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Loss of Gelsolin expression in human ovarian carcinomas

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Abstract

The ubiquitously expressed actin-binding protein, gelsolin, is known to play a role in the modulation of the actin network and in the regulation of cell growth and cell motility. In the present study, we analysed the expression of gelsolin in 241 matched cDNA pairs from human normal and tumour tissues using a Cancer Profiling Array. We found a decreased expression of gelsolin in cancer tissue from female reproductive organs, including the ovary. On a protein level, we examined the expression of gelsolin in human ovarian cancer cell lines and in a set of 110 cases of human benign and malignant ovarian tumours. Low levels of gelsolin protein were observed in four of six ovarian carcinoma cell lines, in contrast to its expression in normal ovarian surface epithelial cells. In addition, we found a reduced expression of gelsolin in borderline tumours and ovarian carcinomas compared with the epithelium of normal ovaries and benign adenomas. Decreased gelsolin expression was associated with poorly differentiated carcinomas ($p = 0.014$). No significant association between gelsolin expression and other clinicopathological markers or patient survival could be established. In addition, we investigated the growth regulatory function of gelsolin in human ovarian cancer cell lines using cDNA transfections. Re-expression of gelsolin in OAW42 and ES-2 cells resulted in a suppression of tumour cell survival *in vitro*. To explore the mechanism responsible for the downregulation of gelsolin expression in ovarian carcinoma cells, we treated cells with inhibitors of DNA methylation and histone deacetylation. We observed an upregulation of gelsolin in ovarian cancer cells after treatment with both types of inhibitor. Our results suggest that gelsolin might be involved in the growth regulation of human ovarian cancer.

Keywords: Ovarian cancer; Gelsolin

1. Introduction

Ovarian carcinoma is characterised by an unfavourable prognosis and this depends on the stage of disease and to some extent, on the patient's age, histological type and tumour grade. At present, the molecular basis of ovarian carcinogenesis remains poorly understood. Experiments using cDNA microarray-based technologies have shown a large number of genes with differential expression profiles in ovarian carcinomas compared with normal ovaries [1,2]. Nevertheless, there is a lack of diagnostic and prognostic factors for this disease.

To identify novel genes involved in ovarian carcinogenesis, we recently hybridised microarrays with cDNAs derived from normal human ovaries and advanced stage ovarian carcinomas [3]. This analysis revealed a downregulation of the gelsolin gene in ovarian carcinoma samples.

Gelsolin is an actin-binding protein involved in dynamic changes of the actin cytoskeleton. The protein regulates the length of actin filaments by severing and capping the fast-growing (barbed) filament ends and

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promotes actin nucleation. Gelsolin is activated by calcium and inhibited by polyphosphoinositides, particularly phosphatidylinositol 4,5-bisphosphate (PIP2). Actin assembly and disassembly plays a critical role in cell shape, motility, cell growth, and differentiation [4]. In addition, gelsolin interacts with several signal transduction pathways [5–7]. Gelsolin exists in a cytoplasmic and an extracellular form. Both variants are derived from a single gene by alternative transcription initiation sites and differential splicing [8].

In normal tissues, gelsolin is widely expressed [9]. In mouse ovary, it is predominantly expressed in superficial epithelial cells, endothelium and in cells of the theca externa and stroma [10]. Little is known about its expression in human normal and malignant ovarian tissues. Decreased gelsolin expression occurs in many transformed cell types [11] and in carcinomas of the colon, bladder, breast, lung and prostate, suggesting gelsolin may act as a tumour supressor [12–16].

To study the role of gelsolin in the biology of human ovarian cancer, we investigated the expression and regulation in human normal and malignant ovarian tissue samples, as well as in human ovarian cancer cell lines. The aim of our study was to evaluate the association between the expression pattern of gelsolin and clinicopathological features, as well as any association with patient survival.

2. Materials and methods

2.1. Cancer profiling array

The Cancer Profiling Array (Clontech™) contains 241 matched cDNA pairs from normal and corresponding tumour tissues, including breast, uterus, colon, stomach, ovary, lung, kidney, rectum, thyroid gland, cervix, small intestine, pancreas and prostate [17]. Total RNAs were isolated from histologically validated tissue samples by microdissection. The cDNA samples were generated using SMART technology and represent the entire mRNA population from an individual sample. All cDNA probes for a tissue type are normalised based on the expression of four housekeeping genes (ubiquitin, 23-kDa highly basic protein, β-actin, glutamase dehydrogenase) and spotted on a nylon membrane. A gelsolin-specific cDNA was radiolabelled, hybridised overnight, washed and exposed to X-ray film, as described by Sers and colleagues [18]. Signal intensities for individual spots were quantified using a phosphorimager (BioRad, Hercules, CA).

2.2. Study population and tissue samples

Immunohistochemical examination was performed retrospectively on the tissue samples taken for routine diagnostic and therapeutic purposes. Formalin-fixed, paraffin-embedded specimens of 110 patients with normal ovaries and benign or malignant epithelial ovarian tumours, who were diagnosed at the Institute of Pathology, University Hospital Charité Berlin, Germany, between 1991 and 2003, were investigated. A histopathological diagnosis of the tumours was performed according to the World Health Organisation criteria. The tissues included 7 normal ovaries, 18 benign cystadenomas, 10 borderline tumours and 75 invasive ovarian carcinomas. Tumour grading was assessed according to the Silverberg system based on architectural, nuclear, and mitotic features [19].

At the time of diagnosis, the mean age of the patients was 58.9 years (range 22–90 years). Of the 75 invasive carcinomas, 48 (64%) were serous carcinomas, 5 (7%) mucinous carcinomas, 8 (11%) endometrioid carcinomas, 4 (5%) clear cell carcinomas, 2 (2%) transitional cell carcinomas, and 8 (11%) undifferentiated carcinomas. Of the patients with invasive carcinomas, 14 (19%) were International Federation of Gynecology and Obstetrics (FIGO) stage I, 11 (15%) were stage II, 47 (62%) were stage III, and 3 (4%) were stage IV. For our statistical evaluation and survival analysis, only the patients with invasive ovarian carcinomas were included. The duration of follow-up ranged from 1.70 to 120.70 months (mean of 35.6 months). For 18 cases of invasive carcinomas, no follow-up data were available.

2.3. Immunohistochemistry

Deparaffinised and rehydrated sections were boiled for 5 min in 0.01 M citrate buffer (pH 6.0) for antigen retrieval. The monoclonal anti-gelsolin mouse antibody (clone GS-2C4, Sigma–Aldrich, St. Louis, USA) was applied for 1 h at a dilution of 1:2000. Normal ovarian surface epithelium, stromal cells, macrophages, endothelial cells served as internal positive controls. Immunostaining was accomplished using a DAKO-ChemMate (Dako, Hamburg, Germany), as recommended by the manufacturer. Sections were counterstained with haematoxylin and mounted in Clarion Mounting Medium (Biomeda corp. Foster City, CA). The specimens were independently evaluated by two pathologists. Immunostaining of gelsolin was semi-quantitatively scored using a composite score obtained by multiplying the values of the mean staining intensity and the percentage of gelsolin-positive cells. The intensity was graded as absent (0), weakly positive (1), moderately positive (2) or strongly positive (3) compared to normal epithelial and stromal cells. The percentage of positive cells was assessed as either no cells (0), less then 10% of the cells (1), 10–50% of the cells (2), 51–80% of the cells (3), and more then 80% of the cells (4). Finally, cases were grouped

as gelsolin-negative (scores 1–6) and -positive (scores 7–12).

2.4. Cell culture

SK-OV-3, ES-2, OVCAR3, CaOV3 and MDA H2774 ovarian carcinoma cell lines were obtained from the American Type Culture Collection and OAW42 cells from the European Collection of Cell Cultures. Cancer cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in Dulbecco's modified Eagles Medium (DMEM) (BioWhittaker, Walkersville, MD) containing 10% foetal calf serum and 2 mM L-glutamine. The HOSE cell line, an immortalised human ovarian surface epithelium cell line [20], was cultivated in a 1:1 mixture of medium 199 (Sigma Chemical Co., St. Louis, MO) and MCDB 105 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% foetal calf serum and 2 mM L-glutamine. The inhibitor of DNA methylation, 5-aza-2′-deoxycytidine (Sigma, St. Louis, USA), was dissolved in phosphate buffer, pH 6.0 and applied at a concentration of 5 μM for 72 h. The inhibitior of the histone deacetylase, Trichostatin A (Sigma, St. Louis, USA), was dissolved in ethanol and applied at 25 ng/ml for 72 h. Appropriate solvent controls were performed in all of the experiments.

2.5. Western blot analysis

To obtain total protein lysates, 2×10^6 cells plated on 10-cm dishes, were washed twice with cold phosphate-buffered saline and incubated on ice for 30 min in RIPA-buffer (150 mmol/l NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 50 mmol/l Tris–HCl, pH 8.0, 2 μg/ml aprotinin). Cells were scraped off the plates and the lysates were mixed with 2× SDS sample buffer (120 mmol/l Tris–HCl, pH 6.8, 0.2 mol/l dithiothreitol, 4% SDS, 20% glycerol, 0.002% bromophenol blue), boiled for 10 min, and centrifuged for 5 min at 12,000*g*. The protein concentration of the supernatants was measured using the amido-black method, as described by Schaffner and Weissmann [21]. Equal amounts of protein (10 μg) were separated on 7.5% polyacrylamide gels by SDS-gel electrophoresis and transferred to polyvinylidene difluoride membranes (Hybond-P; Amersham, Freiburg, Germany). Immunodetection was performed using the gelsolin monoclonal antibody (clone GS-2C4, Sigma–Aldrich, St. Louis, USA) at a dilution of 1:1500 followed by detection with the enhanced chemiluminescence system (Amersham). To ensure equal loading amounts, the blots were stripped in 200 mmol/l glycine, 1% Tween 20, 0.1% SDS, pH 2.2, for 2 h at room temperature and reprobed using a monoclonal antibody to actin (clone C4, Chemicon, Temecula, CA) at a dilution of 1:5000.

2.6. Colony formation assay

OAW42 and ES-2 cells (2×10^5) were plated onto 25 cm^2 tissue culture flasks and transfected after 24 h with 1.5 μg of the LKCG gelsolin expression plasmid (kindly provided by Dr. D.J. Kwiatkowski, Harvard University, Boston, MA) or the empty pHβ vector as a control using Fugene 6 transfection reagent (Roche), according to the manufacturer's instructions [22,23]. Forty-eight hours after transfection, 800 μg/ml (OAW42) and 1000 μg/ml (ES-2) of geneticin (G418) was added to the culture medium and the colonies were stained and counted after 10 days of selection. To evaluate the transfection efficiency, OAW42 and ES-2 cells were transfected with an EGFP plasmid (enhanced green fluorescent protein, BD Biosciences Clontech, Paolo Alto, USA) and viewed under ultraviolet (UV) light 48 h after the transfection.

2.7. Statistics

Statistical analyses were performed using the Chi-square and Fisher's exact tests. The probability of overall survival as a function of time was determined by the Kaplan–Meier method and the log-rank test. Differences were considered significant when the Confidence Intervals (CIs) were >95% ($p < 0.05$). For the statistical evaluation, the Statistical Package for the Social Sciences (SPSS) software version 11.0 was used.

3. Results

3.1. Gelsolin is differentially expressed in ovarian carcinomas

We examined the cancer-specific changes of gelsolin expression in 241 matched normal and cancerous cDNA pairs using a Cancer Profiling Array (Clontech™) (Fig. 1(a)). This analysis revealed a downregulation of gelsolin in 9 of 14 ovarian tumour cDNAs compared with the corresponding cDNA from normal tissues. In Fig. 1(b), both an enlarged panel of cDNA pairs for ovarian samples and the calculated ratio of each individual patient is indicated. Additionally, two cDNA samples derived from a metastasis of the preceding tumour pair are shown. A similar downregulation was observed in 49 of 50 breast tumours, as well as in 41 of 42 uterine tumours. In carcinomas of the colon and lung, a downregulation was also found. In contrast, cDNA pairs derived from stomach, kidney, rectum, thyroid gland, cervix, small intestine, pancreas and prostate exhibited no such differential expression patterns.

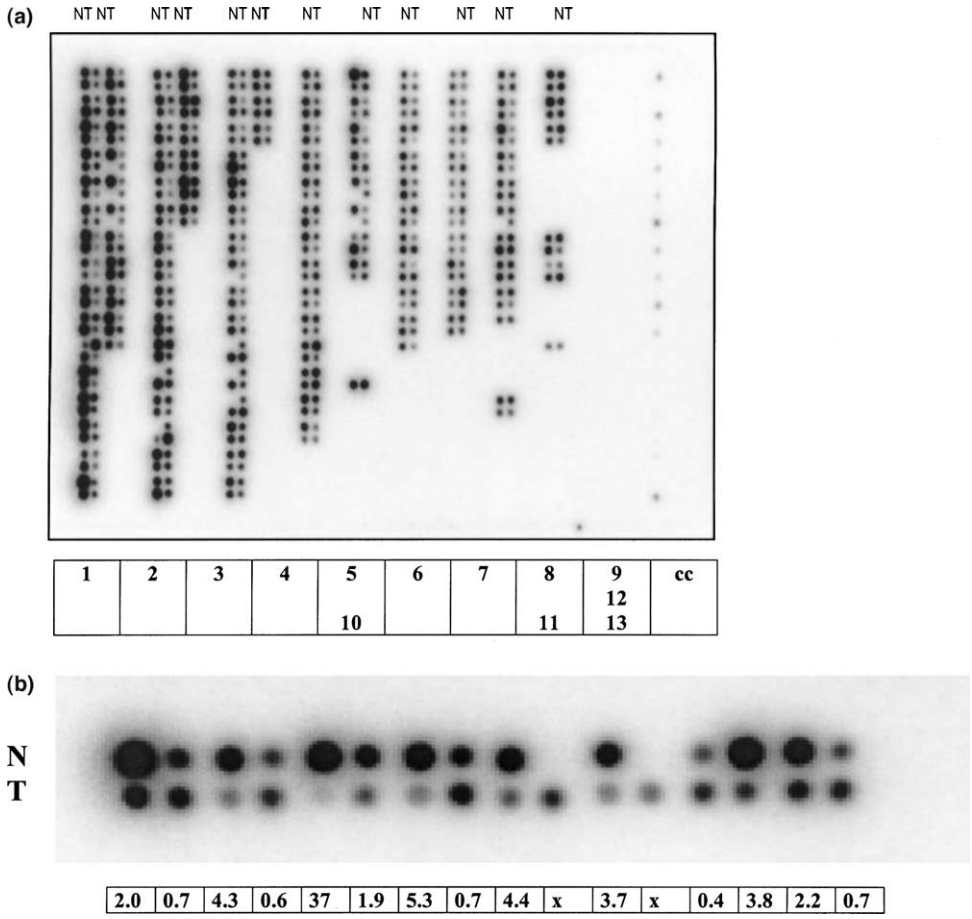

Fig. 1. (a) A Cancer Profiling Array representing cDNA pools from matched tumours and normal tissues was hybridised with a gelsolin-specific probe. Individual spots were quantified by phosphorimaging. Numbers indicate the tissue types in columns. 1, breast; 2, uterus; 3, colon; 4, stomach; 5, ovary; 6, lung; 7, kidney; 8, rectum; 9, thyroid gland; 10, cervix; 11, small intestine; 12, pancreas; 13, prostate. N, normal; T, tumour; cc, cancer cell line cDNAs. (b) Enlarged panel of cDNA pairs for ovary – demonstrating downregulation of gelsolin in ovarian carcinomas. cDNAs representing normal ovarian tissues are spotted in the upper row (N), cDNAs prepared from the corresponding ovarian carcinomas are spotted in the lower row (T), "x" indicates a cDNA derived from a metastasis of the preceding tumour pair. A ratio for each of the 14 ovarian cDNA pairs (normal/ tumour) was calculated and is shown below.

3.2. Gelsolin is downregulated in ovarian carcinomas and cell lines

Samples from a total of 110 patients were investigated for gelsolin immunoreactivity (Table 1). Our analysis revealed a strong cytoplasmic expression of the protein in normal ovarian surface epithelium (Fig. 2(a)), cortical stroma and endothelium. In benign serous cysts, as well as in serous and mucinous cystadenomas, a comparable strong epithelial expression was detectable (Fig. 2(b)). In contrast, tumours of borderline malignancy, and particularly carcinomas, displayed a reduced expression level of gelsolin (Fig. 2(c)). Most of the invasive carcinomas (85%) exhibited no or only weak expression (scores 1–6), whereas a moderate or strong expression level was found in 15% of the carcinomas (scores 7–12) (Fig. 2(d)).

We used Western blotting analysis to examine the expression of gelsolin in immortalised ovarian surface epithelium (HOSE) cells and six human ovarian cancer cell lines (Fig. 3). Expression was observed in HOSE cells, as well as in SK-OV-3 and CaOV3 cancer cell

Table 1
Expression of gelsolin in normal ovaries, ovarian adenomas and carcinomas

Gelsolin expression	Normal ovaries	Benign adenomas	Borderline tumours	Invasive carcinomas	*p*-value (χ^2 test)
Negative	0/7 (0%)	0/18 (0%)	8/10 (80%)	64/75 (85%)	0.0001
Positive	7/7 (100%)	18/18 (100%)	2/10 (20%)	11/75 (15%)	

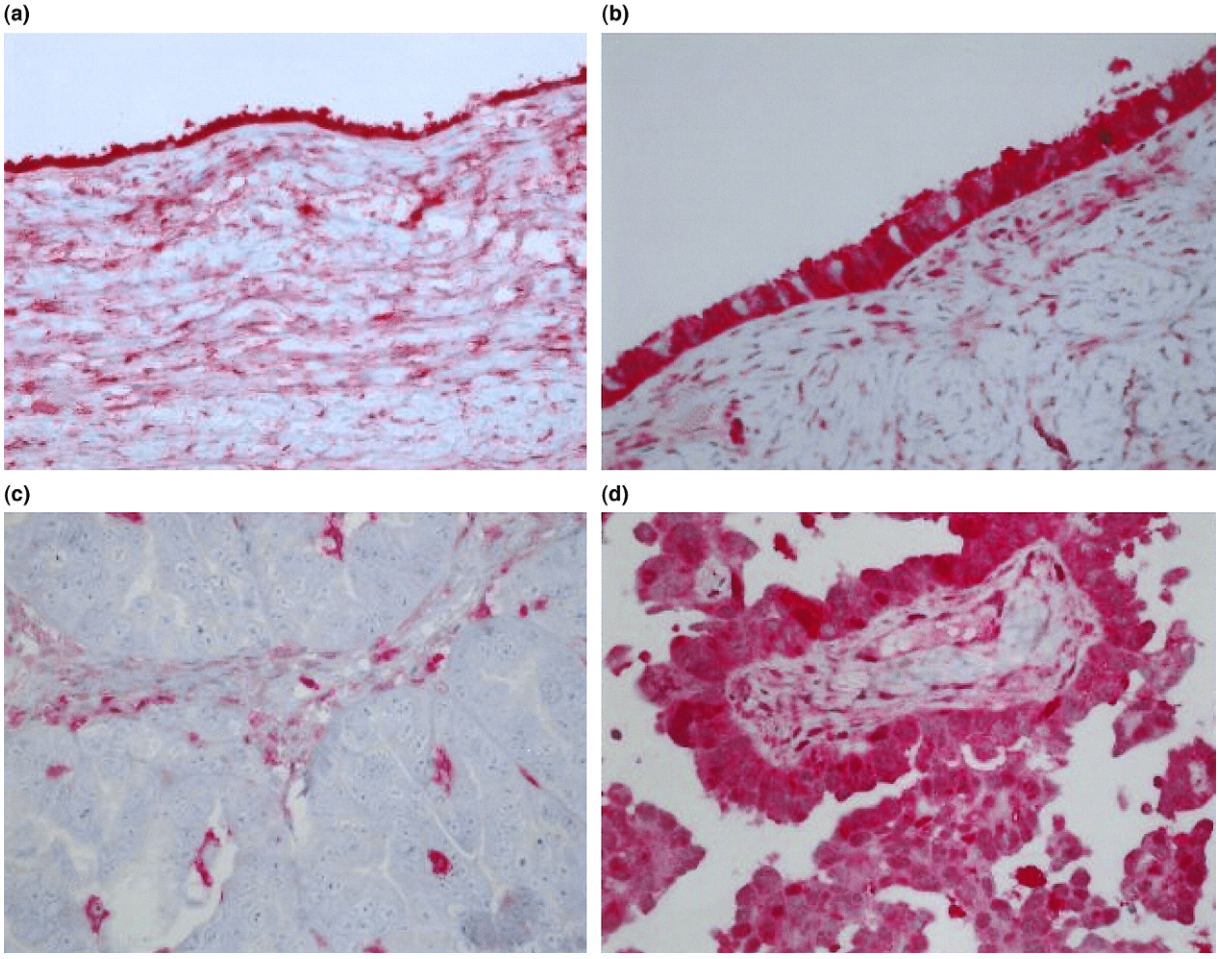

Fig. 2. Immunohistochemical analysis of gelsolin expression. (a) The ovarian surface epithelium and the underlying stroma of normal ovary tissue shows strong gelsolin expression. (b) A similar staining can be observed in the epithelial lining of a serous cystadenoma and in the underlying stromal tissue. (c) Complete loss of gelsolin expression in tumour cells of a serous ovarian carcinoma. Intratumoral stromal cells and scattered macrophages are strongly positive. (d) In contrast, strong expression of gelsolin is seen in a well-differentiated serous carcinoma of the ovary.

lines. In contrast, only low amounts of gelsolin were found in ES-2, OAW42, OVCAR3 and MDAH2774 cells.

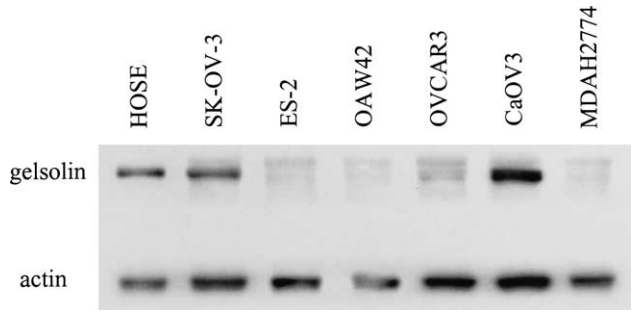

Fig. 3. Western Blotting analysis of gelsolin expression in human ovarian cancer cell lines. In the ovarian surface epithelium cells (HOSE) and in two ovarian carcinoma cell lines (SK-OV-3, CaOV3), gelsolin was present, whereas decreased expression was observed in the other cell lines. The blot was stripped and reprobed with an antibody raised against actin to control for equal loading.

3.3. Gelsolin expression and clinicopathological parameters

Decreased gelsolin expression was associated with tumour grade. A reduced gelsolin expression was observed in 9 of 12 G1-carcinomas, in 24 of 31 G2-carcinomas and in 31 of 32 G3-carcinomas. Poorly differentiated ovarian carcinomas were more frequently gelsolin-negative ($p = 0.014$). All undifferentiated carcinomas were gelsolin-negative. Concerning the other clinicopathological factors, no difference between gelsolin-positive and -negative tumours was noted (Table 2). No impact on progressive-free and overall survival in univariate analyses was seen (data not shown).

3.4. Downregulation of gelsolin involves hypermethylation and histone acetylation

To evaluate whether epigenetic modification is responsible for the inactivation of gelsolin, we treated ovarian cancer cells with inhibitors of DNA methylation

Table 2
Association of gelsolin expression with clinicopathological features in patients with invasive ovarian carcinomas

Characteristics	*n*	Gelsolin-negative	Gelsolin-positive	*p*-value
All carcinomas	75	64 (85%)	11 (15%)	
Age at diagnosis (years)				0.59[a]
<60	35	30 (86%)	5 (14%)	
>60	40	34 (85%)	6 (12%)	
Histological type				0.16[b]
Serous	48	42 (88%)	6 (13%)	
Non-serous	19	14 (74%)	5 (26%)	
Undifferentiated	8	8 (100%)	0 (0%)	
FIGO Stage				0.26[b]
I	14	10 (71%)	4 (29%)	
II	11	10 (91%)	1 (9%)	
III+IV	50	44 (88%)	6 (12%)	
pT				0.18[b]
1	18	13 (72%)	5 (28%)	
2	13	12 (92%)	1 (8%)	
3	44	39 (89%)	5 (11%)	
pN				0.38[a]
0	21	17 (81%)	4 (19%)	
1	28	26 (93%)	2 (7%)	
Grading				0.014[a]
1–2	43	33 (77%)	10 (23%)	
3	32	31 (97%)	1 (3%)	

FIGO, International Federation of Gynecology and Obstetrics.
[a] Fisher's test.
[b] χ^2 test.

(5-aza-2′deoxycytidine) and histone deacetylation (Trichostatin A). To reveal a induction of gelsolin re-expression, we carried out analyses at different incubation times and concentrations of the inhibitors, as well as at different cell densities. By 72 h of treatment with 5 μM 5-aza-2′deoxycytidine and 25 ng/ml Trichostatin A, a strong upregulation of gelsolin was observed in OAW42 and OVCAR3 cells (0.5×10^6) (Fig. 4). After 72 h of exposure and higher concentrations, a decreased viability of the cells was observed and most of the cells died within 96 h.

3.5. Expression of gelsolin reduces the growth of ovarian carcinoma cells

To explore whether gelsolin affects the growth of human ovarian carcinoma cells, we transfected OAW42 and ES-2 cells with a gelsolin expression vector (LKCG). In a colony formation assay, growth of G418-resistant clones harbouring the gelsolin expression vector or a control vector (pHβ) was measured. Gelsolin re-expression resulted in a reduction of colony formation in OAW42 cells by 74% and in ES-2 cells by 28% in four independent experiments (Fig. 5).

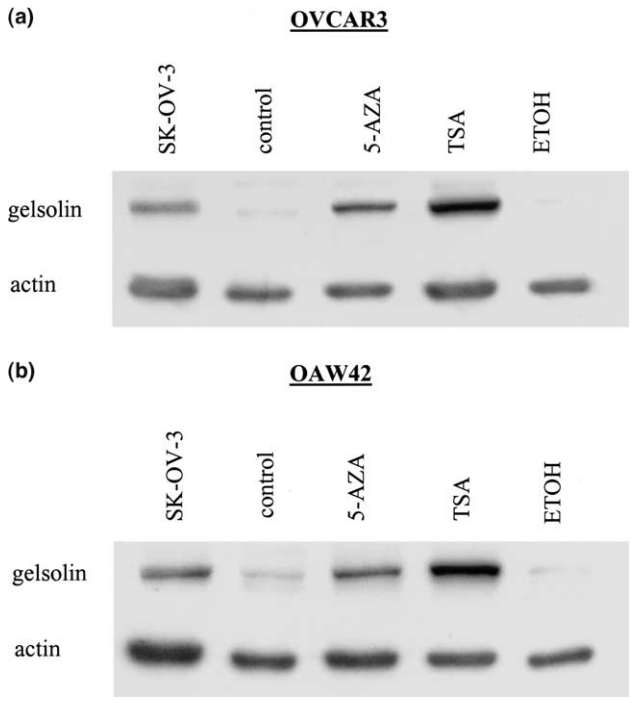

Fig. 4. Gelsolin expression can be reconstituted in OVCAR3 (a) and OAW42 (b) ovarian carcinoma cells by treatment with the DNA methylation inhibitor, 5-aza-2′deoxycytidine, and the histone deacetylase inhibitor, Trichostatin A (TSA). Western blotting analysis was performed using SK-OV-3 cells as a positive control (lane 1), extracts from untreated cells (lane 2), 72 h after incubation with 5 μM 5-aza-2′deoxycytidine (lane 3), with 25 ng/ml TSA (lane 4) and with the solvent ethanol (lane 5). To ensure equal loading, the blots were stripped and reprobed with an antibody raised against actin.

4. Discussion

In the present study, we investigated the expression pattern of the actin filament regulatory protein gelsolin

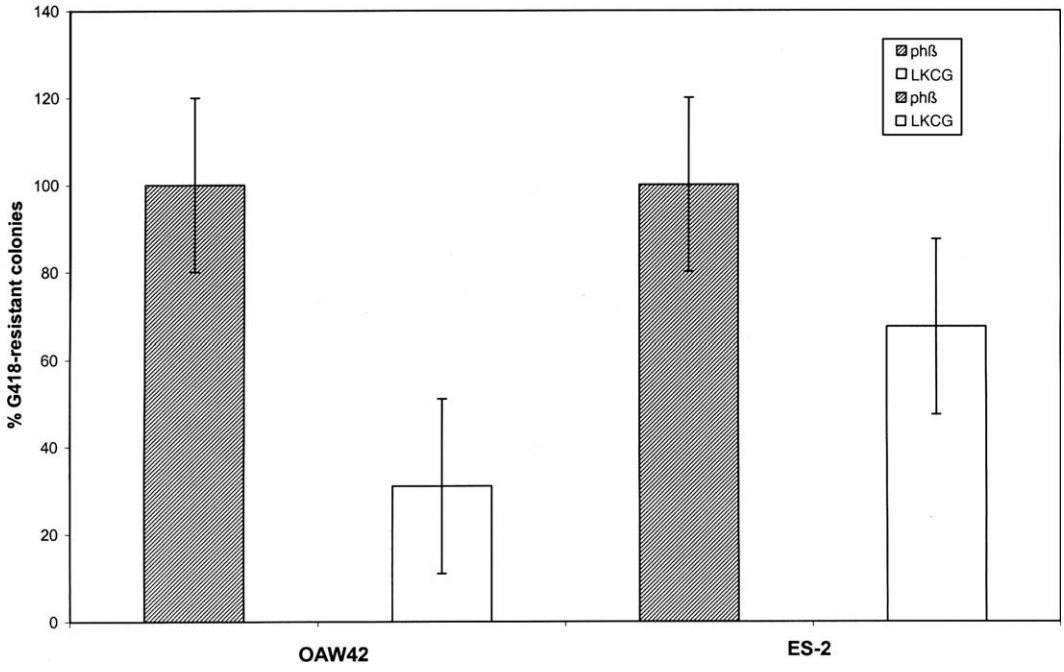

Fig. 5. Colony formation assay demonstrating growth suppression of OAW42 and ES-2 cells following the expression of gelsolin. Both cell lines were transfected with either gelsolin expression vector (LKCG) or an empty vector (pHβ) as a control. The formation of G418-resistant colonies was measured. The mean values of the counted colonies from four independent experiments are shown. The colonies/cm^2 obtained after transfection with the control plasmid were set at 100% in each experiment.

in human ovarian carcinomas. Recently, we hybridised microarrays with cDNAs derived from normal human ovaries and advanced stage ovarian carcinomas and discovered a downregulation of gelsolin in ovarian cancer samples [3]. Here, we confirm the loss of gelsolin expression using a Cancer Profiling Array. Based on these findings, we analysed gelsolin expression in a larger tumour collection by immunohistochemistry and confirmed the loss of or reduced levels of gelsolin protein in 85% of the ovarian carcinomas compared with normal ovarian tissues and benign ovarian lesions. These observations are to a large extent similar to the results described previously by Afify and Werness [24] who investigated a limited set of ovarian carcinomas. However, the authors found an increased gelsolin expression in ovarian clear cell carcinomas which is not consistent with our findings. In our study, ovarian carcinomas with reduced gelsolin expression had sometimes few positive cell clusters with a heterogenous intratumoral distribution. We did not observe single positive cells at the leading edge of the tumour, as has been described in breast carcinomas [25].

In our analysis, the reduced expression of gelsolin in ovarian carcinomas was significantly associated with a higher tumour grade. No association of gelsolin with other clinicopathological factors, including patient survival, could be established. In contrast, other authors have demonstrated that gelsolin expression is positively linked to high-grade urothelial and renal cell carcinomas [26,27]. Gelsolin displayed a biphasic expression pattern in urothelial carcinomas, where a reduced expression in non-invasive lesions and low-grade tumours, as well as an increased expression in invasive tumours, was observed [26]. In addition, loss of gelsolin expression was associated with decreased patient survival in invasive breast carcinomas [28].

Decreased expression of gelsolin has been shown in several types of human cancers (colon, bladder, breast, lung and prostate) suggesting a possible role as a tumour suppressor [12–16]. We transfected gelsolin into ovarian carcinoma cells, OAW42 and ES-2, and found a reduction in colony formation which suggests it has a growth inhibitory function. This observation is consistent with previous studies showing a strong inhibitory effect of re-expressed gelsolin on colony formation in melanoma and bladder cancer cells [13,29]. The authors also reported a reduction in chemotactic cell migration and tumorigenicity. Furthermore, tumour growth and the formation of lung metastasis have been repressed by gelsolin *in vivo* [29]. However, effects related to different transfection efficiencies cannot be completely excluded in our transfection system. To further investigate the effects of gelsolin on cell proliferation, it would be interesting to use dominant-negative expression vectors for gelsolin.

Although gelsolin can suppress tumour progression, it was also shown that malignant tumours overexpressing gelsolin were associated with a poor prognosis

[25,30]. In our study, a subset of the ovarian carcinomas (15%) displayed a moderate or strong gelsolin expression. By contrast, Thor and colleagues described an overexpression of gelsolin in 56% of breast cancers which was associated with the overexpression of c-erbB-2 and EGFR, as well as a more aggressive tumour phenotype [25]. An upregulation of gelsolin and a negative effect on prognosis have been reported in non-small lung cancer [30]. High gelsolin expression is an independent marker for tumour recurrence and progression in urothelial tumours, particularly for high-grade variants [26].

It is known that gelsolin overexpression enhances the motility of fibroblasts [22]. This was demonstrated using NIH3T3 cells and fibroblasts derived from gelsolin-deficient mice. Overexpression of gelsolin in immortalised NIH3T3 cells causes increased motility, but fibroblasts from gelsolin knock-out mice showed a reduced cell motility [5,22,31].

Gelsolin is involved in c-erbB-2/ EGFR signalling, via Rac, phosphatidylinositol-3-kinase (PI3K) and phospholipase C (PLC), which results in dynamic changes of the actin cytoskeleton and increased cell motility [5–7]. Such enhanced motility is important for tumour cell migration and is associated with invasive growth. This suggests that during malignant transformation a proportion of tumours overexpress gelsolin resulting in invasion and metastasis.

The exact mechanism of downregulation of gelsolin has yet to be defined. Until now, mutations within the gelsolin gene – located on chromosome 9q33 – have not been identified in malignancies [13,15,16,32]. In contrast, loss of heterozygosity (LOH) has been observed in different tumours [13,33], but neither mutations nor LOH have been detected in the gelsolin gene in human breast cancer cell lines and tissues [34,35]. Alternatively, several tumour suppressor genes are silenced by aberrant promoter methylation associated with transcriptional loss and the absence of mutations within the coding regions. To our knowledge, the type of inactivation of gelsolin has not yet been analysed in ovarian carcinomas. The gelsolin gene encodes both a cytoplasmic and a secreted form from alternative promoters. Only the promoter for the cytoplasmic form of gelsolin is embedded within the CpG islands. In our study, the incubation of ovarian carcinoma cell lines with inhibitors of DNA methylation (5-aza-2′deoxycytidine) and histone deacetylation (TSA) revealed a strong upregulation of gelsolin protein, where the effect of TSA seems to be slightly stronger. The concerted action of methylation of regulatory gene regions and histone deacetylation is one possible mechanism to suppress gelsolin expression in ovarian carcinoma.

Mielnicki and colleagues [34] demonstrated an upregulation of gelsolin RNA and protein in breast cancer cell lines after treatment with TSA and, to a lesser extent with the demethylating agent 5′ azacytidine, suggesting a downregulation of gelsolin in breast cancer cells by epigenetic modifications, primarily through changes in histone acetylation. In addition, a strong association exists between various external stimuli (e.g. growth factors, chemotactic molecules), signalling pathways, such as c-erbB-2, Rac, Rho, PI3K, and gelsolin expression [4,6,25]. Dong and colleagues [36] also showed that reduced gelsolin is due to decreased promoter activity resulting, in part, from negative regulation by the activating transcription factor 1 (ATF1).

In summary, our study shows a significant reduction of gelsolin expression in human ovarian carcinomas, predominantly in high-grade variants. The protein revealed growth suppressive activity in ovarian cancer cells. Therefore, our results suggest a role for gelsolin in the progression of ovarian cancer. Inactivation of gelsolin might be mediated by epigenetic modification. It is widely accepted that genes affected by epigenetic events can serve as new therapeutic targets for tumours [32]. Some inhibitors of histone deacetylase are already entered in clinical trials in patients with cancer [37,38]. Thus, reconstitution of gelsolin by inhibitors of histone deacetylase could be a promising therapeutic intervention in ovarian cancer.

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