to ASAH1 expression resulting in reduced OS ($P = 0.016$, Supplementary Fig. S5).

Relationship between ASAH1 and estrogen receptor

In breast cancer, ASAH1 expression is associated with ER positivity and "luminal" subtypes [12–14]. In ovarian cancer, ER is most frequently expressed in the endometrioid and HGSC subtypes (in ≈ 80% of cases), with a prognostic value only in the endometrioid histotype [9, 21]. In our moderately sized cohort, we observed similar, yet statistically non-significant effects among endometrioid tumors

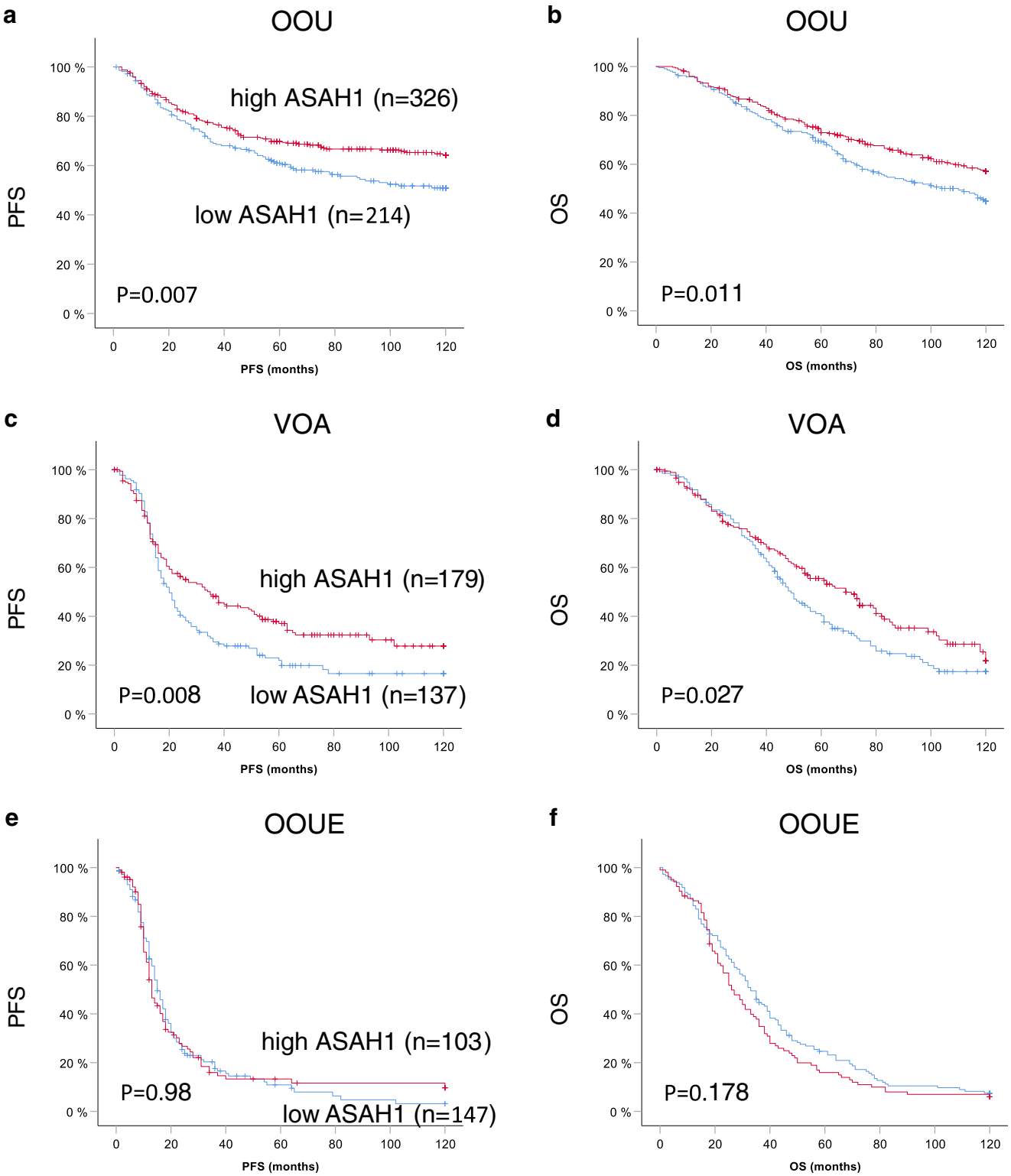

Fig. 2 Overall prognostic effect of ASAH1 expression in cohorts differing in tumor debulking. Kaplan-Meier analyses of progression-free survival (**a**, **c**, **e**) and overall survival (**b**, **d**, **f**) according to ASAH1 expression among all patients in the three different cohorts OOU (**a**, **b**), VOA (**c**, **d**), and OOUE (**e**, **f**)

(Supplementary Fig. S6). However, considerably larger cohorts have clearly demonstrated that ER is highly prognostic especially in the endometrioid subtype [9].

We compared ER and ASAH1 expression among 487 samples for which data for both markers and the histological subtype were available (Table 3). Overall, we

Table 2 ASAH1 expression among histological subtypes

Histotype	Low ASAH1	High ASAH1	Total
Endometrioid OC	26 (20.2%)	103 (79.8%)	129
Mucinous OC	13 (37.1%)	22 (62.9%)	35
Clear cell OC	22 (16.9%)	108 (83.1%)	130
High-grade serous OC	136 (65.1%)	73 (34.9%)	209
Other	17 (47.2%)	19 (52.8%)	36
Total	214 (39.7%)	325 (60.3%)	539

detected a slight negative association ($P < 0.001$), which was mainly driven by the subtype of HGSC ($P = 0.081$), while some positive association was observed within the endometrioid subtype ($P = 0.100$; Table 3).

We saw only a modest agreement between ER and ASAH1 expression within the endometrioid subtype; therefore, in an exploratory analysis, we tested whether both factors added independent prognostic value. Prognostic value of ASHA1 was assessed in endometrioid ovarian cancers that were either ER positive or ER negative (Fig. 3). ASAH1 did not add prognostic value in ER-positive endometrioid OC, and most samples expressed both markers (Fig. 3a, b). In contrast, in endometrioid OC that lacks ER expression (Fig. 3c, d), ASAH1 had a highly significant prognostic value for overall survival even within this small subgroup ($P = 0.007$; Fig. 3d). We also performed a similar analysis vice versa: This demonstrated that ER also has a prognostic value only in those endometrioid OCs that do not express ASAH1 (Supplementary Fig. S7). These results suggest that both biomarkers could add independent value for prognosis of endometrioid OC.

Discussion

In the present work, we analyzed the expression of acid ceramidase (ASAH1) in a cohort of 1106 ovarian cancer patients. While our overall observation was that of better prognosis, we found that this result was confounded by both residual disease and multi-histotype analysis, underscoring the importance of considering these factors in molecular studies. Analysis of histotype-specific ASAH1 expression demonstrated most frequent expression in endometrioid and clear cell ovarian cancer (Table 2), which may in part contribute to our overall results on prognosis. Histotype-stratified analyses of ASAH1 did not reveal significant survival differences (Supplementary Fig. S5). However, this may be due to small sample sizes, in fact, despite previous analysis showing prognostic value for ER in endometrioid carcinomas [9], our subset was insufficient to show this as a significant difference.

Previous data from breast cancers have demonstrated an association of ASAH1 and ER positivity [12–14]; however, we found only a modest association between ER and ASAH1 expression within the endometrioid ovarian cancer subtype (Table 3). In an exploratory analysis among ER-negative endometrioid OC, we found that strong ASAH1 expression was associated with significantly better overall survival ($P = 0.007$; Fig. 3d).

Strengths of our study includes the overall large cohort, centralized histological review and quality control by study pathologists with expertise in gynecological cancers, the centralized IHC in TMA format, and biomarker scoring blinded to clinical data. Another strength of our study is the absence of patients with either residual disease or neoadjuvant chemotherapy as this may affect

Table 3 Correlation of ASAH1 and ER expression

Histotype	ER status	Low ASAH1	High ASAH1	Total	*P* value
Endometrioid OC	Negative	9 (33.3%)	18 (66.7%)	27	
	Positive	15 (16.7%)	75 (83.3%)	90	0.100
Mucinous OC	Negative	12 (41.4%)	17 (58.6%)	29	
	Positive	1 (33.3%)	2 (66.7%)	3	1.00
Clear cell OC	Negative	18 (15.8%)	96 (84.2%)	114	
	Positive	1 (10.0%)	9 (90.0%)	10	1.00
High-grade serous OC	Negative	26 (54.2%)	22 (45.8%)	48	
	Positive	107 (69.0%)	48 (31.0%)	155	0.081
Other	Negative	1 (33.3%)	2 (66.7%)	3	
	Positive	1 (12.5%)	7 (87.5%)	8	0.49
Total	Negative	66 (29.9%)	155 (70.1%)	221	
	Positive	125 (47.0%)	141 (53.0%)	266	< 0.001

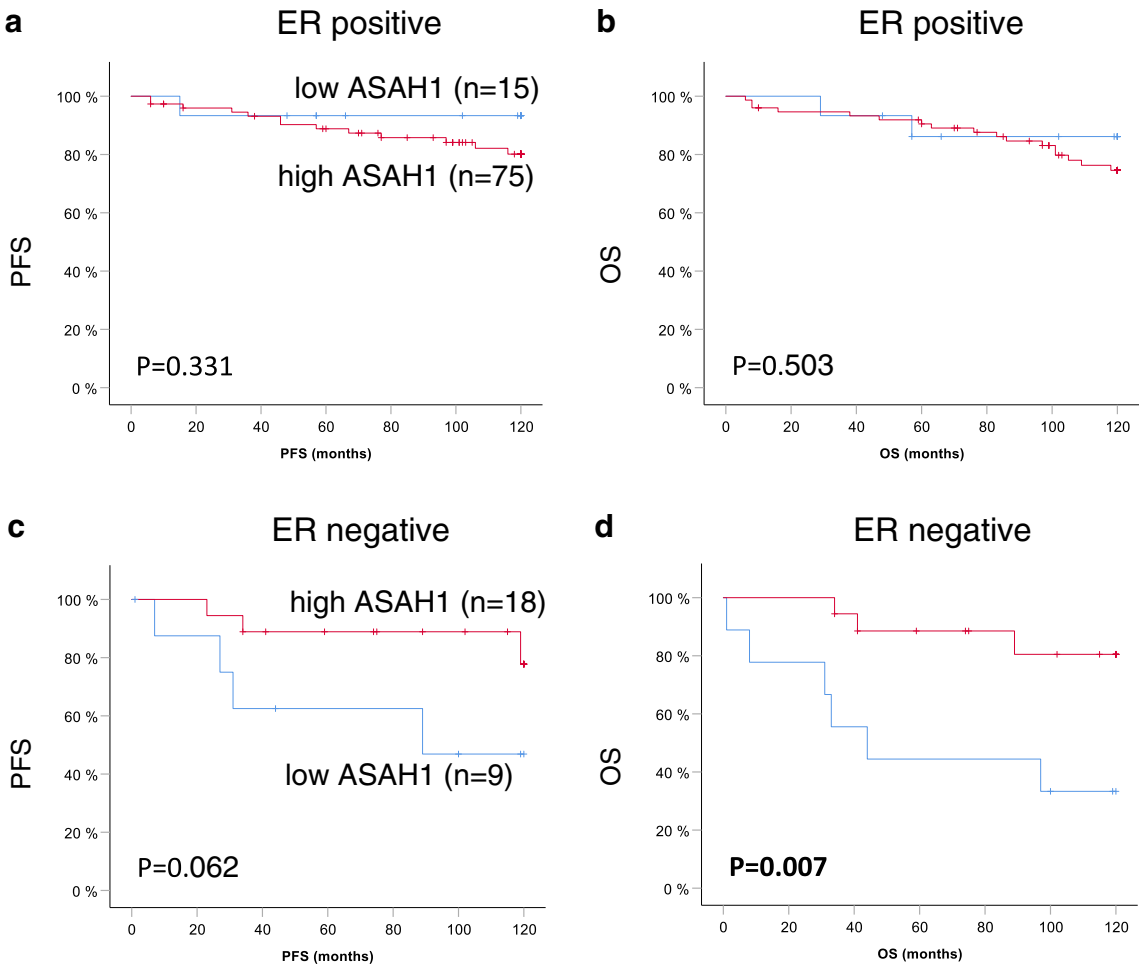

Fig. 3 Prognostic value of ASAH1 expression endometrioid OC stratified by ER status. Kaplan-Meier analyses of progression-free survival (**a**, **c**) and overall survival (**b**, **d**) according to ASAH1 expression among subgroups of endometrioid ovarian cancer, stratified by estrogen receptor status as either ER positive (**a**, **b**), or ER negative (**c**, **d**)

morphology and prognosis [22–24]. Limitations include the retrospective design: clinical information was retrospectively obtained from medical records. Because most patients received some type of adjuvant therapy, we cannot discriminate pure prognostic and potential predictive value of these biomarkers. Further, despite the use of a large overall cohort, our histotype-stratified analysis was often too small to obtain robust results, especially in less-frequent histotypes. Nonetheless, the associations we have described, especially those related to ER-negative endometrioid carcinoma, warrant additional study and validation.

In conclusion, we demonstrate that strong ASAH1 expression in ovarian cancer is preferentially associated with the clear cell and endometrioid subtypes. High ASAH1 expression was associated with a better prognosis in ER-negative endometrioid ovarian cancer and could add independent prognostic value, which may help to further individualize prognostic classifiers for ovarian cancer. Our results also suggest that modulating sphingolipid metabolism could lead to novel therapeutic intervention strategies for this disease [11].

Authors' contributions ZD, LH, and AR designed the study. DH, MA, and SK provided tumor samples and clinical data. UH performed immunohistochemical analyses of the samples and coordinated the study conduct. HG evaluated and scored stained tissue slides and helped finalizing the manuscript. TK and BG carried out the statistical analyses and interpreted the data. AEB, UH, TK, and SB drafted and finalized the manuscript. All authors approved the final manuscript.

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Compliance with ethical standards Collection of patient specimens and data from the British Columbia cohort was done under approved research protocols reviewed by the British Columbia Cancer Agency and University of British Columbia research ethics board (H05–60119).

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL (2018) Ovarian cancer statistics, 2018. CA Cancer J Clin 68(4):284–296. https://doi.org/10.3322/caac.21456
2. Feeley KM, Wells M (2001) Precursor lesions of ovarian epithelial malignancy. Histopathology 38(2):87–95
3. Bell DA (2005) Origins and molecular pathology of ovarian cancer. Mod Pathol 18(Suppl 2):S19–S32. https://doi.org/10.1038/modpathol.3800306
4. Kurman RJ, Shih I-M (2010) The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol 34(3):433–443. https://doi.org/10.1097/PAS.0b013e3181cf3d79
5. Kroeger PT, Drapkin R (2017) Pathogenesis and heterogeneity of ovarian cancer. Curr Opin Obstet Gynecol 29(1):26–34. https://doi.org/10.1097/GCO.0000000000000340
6. Piek JMJ, Verheijen RHM, Kenemans P, Massuger LF, Bulten H, van Diest P (2003) BRCA1/2-related ovarian cancers are of tubal origin: a hypothesis. Gynecol Oncol 90(2):491
7. Kurman RJ, Shih I-M (2016) The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded. Am J Pathol 186(4):733–747. https://doi.org/10.1016/j.ajpath.2015.11.011
8. Köbel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, Leung S, Bowen NJ, Ionescu DN, Rajput A, Prentice LM, Miller D, Santos J, Swenerton K, Gilks CB, Huntsman D (2008) Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med 5(12):e232. https://doi.org/10.1371/journal.pmed.0050232
9. Sieh W, Köbel M, Longacre TA, Bowtell DD, deFazio A, Goodman MT, Høgdall E, Deen S, Wentzensen N, Moysich KB, Brenton JD, Clarke BA, Menon U, Gilks CB, Kim A, Madore J, Fereday S, George J, Galletta L, Lurie G, Wilkens LR, Carney ME, Thompson PJ, Matsuno RK, Kjær SK, Jensen A, Høgdall C, Kalli KR, Fridley BL, Keeney GL, Vierkant RA, Cunningham JM, Brinton LA, Yang HP, Sherman ME, García-Closas M, Lissowska J, Odunsi K, Morrison C, Lele S, Bshara W, Sucheston L, Jimenez-Linan M, Driver K, Alsop J, Mack M, McGuire V, Rothstein JH, Rosen BP, Bernardini MQ, Mackay H, Oza A, Wozniak EL, Benjamin E, Gentry-Maharaj A, Gayther SA, Tinker AV, Prentice LM, Chow C, Anglesio MS, Johnatty SE, Chenevix-Trench G, Whittemore AS, Pharoah PD, Goode EL, Huntsman DG, Ramus SJ (2013) Hormone-receptor expression and ovarian cancer survival: an ovarian tumor tissue analysis consortium study. Lancet Oncol 14(9):853–862. https://doi.org/10.1016/S1470-2045(13)70253-5
10. Peres LC, Cushing-Haugen KL, Köbel M, Harris HR, Berchuck A, Rossing MA, Schildkraut JM, Doherty JA (2019) Invasive epithelial ovarian cancer survival by histotype and disease stage. J Natl Cancer Inst 111(1):60–68. https://doi.org/10.1093/jnci/djy071
11. Ogretmen B (2018) Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 18(1):33–50. https://doi.org/10.1038/nrc.2017.96
12. Ruckhäberle E, Rody A, Engels K, Gaetje R, von Minckwitz G, Schiffmann S, Grösch S, Geisslinger G, Holtrich U, Karn T, Kaufmann M (2008) Microarray analysis of altered sphingolipid metabolism reveals prognostic significance of sphingosine kinase 1 in breast cancer. Breast Cancer Res Treat 112(1):41–52. https://doi.org/10.1007/s10549-007-9836-9
13. Ruckhäberle E, Holtrich U, Engels K, Hanker L, Gätje R, Metzler D, Karn T, Kaufmann M, Rody A (2009) Acid ceramidase 1 expression correlates with a better prognosis in ER-positive breast cancer. Climacteric 12(6):502–513. https://doi.org/10.3109/13697130902939913
14. Sänger N, Ruckhäberle E, Györffy B, Engels K, Heinrich T, Fehm T, Graf A, Holtrich U, Becker S, Karn T (2015) Acid ceramidase is associated with an improved prognosis in both DCIS and invasive breast cancer. Mol Oncol 9(1):58–67. https://doi.org/10.1016/j.molonc.2014.07.016
15. Hanker LC, Karn T, Holtrich U, Gätje R, Rody A, Heinrich T, Ruckhäberle E, Engels K (2013) Acid ceramidase (AC)–a key enzyme of sphingolipid metabolism—correlates with better prognosis in epithelial ovarian cancer. Int J Gynecol Pathol 32(3):249–257. https://doi.org/10.1097/PGP.0b013e3182673982
16. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, Statistics Subcommittee of the NCI-EORTC Working Group on Cancer Diagnostics (2005) Reporting recommendations for tumor marker prognostic studies (REMARK). J Natl Cancer Inst 97(16):1180–1184. https://doi.org/10.1093/jnci/dji237
17. Köbel M, Reuss A, Du Bois A et al (2010) The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. J Pathol 222(2):191–198. https://doi.org/10.1002/path.2744
18. Prentice LM, Klausen C, Kalloger S, Köbel M, McKinney S, Santos JL, Kenney C, Mehl E, Gilks CB, Leung P, Swenerton K, Huntsman DG, Aparicio SA (2007) Kisspeptin and GPR54 immunoreactivity in a cohort of 518 patients defines favourable prognosis and clear cell subtype in ovarian carcinoma. BMC Med 5:33. https://doi.org/10.1186/1741-7015-5-33
19. Kalloger SE, Köbel M, Leung S, Mehl E, Gao D, Marcon KM, Chow C, Clarke BA, Huntsman DG, Gilks CB (2011) Calculator for ovarian carcinoma subtype prediction. Mod Pathol 24(4):512–521. https://doi.org/10.1038/modpathol.2010.215
20. Gyorffy B, Lánczky A, Szállási Z (2012) Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. Endocr Relat Cancer 19(2):197–208. https://doi.org/10.1530/ERC-11-0329
21. Rambau P, Kelemen LE, Steed H, Quan ML, Ghatage P, Köbel M (2017) Association of hormone receptor expression with survival in ovarian endometrioid carcinoma: biological validation and clinical implications. Int J Mol Sci 18(3). https://doi.org/10.3390/ijms18030515
22. McCluggage WG, Lyness RW, Atkinson RJ, Dobbs SP, Harley I, McClelland H, Price JH (2002) Morphological effect of chemotherapy on ovarian carcinoma. J Clin Pathol 55(1):27–31. https://doi.org/10.1136/jcp.55.1.27
23. Böhm S, Faruqi A, Said I, Lockley M, Brockbank E, Jeyarajah A, Fitzpatrick A, Ennis D, Dowe T, Santos JL, Cook LS, Tinker AV, le ND, Gilks CB, Singh N (2015) Chemotherapy response score: development and validation of a system to quantify histopathologic response to neoadjuvant chemotherapy in tubo-ovarian high grade serous carcinoma. J Clin Oncol 33(22):2457–2463. https://doi.org/10.1200/JCO.2014.60.5212
24. Santoro A, Angelico G, Piermattei A, Inzani F, Valente M, Arciuolo D, Spadola S, Mulè A, Zorzato P, Fagotti A, Scambia G, Zannoni GF (2019) Pathological chemotherapy response score in patients affected by high grade serous ovarian carcinoma: the prognostic role of omental and ovarian residual disease. Front Oncol 9:778. https://doi.org/10.3389/fonc.2019.00778