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Inhibin-alpha, -betaA and -betaB subunits in uterine non-endometrioid carcinomas: Prognostic significance and clinical implications

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ABSTRACT

Inhibins, dimeric peptide hormones composed of an alpha-subunit and one of two possible beta-subunits (betaA or betaB), exhibit substantial roles in human reproduction and in endocrine-responsive tumours. However, the prognostic significance and clinical implications of the inhibin-alpha, -betaA and -betaB subunits in uterine non-endometrioid cancers are still quite unclear. A series of 41 uterine non-endometrioid carcinomas were immunohistochemically analysed with monoclonal antibodies against inhibin-subunits. The staining reactions were correlated with several clinicopathological characteristics and clinical outcome. The inhibin-alpha subunit showed a significant association with age although the loss of this subunit did not affect the survival of patients with non-endometrioid carcinomas and did not constitute an independent prognostic parameter. The inhibin-betaA expression was not associated with any of the analysed clinicopathological parameters and did not affect patients' survival. In contrast, a low betaB-subunit demonstrated a significant better cause-specific survival. Moreover, inhibin-βB did constitute an independent prognostic parameter in uterine non-endometrioid cancer patients. In contrast to inhibin-alpha and -betaA subunits, the inhibin-betaB subunit seems to have a substantial role in the carcinogenesis and pathology of uterine non-endometrioid carcinomas and might be used as a marker to identify high-risk patients and may aid in the selection of patients for a more aggressive adjuvant therapy.

1. Introduction

Endometrial cancer has become the most frequent gynaecologic malignancy in the Western World.[1–3] An incidence of 15–20 to 100,000 women per year has been estimated with a life time risk to develop this type of cancer being approximately 2.5%.[4] Meanwhile, several prognostic factors like histological type, histologic grade, surgical stage, pelvic lymph node involvement and myometrial invasion have been established.[1,2]

Meanwhile, endometrial cancer has been described as consisting of two different clinicopathological categories with distinct biological and molecular characteristics.[2,5–7] Type I endometrial cancers are the most common histopathological form, being usually endometrioid adenocarcinomas, well-differentiated with a more favourable outcome compared to endometrial cancer of the second group.[1–6] Contrarily, type II endometrial cancers are often of the non-endometrioid type, poorly differentiated with a poor prognosis.[6–8] These observations lead to the postulation of a dualistic model for

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the molecular carcinogenesis in endometrial carcinomas,[7] although common characteristics of these groups of endometrial cancer exist. The carcinogenesis of type I endometrial carcinomas is thought to be due to oestrogenic risk factors,[1,2,7] demonstrating genetic alterations like mutations in PTEN and K-ras.[7] Type II cancers more often exhibit p53 mutations,[9] HER-2/neu amplification[10] and chromosomal instability.[7]

Inhibins and activins are secreted polypeptides, representing a subgroup of the TGF-β superfamily of growth and differentiation factors.[11–13] Inhibins are heterodimers that consist of an α-subunit and one of two possible β-subunits (βA or βB), resulting in the formation of either inhibin A (α–βA) or B (α–βB), respectively. On the contrary, activins are homodimers of β-subunits linked by a disulphide bond, leading to the formation of activin A (βA–βA), activin B (βB–βB) or activin AB (βA–βB).[11–13] The inhibin-subunits have been primarily detected in endocrine tumours[14] and their differential expression has suggested an important role in malignant cell transformation in human endometrium.[15–17]

Interestingly, TGF-β has been recognised as a tumour suppressor in premalignant stages of carcinogenesis with an additional dual role as a pro-oncogene in later stages of the disease, leading to metastasis.[18] The tumour suppressive activity of the inhibin-α subunit was first identified after functional deletion of the inhibin-α gene in male and female mice, resulting in primary gonadal sex cord-stromal tumours.[19,20] Recently, a significantly lower inhibin-α expression in well-differentiated adenocarcinomas compared to normal and hyperplastic endometrial tissue was demonstrated.[21,22] These results also led to the hypothesis that inhibin-α might be a tumour suppressor with crucial functions in endometrial carcinoma development.[16] This assumption is underlined by demonstrating that inhibin-α subunit was an independent prognostic parameter in a large cohort analysis of human endometrial carcinomas.[17] However, the prognostic significance and clinical implications of the inhibin-α, -βA and -βB subunits in non-endometrioid cancers are still quite unclear.

2. Material and methods

2.1. Tissue samples

Pathological and surgical records of 41 patients who have been operated in the 1st Department of Obstetrics and Gynecology, Ludwig-Maximilians-University Munich, between 1990 and 2002 were reviewed for this retrospective analysis. The evaluated patient group has been previously well characterised.[17,23] In a previous large cohort study 302 endometrial cancer specimens were analysed for the expression of the inhibin-α, -βA and -βB subunits, including 265 endometrioid adenocarcinoma and 37 non-endometrioid carcinomas.[17] In this study four additional cases have been included and the inhibin-subunit expression was evaluated in regard to the non-endometrioid histology. Of the 41 patients analysed, 29 (56.1%) had a serous carcinoma, 7 (17.1%) a clear cell and 5 (12.2%) an undifferentiated carcinoma. Although undifferentiated carcinomas are not clearly defined as a non-endometrioid tumour type but have, similar to serous and clear cell carcinomas, a worse prognosis compared to endometrioid adenocarcinomas.[1,2,24] Therefore, this endometrial cancer subtype is often considered to belong to non-endometrioid histology.[24] Pathological stage and histological subtype were determined for each surgical specimen according to 1988 International Federation of Gynecology and Obstetrics (FIGO) criteria.[25]

Patient data were obtained from three sources: hospital tumour registry, automated database and chart review as previously described.[17,23] All cases of recurrence had radiographic evidence of disease or biopsy-proven progression of disease. Only the records of patients who died of disease were considered to be uncensored; the records of all patients who were alive at follow-up or who did not die of disease (or a related cause) were considered to be censored. Additionally, censored cases were also considered those cases where the exact cause of death was unknown but died within 2 years after the diagnosis of a metastatic lesion.[17,23]

2.2. Immunohistochemistry

Immunohistochemistry was performed using a combination of pressure cooker heating and the standard streptavidin–biotin–peroxidase complex by using the mouse-IgG-Vectastain Elite ABC kit (Vector Laboratories, Burlingame, California, USA) as previously described.[17,26] Mouse monoclonal antibodies used for the experiments were inhibin-α (clone R1, diluted in PBS 1:50; Serotec – Oxford – United Kingdom), inhibin-βA (clone, E4, diluted in PBS 1:50; Serotec – Oxford – United Kingdom) and inhibin-βB (clone C5, diluted in PBS 1:10; Serotec – Oxford – United Kingdom).[17,26]

2.3. Statistical analysis

The intensity and distribution patterns of specific inhibin-subunit immunohistochemical cytoplasmatic staining reaction was evaluated by two blinded, independent observers using a semi-quantitative score and an internal colorimetric scale as previously described and used to assess the expression pattern of inhibin-subunits.[17,26] The IRS score was calculated by multiplication of optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining) and the percentage of positive-stained cells (0 = no staining, 1 = <10% of the cells, 2 = 11–50% of the cells, 3 = 51–80% of the cells and 4 = >81% of the cells). The IRS-scores of inhibin-βA and -βB immunohistochemical expression levels were compared using the non-parametric Kruskal–Wallis test and the Mann–Whitney *U* test where applicable. Correlations of the immunoreactive scores and staining intensities were assessed using the Spearman rank correlation test. Significance was assumed at $p \leqslant 0.05$ at the two-sided test (SPSS version 16.0; SPSS Inc., Chicago, IL).

For the purposes of statistical survival analysis, the median of the inhibin-α staining intensity for all tumour samples was used (median for inhibin-α = 0) as previously described.[17] ROC analysis revealed that the area under the curve was higher by using staining intensity with a cut-off value of 1, instead of the IRS with the previously described cut-offs of 4 and 6 for inhibin-βA and -βB, respectively.[17] Therefore, staining intensity with the value $\leqslant 1$ for inhibin-βA and -βB was considered to be a negative expression. For the evaluation of increased/

positive versus not increased/negative immunostaining in tumour samples was compared using the χ^2 test and the exact Fisher's test where applicable.

The outcomes analysed were progression-free survival, cause-specific survival and overall survival. Univariate analysis was performed with Kaplan–Meier life-table curves to estimate survival[27] and was compared using the log-rank test. Prognostic models used multivariate Cox regression analysis for multivariate analyses of survival. Data were adjusted for age (⩽65 years versus >65 years), histology (categorical variable), FIGO stage (FIGO I/II versus FIGO III/IV), lymph node involvement (categorical variable), lymphovascular space invasion (positive versus negative), inhibin-α (positive versus negative), inhibin-βA (positive versus negative) and inhibin-βB (positive versus negative). The variables were entered in a forward stepwise manner.[28] Significance of differences was assumed at $p \leqslant 0.05$ (SPSS version 16.0; SPSS Inc., Chicago, IL).

3. Results

3.1. Clinicopathological characterisation

The clinicopathological features of the endometrial carcinomas are summarised in Table 1. The median patient's age at the time of diagnosis was 65.98 years (range, 45.83–88.37 years). Twenty-four (58.5%) and 3 (7.3%) patients were diagnosed in FIGO stages I and II, respectively, while 13 (31.7%) patients had FIGO stage III and 1 patient (2.4%) presented with metastatic disease (FIGO IV). Pelvic and/or para-aortic lymph node sampling was performed for 32 patients (78%) while 6 patients (14.6%) demonstrated lymph node metastasis. A low FIGO stage (FIGO Ia), obesity, advanced age and excessive comorbidity were factors against a full surgical staging in 9 patients (22%). Obesity was observed in 28 (68.3%) cases, while 27 (65.9%) and 4 (9.8%) patients presented with hypertension and diabetes, respectively. Of the analysed 41 patients, 22 patients (53.7%) received a radiation therapy, while 2 patients (4.9%) and 1 patient (2.4%) received an anti-hormone therapy and chemotherapy, respectively. During the follow-up interval, tumour recurrence was observed in 11 patients (26.8%), and 11 patients (26.8%) died of disease. Nineteen (46.3%) died during the entire observation period.

3.2. Endometrial carcinoma samples

The mean of the IRS score was 0.44 ± 0.16, 6.56 ± 0.445 and 6.68 ± 0.564 for inhibin-α, -βA and -βB, respectively (mean ± SEM). Positive inhibin-α immunostaining was observed in 9 of 41 endometrial carcinoma samples (22.0%), while 28

Table 1 – Clinicopathological characteristics of the analysed uterine non-endometrioid carcinomas.

Parameter	Definition	N = 41
Age (years)	⩽65	18 (43.9%)
	>65	23 (56.1%)
Histology	Serous/papillary	29 (70.7%)
	Clear cell	7 (17.1%)
	Undifferentiated	5 (12.2%)
FIGO stage	FIGO I	24 (58.5%)
	FIGO Ia	7 (17.1%)
	FIGO Ib	17 (41.5%)
	FIGO Ic	0 (0%)
	FIGO II	3 (7.3%)
	FIGO 2a	2 (4.9%)
	FIGO 2b	1 (2.4%)
	FIGO III	13 (31.7%)
	FIGO 3a	7 (17.1%)
	FIGO 3b	1 (2.4%)
	FIGO 3c	5 (12.2%)
	FIGO IV	1 (2.4%)
LN status	Negative	26 (63.4%)
	Positive	6 (14.6%)
	Unknown	9 (22%)
LVSI	Negative	35 (85.4%)
	Positive	6 (14.6%)
Adipositas	Negative	28 (68.3%)
	Positive	13 (31.7%)
Diabetes	Negative	37 (90.2%)
	Positive	4 (9.8%)
Hypertension	Negative	27 (65.9%)
	Positive	14 (34.1%)
Chemotherapy	Negative	40 (97.6%)
	Positive	1 (2.4%)
Radiotherapy	Negative	22 (53.7%)
	Positive	19 (46.3%)
Anti-hormonal therapy	Negative	39 (95.1%)
	Positive	2 (4.9%)

(68.3%) and 24 (58.5%) tumour samples were positive for inhibin-βA and -βB, respectively (Fig. 1). No significant differences of the immunoreactive scores of all analysed inhibin-subunits were found among the various subtypes of non-endometrioid carcinomas and FIGO stages. A significant correlation between the immunoreactive scores ($p = 0.002$) and staining intensities ($p = 0.001$) for βA- and βB-subunits was also observed. By analysing positive and negative expression univariate analysis (χ^2 test) revealed a significant association of inhibin-α with patient age ($p = 0.005$) (Table 2).

3.3. *Survival analysis*

The median time to progression for the uncensored subgroup was 10.87 months (range 0.01–116.67 months), whereas the median follow-up of censored patients was 85.87 months (range 5.33–178.87 months). The median for the cause-associated death for the uncensored subgroup was 29.93 months (range 4.97–137.73 months), whereas the median follow-up of censored patients was 89.00 months (range 7.50–178.87 months).

Univariate survival analysis demonstrated no differences in the progression-free survival, cause-specific survival and overall survival for inhibin-α (Fig. 2) and inhibin-βA (Fig. 3) expression. Additionally, patients with a positive inhibin-βB expression demonstrated a significant cause-specific survival ($p = 0.028$, log-rank test) (Fig. 4).

The Cox regression led to a model containing three independent terms that were predictive of progression-free survival: FIGO stage ($p = 0.001$; RR = 8.956 confidence interval

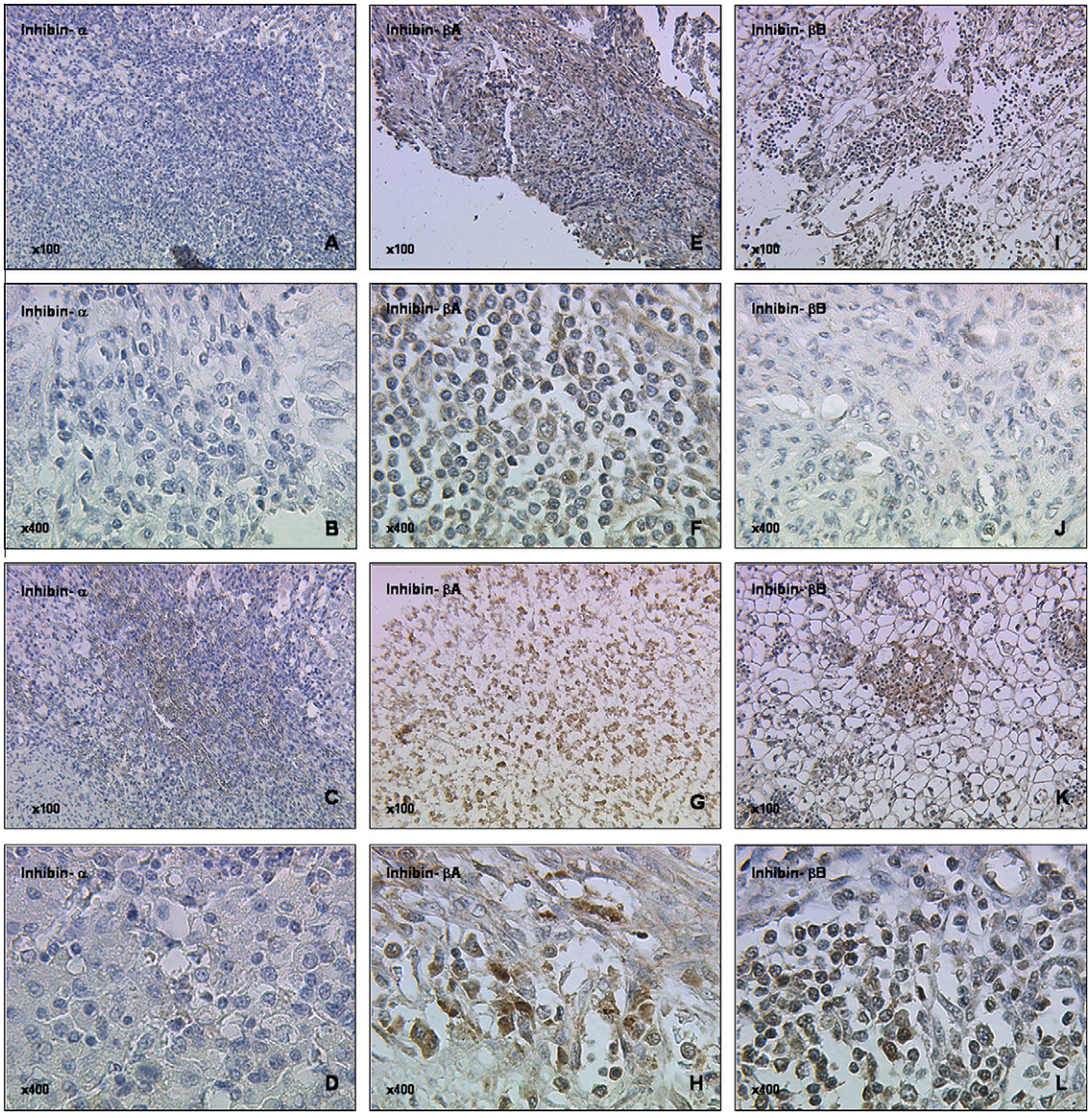

Fig. 1 – Expression of inhibin-α, -βA and -βB subunits in human non-endometrioid carcinomas. Inhibin-α showed no ((A) ×100; (B) ×400) to minimal expression ((C) ×100; (D) ×400) in non-endometrioid endometrial carcinomas, whereas inhibin-βA demonstrated moderate ((E) ×100; (F) ×400) to strong intensity ((G) ×100; (H) ×400). Inhibin-βB reacted with a minimal ((I) ×100; (J) ×400) to moderate staining intensity ((K) ×100; (L) ×400) in uterine non-endometrioid carcinomas.

Table 2 – Univariate statistical analysis for positive inhibin-α, -βA and -βB subunits according to various clinicopathological features. NS = not significant.

			Inhibin-α		Inhibin-βA		Inhibin-βB	
			Negative	Positive	Negative	Positive	Negative	Positive
Age (years)	⩽65	18 (43.9%)	10 (55.6%)	8 (44.4%)	8 (44.4%)	10 (55.6%)	8 (44.4%)	10 (55.6%)
	>65	23 (56.1%)	22 (95.7%)	1 (4.3%)	5 (21.7%)	18 (78.3%)	9 (39.1%)	14 (60.9%)
	χ^2		0.005		NS		NS	
Histology	Serous/papillary	29 (70.7%)	21 (72.4%)	8 (27.6%)	9 (31%)	20 (69%)	13 (44.8%)	16 (55.2%)
	Clear cell	7 (17.1%)	6 (85.7%)	1 (14.3%)	2 (28.6%)	5 (71.4%)	3 (42.9%)	4 (57.1%)
	Undifferentiated	5 (12.2%)	5 (100%)	0 (0%)	2 (40%)	3 (60%)	1 (20%)	4 (80%)
	χ^2		NS		NS		NS	
FIGO stage	FIGO I + II	27 (65.9%)	20 (74.1%)	7 (25.9%)	8 (29.6%)	19 (70.4%)	12 (44.4%)	15 (55.6%)
	FIGO III + IV	14 (34.1%)	12 (85.7%)	2 (14.3%)	5 (35.7%)	9 (64.3%)	5 (35.7%)	9 (64.3%)
	χ^2		NS		NS		NS	
LN status	Negative	26 (63.4%)	20 (76.9%)	6 (23.1%)	7 (26.9%)	19 (73.1%)	10 (38.5%)	16 (61.5%)
	Positive	6 (14.6%)	5 (83.3%)	1 (16.7%)	2 (33.3%)	4 (66.7%)	2 (33.3%)	4 (66.7%)
	Unknown	9 (22%)	7 (77.8%)	2 (22.2%)	4 (44.4%)	5 (55.6%)	5 (55.6%)	4 (44.4%)
	χ^2		NS		NS		NS	
LVSI	Negative	35 (85.4%)	26 (74.3%)	9 (25.7%)	10 (28.6%)	25 (71.4%)	15 (42.9%)	20 (57.1%)
	Positive	6 (14.6%)	6 (100%)	0 (0%)	3 (50%)	3 (50%)	2 (33.3%)	4 (66.7%)
	χ^2		NS		NS		NS	
Adipositas	Negative	28 (68.3%)	23 (82.1%)	5 (17.9%)	6 (21.4%)	22 (78.6%)	9 (32.1%)	19 (67.9%)
	Positive	13 (31.7%)	9 (69.2%)	4 (30.8%)	7 (53.8%)	6 (46.2%)	8 (61.5%)	5 (38.5%)
	χ^2		NS		NS		NS	
Diabetes	Negative	37 (90.2%)	28 (75.7%)	9 (24.3%)	12 (32.4%)	25 (67.6%)	15 (40.5%)	22 (59.5%)
	Positive	4 (9.8%)	4 (100%)	0 (0%)	1 (25%)	3 (75%)	2 (50%)	2 (50%)
	χ^2		NS		NS		NS	
Hypertension	Negative	27 (65.9%)	21 (77.8%)	6 (22.2%)	7 (25.9%)	20 (74.1%)	10 (37%)	17 (63%)
	Positive	14 (34.1%)	11 (78.6%)	3 (21.4%)	6 (42.9%)	8 (57.1%)	7 (50%)	7 (50%)
	χ^2		NS		NS		NS	
Radiotherapy	Negative	22 (53.7%)	16 (72.7%)	6 (27.3%)	5 (22.7%)	17 (77.3%)	10 (45.5%)	12 (54.5%)
	Positive	19 (46.3%)	16 (84.2%)	3 (15.8%)	8 (42.1%)	11 (57.9%)	7 (36.8%)	12 (63.2%)
	χ^2		NS		NS		NS	
Chemotherapy	Negative	40 (97.6%)	31 (77.5%)	9 (22.5%)	13 (32.5%)	27 (67.5%)	17 (42.5%)	23 (57.5%)
	Positive	1 (2.4%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
	χ^2		NS		NS		NS	
Anti-hormonal therapy	Negative	39 (95.1%)	31 (79.5%)	8 (20.5%)	12 (30.8%)	27 (69.2%)	16 (41%)	23 (59%)
	Positive	2 (4.9%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
	χ^2		NS		NS		NS	

(CI) 95% 2.334–34.365). Independent prognostic factors for cause-specific survival was FIGO stage ($p = 0.001$; RR = 12.749 CI 95% 2.897–56.112) and inhibin-βB ($p = 0.036$; RR = 9.494 CI 95% 1.161–77.625). The overall survival was only influenced by FIGO stage ($p < 0.001$; RR = 18.745 CI 95% 5.182–67.801).

4. Discussion

Endometrial cancer is the most frequent gynaecologic malignancy in the Western World.[1,2,4] Although more than 50% of patients with endometrial carcinoma are diagnosed with FIGO stage I, as many as 20% die of their disease.[3] This is an unusual situation, compared to other solid tumours, and may reflect the failure of current diagnostic methods for identifying endometrial cancer patients with a poor prognosis. Especially non-endometrioid carcinomas pose a problem, since they are mostly poorly differentiated, have a poor prognosis and limited prognostic parameters are available.[6–10]

The inhibin/activin-subunits belong to the TGF-β superfamily and have been demonstrated in normal female tissue and endocrine tumours,[14] including normal and pathological endometrial tissue.[15,17,22,26] Interestingly, TGF-β has been recognised as a tumour suppressor in premalignant stages of carcinogenesis with an additional dual role as a pro-oncogene in later stages of disease, leading to metastasis.[18] Regarding metastasis, inhibition of TGF-β suppresses experimental metastasis to multiple organs.[29,30]

However, the most interesting function of inhibin-α might be its action as a tumour suppressor gene, according to results from transgenic mouse models for ovarian cancer.[19,20] The loss of the inhibin-α subunit production in mice resulted in a marked increase of activin production in the ovary. The inhibin-α sufficient mice presented with cachectic symptoms that were associated with the compensatory excessive secretion of activin.[20] Additionally, inhibin-α might be a tumour suppressor with crucial functions in endometrial carcinoma

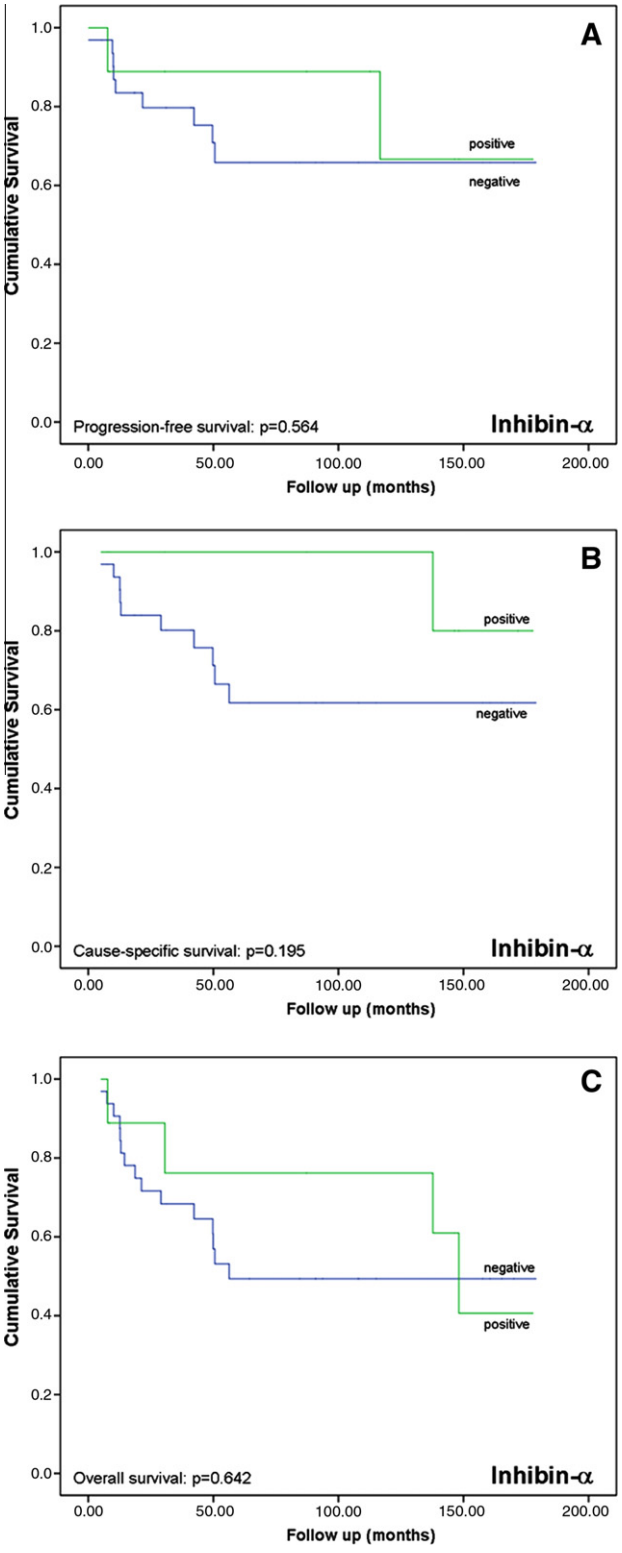

Fig. 2 – Kaplan–Meier curves of clinical outcome regarding inhibin-α expression for progression-free-survival (A), cause-specific survival (B) and overall survival (C).

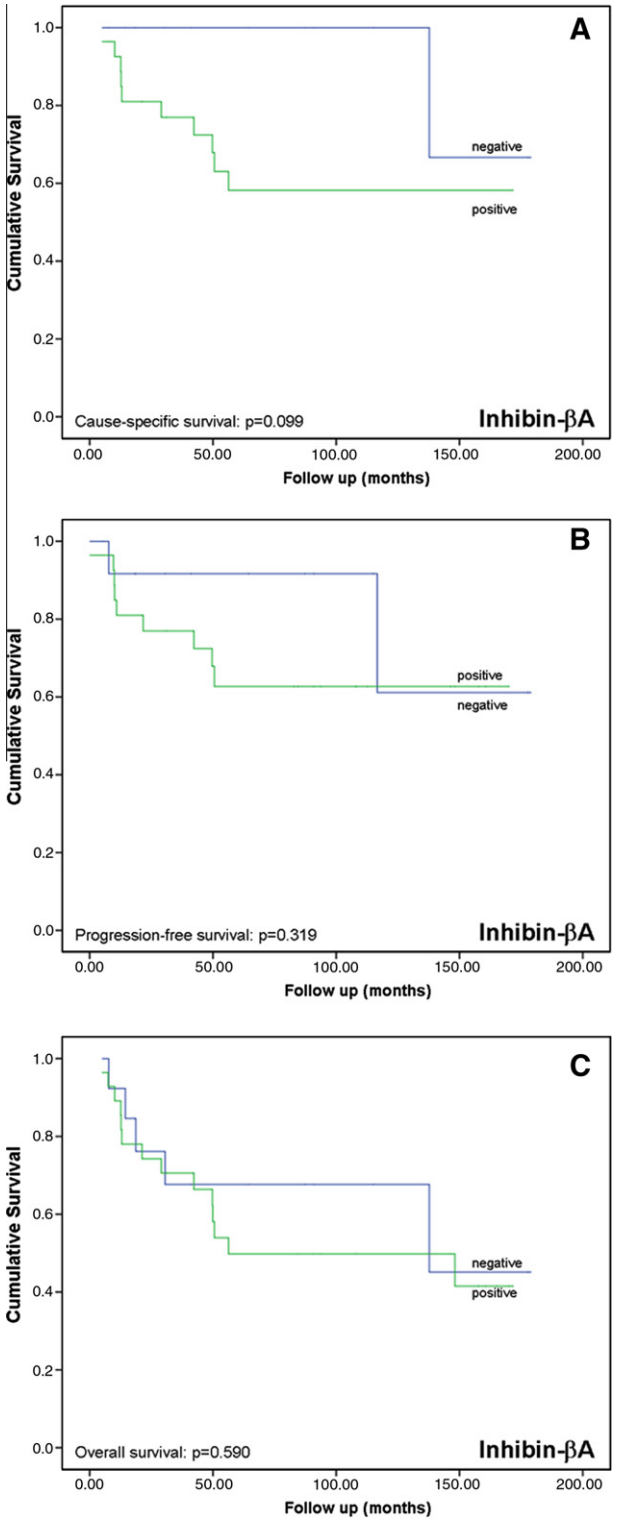

Fig. 3 – Kaplan–Meier curves of clinical outcome regarding inhibin-βA expression for progression-free-survival (A), cause-specific survival (B) and overall survival (C).

development,[16,17,22] since it constitutes an independent prognostic parameter in a large cohort study of over 300 analysed endometrial cancer patients.[17] The inhibin-α subunit showed a significant association with age in uterine non-endometrioid cancer patients. Additionally, no correlation between the inhibin-α and -β subunits was observed, with a significant correlation of both β-subunits. Therefore, different molecular mechanisms and pathways might exist that regulate the β-

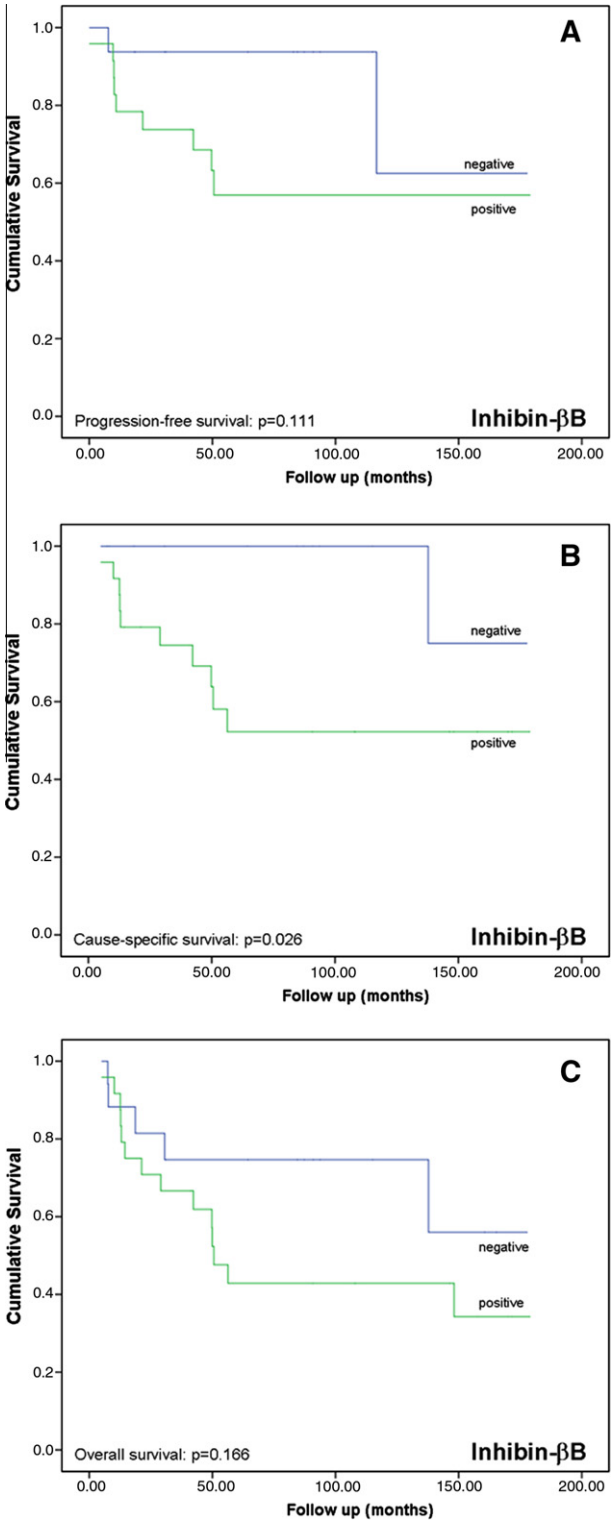

Fig. 4 – Kaplan–Meier curves of clinical outcome regarding inhibin-βB expression for progression-free-survival (A), cause-specific survival (B) and overall survival (C).

subunit production compared to the α-subunit. However, the loss of inhibin-α immunoreactivity did not constitute an independent prognostic parameter and also did not lead to a poorer survival of the affected patients. Therefore, this subunit might not have a substantial role in the carcinogenesis and pathology of uterine non-endometrioid carcinomas.

The expression of inhibin-β subunits in endometrial cancer is of extreme importance, since activin signalling might be a promising target for therapeutic interventions.[31] Interestingly, activin A inhibits cancer cell proliferation in various experimental models *in vitro* and *in vivo*.[32–36] Activin A can induce an inhibition of the telomerase activity in cancer cell lines and therefore contribute to the inhibition of cancer cell proliferation.[37] However, it was demonstrated that activin A is also capable of enhancing proliferation in certain cancer cell lines.[38,39] Therefore, the function of activins in different tissue and cell lines remains still controversial.[14] Moreover, if the inhibin-β subunits have similar tumour suppressive properties, as observed for the inhibin-α subunit, remains also unclear.[14,40] The role of activins is further complicated since they have been recognised as important cytokines that can regulate cell growth and differentiation[41] and act as growth inhibitors of vascular endothelial cells.[42] Interestingly, inhibin-βA is overexpressed in lung adenocarcinomas and this overexpression is associated with a poorer survival, probably affecting promoter methylation and histone acetylation.[43] In this study, the inhibin-βA expression was not associated with any of the analysed clinicopathological parameters and did not affect patients' survival, suggesting a minor role in the pathogenesis and the prognostic value in uterine non-endometrioid cancers.

Whether the inhibin-βB subunit has a similar function as suggested for inhibin-βA subunit is still not clear yet. When the inhibin-βB gene is knocked into the inhibin-βA gene locus, the phenotypes in the inhibin-βA knockout mouse are partially restored, but also results in novel phenotypes,[44] indicating that the two subunits are not functionally equivalent and exert different functions.[44–47] Interestingly, activin B has been recently suggested as a marker for patients with ovarian granulosa cell tumours[48] and inhibin B might be a more specific marker than inhibin A.[49] In this analysis, the βB-subunit demonstrated a significant association with cause-specific survival and might therefore have an important function in the carcinogenesis of non-endometrioid carcinomas.

In conclusion, we demonstrated an expression of inhibin-α, -βA and -βB subunits in uterine non-endometrioid cancer tissue. The loss of inhibin-α immunoreactivity did not constitute an independent prognostic parameter and also did not lead to a poorer survival of the affected patients. Additionally, the inhibin-βA subunit expression was not associated with any of the analysed clinicopathological parameters and did not affect patients' survival. In contrast, the βB-subunit demonstrated a significant association cause-specific survival. Moreover, inhibin-βB did constitute an independent prognostic parameter in uterine non-endometrioid cancer patients. Therefore, the inhibin-βB subunit seems, in contrast to inhibin-α and -βA, to have a substantial role in the pathology of non-endometrioid carcinomas and might be used as an easy, simple and highly efficient marker to identify high-risk patients and may aid in the selection of patients for a more aggressive adjuvant therapy. However, the observed prognostic significance of the inhibin-βB-subunit should be analysed in further studies.

Conflict of interest statement

The author declares that he has no competing interests. He received once a lecture fee in the year 2006 with the title 'Endometrial cancer and inhibin-subunits'.

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