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ORIGINAL ARTICLE

Comprehensive in situ analysis of ALDH1 and SOX2 reveals increased expression of stem cell markers in high-grade serous carcinomas compared to low-grade serous carcinomas and atypical proliferative serous tumors

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Abstract

Recent studies have shown that re-expression of stem cell factors contribute to pathogenesis, therapy resistance, and recurrent disease in ovarian carcinomas. In this study, we compare the expression and co-expression of stem cell markers ALDH1 and SOX2 in different types of serous ovarian tumors. A total of 215 serous ovarian tumors (161 high-grade serous carcinomas (HGSC), 17 low-grade serous carcinomas (LGSC), 37 atypical proliferative serous tumors (APST)), and 10 cases of serous tubal intraepithelial carcinoma (STIC) were analyzed. Double immunostaining experiments addressed the association of cell proliferation (Ki67) with ALDH1 and the potential co-expression of SOX2 and ALDH1. The prognostic effect was analyzed in the cohort of HGSC. Expression of ALDH1and/or SOX2 was detected with increased frequency in HGSC (88.8%), compared with LGSC (70.5%) and APST (36.4%), while ALDH1 alone was significantly more frequently expressed in LGSC. The majority of all tumor types showed expression of ALDH1 and SOX2 in different cells. Only a minority of HGSC (4.6%) and STIC (20%) showed SOX2/ALDH1 co-expression in > 10% of tumor cells. Double staining also revealed that ALDH1 is associated with the non-proliferating Ki67-negative fraction consistent with a stem cell phenotype. Co-expression of ALDH1 and SOX2 or ALDH1 and Ki67 has no effect on survival. Expression of stem cell factors ALDH1 and/or SOX2 shows increased frequency in high-grade serous ovarian carcinomas compared to low-grade carcinomas and borderline tumors, supporting the concept that stem cell markers play different biological roles in low-grade versus high-grade serous neoplasia of the ovary.

Keywords Ovarian neoplasms · Cancer stem cells · Proliferation · SOX2 · ALDH1

Introduction

Ovarian cancer is one of the most lethal malignancies in women worldwide [1–3]. More than 70% of patients with high-grade serous carcinoma (HGSC), the most common histological type of ovarian cancer, present with advanced stage at diagnosis and wide spread peritoneal metastasis [4–6]. Most patients will respond initially to radical surgery and platinum-

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containing chemotherapy. However, many of them will experience relapse eventually, resulting in a 5-year survival rate of 30–40% [4, 7–9].

Cancer stem cells provide a potential explanation for this phenomenon, as they are thought to contribute to chemoresistance. Therefore, they have been extensively studied in a variety of tumor entities in the recent years. By definition, stem cells are capable to self-renew, regenerate, and differentiate into the full spectrum of cell types [10, 11]. Cancer stem cells have been identified and isolated from malignancies of the hematopoietic system and solid tumors like carcinomas of the breast, ovary, lung, prostate, colon, brain, head and neck, and pancreas [12, 13].

Niche factors that support stem cells non-autonomously have been identified by genetic and molecular analysis. They include components of the Notch, Wnt, and Shh signaling pathways, pluripotency-associated transcription factors, such as SOX2 and detoxifying enzymes, e.g. ALDH1 [4, 14]. Aldehyde dehydrogenase (ALDH) catalyzes the oxidation of aldehyde and is present in many isoenzymes, among which ALDH1 plays an important role in cellular homeostasis. Cells with high ALDH1-activity show increased potential of self-renewal and stress resistance [10, 15].

The SOX (sex-determining region Y-box) gene family, consisting of more than 30 SOX genes, is important for regulation of organ development, determination of cell fate, and maintenance [7, 16, 17]. SOX2 expression has been reported in several solid tumors, including squamous cell carcinoma [18], gastric [19], breast [20], and ovarian cancer [21].

Over the last decade, new concepts for the pathogenesis of serous ovarian carcinomas have been proposed. Ovarian carcinomas can be divided into two major groups. Type I tumors which progress from borderline tumors to low-grade carcinomas include low-grade serous carcinomas as well as endometrioid, clear cell, and mucinous tumors [6]. On the other hand, there are type II tumors, which form highly aggressive, rapidly growing tumors and include high-grade serous carcinomas and mixed mullerian type tumors [7, 22, 23]. Specifically, there is recent evidence that high-grade serous carcinomas develop in precursor lesions in the fallopian tube, which show a loss of ALDH1 with consecutive gain of expression in the invasive carcinoma [2, 24]. However, the pattern of expression and co-expression of stem cell markers in these tumor entities has not been evaluated with special reference to type I vs. type II tumors. Especially, there is limited data on expression of stem cell markers in atypical proliferative serous tumors/serous borderline tumors (APST/SBOT) and low-grade serous carcinomas (LGSC) in comparison to high-grade serous carcinomas (HGSC).

Therefore, our study had two main objectives: First, we examined the expression of stem cell marker ALDH1 in a tissue microarray (TMA) series of serous ovarian tumors including APST, LGSC, and HGSC and explored the correlation with tumor type and cell proliferation. Furthermore, we analyzed the in situ co-expression patterns of SOX2 and ALDH1 within the heterogeneous tumor context by performing double immunohistochemistry to potentially identify specific subsets of cancer stem cell-like populations.

Materials and methods

Patients and tumor samples

A database was constructed from 215 consecutive patients diagnosed with serous ovarian tumors treated at the University Women's hospital in Tuebingen, Germany, between the years 2000 and 2008 and with sufficient material for construction of a tissue microarray (APST = 37, low-grade carcinomas = 6, high-grade carcinomas = 161). Furthermore, a set of 11 LGSC and 10 serous tubal intraepithelial carcinomas from patients with associated HGSC was included in the study on whole sections. The patients were treated at the University Women's hospital in Tuebingen, Germany, between the years 2000 and 2008. Follow-up data was obtained from the patient registry of the Tuebingen Cancer Center. All slides were retrieved from the archives of the Institute of Pathology and reviewed by a pathologist with subspecialty training in Gynecologic Pathology (A.S). Statistics were performed using SPSS software (SPSS Inc., Chicago, IL, USA). This study was approved by the institutional ethics review board of the University Hospital Tuebingen.

Tissue microarray construction

The tissue microarray was constructed as described previously. Each case was represented with up to 6 cores. Cases with a minimum of one interpretable core were accepted in the study. In cases with bilateral ovarian carcinoma, 3 cores were taken for each side.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 4-μm-thick sections with a Ventana Discovery automated immunostaining system using Ventana reagents (Ventana Medical Systems, Tucson, AZ, USA). Pretreatment for Ki67 as well as for SOX2 was conducted with an EDTA-based buffer at pH 8.4, and heat-induced epitope retrieval (HIER) was performed before the primary antibody was applied (monoclonal mouse anti-human Ki-67 Antigen, clone MIB-1, M7240, DakoCytomation Denmark, dilution 1:200 Antibody diluent Zytomed Systems and rabbit monoclonal antibody SOX-2 (SP76), Cell Marque USA, dilution 1:100 Antibody diluents Zytomed Systems). A biotinylated detection kit containing diaminobenzidine and horseradish peroxidase (DABMap Kit Ventana) was applied for both staining procedures.

Pretreatment for ALDH1 was the same as for Ki67, followed by an endogenous biotin-block before the primary antibody was applied (ALDH1a1 Rabbit monoclonal Antibody, EP1933Y, Epitomics, 1:100 diluent Zytomed Systems). Finally, a biotin-free detection kit containing diaminobenzidine and horseradish peroxidase was used (ultraView Universal DAB detection kit, Roche).

The double-staining for ALDH1 and Ki67 was performed by using the same pretreatment, before the primary antibody (MIB1/ALDH1, Epitopics, MIB1 dilution 1:100, ALDH1 dilution 1:100, Zytomed Systems) followed by UV Red Enhancer marking ALDH1-positive cells (ultraView Universal Alkaline Phosphatase Red Detection kit, Roche), biotin-free, and using a biotin-free detection kit containing diaminobenzidine and horseradish (ultraView Universal DAB Detection kit).

The double-staining for SOX2 and ALDH1 followed the same basic protocol like ALDH1/Ki67 double staining using a pretreatment (TBE buffer at pH 8.4, incubation for 32 min. at 37 °C), before the primary antibodies were applied (SOX2/ALDH1, SOX2 rabbit monoclonal antibody (SP76), Cell Marque, dilution 1:100, Zytomed Systems, and ALDH1a1 rabbit monoclonal antibody, EP1933Y, Epitomics, 1:100 diluent Zytomed Systems). Subsequent steps were performed according to the ALDH1/Ki67 double-staining protocol as mentioned above.

Evaluation of immunohistochemistry

The immunostaining of ALDH1 as well as SOX2 was evaluated using a semiquantitative scoring system. For ALDH1, the relative number of cells with cytoplasmic staining was estimated, as previously published for SOX2 nuclear stain: complete negative staining equals score 0, 0–10% score 1, 10–50% score 2, and > 50% score 3 (Fig. 1). Cases were included into the analysis if at least one core was assessable. This analysis was independently performed by 2 reviewers (A.S., A.F.).

Ki67 immunostaining was assessed by counting 100 cells and determining the percentage of cells with nuclear positivity. The average percentage of at least two cores was determined.

When evaluating the double-stain of ALDH1/Ki67, we compared the fraction of Ki67-positive nuclei in ALDH1-positive cells and ALDH1-negative cells case by case. Regarding the double-stain of SOX2/ALDH1, cases with any positive cell for either marker or co-expression of both markers were counted.

Statistics

Results of immunohistochemistry were analyzed in subgroups, compared to clinical parameters, and tumor type using χ^2 or Fisher's exact test. The average proliferation index was compared with the *t* test. Overall survival curves were generated using the Kaplan-Meier method.

Results

Expression of ALDH1 serous ovarian tumors

In almost half of tumors (45.6%), immunohistochemistry showed cytoplasmic ALDH1 expression, at least in a small number of cells (Fig. 1a–d). There was a significant difference between APST, LGSC, and HGCS, with regard to frequency of positive cases or quantity of positive cells. Specifically, ALDH1 was detected in monostaining experiments in 18 of 37 APST (48.6%), 12 of 17 LGSC (70.6%), and 68 of 161 HGSC (42.2%) (Table 1). In all three entities (APST/LGSC/HGSC), there was no significant difference in the number of cases with less than 10% positive cells (score 1 21.6/29.4/25.5%). However, LGSC showed significantly more cases with > 10% positive cells (scores 2 and 3 27/41.2/16.8%) and > 50% (5.4%, 23.5%, and 7.5%). There was no impact of ALDH1 expression on overall or disease-free survival (Supplemental data). Differences in number of positive cases and correlation of immunostaining scores with APST, LGSC, and HGSC were detected also for SOX2, confirming previously published data from our group (Table 2).

Highly proliferating carcinomas show focal ALDH1-positive cells, which reside predominantly in a slowly proliferating compartment

Performing immunohistochemical staining of consecutive sections of the same cases, we found that for HGSC, the presence of a large fraction of ALDH1-positive cells (score 3 > 50%) correlated with a high Ki67 proliferation index (up to 40% Ki67-positive cells) (Fig. 2a, b). However, double-staining experiments for ALDH1/Ki67 showed that ALDH1-positive cells diplayed none or only very little Ki67 expression (average 4.4%), whereas the ALDH1-negative cell fraction presented with a high Ki67 index of 23.6% (mean difference between the two compartments 19.1%, 95% confidence interval 16.6 to 21.7%, $p < 0.001$ paired *t* test) (Fig. 2c, d). There was no significant impact on overall or disease-free survival. These data were not influenced by the status of resection in optimally debulked patients (data not shown).

Despite common co-occurrence of ALDH1 and SOX2-positive populations, co-expression in the same cell is rare and limited to high-grade serous ovarian carcinomas

Next, we analyzed the corresponding patterns for ALDH1 and SOX2, with double-staining of both markers. Comparison of

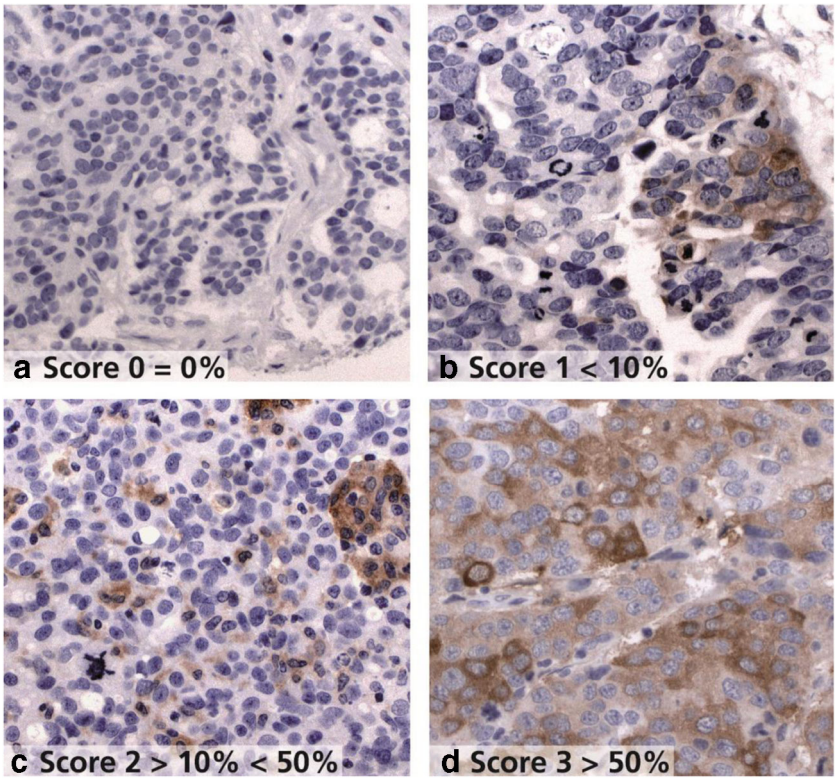

Fig. 1 ALDH1 by Immunohistochemistry. **a** Score 0 = 0% positive cells. **b** Score 1 ≤ 10%. **c** Score 2 ≤ 50. **d** Score 3 > 50% positive cells. Cells were counted positive if intensity of staining was moderate or strong

high-grade serous carcinomas with low-grade serous carcinomas and borderline tumors showed significantly more cases with expression of any of both markers SOX2 or ALDH1 (HGSC 88.8%, LGSC 70.5% vs. APST 36.4%. *p* value < 0.001). Furthermore, co-expression of markers was also more frequent in high-grade serous carcinomas than in LGSC or borderline tumors (HGSC 34.9%, LGSC 11.7% vs. APST 9.1%. *p* value 0.003). Double expression of both markers within the same cells was detected in very few cases, predominantly within carcinomas. Only high-grade serous ovarian carcinomas presented ALDH1/SOX2 double staining in 20 cases (13.2%). Further, two cases of low-grade serous carcinoma (11.7%) and two APSTs (6.1%) were positive within the same cells (*p* value 0.490). Therefore, in most cases, including borderline tumors, ALDH1 and SOX2 were expressed by different cell fractions. Only 4.6% of HGSC and none of the LGSC or APST showed co-staining in more than 10% of cells (*p* value 0.261) (Figs. 3 and 4; Table 3). Survival data was available for the patients with carcinomas. There was no impact on overall survival for expression of ALDH alone or co-expression of both markers in HGSC (Supplemental data).

Expression of ALDH1 and SOX2 in serous tubal intraepithelial carcinomas

We further investigated a cohort of ten serous tubal intraepithelial carcinomas by single- as well as double-staining experiments for SOX2 and ALDH1. SOX2 was

Table 1 ALDH1 distribution according to entities, expression patterns 0%, < 10%. > 10–50%, and > 50%

	ALDH 0%	ALDH < 10%	ALDH > 10%	ALDH > 50%	Total	*n*/ %
APST	19	8	8	2	37	*n*
	51.4%	21.6%	21.6%	5.4%	100%	%
LGSC	5	5	3	4	17	*n*
	29.4%	29.4%	17.6%	23.5%	100%	%
HGSC	93	41	15	12	161	*n*
	57.8%	25.5%	9.3%	7.5%	100%	%
Total	117	54	26	18	215	*n*
	54.4%	25.1%	12.1%	8.4%	100%	%

p value APST vs. invasive = 0.006. *p* value LG vs. HG = 0.003 (χ^2 test)

Table 2 SOX2 distribution according to entities, expression patterns 0%, < 10%, > 10–50%, and > 50%

	SOX2 0%	SOX2 < 10%	SOX2 > 10%	SOX2 > 50%	Total	*n*/%
APST	29	8	0	0	37	*n*
	78.4%	21.6%	0%	0%	100%	%
LGSC	15	1	1	0	17	*n*
	88.2%	5.9%	5.9%	0%	100%	%
HGSC	81	34	31	13	159	*n*
	50.9%	21.4%	19.5%	8.2%	100%	%
Total	125	43	32	13	213	*n*
	58.7%	20.2%	15.0%	6.1%	100%	%

p value APST vs. invasive < 0.001. *p* value LG vs. HG = 0.005 (χ^2 test)

expressed in three cases (*n* = 3/1; 33.3%) of all STIC cases by mono stain. Thereof, two cases showed SOX2 expression of 10–50% (*n* = 2/10; 20.0%) of tumor cells and one case < 10% of tumor cells (*n* = 1/10; 10.0%). Seventy percent of cases were SOX2 negative. ALDH1-monostaining was negative in three cases (*n* = 3/10; 33.3%), expressed within < 10% of tumor cells in three cases (*n* = 3/10; 33.3%) and 10–50% of tumor cells in two cases (*n* = 2/10; 20%). Only two STIC cases showed ALDH1 expression in > 50% of tumor cells (*n* = 2/10; 20.0%). In summary, this result describes a decrease in ALDH1 expression in most cases of STIC relative to the surrounding normal epithelium, which shows consistently diffuse and strong expression. Via SOX2/ALDH1 double staining, two cases were completely negative (20%), whereas 80% showed either mono-positivity or even double positivity for SOX2 and ALDH1. In two cases (*n* = 2/10; 20.0%), SOX2/ALDH1 double positivity was found within the same cells, even in more than 10% of tumor cells.

Further, we noticed SOX2—as well as ALDH1—expression in normal tubal epithelium located next to STIC lesions, mostly either ALDH1 or SOX2 in separate cells and focally within the same cell (Table 3; Fig. 5).

Discussion

Stem cell marker ALDH1 can be expressed in a broad range of serous epithelial ovarian neoplasms, including atypical

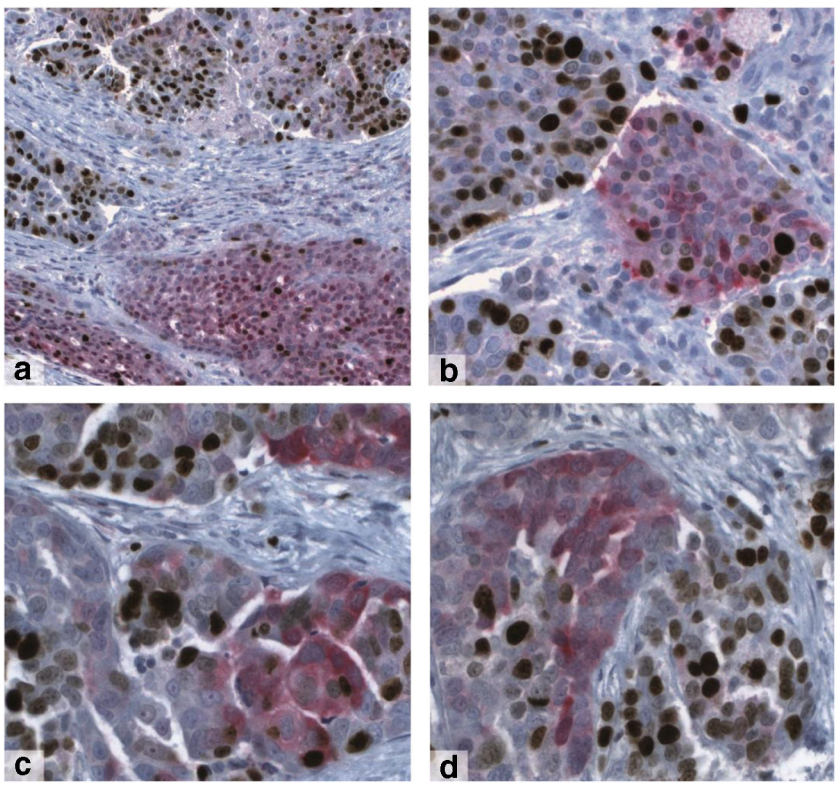

Fig. 2 **a** ALDH1/Ki67 double-staining shows ALDH1-positive cell-fractions (red) with no or low proliferation rate, whereas Ki67-expressing cells (brown) are ALDH1-negative. **b** There is a significant difference in the average Ki67-index between tumors with > 50% ALDH1 pos. cells and < 50% pos. cells (39.2% vs 26.11%, *p* = 0.029, *t* test). **c**, **d** High Ki67-Index dominates in ALDH1-negative cell fraction and not in the ALDH1-positive cell fraction

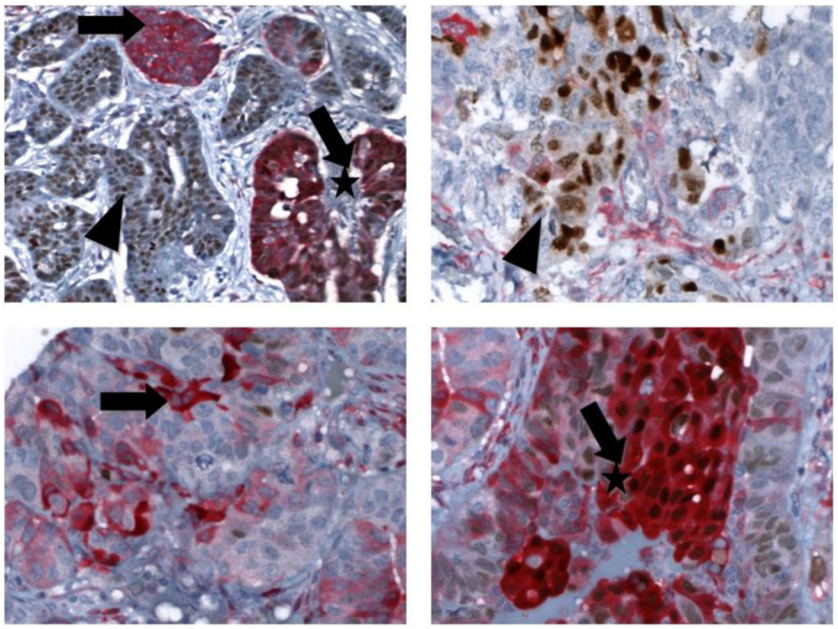

Fig. 3 ALDH1/SOX2 double-staining of HGSC shows different cell fractions: ALDH1-expressing (arrow), SOX2-positive (arrow-head), and double-stained cell conglomerates (arrow with asterisk)

proliferative serous tumors (APST/serous borderline tumors), low-grade serous carcinomas (LGSC), and high-grade serous carcinomas (HGSC) with similar frequency and is therefore found in both high- and low-grade tumors. Despite the overall

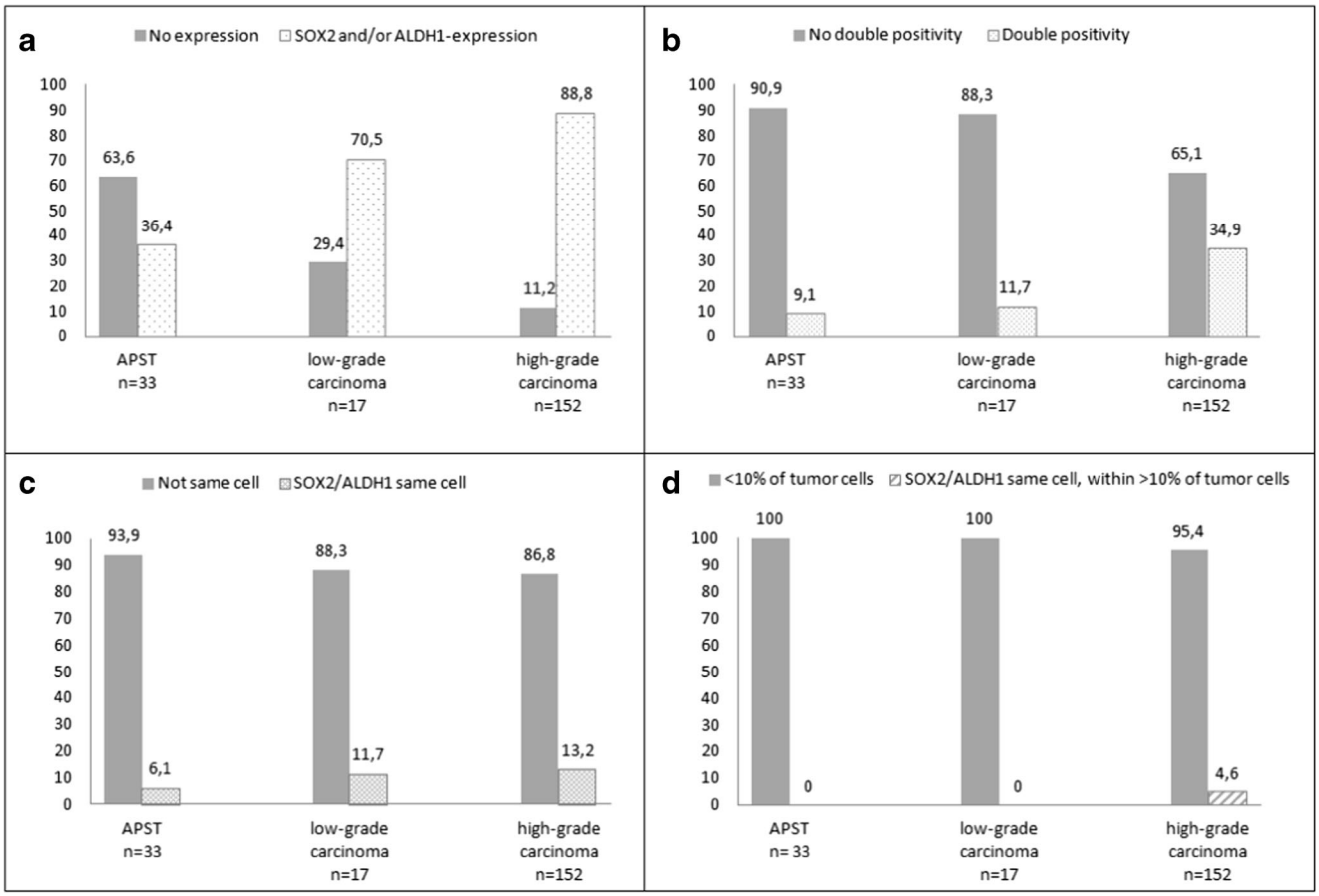

Fig. 4 Double-staining experiments **a** SOX2 and/or ALDH1 expression vs. no expression; **b** SOX2/ALDH1 double positivity vs. no double positivity; **c** SOX2/ALDH1double positivity within the same cell vs. not the same cell; **d** SOX2/ALDH1double positivity within the same cell within > 10% of tumor cells vs. < 10% of tumor cells

Table 3 Double-staining experiments for ALDH1 and SOX2: APST, low-grade serous carcinomas, and high-grade serous carcinomas

Tumor	Measurement						
	SOX2 and ALDH1 negative, *n*/%	ALDH1 positive, *n*/%	SOX2 positive, *n*/%	ALDH1 and/or SOX2 positive, *n*/%	ALDH1 and SOX2 positive (same case), *n*/%	ALDH1 and SOX2 positive (double-positive cells), *n*/%	ALDH1 and SOX2 positive (> 10% double- positive cells), *n*/%
APST ($n = 33$)	21/63.6%	10/30.3%	5/15.2%	12/36.4%	3/9.1%	2/6.1%	0/0%
Low-grade ($n = 17$)	5/29.4%	12/70.5%	2/11.7%	12/70.5%	2/11.7%	2/11.7%	0/0%
High-grade ($n = 152$)	17/11.2%	118/77.6%	69/45.4%	135/88.8%	53/34.9%	20/13.2%	7/4.6%
STIC ($n = 10$)	2/20%	7/70%	3/30%	8/80%	2/20%	2/20%	2/20%
Total without STIC ($n = 202$)	43/21.3%	140/69.3%	76/37.6%	159/78.7%	58/28.7%	24/11.9%	7/3.4%
p value	< 0.001	< 0.001	< 0.001	< 0.001	0.003	0.490	0.261

p value was calculated without STIC lesions (χ^2 test)

association of ALDH1 with highly malignant tumors, within each individual tumor, ALDH1 is expressed in a slowly proliferating compartment, consistent with a stem cell-like population seeming to rest but still relying on detoxifying

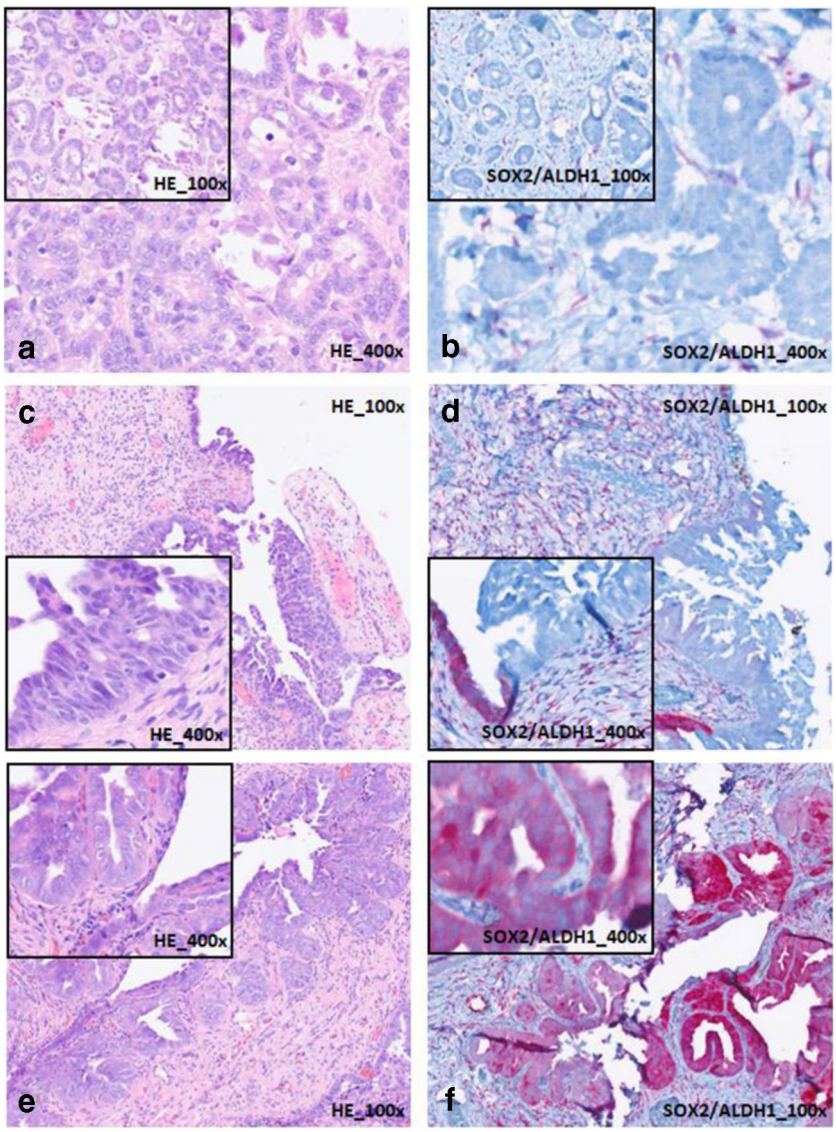

Fig. 5 Double-staining experiments for ALDH1 (red, cytoplasm) and SOX2 (brown, nuclear). **a**, **b** LGSC, HE of × 100 and × 400 magnifications. **c**, **d** Serous tubal intraepithelial carcinoma (STIC), HE of × 100 and × 400 magnifications. **e**, **f** Serous tubal intraepithelial carcinoma (STIC), HE of × 100 and × 400 magnifications

qualities. Finally, based on simultaneous analysis in double-staining experiments, we hypothesize that stem cell factors ALDH1 and SOX2 are expressed in different cell populations. Both factors seem to have a relevant role in tumor cell differentiation and cell cycle regulation, either by protection of toxicity and mediating a self-renewal reservoir of tumor cells or by controlling cell proliferation and tumor cell differentiation predominantly in high-grade tumors, because almost 90% of high-grade serous ovarian carcinomas stained positive for either SOX2 and/or ALDH1.

Our data are consistent with previous studies, which reported ALDH1 expression in serous ovarian carcinomas [10, 25, 26]. Depending on the cutoff for ALDH-positive tumors at 10% or 1% ALDH1 expressing cells, these studies report between 40 [27] and 74% positive serous carcinomas [28]. Our results for HGSC are within this range at 42.2% with a cutoff at the presence of any positive cell. In addition, we report the first study specifically on type I tumors including serous borderline tumors (APST/SBOT) and LGSC with ALDH1 expression in 48.6% and 70.6%, which is significantly different from HGSC. Studies from other groups report that ALDH1 is also highly expressed in normal ovarian surface epithelium and normal serous fallopian tube epithelium and decreases in serous ovarian carcinoma and in its precursor lesion serous tubal intraepithelial carcinoma (STIC) [29] which was confirmed by our study. Initial loss of the protective ALDH1 activity may contribute to increased genomic instability if a normal cell is transformed into a high-grade cancer cell [7, 14].

Upon tumor progression, some cells may regain the activity of ALDH1, which may mediate protection during cell division and promote disease aggressiveness [30]. In support of this hypothesis, we observed a striking correlation of high numbers of ALDH1-positive cells with a higher proliferation rate, which seems to question the assumption that cancer stem cells reside in a resting compartment. To resolve this contradiction, we were able to show that Ki67 is expressed mostly in the ALDH-negative cell population. This finding is consistent with in vitro experiments, which showed that ALDH1 has an inhibitory function in cell cycle regulation [31].

Furthermore, almost 90% of high-grade serous ovarian carcinoma stained positive for either SOX2 or ALDH1, indicating that aggressive and actively proliferating tumors increase the frequency of tumor cells with stem cell marker expression. In comparison, 70.5% of low-grade carcinomas and only 36.4% of borderline tumors showed either SOX2 or ALDH1 (Fig. 2a). Only a minority of cases shows co-expression of both markers and very few cases of HGSC show co-expression in the same cell population. SOX2 and ALDH1 are expressed independently from one another in many cases.

We could not demonstrate any effect of SOX2 or ALDH1 on survival or relapse from disease. The prognostic relevance of ALDH1 alone in ovarian carcinoma has been discussed controversially with one study showing a positive effect [32], while most studies that focus on HGSC show a negative impact on prognosis [27, 33] which has recently been summarized in small meta-analysis. Our study did not indicate a prognostic significance for ALDH1 expression, which is consistent with controversial data in the literature with either positive or negative prognostic impact. Upregulation of SOX2 appears to be an early event in the pathogenesis of high-grade serous carcinoma of the ovary and fallopian tube. A current study from Hellner et al. (2016) found upregulated SOX2 expression in histologically benign fallopian tube surface epithelium in patients with high-grade serous ovarian carcinoma diagnosis. Fallopian tube epithelium in patients without HGSC of the ovary was SOX2 negative. Furthermore, tissue samples of fallopian tubes of BRCA1 and BRCA2 positive women showed also SOX2-positive tubal epithelium, irrespective of neoadjuvant chemotherapy, prior tumor excision or surgery [34]. This is consistent also with observations in breast carcinoma, where SOX2 was reported to be expressed already in ductal carcinoma in situ [35].

However, the role of SOX2 may vary according to tumor type. Expression of SOX2 in HGSC was mostly limited to a small subset of cells. In our previous study [16], we showed that the SOX2-positive population in HGSC exhibits increased expression of cancer stem cell markers and enhanced in vivo tumor-initiating capacity, as well as resistance to apoptosis, suggestive of stem cell like properties, whereas cellular proliferation remains unaltered. Our study further supports the hypothesis that SOX2 expression levels and proliferation rate assessed by Ki67 staining are not correlated in HGSC.

Our study is the first to compare the expression of ALDH1 and SOX2 in APST/serous borderline tumors and low-grade carcinomas with high-grade serous carcinomas. Although we found an increase in stem cell marker expression from low-grade to high-grade tumors, a significant minority of APSTs also revealed either ALDH1 or SOX2 staining. Future studies will examine the clinical relevance of the small subgroup of APSTs with expression of stem cell markers and correlate this finding with extent of disease, presence of invasive or non-invasive implants, recurrence, and potential progression to low-grade carcinoma.

Expression of stem cell factors ALDH1 and/or SOX2 shows increasing frequencies in high-grade serous ovarian carcinomas compared to low-grade carcinomas and borderline tumors, supporting the concept of LGSC and APST being separate pathologically and molecularly from HGSC. In addition, we conclude that stem cell factors in serous ovarian neoplasms do not reflect a unique stem cell phenotype but rather represent different cell populations with alternating expression patterns in HGSC, LGSC, and serous borderline tumors. Consequently, future attempts to target tumor stem cells should consider the heterogeneity of stem cell features in ovarian carcinoma and their distribution in compartments by using a multitarget approach.

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Contributions AF and AS were involved in all aspects of the study including collecting and choosing material for TMA construction, analyzing immunohistochemistry, interpreting the data, statistical analysis, and writing the manuscript. They were expanding TMAs previously constructed by Deborah Pham.

CB constructed the TMAs.

PW, SK, and CB provided patient tissue and clinical data.

SP and FF were involved in establishing immunohistochemistry, study design, and writing of the manuscript.

CL and HB were involved in the study design and writing of the manuscript.

AS oversaw and coordinated the work performed.

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Compliance with ethical standards The study is in agreement with the guidelines of local ethics committee and was approved (Nr. 645/2012/BO2). This study was approved by the institutional ethics review board of the University Hospital Tuebingen.

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

References

1. Bowen NJ, Walker LD, Matyunina LV, Logani S, Totten KA, Benigno BB, McDonald JF (2009) Gene expression profiling supports the hypothesis that human ovarian surface epithelia are multipotent and capable of serving as ovarian cancer initiating cells. BMC Med Genet 2:71. https://doi.org/10.1186/1755-8794-2-71
2. Crum CP, Herfs M, Ning G, Bijron JG, Howitt BE, Jimenez CA, Hanamornroongruang S, McKeon FD, Xian W (2013) Through the glass darkly: intraepithelial neoplasia, top-down differentiation, and the road to ovarian cancer. J Pathol 231(4):402–412. https://doi.org/10.1002/path.4263
3. A snapshot of ovarian cancer. http://planning.cancer.gov/disease/ovarian-snapshot.pdf%5D
4. McAuliffe SM, Morgan SL, Wyant GA, Tran LT, Muto KW, Chen YS, Chin KT, Partridge JC, Poole BB, Cheng KH, Daggett J Jr, Cullen K, Kantoff E, Hasselbatt K, Berkowitz J, Muto MG, Berkowitz RS, Aster JC, Matulonis UA, Dinulescu DM (2012) Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy. Proc Natl Acad Sci U S A 109(43):E2939–E2948. https://doi.org/10.1073/pnas.1206400109
5. Cannistra SA (2004) Cancer of the ovary. N Engl J Med 351(24):2519–2529. https://doi.org/10.1056/NEJMra041842
6. Kurman RJ, Shih Ie M (2008) Pathogenesis of ovarian cancer: lessons from morphology and molecular biology and their clinical implications. Int J Gynecol Pathol 27(2):151–160. https://doi.org/10.1097/PGP.0b013e318161e4f5
7. Ahmed N, Abubaker K, Findlay J, Quinn M (2013) Cancerous ovarian stem cells: obscure targets for therapy but relevant to chemoresistance. J Cell Biochem 114(1):21–34. https://doi.org/10.1002/jcb.24317
8. Folkins AK, Saleemuddin A, Garrett LA, Garber JE, Muto MG, Tworoger SS, Crum CP (2009) Epidemiologic correlates of ovarian cortical inclusion cysts (CICs) support a dual precursor pathway to pelvic epithelial cancer. Gynecol Oncol 115(1):108–111. https://doi.org/10.1016/j.ygyno.2009.06.032
9. Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, Ginestier C, Johnston C, Kueck A, Reynolds RK, Wicha MS, Buckanovich RJ (2011) Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Res 71(11):3991–4001. https://doi.org/10.1158/0008-5472.CAN-10-3175
10. Abelson S, Shamai Y, Berger L, Shouval R, Skorecki K, Tzukerman M (2012) Intratumoral heterogeneity in the self-renewal and tumorigenic differentiation of ovarian cancer. Stem Cells 30(3):415–424. https://doi.org/10.1002/stem.1029
11. Flesken-Nikitin A, Hwang CI, Cheng CY, Michurina TV, Enikolopov G, Nikitin AY (2013) Ovarian surface epithelium at the junction area contains a cancer-prone stem cell niche. Nature 495(7440):241–245. https://doi.org/10.1038/nature11979
12. Cariati M, Purushotham AD (2008) Stem cells and breast cancer. Histopathology 52(1):99–107. https://doi.org/10.1111/j.1365-2559.2007.02895.x
13. Tang C, Ang BT, Pervaiz S (2007) Cancer stem cell: target for anti-cancer therapy. FASEB J 21(14):3777–3785. https://doi.org/10.1096/fj.07-8560rev
14. Hu Y, Fu L (2012) Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res 2(3):340–356
15. Kuroda T, Hirohashi Y, Torigoe T, Yasuda K, Takahashi A, Asanuma H, Morita R, Mariya T, Asano T, Mizuuchi M, Saito T, Sato N (2013) ALDH1-high ovarian cancer stem-like cells can be isolated from serous and clear cell adenocarcinoma cells, and ALDH1 high expression is associated with poor prognosis. PLoS One 8(6):e65158. https://doi.org/10.1371/journal.pone.0065158
16. Bareiss PM, Paczulla A, Wang H, Schairer R, Wiehr S, Kohlhofer U, Rothfuss OC, Fischer A, Perner S, Staebler A, Wallwiener D, Fend F, Fehm T, Pichler B, Kanz L, Quintanilla-Martinez L, Schulze-Osthoff K, Essmann F, Lengerke C (2013) SOX2 expression associates with stem cell state in human ovarian carcinoma. Cancer Res 73(17):5544–5555. https://doi.org/10.1158/0008-5472.CAN-12-4177
17. Ye F, Li Y, Hu Y, Zhou C, Hu Y, Chen H (2011) Expression of Sox2 in human ovarian epithelial carcinoma. J Cancer Res Clin Oncol 137(1):131–137. https://doi.org/10.1007/s00432-010-0867-y
18. Long KB, Hornick JL (2009) SOX2 is highly expressed in squamous cell carcinomas of the gastrointestinal tract. Hum Pathol 40(12):1768–1773. https://doi.org/10.1016/j.humpath.2009.06.006
19. Sanada Y, Yoshida K, Konishi K, Oeda M, Ohara M, Tsutani Y (2006) Expression of gastric mucin MUC5AC and gastric transcription factor SOX2 in ampulla of vater adenocarcinoma: comparison between expression patterns and histologic subtypes. Oncol Rep 15(5):1157–1161
20. Rodriguez-Pinilla SM, Sarrio D, Moreno-Bueno G, Rodriguez-Gil Y, Martinez MA, Hernandez L, Hardisson D, Reis-Filho JS, Palacios J (2007) Sox2: a possible driver of the basal-like phenotype in sporadic breast cancer. Mod Pathol 20(4):474–481. https://doi.org/10.1038/modpathol.3800760
21. Pham DL, Scheble V, Bareiss P, Fischer A, Beschorner C, Adam A, Bachmann C, Neubauer H, Boesmueller H, Kanz L, Wallwiener D, Fend F, Lengerke C, Perner S, Fehm T, Staebler A (2013) SOX2 expression and prognostic significance in ovarian carcinoma. Int J Gynecol Pathol 32(4):358–367. https://doi.org/10.1097/PGP.0b013e31826a642b
22. He QZ, Luo XZ, Wang K, Zhou Q, Ao H, Yang Y, Li SX, Li Y, Zhu HT, Duan T (2014) Isolation and characterization of cancer stem cells from high-grade serous ovarian carcinomas. Cell Physiol Biochem 33(1):173–184. https://doi.org/10.1159/000356660
23. Orford KW, Scadden DT (2008) Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. Nat Rev Genet 9(2):115–128. https://doi.org/10.1038/nrg2269

24. Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM (2012) High-grade serous ovarian cancer arises from fallopian tube in a mouse model. Proc Natl Acad Sci U S A 109(10):3921–3926. https://doi.org/10.1073/pnas.1117135109
25. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90. https://doi.org/10.3322/caac.20107
26. Chen X, Zhang J, Zhang Z, Li H, Cheng W, Liu J (2013) Cancer stem cells, epithelial-mesenchymal transition, and drug resistance in high-grade ovarian serous carcinoma. Hum Pathol 44(11):2373–2384. https://doi.org/10.1016/j.humpath.2013.05.001
27. Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, Li C, Wang LP, Roby KF, Orsulic S, Connolly DC, Zhang Y, Montone K, Butzow R, Coukos G, Zhang L (2010) Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS One 5(4):e10277. https://doi.org/10.1371/journal.pone.0010277
28. Landen CN Jr, Goodman B, Katre AA, Steg AD, Nick AM, Stone RL, Miller LD, Mejia PV, Jennings NB, Gershenson DM, Bast RC Jr, Coleman RL, Lopez-Berestein G, Sood AK (2010) Targeting aldehyde dehydrogenase cancer stem cells in ovarian cancer. Mol Cancer Ther 9(12):3186–3199. https://doi.org/10.1158/1535-7163.MCT-10-0563
29. Chui MH, Wang Y, Wu RC, Seidman J, Kurman RJ, Wang TL, Shih Ie M (2015) Loss of ALDH1A1 expression is an early event in the pathogenesis of ovarian high-grade serous carcinoma. Mod Pathol 28(3):437–445. https://doi.org/10.1038/modpathol.2014.89
30. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ (2008) Efficient tumour formation by single human melanoma cells. Nature 456(7222):593–598. https://doi.org/10.1038/nature07567
31. Li H, Bitler BG, Vathipadiekal V, Maradeo ME, Slifker M, Creasy CL, Tummino PJ, Cairns P, Birrer MJ, Zhang R (2012) ALDH1A1 is a novel EZH2 target gene in epithelial ovarian cancer identified by genome-wide approaches. Cancer Prev Res (Phila) 5(3):484–491. https://doi.org/10.1158/1940-6207.CAPR-11-0414
32. Chang B, Liu G, Xue F, Rosen DG, Xiao L, Wang X, Liu J (2009) ALDH1 expression correlates with favorable prognosis in ovarian cancers. Mod Pathol 22(6):817–823. https://doi.org/10.1038/modpathol.2009.35
33. Liebscher CA, Prinzler J, Sinn BV, Budczies J, Denkert C, Noske A, Sehouli J, Braicu EI, Dietel M, Darb-Esfahani S (2013) Aldehyde dehydrogenase 1/epidermal growth factor receptor coexpression is characteristic of a highly aggressive, poor-prognosis subgroup of high-grade serous ovarian carcinoma. Hum Pathol 44(8):1465–1471. https://doi.org/10.1016/j.humpath.2012.12.016
34. Hellner K, Miranda F, Fotso Chedom D, Herrero-Gonzalez S, Hayden DM, Tearle R, Artibani M, KaramiNejadRanjbar M, Williams R, Gaitskell K, Elorbany S, Xu R, Laios A, Buiga P, Ahmed K, Dhar S, Zhang RY, Campo L, Myers KA, Lozano M, Ruiz-Miro M, Gatius S, Mota A, Moreno-Bueno G, Matias-Guiu X, Benitez J, Witty L, McVean G, Leedham S, Tomlinson I, Drmanac R, Cazier JB, Klein R, Dunne K, Bast RC Jr, Kennedy SH, Hassan B, Lise S, Garcia MJ, Peters BA, Yau C, Sauka-Spengler T, Ahmed AA (2016) Premalignant SOX2 overexpression in the fallopian tubes of ovarian cancer patients: discovery and validation studies. EBioMedicine 10:137–149. https://doi.org/10.1016/j.ebiom.2016.06.048
35. Lengerke C, Fehm T, Kurth R, Neubauer H, Scheble V, Muller F, Schneider F, Petersen K, Wallwiener D, Kanz L, Fend F, Perner S, Bareiss PM, Staebler A (2011) Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma. BMC Cancer 11:42. https://doi.org/10.1186/1471-2407-11-42