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# Downregulation of ALDH5A1 Promotes Tumor Metastasis and Contributes to Poor Prognosis in Ovarian Cancer

**Chen Cao**
The central hospital of Wuhan https://orcid.org/0000-0002-3739-5240
**Xin Wang**
Huazhong University of Science and Technology Tongji Medical College
**Rui Li**
The central hospital of Wuhan
**Ping Jin**
The central hospital of Wuhan
**Hongwei Chen**
The central hospital of Wuhan
**Meng Xia**
The central hospital of Wuhan
**Qinghua Zhang**
The central hospital of Wuhan
**Xun Tian** ( tianxun@zxhospital.com )

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# Downregulation of ALDH5A1 Promotes Tumor Metastasis and Contributes to Poor Prognosis in Ovarian Cancer

**Chen Cao[1,#], Xin Wang[2,#], Rui Li[1,#], Ping Jin[1], Hongwei Chen[1], Meng Xia[1], Qinghua Zhang[3,*], Xun Tian[1,*]**

[1] Department of Obstetrics and Gynecology, Academician expert workstation, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.

[2] Department of Gynecology and Obstetrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.

[3] Department of Obstetrics and Gynecology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

[#] These authors contributed equally to this work.

** Correspondence:*

Xun Tian. Department of Obstetrics and Gynecology, Academician expert workstation, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, ShenLi Avenue 26#, Jang'an District, Wuhan 430030, Hubei, China. Email: tianxun@zxhospital.com, Tel: 00-86-027-82205010;

Qinghua Zhang. Department of Obstetrics and Gynecology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology,

ShenLi Avenue 26#, Jang'an District, Wuhan 430030, Hubei, China.

Email:2019zx0005@hust.edu.cn, Tel: 00-86-027-82205010.

**Abstract**

**Background:** Despite modern therapies, ovarian cancer (OC) remains a major clinical problem with a high risk of mortality. We previously reported that low expression of ALDH5A1 could serve as an indicator for predicting poor prognosis in OC. However, the function of ALDH5A1 in OC progression has not been elucidated yet.

**Methods:** We firstly compared ALDH5A1 expression in metastatic tissues to primary site of OC based on the Oncomine database. Then wound healing assay and Transwell assay were utilized to determine the biological role of OC cells transfected with ALDH5A1 siRNA. To unravel the potential mechanism of ALDH5A1 meditating metastasis of OC, the co-expression profile of ALDH5A1 in OC cell lines and OC patients were generated using cBioPortal. Moreover, qRT-PCR and WB analysis were used to detect the expression levels of metastasis-related genes after ALDH5A1 suppression, HPA database was used to confirm the relative expression of ALDH5A1 and MMP in OC patients. In addition, KM survival plots in 578 OC patients from the TCGA database were analyzed.

**Results:** We proved lower ALDH5A1 expression in metastatic tissues compared to primary site of OC, and knockdown of ALDH5A1 promoted the malignant behavior of OC cells. Additionally, the co-expression profile of ALDH5A1 was significantly enriched in extracellular matrix (ECM) organization pathway. We further confirmed ALDH5A1 was negatively associated with MMP expression in OC, indicating that ALDH5A1 was closely related to OC metastasis via ECM organization pathway. Finally, KM survival plots revealed that low ALDH5A1 expression contributed to poor OC survival.

**Conclusions:** These results suggested a key role of ALDH5A1 in driving the progression of OC and identified ALDH5A1 as a robust therapeutic target of OC.

**Keywords:** ALDH5A1; ovarian cancer; metastasis; prognosis; therapeutic target

**Abbreviations**

OC: ovarian cancer; ECM: extracellular matrix; ALDHs: Aldehyde dehydrogenases; ALDH5A1: Aldehyde dehydrogenase 5 family member A1; SSADH: succinic semialdehyde dehydrogenase; GABA: gamma-aminobutyric acid; SNPs: single nucleotide polymorphisms; TMA : tissue microarray; HR: Hazard ratio; MMPs: matrix metalloproteinases; OS: overall survival; TCA: tricarboxylic acid; HBA: hydroxybutyric acid; HGSOC: high-grade serous ovarian cancer; EMT: epithelial-mesenchymal transition

## Background

Ovarian cancer (OC) is the fifth most common cause of cancer death among US women and the leading cause of death from gynecologic cancer(1). In 2015, it was estimated that 52,100 new cases of OC and 22,500 deaths occurred due to OC in China(2). Despite ongoing efforts to develop effective treatment, the overall survival rate remains fewer than one-half, which largely results from early stages of OC is usually asymptomatically(3). OC associated death can primarily be attributed to cancer metastasis, because over 70% patients are diagnosed at late stage with metastatic disease(4). Since a majority of patients will be treated for metastatic disease at the time of diagnosis, it is important to increase our knowledge of the mechanisms of OC metastasis to improve treatment outcome.

Aldehyde dehydrogenases (ALDHs) are a group of intracellular enzymes participate in maintaining cellular detoxification and drug resistance through oxidizing reaction of cellular aldehydes(5). As a member of the superfamily of ALDHs, Aldehyde dehydrogenase 5 family member A1 (ALDH5A1)

encodes for succinic semialdehyde dehydrogenase (SSADH), which degrading gamma-aminobutyric acid (GABA) by catalyzing the oxidantion of succinic semialdehyde(6). As early as 2001, Nicholson-Guthrie and colleagues(7) reported the elevated concentration of urine GABA in OC patients. The GABA elevation supported the deregulation of the SSADH pathway in OC. Meanwhile, A miRNA-related single nucleotide polymorphisms (SNPs) study(8) revealed that ALDH5A1 SNPs were significantly associated with treatment response of OC. Our previous results also showed ALDH5A1 expression was downregulated in OC samples compared with that in normal ovarian tissues, and low expression of ALDH5A1 was associated with worse clinical prognosis of OC patients(9).

Prior research confirmed ALDH5A1 could be used as a predictive biomarker of OC and might play a crucial role in OC progression. However, the underlying mechanism of ALDH5A1 downregulation leading to poor prognosis in OC was still not clear. In the current study, to provide an extension of our previous results, we planned to investigate the biological role of ALDH5A1 in the progression of OC, and to find out whether ALDH5A1 can serve as a potential therapeutic target of OC.

## Methods

### *Cell culture and reagents*

Human epithelial OC cell line SKOV3 were purchased from American Type Culture Collection (ATCC). This cell line was cultured in McCoy's 5A (Lonza), supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco), in a humidified incubator under 5% $CO_2$ at 37°C.

### *Oncomine database analysis and TMA cohorts*

Anglesio Ovarian data(10) was downloaded from Oncomine database, interpreted, normalized, and

log2-scaled using the online analysis to compare the mRNA expression level of ALDH5A1. To further confirm the proteomic expression of ALDH5A1 and co-expression genes, we analyzed an OC tissue microarray (TMA) cohort obtained from the Human Protein Atlas database (https://www.proteinatlas.org/).

*Small interfering RNA*

Two different siRNA sequences of ALDH5A1 were purchase from Sangon Biotech (Shanghai, China). The target sequences are as follows:

siRNA1: CGGAAGTGGTACAATTTAATG;

siRNA2: GGTTCAACAACTACAGGAAAG.

The siRNA and the negative control were transfected into SKOV3 cells using lipofectamine$^{TM}$ 3000 (Thermo Fisher) according to the protocols. The efficiency of silence was determined at 48 hours after transfection.

*Cell migration and invasion assays*

SKOV3 cells either native or transfected at the concentration of $1\times 10^6$ cells/well were seeded in a 6-well plate and incubated under 5% $CO_2$ at 37°C. After an overnight incubation the cells grew to 100% confluence. A rectangular lesion on the monolayer cells was generated using a sterile 100ul pipette tip. The debris were removed and the edge of the scratch was smoothed by washing the cells once with PBS and then replaced with 2.5ml of McCoy's 5A medium, after that cells were cultured. Photographic images of the lesion border were acquired using an inverted microscope.

SKOV3 cells invasion assay was evaluated by transwell chambers with Matrigel-coated inserts (BD Biosciences) according to the manufacture's protocol. In brief, Matrigel was thawed and liquefied on ice,

and then 30ul of Matrigel was added to a 24-well transwell insert and solidified. $1\times 10^5$ cells either native or transfected with siRNA were plated in the insert on top of the Matrigel coating and incubated for 10 minutes under 5% $CO_2$ at 37°C to allow the cells to settle down. The lower chamber contained McCoy's 5A medium with 10% FBS as the chemoattractant. After incubation for 24h under 5% $CO_2$ at 37°C, any cells had not penetrated the membrane were removed using cotton swabs. The cells had successfully migrated to the bottom surfaces of the membranes were fixed with 4% polyoxymethylene and stained with 0.2% crystal violet for 10 minutes. The number of cells was counted underneath an inverted microscope.

*Database search and analysis for ALDH5A1 co-expression genes*

Data from OC cell lines (n = 47) in the Cancer Cell Line Encyclopedia (CCLE) and from patients with ovarian serous cystadenocarcinoma (n = 489) in the Cancer Genome Atlas were analyzed by cBioPortal(11) online platform. The genes were considered as ALDH5A1 co-expression genes when the |Spearman's correlation|$>$0.2 and $P<0.05$. The common co-expression genes of ALDH5A1 in both databases were conducted for pathway enrichment analysis using the g:Profiler(12) and Metascape(13) online platform. Significant GO terms with similar function were visualized as interaction networks using the Metascape online platform to further determine the relationship among terms, where terms with $P<0.01$ and similarity score $>0.3$ were connected by edges.

In addition, the co-expression analysis between ALDH5A1 and key genes in the ECM organization pathway were also performed using cBioPortal.

*Quantitative RT-PCR (qRT-PCR) and Western blotting*

Total RNAs were extracted using TRIzol reagent (Invitrogen) according to the instructions. cDNA

was synthesized with 1ug of total RNA by reverse transcriptase (Transgen). For quantitative determination of gene expression, qRT-PCR analysis was performed with SYBR Green PCR Master Mix (Takara Bio) using LightCycler (Roche). The results were calculated by 2-$\Delta\Delta Ct$ method. The primer sets used for qRT-PCR are as follows: ALDH5A1-F: GTGGTTCTCTGAGGAAGCCC, ALDH5A1-R: TTCACCACGACAGTACAGCC; GAPHD-F: GATTTGGTCGTATTGGGCGC, GAPHD-R: TTCCCGTTCTCAGCCTTGAC; MMP-2-F: TGATGGCATCGCTCAGATCC, MMP-2-R: GGCCTCGTATACCGCATCAA; MMP-3-F: TGAGGACACCAGCATGAACC, MMP-3-R: ATCACCTCCAGAGTGTCGGA; MMP-14-F: GGCTGCCTACCGACAAGATT, MMP-14-R: GGGAGACTCAGGGATCCCTT.

SKOV3 cells were collected and lysed in cell lysis buffer supplemented with protease inhibitors (abcam) according to standard instructions. The lysates were resolved using 10% SDS-PAGE, transferred to nitrocellulose membranes and immunoblotted with primary antibodies against ALDH5A1, GAPHD, MMP-2, MMP-3 and MMP-14. Following incubation with secondary antibodies, the protein bands were visualized using a chemiluminescence reagent (Thermo Fisher).

*Prognostic implications of ALDH5A1 in OC*

A web-based tool PROGgeneV2(14) was used to assess the prognostic implication of ALDH5A1 in OC. The KM survival plots were established using ALDH5A1 mRNA expression data and overall survival information of the 578 OC patients from the TCGA database. ALDH5A1 was entered in the database to get the KM survival plots, Hazard ratio (HR), 95% confidence intervals and *P* value were presented on the main plots.

*Statistical analysis*

All data were analyzed using GraphPad Prism 7.0 and were presented as mean±SD of triplicates. Quantitative data were analyzed using Student's *t*-test between two groups. For all analyses, a $P<0.05$ was considered statistically significant and were indicated with an asterisk.

**Results**

*Down-regulation of ALDH5A1 Correlated with tumor malignant features*

To explore whether the expression of ALDH5A1 was correlated with malignancy in human OC, we firstly evaluated the expression level of ALDH5A1 in primary and in metastatic tissues of OC patients. Clinical data of OC patients were obtained from the Anglesio Ovarian data in Oncomine dataset. As shown in Fig.1A, the transcription level of ALDH5A1 in metastatic site of OC (n = 16) was markedly downregulated compared with that in primary site (n = 74) ( ** $P<0.01$).

Next, we further understood the relationship between ALDH5A1 expression and tumor malignancy in vitro. ALDH5A1 gene was knocked down by siRNA to test the possible roles in tumor aggressiveness. qRT-PCR analysis confirmed that ALDH5A1 expression was successfully down-regulated in SKOV3 cells (Fig.1B). Then we performed a scratch-wound healing assay and a transwell assay to determine the effects of ALDH5A1 on OC cell migration and invasion. After down-regulation of ALDH5A1, the migratory and invasion abilities of OC cells were dramatically increased (Fig.1C-F).

*Gene Ontology Analysis of ALDH5A1 and co-expressed genes revealed Relationship between ALDH5A1 and ECM signaling pathways in OC*

To unravel the potential mechanism meditating the biological functions of ALDH5A1, we extracted 1575 co-expression genes of ALDH5A1 from 47 OC cell lines in CCLE database (supplement table 1),

and 1220 co-expression genes of ALDH5A1 from 489 OC patients in TCGA database (supplement table 2) using the cBioPortal online platform. In total, 128 common co-expression genes were found to be overlaped through taking the intersection of these two co-expression gene sets (Fig.2A and supplement table 3).

To explore the aim of identifying possible signaling pathways from the list of co-expression genes of ALDH5A1 in OC, we performed functional enrichment analysis with these 128 common co-expression genes of ALDH5A1 obtained from CCLE and TCGA database. Firstly g:Profiler was used to identify functional information and enriched pathways and processes of ALDH5A1 and the 128 common co-expression genes. As shown in Fig.2B, these genes were mainly enriched in biological processes (BPs), including ECM organization (GO: 0030198) and extracellular structure organization (GO: 0043062). For cellular components (CCs), these genes were mostly enriched in ECM (GO:0031012). In the meantime, molecular functions (MFs) analysis and Reactome (REAC) analysis also showed these genes were significantly enriched in ECM structural constituent (GO: 0005201) and ECM organization (REAC: R-HSA-1474244). We then performed ontology analysis again using the Metascape platform to confirm these results. Fig.2C showed the top 20 putative biological processes, and the most significantly enriched gene set was ECM organization pathway (GO: 0030198). This analysis also revealed ALDH5A1 and correlated genes were largely related to tissue morphogenesis (GO: 0048729) and skeletal system development (GO: 0001501). Fig.2D showed all these biological processes identified with a significant P value were closely inter-related. All the above results indicated that ALDH5A1 and correlated genes were mainly related to the ECM and influence the development of cancer.

*ALDH5A1 was negatively associated with MMP expression in OC*

Nextly, we checked the key genes participated in the ECM organization pathway which may be correlated with ALDH5A1 using g:Profiler. Among these genes, a negative correlation was found between ALDH5A1 and matrix metalloproteinases (MMPs). The negative correlation between ALDH5A1 and MMP2 expression (R = 0.33), between ALDH5A1 and MMP3 expression (R = 0.25), between ALDH5A1 and MMP14 expression (R = 0.30) were confirmed using Spearman and Pearson correlation analyses (Fig.3A).

We confirmed that both MMP mRNA and protein expression levels were upregulated by siRNA-mediated ALDH5A1 knockdown in OC cells. When the expression levels of ALDH5A1 were decreased, MMP2, MMP3, MMP14 mRNA expression was significantly increased in OC cells (Fig.3B). In parallel, western blot results showed that the MMP2, MMP3, MMP14 protein expression were increased in ALDH5A downexpressing OC cells compared with control cells (Fig.3C).

In addition, we analyzed an OC TMA cohort obtained from the HPA database. Immunohistochemical (IHC) staining results also demonstrated the similar expression patterns of ALDH5A1 and MMP in OC patients (Fig.4).

*The poor prognosis of the patients with lower expression of ALDH5A1 in OC*

Finally, to investigate whether ALDH5A1 is associated with OC patient prognosis, a Kaplan-Meier analysis based on the TCGA ovarian adenocarcinoma data was organized by the web-based tool PROGgeneV2. The results showed that the patients with lower expression of ALDH5A1 presented poorer prognosis than those with higher expression in OC. The overall survival (OS) rates of OC patients with $ALDH5A1^{high}$ were obviously higher than those of patients with $ALDH5A1^{low}$ [HR = 0.75 (0.64-0.88), $P$ = 0.0005, Fig.5A]. We also explored the correlation between ALDH5A1 mRNA expression and

pathological grades of OC. As the sample size of patients with pathological grade I was too small (n = 6), we did not analyze the survival curves in this group. As shown in Fig.5B and 5C, the high expression of ALDH5A1 in pathological grade II and III patients were associated with improved OS [HR = 0.54 (0.34-0.88), *P* = 0.0127 / HR = 0.79 (0.66-0.94), *P* = 0.0077].

**Discussion**

Changes in cell metabolism can contribute to tumor progression because tumorigenesis is dependent on the reprogramming of cellular metabolism to acquire necessary nutrients to maintain viability and malignant properties(15). Metabolic profiling is an emerging diagnostic tool enabling the detection of biomarker reflecting alterations in tumor metabolism(16). Studies had demonstrated that reprogrammed metabolism was considered a hallmark of cancer because some altered metabolic features are observed quite generally across many types of cancer(17). ALDH5A1 gene encodes SSADH, which is a mitochondrial NAD(+)-dependent dehydrogenase, works in tandem with GABA transaminase to convert the carbon backbone of GABA to succinic acid, the latter is a source of energy within the tricarboxylic acid (TCA) cycle(18), and has extend its roles into tumorigenesis recently(19).

Previous studies had shown the activity of ALDH genes in various cancers such as prostate cancer(20), leukemia(21), breast cancer(22, 23) and esophageal cancer(24). As a member of ALDH gene family, preliminary characterization of ALDH5A1 in OC had been reported(25, 26). Mika Hilvo and colleagues(25) revealed a distinct metabolic signature characterized by the accumulation of hydroxybutyric acid (HBA) of high-grade serous OC (HGSOC) patients, and they demonstrated that these metabolites accumulation was caused by mutations and lowered activity of ALDH5A1 gene. Thus,

253 the metabolomics analysis of OC patients(25) revealed a prognostic signature of metabolites related to
254 lowered activity of ALDH5A1. Analysis of transcriptome data(26) associated high ALDH5A1
255 expression with stem-like and cancerous behaviors of glioblastoma. In our previous study(9), we also
256 showed ALDH5A1 expression was downregulated in OC samples compared with that in normal ovarian
257 tissues. Although sveral studies had agreed that ALDH5A1 is correlated with prognosis in OC, the
258 relationships and molecular mechanisms through which ALDH5A1 meditated metastasis of OC
259 remained unknown.

260 Here in this work, we found the expression levels of ALDH5A1 was decreased in metastatic tissue
261 of OC patients compared with primary site, and a lower ALDH5A1 expression level enhanced the
262 migration and invasion of OC cells. Metastasis is closely associated with a poor prognosis of OC (4).
263 Meanwhile, migration and invasion of cancer cells into surrounding tissue and vasculature is an important
264 initial event in tumor metastasis(27). Therefore, we hypothesized that ALDH5A1 participates in the
265 modulation of OC cell metastasis. To gain insights into the function of ALDH5A1, we constructed the
266 functional enrichment analysis and found out ALDH5A1 and correlated genes were mainly related to the
267 ECM pathway and influence the development of OC.

268 The ECM is a dynamic structure influences tumour progression(28), which is commonly
269 deregulated and becomes disorganized in cancer. Deregulated ECM dynamics disrupt tissue polarity,
270 architecture, and integrity and promote epithelial-mesenchymal transition (EMT) and metastasis(29).
271 The MMPs are a family of zinc-dependent enzymes, and MMP-mediated ECM degradation leads to
272 disrupt the physiological barrier and cancer cell metastasis has been a guiding principle in MMP
273 research(30). Using g:Profiler, we extracted MMP2, MMP3, MMP14 from the key genes participated in

the ECM organization pathway, verified a negative correlation between ALDH5A1 and MMP expression. In terms of MMP2 and MMP3, both of them were found to function as early response proteins in OC metastasis(31-33). In recent years, several studies revealed that MMP14 plays a central role in pericellular matrix degradation during basement membrane and interstitial tissue transmigration programs(34), which stimulates a tumor-stromal signaling pathway and promotes angiogenesis and tumor growth on OC cells(35). All the growing evidence verified our hypothesis that ALDH5A1 was somewhat relative to the metastasis of OC.

Moreover, the OS rates of OC patients with ALDH5A1$^{high}$ were obviously higher than those of patients with ALDH5A1$^{low}$, and the high expression of ALDH5A1 in pathological grade II and III patients were associated with improved OS. This indicated low expression of ALDH5A1 was a significant predictor of worse clinical prognosis in OC patient.

## Conclusion

Overall, the present study revealed that ALDH5A1 may play an important role in metastasis of OC, and ALDH5A1 may be a therapeutic target of OC that is potentially effective in treating OC metastasis according to the bioinformatic analyses and verification experiments. Therefore, although much remains to be learned and further studies are needed to fully understand the reciprocal interactions that are essential for OC metastasis, and the precise role of interaction between ALDH5A1 and metastasis of OC needs to be further investigated, our findings confirmed that ALDH5A1 might be a promising molecular target for OC therapeutic intervention.

295 **Declarations**

296 *Ethical approval and consent to participate:* Not applicable.

297 *Consent for publication:* Not applicable.

298 *Availability of data and materials:* All data generated or analyzed during this study are included in this
299 article and its additional files.

300 *Competing interests:* The authors declare that they have no competing interests.

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303 *Authors' contributions:* XT and QHZ contributed to the conception and design of the study. CC, XW and
304 RL performed the study and drafted the article. PJ, HWC and MX conducted data acquisition, data
305 analysis and interpretation. All authors discussed the results and agreed to be accountable for all aspects
306 of the work. All authors read and approved the final manuscript.

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309 **References**

310 1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7-34.

311 2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin.
312 2016;66(2):115-32.

313 3. Aziz M, Agarwal K, Dasari S, et al. Productive Cross-Talk with the Microenvironment: A
314 Critical Step in Ovarian Cancer Metastasis. Cancers (Basel). 2019;11(10).

315 4. Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin.

316 2018;68(4):284-96.

317 5. Jackson B, Brocker C, Thompson DC, et al. Update on the aldehyde dehydrogenase gene

318 (ALDH) superfamily. Human genomics. 2011;5(4):283-303.

319 6. Pearl PL, Novotny EJ, Acosta MT, et al. Succinic semialdehyde dehydrogenase deficiency

320 in children and adults. Ann Neurol. 2003;54 Suppl 6:S73-80.

321 7. Nicholson-Guthrie CS, Guthrie GD, Sutton GP, et al. Urine GABA levels in ovarian cancer

322 patients: elevated GABA in malignancy. Cancer Lett. 2001;162(1):27-30.

323 8. Liang D, Meyer L, Chang DW, et al. Genetic variants in MicroRNA biosynthesis pathways

324 and binding sites modify ovarian cancer risk, survival, and treatment response. Cancer Res.

325 2010;70(23):9765-76.

326 9. Tian X, Han Y, Yu L, et al. Decreased expression of ALDH5A1 predicts prognosis in

327 patients with ovarian cancer. Cancer Biol Ther. 2017;18(4):245-51.

328 10. Anglesio MS, Arnold JM, George J, et al. Mutation of ERBB2 provides a novel alternative

329 mechanism for the ubiquitous activation of RAS-MAPK in ovarian serous low malignant

330 potential tumors. Mol Cancer Res. 2008;6(11):1678-90.

331 11. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform

332 for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.

333 12. Raudvere U, Kolberg L, Kuzmin I, et al. g:Profiler: a web server for functional enrichment

334 analysis and conversions of gene lists (2019 update). Nucleic Acids Res. 2019;47(W1):W191-

335 W8.

336 13. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the

337 analysis of systems-level datasets. Nat Commun. 2019;10(1):1523.

338 14. Goswami CP, Nakshatri H. PROGgeneV2: enhancements on the existing database. BMC

339 Cancer. 2014;14(1):970.

340 15. Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. Cell Metab.

341 2016;23(1):27-47.

342 16. Holmes E, Wilson ID, Nicholson JK. Metabolic phenotyping in health and disease. Cell.

343 2008;134(5):714-7.

344 17. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv.

345 2016;2(5):e1600200.

346 18. Kim KJ, Pearl PL, Jensen K, et al. Succinic semialdehyde dehydrogenase: biochemical-

347 molecular-clinical disease mechanisms, redox regulation, and functional significance. Antioxid

348 Redox Signal. 2011;15(3):691-718.

349 19. Vander Heiden MG, DeBerardinis RJ. Understanding the Intersections between

350 Metabolism and Cancer Biology. Cell. 2017;168(4):657-69.

351 20. van den Hoogen C, van der Horst G, Cheung H, et al. The aldehyde dehydrogenase

352 enzyme 7A1 is functionally involved in prostate cancer bone metastasis. Clinical &

353 Experimental Metastasis. 2011;28(7):615-25.

354 21. Gerber JM, Smith BD, Ngwang B, et al. A clinically relevant population of leukemic

355 CD34(+)CD38(-) cells in acute myeloid leukemia. Blood. 2012;119(15):3571-7.

356 22. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant

357 human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell.

2007;1(5):555-67.

23. Charafe-Jauffret E, Ginestier C, Iovino F, et al. Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer. Clin Cancer Res. 2010;16(1):45-55.

24. Ajani JA, Wang X, Song S, et al. ALDH-1 expression levels predict response or resistance to preoperative chemoradiation in resectable esophageal cancer patients. Mol Oncol. 2014;8(1):142-9.

25. Hilvo M, de Santiago I, Gopalacharyulu P, et al. Accumulated Metabolites of Hydroxybutyric Acid Serve as Diagnostic and Prognostic Biomarkers of Ovarian High-Grade Serous Carcinomas. Cancer Res. 2016;76(4):796-804.

26. El-Habr EA, Dubois LG, Burel-Vandenbos F, et al. A driver role for GABA metabolism in controlling stem and proliferative cell state through GHB production in glioma. Acta Neuropathol. 2017;133(4):645-60.

27. Welch DR, Hurst DR. Defining the Hallmarks of Metastasis. Cancer Res. 2019;79(12):3011-27.

28. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. Nat Rev Cancer. 2014;14(6):430-9.

29. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012;196(4):395-406.

30. Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. Cell. 2010;141(1):52-67.

379 31. Wang X, Yang B, She Y, et al. The lncRNA TP73-AS1 promotes ovarian cancer cell
380 proliferation and metastasis via modulation of MMP2 and MMP9. J Cell Biochem.
381 2018;119(9):7790-9.
382 32. Gonzalez-Villasana V, Fuentes-Mattei E, Ivan C, et al. Rac1/Pak1/p38/MMP-2 Axis
383 Regulates Angiogenesis in Ovarian Cancer. Clin Cancer Res. 2015;21(9):2127-37.
384 33. Qiu J, Ye L, Ding J, et al. Effects of oestrogen on long noncoding RNA expression in
385 oestrogen receptor alpha-positive ovarian cancer cells. J Steroid Biochem Mol Biol.
386 2014;141:60-70.
387 34. Castro-Castro A, Marchesin V, Monteiro P, et al. Cellular and Molecular Mechanisms of
388 MT1-MMP-Dependent Cancer Cell Invasion. Annu Rev Cell Dev Biol. 2016;32:555-76.
389 35. Kaimal R, Aljumaily R, Tressel SL, et al. Selective blockade of matrix metalloprotease-14
390 with a monoclonal antibody abrogates invasion, angiogenesis, and tumor growth in ovarian
391 cancer. Cancer Res. 2013;73(8):2457-67.

392

393 **Figure legends**

394 **Fig.1** Downregulation of ALDH5A1 promoted cell invasion and migration in OC cells. (**A**) Microarray
395 data analysis of ALDH5A1 mRNA expression level from the Oncomine database showed decreased
396 expression of ALDH5A1 in metastasis site of OC than in primary site. (**B**) ALDH5A1 expression was
397 interfered by siRNA and confirmed by qRT-PCR. (**C**) Invasion assay was conducted to measure the
398 invasive capacity of OC cells after ALDH5A1 depletion. (**D**) Quantitative results are illustrated for
399 invasion assay. (**E**) Wound healing assay was conducted to detect the motility of OC cells after

ALDH5A1 depletion. (**F**) Quantitative results are illustrated for wound healing assay. ** $P<0.01$, *** $P<0.005$, **** $P<0.001$

**Fig.2** Functional enrichment analysis of ALDH5A1 and the co-expression genes in OC. (**A**) The Venn diagram representing the intersection of the co-expression gene sets extracted from OC cell lines in CCLE and OC patients in TCGA. (**B**) The functional enrichment analysis of ALDH5A1 and the 128 common co-expression genes from g:Profiler. (**C**) Top 20 clusters from Metascape pathway enrichment analysis of ALDH5A1 and the 128 common co-expression genes. Length of bars represent $\log_{10}(P)$ based on the best-scoring term within each cluster. (**D**) The enrichment network created by Metascape colored by *P*-values

**Fig.3** The co-expression and interaction analysis of ALDH5A1 and the ECM organization pathway. (**A**) The inversely correlations between mRNA expression level of ALDH5A1 and MMP2, MMP3, MMP14 in cBioPortal database. (**B**) ALDH5A1 and MMP mRNA were detected by quantitative RT-PCR analysis. (**C**) ALDH5A1 and MMP protein were detected by western blot analysis. * $P<0.05$, *** $P<0.0005$

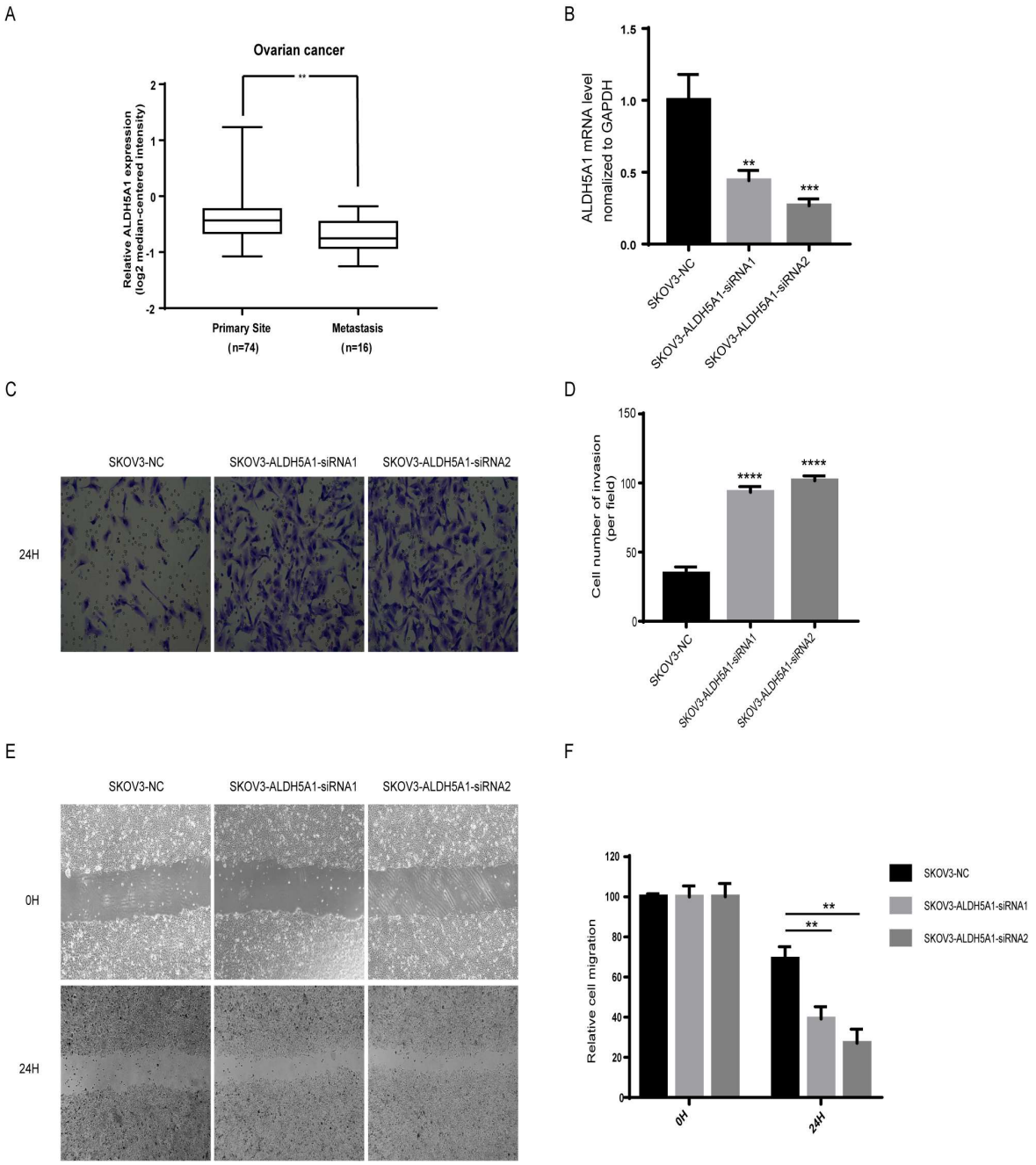
**Fig.4** The proteomic expression of ALDH5A1 and the ECM organization pathway in OC patients. Data from HPA database are determined by IHC staining. Representative IHC staining of ALDH5A1, MMP2, MMP3, MMP14 from two OC patients showed the negative correlations between proteomic expression level of ALDH5A1 and MMP2, MMP3, MMP14

421 **Fig.5** The prognostic effect of the ALDH5A1 mRNA expression in OC. The correlation of ALDH5A1

422 mRNA with pathological grades of OC patients. Survival curves are plotted for all patients (n = 578) (**A**),

423 for cases in grade II (n = 78) (**B**) and for cases in grade III (n = 481) (**C**)

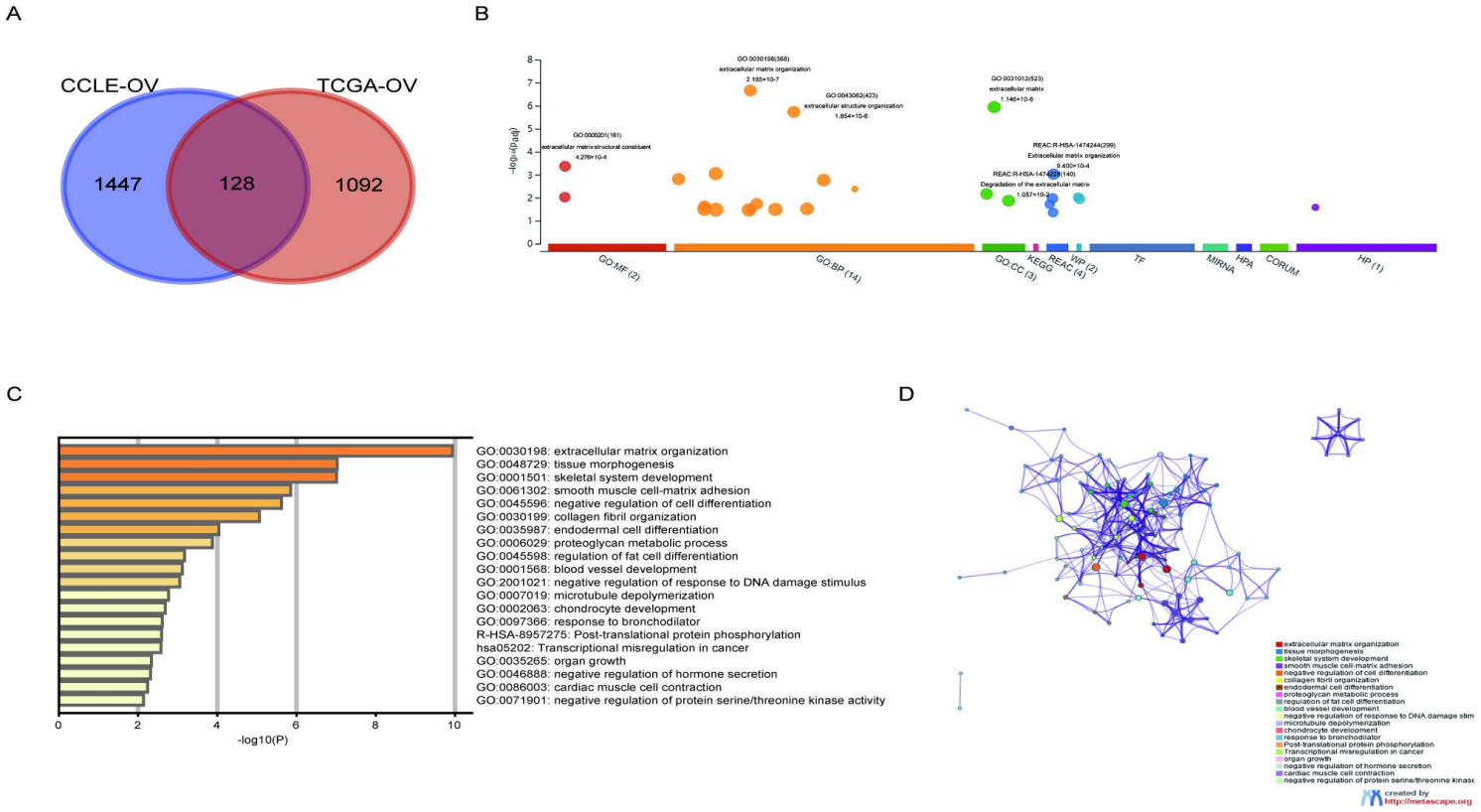
# Figures




## Figure 1

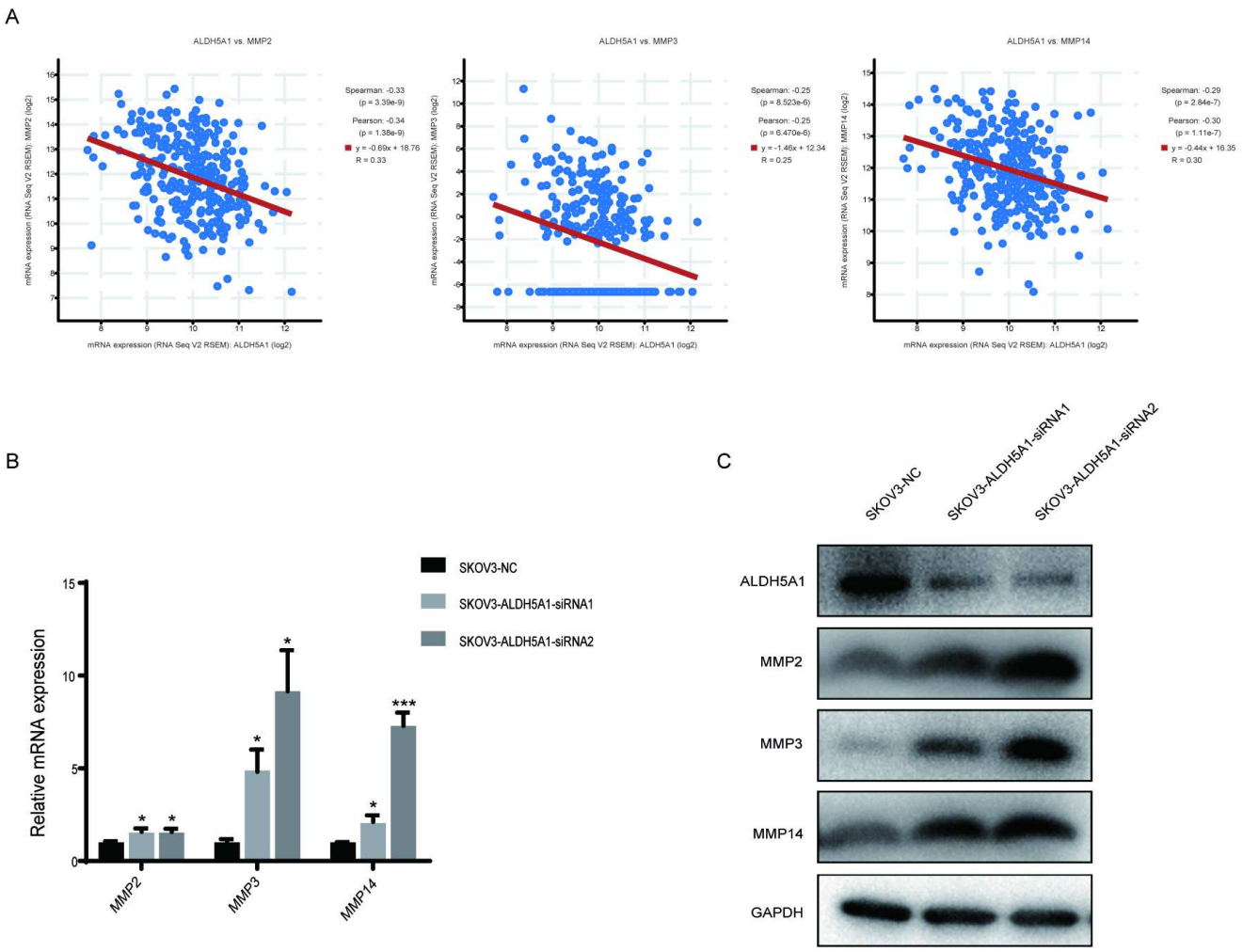
Downregulation of ALDH5A1 promoted cell invasion and migration in OC cells. (A) Microarray data analysis of ALDH5A1 mRNA expression level from the Oncomine database showed decreased expression of ALDH5A1 in metastasis site of OC than in primary site. (B) ALDH5A1 expression was interfered by

siRNA and confirmed by qRT-PCR. (C) Invasion assay was conducted to measure the invasive capacity of OC cells after ALDH5A1 depletion. (D) Quantitative results are illustrated for invasion assay. (E) Wound healing assay was conducted to detect the motility of OC cells after ALDH5A1 depletion. (F) Quantitative results are illustrated for wound healing assay. ** P<0.01, *** P<0.005, **** P<0.001



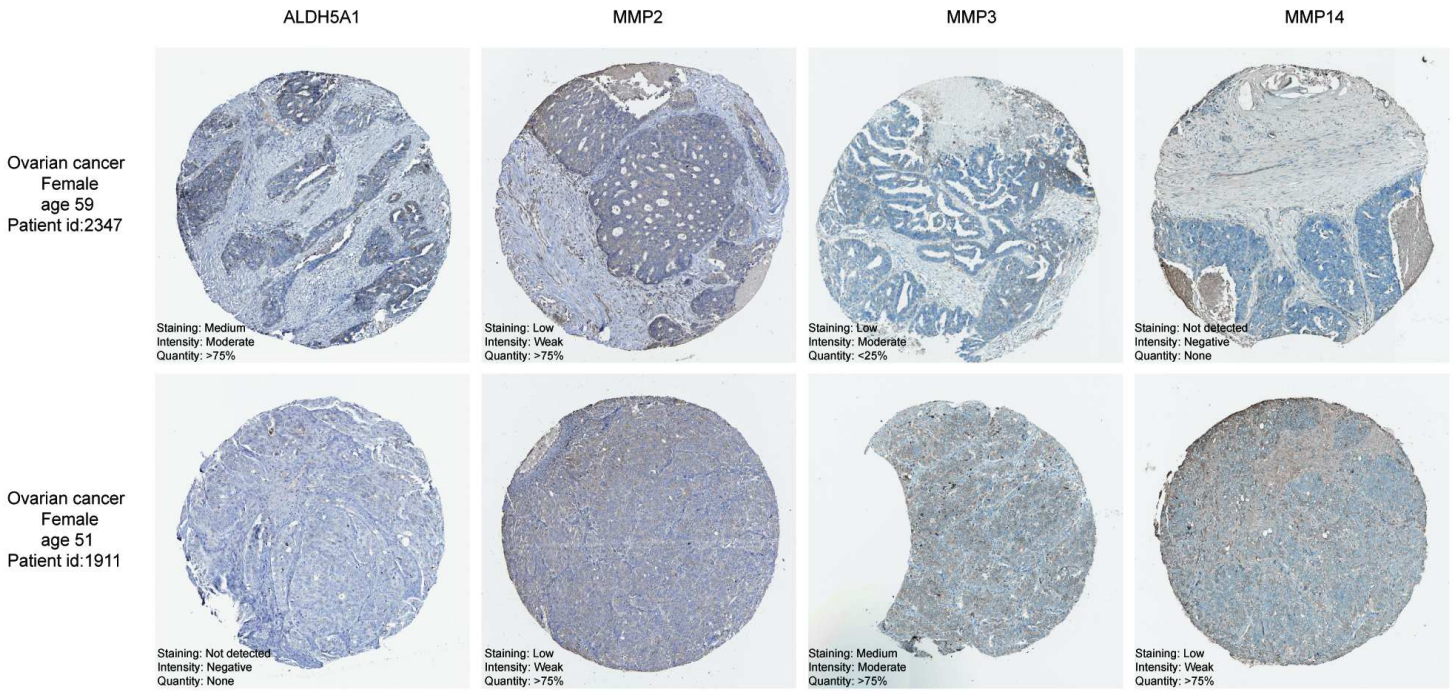

## Figure 2

Functional enrichment analysis of ALDH5A1 and the co-expression genes in OC. (A) The Venn diagram representing the intersection of the co-expression gene sets extracted from OC cell lines in CCLE and OC patients in TCGA. (B) The functional enrichment analysis of ALDH5A1 and the 128 common co-expression genes from g:Profiler. (C) Top 20 clusters from Metascape pathway enrichment analysis of ALDH5A1 and the 128 common co-expression genes. Length of bars represent log10(P) based on the best-scoring term within each cluster. (D) The enrichment network created by Metascape colored by P-values



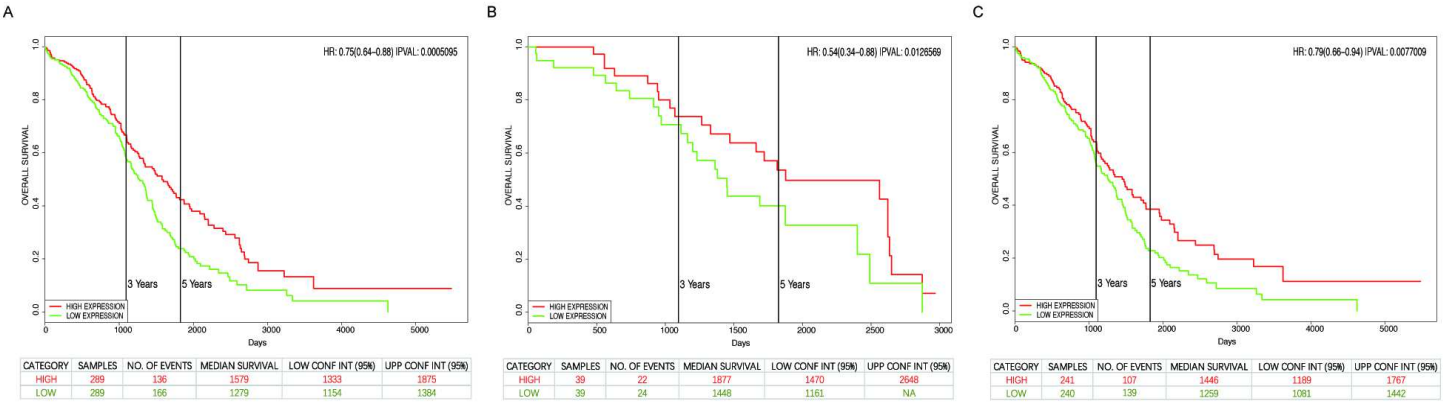

## Figure 3

The co-expression and interaction analysis of ALDH5A1 and the ECM organization pathway. (A) The inversely correlations between mRNA expression level of ALDH5A1 and MMP2, MMP3, MMP14 in cBioPortal database. (B) ALDH5A1 and MMP mRNA were detected by quantitative RT-PCR analysis. (C) ALDH5A1 and MMP protein were detected by western blot analysis. * P<0.05, *** P<0.0005




## Figure 4

The proteomic expression of ALDH5A1 and the ECM organization pathway in OC patients. Data from HPA database are determined by IHC staining. Representative IHC staining of ALDH5A1, MMP2, MMP3, MMP14 from two OC patients showed the negative correlations between proteomic expression level of ALDH5A1 and MMP2, MMP3, MMP14




| CATEGORY | SAMPLES | NO. OF EVENTS | MEDIAN SURVIVAL | LOW CONF INT (95%) | UPP CONF INT (95%) |
|---|---|---|---|---|---|
| HIGH | 289 | 136 | 1579 | 1333 | 1875 |
| LOW | 289 | 166 | 1279 | 1154 | 1384 |

| CATEGORY | SAMPLES | NO. OF EVENTS | MEDIAN SURVIVAL | LOW CONF INT (95%) | UPP CONF INT (95%) |
|---|---|---|---|---|---|
| HIGH | 39 | 22 | 1877 | 1470 | 2648 |
| LOW | 39 | 24 | 1448 | 1161 | NA |

| CATEGORY | SAMPLES | NO. OF EVENTS | MEDIAN SURVIVAL | LOW CONF INT (95%) | UPP CONF INT (95%) |
|---|---|---|---|---|---|
| HIGH | 241 | 107 | 1446 | 1189 | 1767 |
| LOW | 240 | 139 | 1259 | 1081 | 1442 |

## Figure 5

The prognostic effect of the ALDH5A1 mRNA expression in OC. The correlation of ALDH5A1 mRNA with pathological grades of OC patients. Survival curves are plotted for all patients (n = 578) (A), for cases in grade II (n = 78) (B) and for cases in grade III (n = 481) (C)

# Supplementary Files

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