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Actinin-4 gene amplification in ovarian cancer: a candidate oncogene associated with poor patient prognosis and tumor chemoresistance

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Actinin-4, an isoform of non-muscular α-actinin, enhances cell motility by bundling the actin cytoskeleton. We previously reported a prognostic implication of high immunohistochemical expression of actinin-4 protein in ovarian cancers. Chromosomal gain or amplification of the 19q12–q13 region has been reported in ovarian cancer. We hypothesized that the actinin-4 (*ACTN4*) gene might be a target of the 19q12–q13 amplicon and play an essential role of ovarian cancer progression. In total, 136 advanced-stage ovarian cancers were investigated for the copy number of the *ACTN4* gene on chromosome 19q13, using fluorescence *in situ* hybridization, and the correlation of the *ACTN4* copy number with actinin-4 protein immunoreactivity and major clinicopathological factors was investigated. A higher copy number (≧4 copies) of the *ACTN4* gene was detected in 29 (21%) cases and was highly associated with the intensity of actinin-4 immunoreactivity ($P<0.0001$), a high histological tumor grade ($P=0.030$), a clear-cell adenocarcinoma histology ($P=0.012$), resistance to first-line chemotherapies ($P=0.028$), and poor patient outcome ($P=0.0011$). Univariate analyses using the Cox regression model showed that a higher *ACTN4* gene copy number was able to predict patient outcome more accurately than high actinin-4 immunoreactivity (relative risk: 2.48 *vs* 1.55). Multivariate analysis showed that a higher copy number of the *ACTN4* gene and the degree of residual disease were independent prognostic factors for overall patient survival. The actinin-4 gene may be a target of the 19q amplicon, acting as a candidate oncogene, and serve as a predictor of poor outcome and tumor chemoresistance in patients with advanced-stage ovarian cancers.
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Epithelial ovarian cancer, accounting for 90% of all ovarian malignant tumors, is the leading cause of death among female genital malignancies.[1] Because of its insidious onset and lack of an effective early diagnostic test, up to 70% of cases are diagnosed at an advanced stage with extensive peritoneal dissemination and/or distant metastasis, resulting in an extremely poor prognosis.[1] Although ovarian cancer progression is a multistep process, involving local invasion, infiltration into vessels, survival in the circulation, extravasation, and growth at secondary sites, enhancement of cancer cell motility is also considered necessary.[2,3]

α-Actinin crosslinks the actin cytoskeleton, and two types of non-muscle α-actinins—actinin-1 and actinin-4—have been identified.[4,5] Actinin-1, localized at the ends of actin stress fibers, plays an important role in stabilizing cell adhesion through association with cell adhesion molecules such as integrin-β and α-catenin.[6,7] On the other hand, actinin-4 protein is highly concentrated at the leading edge of the cytoplasm of motile cells or actively moving structures such as cell surface

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ruffles, and is not localized to focal adhesion plaques or adherent junctions.[5,8–10]

In human cancer cells showing enhanced motility, the cytoplasmic expression levels of actinin-4 protein are significantly increased.[5,10] Recent studies have shown that cytoplasmic overexpression of actinin-4 is associated with various clinicopathological parameters in some human carcinomas: histologically invasive phenotypes of breast cancer,[5] lymph node metastasis of colorectal cancer,[10] poor prognosis of breast cancers, non-small cell lung cancers, and pancreatic cancer.[5,11,12]

Previously, using immunohistochemistry, we detected high actinin-4 protein expression in the cytoplasm in 52% (137 of 265 cases) of ovarian cancers, and this was significantly associated with a clinically advanced tumor stage, a serous adenocarcinoma histology, high-grade tumor histology, a high degree of residual tumor after initial surgery, and a poor patient outcome.[13] Multivariate analysis indicated that high actinin-4 expression can be a prognostic indicator that is independent of clinical stage and histologic subtype.[13] Therefore, as is the case in other solid cancers, the cytoplasmic accumulation of actinin-4 protein in ovarian cancer cells is suggested to be associated with tumor cell motility, invasiveness, and metastatic potential.

The actinin-4 (*ACTN4*) gene is localized to chromosome region 19q13.2 (http://www.ncbi.nlm.nih.gov/mapview/). In ovarian cancer, chromosomal gain or high copy-number amplification of 19q12–q13 in the form of a homogeneously staining region has been reported, and this region contains several candidate oncogenes such as *cyclin E*, *AKT2*, and *SEI-1*.[14–18] In the present study, we hypothesized that amplification of the *ACTN4* gene might be a target of the 19q amplicon and play an essential role in ovarian cancer progression. Therefore, we investigated the copy number of the *ACTN4* gene in chromosome 19q13.2 in 136 advanced-stage ovarian cancers using fluorescence *in situ* hybridization (FISH), and compared it with the intensity of actinin-4 immunoreactivity and major clinicopathological factors.

Materials and methods

Patients and Tissue Samples

This study was performed with the approval of the Internal Review Board on ethical issues. All patients involved in the study gave their informed consent to participate. We reviewed the clinicopathological records of 136 patients who had undergone initial surgery followed by platinum-based chemotherapies for stage III or IV primary ovarian cancer at the Department of Obstetrics and Gynecology, National Defense Medical College Hospital, Tokorozawa, Japan, between 1987 and 2005. None of the patients had undergone neoadjuvant chemotherapy or radiation therapy before surgery.

Formalin-fixed paraffin-embedded tissue samples were obtained at the Department of Laboratory Medicine. All pathology specimens were reviewed in our institution, and the histological types of the tumors were classified according to the WHO criteria.[19] Histological grading, with reference to the grading system proposed by Shimizu *et al*[20] and Silverberg,[21] was performed as described previously.[13] The staging of tumors was assigned according to the International Federation of Gynecology and Obstetrics (FIGO) system. The chemotherapeutic regimens comprised cyclophosphamide, doxorubicin, and cisplatin in 80 patients, paclitaxel and carboplatin in 30, irinotecan and cisplatin in 8, etoposide and cisplatin in 7, docetaxel and carboplatin in 6, cyclophosphamide and cisplatin in 3, irinotecan and carboplatin in 1, and cyclophosphamide and carboplatin in 1.

Clinical response to chemotherapy was evaluated by ultrasonography or computed tomography and classified into complete response, partial response, stable disease, or progressive disease according to the new Response Evaluation Criteria for Solid Tumours (RECIST) guidelines.[22]

Clinicopathological details such as patient age, FIGO clinical stage, histological type and grade of the tumor, residual tumor after initial cytoreductive surgery, response to chemotherapy, and overall survival were assessed for all patients. Clinical response to chemotherapy was assessed for the 92 patients with residual tumors $\geqq$1 cm in size after initial surgery. Details of lymph node status at initial surgery were obtained for 53 cases.

Follow-up was calculated from the date of initial definitive surgery to the date of either last follow-up or death. The average follow-up period after initial surgery was 40 months, ranging between 2 and 168 months. In total, 66 (49%) of 136 patients died because of their tumor burden, and 3 due to other causes.

Tissue Microarray, FISH, and Immunohistochemistry

To construct tissue microarray blocks, we selected formalin-fixed paraffin-embedded cancer tissue blocks from all cases containing the areas that had been used for histological grading. Two core specimens, 2.0 mm in diameter, for each case were taken from these blocks and transferred to recipient blocks using a Tissue Microarrayer (Beecher Instrument, Silver Spring, MD, USA). As a control for FISH analysis described below, non-neoplastic lymphoid tissue was added to each tissue microarray block. These tissue microarray blocks were then cut into 4-μm-thick sections and subjected to both FISH and immunohistochemical analyses.

A bacterial artificial chromosome (BAC) clone RP11-118P21 containing the *ACTN4* gene was selected from a RP11 BAC library. DNA from the BAC clone was isolated and labeled with Spectrum

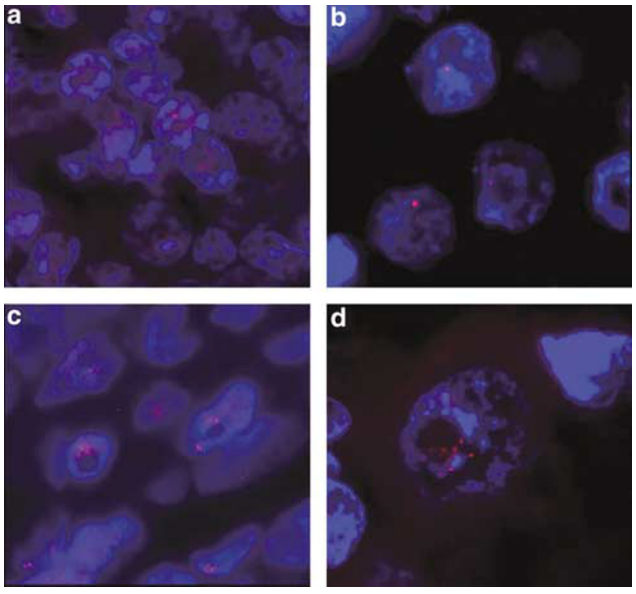

Figure 1 Copy-number status of the *ACTN4* gene determined by fluorescence *in situ* hybridization (FISH) in non-neoplastic lymphocytes and ovarian cancers. (**a**) Lymphocytes, used as a control tissue for FISH analysis. One to two red signals are noted. (**b**) A case of ovarian cancer shows a lower copy number of the *ACTN4* gene. Two to four signals are noted per nucleus. (**c**, **d**) Cases of ovarian cancer showing a higher copy number of the *ACTN4* gene. More than 10 signals are noted in **d**. DAPI-stained interphase nuclei are shown for each specimen (original magnification, ×1000).

Orange (Abbott Molecular, Des Plaines, IL) using a Nick Translation Reagent kit (Abbott Molecular). The labeled BAC clone DNA was subjected to FISH using methods that were the same as those for the PathVysion DNA probe kit (Abbott Molecular), as described previously.[23] Hybridization was performed between the denatured probe and denatured tissue microarray sections at 37°C for 18–24 h. The sections were counterstained with 4,6-diamidino-2-phenylindone. The number of fluorescence signals, corresponding to the copy number of the *ACTN4* gene, in 20 interphase tumor cell nuclei were counted and averaged independently by two observers (SY and HT). These tentative averages of *ACTN4* gene copy numbers in a tumor were compared, and when they differed significantly, the third observer (KO) independently counted a further 20 nuclei in the tumor and averaged the values. The average number among the two observers (40 nuclei), or three observers (60 nuclei) if there was a significant discrepancy, was acquired as the representative *ACTN4* gene copy number of the tumor. Consequently, the *ACTN4* copy numbers of the tumor were classified into two grades: higher and lower. The copy number of the *ACTN4* gene was defined as higher and lower if the representative copy number was ≧4.0 and <4.0, respectively (Figure 1).

The anti-actinin-4 rabbit polyclonal antibody (Ab-2, diluted 1:500) we employed had been raised against the synthetic peptide NQSYQYGPSSA GNGAGC, a unique amino-terminal sequence of actinin-4, as described previously.[10,13] Immunohistochemistry was performed as described previously.[13] Endothelium contained in the tissue microarray cores was used as an internal control for the actinin-4 immunoreaction (Figure 2a and b). As negative controls, the primary antibodies were omitted from each reaction process, and absence of immunoreactivity was confirmed.

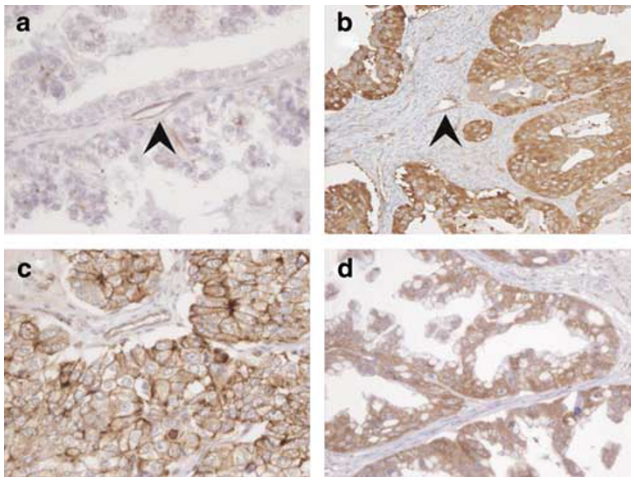

Figure 2 Representative immunohistochemistry for actinin-4 protein in ovarian cancers. (**a**) A case of clear-cell adenocarcinoma showing low actinin-4 expression. Cases showing high cytoplasmic and/or membranous actinin-4 expression with a histology of (**b**) serous adenocarcinoma; (**c**) clear-cell adenocarcinoma; and (**d**) mucinous adenocarcinoma. Note the immunoreactivity in capillary endothelial cells (arrowheads in **a** and **b**) for comparison with that in tumor cells. Original magnification: ×400 in (**a**, **c**); ×200 in (**b**, **d**).

By referring to the intensity of endothelial immunoreactivity as a standard, the intensity of cytoplasmic and/or membranous immunoreactivity was divided into one of two grades: low or high. Actinin-4 expression was considered to be high if the intensity of the cytoplasmic and/or membranous immunoreactivity was equal to or higher than that in the endothelial cells. Otherwise, actinin-4 expression was defined as low. Nuclear immunoreactivity for actinin-4 was not considered in the present study.

Statistical Analysis

Statistical analyses were performed using StatMate III software (ATMS, Tokyo, Japan). Comparisons between parameters were computed by the χ^2 test or Student's *t*-test for unpaired data. For survival analysis, Kaplan–Meier curves were drawn and differences between the curves were calculated by the log-rank test. Independent prognostic significance was computed by the Cox proportional hazards general linear model. Differences at

Table 1 Correlation of the copy number of the *ACTN4* gene with actinin-4 immunoreactivity and clinicopathological parameters in advanced-stage ovarian cancers

		Number of cases (%)		
		Copy number of the ACTN4 gene		
Factor	*Total*	*Higher (≧4 copies)* (n = *29*)	*Low (<4 copies)* (n = *107*)	P-*value*
Actinin-4 immunoreactivity				**<0.0001**
Low expression	54	2 (4)	52 (96)	
High expression	82	27 (33)	55 (67)	
Age, years (median ± s.d.)		57.1 (± 11.1)	55.3 (± 10.7)	0.418
FIGO[a] *stage*				0.338
III	103	20 (19)	83 (81)	
IV	33	9 (27)	24 (73)	
Residual tumor (cm)				0.145
<1	43	6 (14)	37 (86)	
≧1	92	23 (25)	69 (75)	
Unknown	1	1		
Histological grade				**0.030**
1 or 2	93	15 (16)	78 (84)	
3	43	14 (33)	29 (67)	
Histological type (1)				0.081
Serous	91	16 (18)	75 (82)	
Clear cell	25	10 (40)	15 (60)	
Endometrioid	10	2 (20)	8 (80)	
Mucinous	10	1 (10)	9 (90)	
Histological type (2)				**0.012**
Non-clear cell	111	19 (17)	92 (83)	
Clear cell	25	10 (40)	15 (60)	
Lymph node metastasis				0.486
Absent	20	3 (15)	17 (85)	
Present	33	9 (27)	24 (73)	
Response to chemotherapies				**0.028**
Complete response/partial response	54	9 (17)	45 (83)	
Stable disease/progressive disease	38	14 (37)	24 (63)	
5-Year survival (%)		20.9	48.5	**0.0011**[b]

Bold values indicate statistical significance.
[a]International Federation of Gynecology and Obstetrics.
[b]Calculated by log-rank test.

$P<0.05$ were considered to be statistically significant.

Results

Correlation between ACTN4 Gene Copy Number and Actinin-4 Immunoreactivity

The copy number of the *ACTN4* gene ranged from 1.2 to 16.7 (mean 2.83 ± 2.22) in the 136 cases studied (Figure 1). A higher *ACTN4* gene copy number was detected in 29 (21%) of the 136 cases. Of the 136 cases, 82 (60%) and 54 (40%) were classified as showing a high and low immunoreactivity, respectively (Figure 2). A higher copy number of *ACTN4* was highly associated with the intensity of actinin-4 immunoreactivity ($P<0.0001$): 27 (33%) of the 82 cases with high actinin-4 immunoreactivity and only 2 (4%) of the 54 cases with low actinin-4 immunoreactivity showed a higher copy number of the *ACTN4* gene, respectively (Table 1).

ACTN4 Gene Copy Number and its Clinicopathological Significance

In comparison with the lower copy number (<4 copies) group, the higher *ACTN4* gene copy number (≧4 copies) group had histologically high-grade tumors (48% (14 of 29) *vs* 27% (29 of 107), $P=0.030$), a clear-cell histology (34% (10 of 29) *vs* 14% (15 of 107), $P=0.012$), and chemoresistant tumors (61% (14 of 23) *vs* 35% (24 of 69), $P=0.028$) (Table 1). Patient survival curves also differed

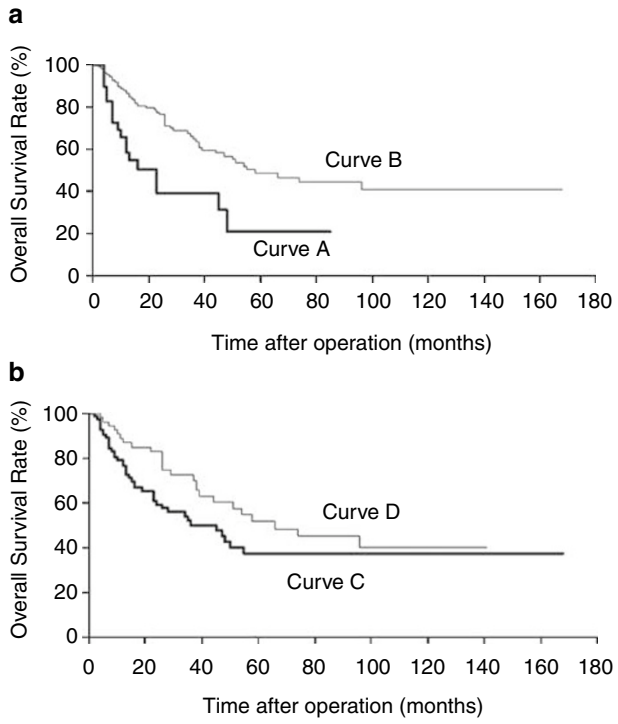

Figure 3 Overall survival curves for 136 patients with advanced-stage ovarian carcinoma, stratified by (**a**) copy number of the *ACTN4* gene, and by (**b**) cytoplasmic actinin-4 immunoreaction. (**a**) Curve A for the group with a higher copy number of the *ACTN4* gene ($n=29$), and curve B for the group with a lower copy number of the *ACTN4* gene ($n=107$). There is a significant difference between these two groups ($P=0.0011$, by log-rank test). (**b**) Curve C for the group with high actinin-4 immunoreactivity ($n=82$), and curve D for the group with low actinin-4 immunoreactivity ($n=54$). There is a significant marginal difference between these two groups ($P=0.089$, by log-rank test).

significantly between the two groups ($P=0.0011$, log-rank test) (Figure 3a). The 5-year survival rates were 20.9 and 48.5% in the higher and lower copy-number groups, respectively. On the other hand, there were no significant differences between the higher (≧4 copies, $n=29$) and lower (<4 copies, $n=107$) copy-number groups with regard to mean patient age, distribution of FIGO stage (stages III *vs* IV), degree of the residual tumor after initial surgery, and frequency of lymph node metastasis (Table 1).

Clinicopathological Characteristics of Tumors showing High Actinin-4 Immunoreactivity

In comparison with the low actinin-4 immunoreactivity group, the high actinin-4 immunoreactivity group had a high degree (≧1 cm) of residual tumor (41% (56 of 137) *vs* 23% (29 of 128), $P=0.0033$). Although not showing statistical significance, in comparison with the low immunoreactivity group, the high actinin-4 immunoreactivity group tended to have histologically high-grade tumors and chemoresistant tumors, and showed a poor patient prognosis with statistically marginal significance (Table 2; Figure 3b). The 5-year survival rates were 37.4 and 51.6% in the high and low immunoreactivity groups, respectively. On the other hand, there were no significant differences between the high ($n=82$) and low ($n=54$) actinin-4 immunoreactivity groups with regard to mean patient age, distribution of FIGO stage (stages III *vs* IV), tumor histology, or frequency of lymph node metastasis (Table 2).

Multivariate Analysis

Univariate analyses using the Cox model including 10 parameters showed that FIGO stage IV ($P=0.038$), the presence of residual tumor ≧1 cm ($P=0.0023$), a clear-cell adenocarcinoma histology ($P=0.00021$), and higher *ACTN4* gene copy number ($P=0.0013$) were correlated with worse patient outcome (Table 3a). Additionally, a serous adenocarcinoma histology was correlated with favorable patient outcome ($P=0.0019$). Univariate analysis also showed that the higher *ACTN4* copy number was able to predict patient outcome more accurately than high actinin-4 immunoreactivity (relative risk (RR): 2.48 *vs* 1.55). Multivariate analysis using the Cox model including five variables demonstrated that a higher copy number of the *ACTN4* gene and the presence of residual tumor ≧1 cm ($P=0.00030$) had independent impacts on overall survival ($P=0.031$) (Table 3b). A serous adenocarcinoma histology was also identified as an independent favorable prognostic factor in comparison with other histological types ($P=0.0087$) (Table 3b).

Discussion

α-Actinin crosslinks cytoskeletal actin filaments, and two types of non-muscular α-actinin—actinin-1 and actinin-4—have been identified.[4,5] By enhancing cell motility, actinin-4 shows different biological properties from actinin-1, and variable clinicopathological roles of actinin-4 have been demonstrated in human epithelial malignancies, such as breast carcinoma, non-small cell lung cancer, colorectal adenocarcinoma, and pancreatic ductal adenocarcinoma.[5,10–12] Recently, Kikuchi *et al*[12] reported that actinin-4 protein was overexpressed in 63% of invasive ductal carcinomas of the pancreas, and that increased expression of actinin-4 protein was significantly associated with poor prognosis of patients with pancreatic ductal carcinoma. They also demonstrated that 38% (11 of 29) of pancreatic invasive ductal carcinomas with increased actinin-4 protein expression showed amplification of the *ACTN4* gene on chromosome 19q13.1.[12]

Gain or amplification of chromosomal region 19q13.1–q13.2 has been reported in clinical samples of ovarian cancer or in ovarian cancer cell lines,[14,18] and candidate oncogenes such as *AKT2* and *SEI-1*

Table 2 Correlation of cytoplasmic actinin-4 expression with clinicopathological parameters in advanced-stage ovarian cancers

		Number of cases (%)		
		Cytoplasmic/membrane immunoreactivity		
Factor	*Total*	*High (n = 82)*	*Low (n = 54)*	P-*value*
Age, years (median ± s.d.)		56.0 (± 11.2)	552 (± 10.1)	0.677
FIGO[a] *stage*				0.205
III	103	59 (57)	44 (43)	
IV	33	23 (70)	10 (30)	
Residual tumor (cm)				**0.0033**
<1	43	18 (42)	25 (58)	
≧1	92	63 (68)	29 (32)	
Unknown	1	1		
Histological grade				0.056
1 or 2	93	51 (55)	42 (45)	
3	43	31 (72)	12 (28)	
Histological type				0.126
Serous	91	59 (65)	32 (35)	
Clear cell	25	13 (52)	12 (48)	
Endometrioid	10	3 (30)	7 (70)	
Mucinous	10	7 (70)	3 (30)	
Lymph node metastasis				0.111
Absent	20	7 (35)	13 (65)	
Present	33	19 (58)	14 (42)	
Response to chemotherapies				0.070
Complete response/partial response	54	33 (61)	21 (39)	
Stable disease/progressive disease	38	30 (79)	8 (21)	
5-Year survival (%)		37.4	51.6	0.089[b]

Bold values indicate statistical significance.
[a]International Federation of Gynecology and Obstetrics.
[b]Calculated by log-rank test.

are thought to be targets of the amplicon.[15,18] In the present study, we investigated the copy number of the *ACTN4* gene on chromosome 19q13 in 136 advanced-stage ovarian cancers, and detected a higher copy number (4 copies or higher) of the *ACTN4* gene in 21% of the cases studied, being highly correlated with the intensity of actinin-4 immunoreactivity and poorer patient outcome. Moreover, univariate analyses showed that a higher copy number of the *ACTN4* gene was able to predict patient outcome more accurately than high actinin-4 immunoreactivity (RR: 2.48 *vs* 1.55). These results suggest that the *ACTN4* gene can be a target of the chromosome 19q13 amplicon and play an oncogenic role in ovarian cancer progression. However, two-thirds (55 of 82) of ovarian cancers showing high actinin-4 immunoreactivity, probably reflecting high actinin-4 protein expression, were found to have a normal to slightly high copy number (<4 copies) of the *ACTN4* gene. Therefore, other mechanisms activating the *ACTN4* gene or interaction between actinin-4 and other intracellular molecules are also suspected, and should be analyzed in future.

The present study also demonstrated that a higher copy number of the *ACTN4* gene was significantly associated with tumors showing resistance to platinum-based chemotherapies and a histology of clear-cell adenocarcinoma, the latter being recognized as the most lethal and chemoresistant form of ovarian cancer.[24,25] Therefore, the cases with a clear-cell adenocarcinoma histology, which were associated with increased copy number of the *ACTN4* gene, might have drive the tumor chemoresistance as well as poor prognosis of the patients for the entire study set. Although not statistically significant, tumors showing a high level of actinin-4 immunoreactivity tented to be chemoresistant, with marginal significance ($P = 0.070$). These data were different from those of our previous immunohistochemical study, in which high-level immunoreactivity of actinin-4 was associated with a serous adenocarcinoma histology, and not correlated with tumor chemoresistance.[13] However, in contrast to the previous series including a larger number of early-stage tumors (FIGO stage I or II), cases enrolled in the present study were limited to those at advanced stages (FIGO stages III or IV) among which

Table 3 Cox regression model estimates of the significance of prognostic factors

Variables	P-*value*	*RR (95% CI)*
(a) Univariate		
Age (≧56[a] years *vs* <56 years)	0.252	0.78 (0.45–1.23)
FIGO stage[b] (IV *vs* III)	**0.038**	1.72 (1.03–2.88)
Residual tumor (≧1 cm *vs* <1 cm)	**0.0023**	2.47 (1.38–4.41)
Histological grade (3 *vs* 2 or 1)	0.091	0.61 (0.34–1.08)
Tumor histology		
Serous adenocarcinoma	**0.0019**	0.46 (0.28–0.75)
Clear-cell adenocarcinoma	**0.00021**	2.82 (1.63–4.88)
Endometrioid adenocarcinoma	0.612	0.79 (0.31–1.98)
Mucinous adenocarcinoma	0.183	1.77 (0.76–4.11)
High actinin-4 immunoreaction	0.088	1.55 (0.94–2.56)
Higher copy number of the *ACTN4* gene	**0.0013**	2.48 (1.42–4.31)
(b) Multivariate		
FIGO stage (IV *vs* III)	0.061	1.68 (0.98–2.87)
Residual tumor (≧1 cm *vs* <1 cm)	**0.0003**	3.16 (1.69–5.89)
Tumour histology		
Serous adenocarcinoma	**0.0087**	0.38 (0.18–0.78)
Clear-cell adenocarcinoma	0.299	1.52 (0.69–3.35)
Higher copy number of the *ACTN4* gene	**0.031**	1.89 (1.06–3.37)

Bold values indicate statistical significance.
RR, relative risk; CI, confidence interval. Other abbreviations as in Tables 1 and 2.
[a]International Federation of Gynecology and Obstetrics.
[b]Mean values.

the majority (68% (92 of 136 cases)) had not been treated by optimal cytoreduction. The difference in distribution of clinical stages and the presence of cases with suboptimal cytoreduction might have accounted for the difference in results between the two series. Some reports have indicated that amplification of genes such as *ABCF2* located in chromosome 7q35–q36,[26] *PPM1D* and *APPBP2* located in 17q21–q24,[27] *cyclin E1* located in 19q12,[28] and *MUC1* in 1q21–q22[29] are associated with the chemoresistant nature and/or clear-cell histology of ovarian cancer. Added to these genes, *ACTN4* gene amplification may be a novel indicator of chemoresistance and/or a clear-cell histology of the tumor.

For further comprehension of the functional roles of the *ACTN4* gene and intracellular dynamics of accumulated actinin-4 protein in ovarian cancer, the interaction of actinin-4 with the phosphatidylinositol 3-kinase (PI3K)/AKT pathways is worth considering. Dysregulation of the PI3K/AKT signaling systems has been reported in ovarian cancer, including elevated AKT1 kinase activity, amplification of *AKT2* and *PIK3CA* (encoding the p110 catalytic subunit of PI3K), and mutation of *PIK3R1* (encoding the p85 regulatory subunit of PI3K). Moreover, inhibition of PI3K or AKTs can decrease ovarian cancer cell migration, invasion, and proliferation *in vivo*.[30–34] The PI3K/AKT pathways are also reported to play an important role in mediating multidrug resistance of breast and ovarian cancers.[35,36] Actinin-4 binds the p85 regulatory subunit of PI3K, and translocation of actinin-4 out of the cell membrane can be introduced by treatment with wortmannin, a PI3K inhibitor, or with cytochalasin D, an inhibitor of actin polymerization.[5,37,38] Moreover, Ding *et al*[39] have reported that actinin-4 protein interacts physically and functionally with AKT1, and that siRNA-mediated actinin-4 gene silencing downregulates AKT phosphorylation, blocks AKT translocation to the cell membrane, and inhibits cell proliferation.

In summary, we have demonstrated that the copy number of the actinin-4 (*ACTN4*) gene, located in chromosome 19q13, was amplified in 21% of advanced-stage ovarian cancers we examined, and was highly correlated with high actinin-4 immunoreactivity, high-grade tumor histology, a clear-cell adenocarcinoma histology, tumor chemoresistance, and poor patient outcome. The *ACTN4* gene can be a target of the chromosome 19q13 amplicon and may act as an oncogene in highly aggressive ovarian cancers. Although both high-level expression of actinin-4 protein detected by immunohistochemistry and a higher *ACTN4* gene copy number detected by FISH could well predict the chemoresistant nature of the tumors and poor prognosis of patients with advanced-stage ovarian cancer, FISH analysis was able to predict these events more accurately than the immunohistochemistry. Actinin-4 and its functional association with the PI3K/AKT pathways would be worth investigating to devise promising therapeutic approaches for high-grade, advanced-stage, and chemoresistant ovarian cancers.

Conflict of interest

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