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MEIS and PBX homeobox proteins in ovarian cancer

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ABSTRACT

Three amino-acid loop extension (TALE) homeobox proteins MEIS and PBX are cofactors for HOX-class homeobox proteins, which control growth and differentiation during embryogenesis and homeostasis. We showed that *MEIS* and *PBX* expression are related to cisplatin resistance in ovarian cancer cell lines. Therefore, MEIS1, MEIS2 and PBX expression were investigated immunohistochemically in a tissue microarray ($N = 232$) of ovarian cancers and ovarian surface epithelium ($N = 15$). Results were related to clinicopathologic characteristics and survival. All cancers expressed MEIS1, MEIS2 and PBX in nucleus and cytoplasm. MEIS1 and 2 only stained nuclear in surface epithelium. Nuclear MEIS2 was negatively related to stage, grade and overall survival in univariate analyses. Additionally, MEIS and PBX RNA expression in ovarian surface epithelium and other normal tissues and ovarian cancer versus other tumour types using public array data sets were studied. In ovarian cancer, *MEIS1* is highly expressed compared to other cancer types. In conclusion, MEIS and PBX are extensively expressed in ovarian carcinomas and may play a role in ovarian carcinogenesis.

1. Introduction

HOX homeobox proteins are transcription factors involved in growth control and differentiation during embryogenesis as well as homeostasis.[1] *HOX* genes, when deregulated, play important roles in oncogenesis. Their expression and function in cancers seems to be tissue-specific.[2–7] Three amino-acid loop extension (TALE) homeobox proteins MEIS and PBX function as cofactors for HOX proteins. All vertebrate model organisms seem to have three functional *MEIS* genes. Human *MEIS1* and *MEIS2* genes have been reported *in vivo*, while the *MEIS3* gene has only been identified *in silico*. Furthermore, there are four human *PBX* genes.[8–14]

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In a recent study using cDNA microarrays and reverse transcription polymerase chain reaction, we have shown that the three amino-acid loop extension (TALE) homeobox genes *MEIS1*, *MEIS2* and *PBX3* were down-regulated in three cisplatin resistant sub lines of the cisplatin sensitive parental ovarian cancer cell line A2780.[15] In addition, the *MEIS1* gene has been shown to be amplified and over-expressed in ovarian cancers compared to normal ovarian surface epithelium and is part of an ovary-specific gene expression profile distinguishing primary lung, colon and ovarian adenocarcinomas.[16–18]

As protein expression data on the HOX cofactors in ovarian cancer are lacking, the aim of the present study was to investigate MEIS1, MEIS2 and PBX protein expression in a large set of ovarian cancers. To discover the effect of chemotherapy on MEIS and PBX proteins in ovarian cancers, their expression levels were also compared between paired pre- and post-chemotherapy tumour samples. The results were related to clinicopathologic characteristics and survival. Finally, to compare MEIS and PBX RNA expression between normal ovarian surface epithelium and various other normal tissues and between ovarian cancer and various other tumour types the public Affymetrix data sets N353 and XPO1026 were studied.[19,20]

2. Material and methods

2.1. Tissue microarray

Since the early 1980s, all clinicopathologic and follow-up data of ovarian cancer patients referred to the Department of Gynaecologic Oncology at the University Medical Centre Groningen (Groningen, The Netherlands) were prospectively collected during standard treatment and follow-up and stored in a computerised database. International Federation of Gynaecology and Obstetrics (FIGO) staging was performed. The patients were treated according to regional guidelines on the diagnostic work-up, surgical and medical treatment and follow-up.[21] The surgical guidelines largely resembled FIGO guidelines.[22] New treatment regimens were adopted as follows: platinum-based chemotherapy in early 1980s, debulking surgery at the end of 1980s and platinum/paclitaxel chemotherapy since 1996. Clinical response to chemotherapy was determined according to standard WHO criteria.[23] Optimal and suboptimal debulking were defined as the largest tumour lesions having a diameter <2 cm or $\geqslant$2 cm, respectively. Progression free survival and overall survival were calculated from the date of primary surgery to the date of progression/relapse or last follow-up/death due to ovarian cancer, respectively. The database also contained information on the availability of tumour samples. Patients had given informed consent for collection and storage of tissue samples in a tissue bank for future research. Tumour samples were obtained at the time of surgery and embedded in paraffin blocks and/or frozen in liquid nitrogen and stored at −80 °C.

For the present study, the database was searched for consecutive patients treated for epithelial ovarian cancer between 1985 and 2002 and of whom paraffin-embedded tumour was available. All relevant data were retrieved from the database and transferred into a separate anonymous database. In this separate password protected database, patient identity was protected by study-specific, unique patient codes. The true identity of patients was only known to two dedicated data managers, who also have daily responsibility for the larger database. In case of uncertainties with respect to clinicopathologic and follow-up data, the larger databases could only be checked through the data managers, thereby ascertaining the protection of patients' identity. Owing to these precautions, according to Dutch law no further IRB approval was needed.

Eight tissue microarrays (TMAs) were constructed from tumour samples of 232 ovarian cancer patients. Of 44 patients paired tumour samples before and after first-line chemotherapy were available. Post-chemotherapy samples were collected at surgery after three or six cycles of chemotherapy ($N = 26$) or at surgery for recurrent disease ($N = 20$). TMAs were constructed as described in a previous study.[24] Four separate cores of 0.6 mm were retrieved from each tumour sample (Tissue Arrayer, Beecher Instruments, Silver Spring, MD, USA). Each TMA contained duplicate cores of 10 internal controls to ensure similarity of staining between the slides. As internal controls six tumour samples (serous, mucinous, endometrioid, clear cell and undifferentiated ovarian carcinoma, and an ovarian cystadenoma) and four normal tissue samples (Fallopian tube, endometrial, endocervical and cervical tissue) were present on each TMA. As controls apart from the TMAs, 15 paraffin blocks containing normal ovarian epithelium tissue (pre- ($N = 5$) and post-menopausal ($N = 5$) ovaries, and ovaries prophylactically removed from women with a BRCA1 ($N = 2$) and BRCA2 mutation ($N = 3$)), two blocks containing proliferating endometrial tissue and two blocks containing non-proliferating endometrial tissue were used.[25]

2.2. Immunohistochemistry

For immunohistochemistry 4 μm sections were cut from the ovarian cancer TMAs and paraffin blocks containing normal ovaries or endometrial tissue and mounted on 3-amino-propyl-ethoxy-silane coated glass slides (Sigma–Aldrich, Diesenhofen, Germany). All slides were stained within two weeks from sectioning. After the sections had been dewaxed in xylene, antigen retrieval was performed by autoclave treatment; three times 5 min at 115 °C in blocking reagent (2% block + 0.2% sodium dodecyl sulphate in maleic acid, pH 6.0; Boehringer Mannheim, Mannheim, Germany). Endogenous peroxidase activity was blocked by incubating the slides in hydrogen peroxidase. For MEIS1 and MEIS2, endogenous avidine and biotine activity was also blocked using Blocking kit (Vector laboratories, Burlingame, CA, USA). All primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA): MEIS1/2 (sc-10599), MEIS2 (sc-10600) and PBX1/2/3/4 (sc-28313). MEIS1, MEIS2 and PBX antibodies were diluted 1:25 and sections were incubated overnight at 4 °C. For MEIS1 and MEIS2 the slides were pre-incubated with 1.5% normal rabbit serum for 1 h at room temperature. For all washings and dilutions 0.05 M Tris-buffered saline containing 0.1% Tween-20 was used for MEIS1 and PBX, and PBS containing 1% bovine serum albumin was used for MEIS2. For negative controls the primary antibodies were omitted. PBX was detected using a goat anti-mouse/rabbit secondary antibody

conjugated with a peroxidase labelled polymer (DAKO EnVision+ system; DAKO, Cambridgeshire, UK). Biotinylated rabbit anti-goat IgG ((H + L), Southern Biotechnology, Birmingham, AL, USA) served as a secondary antibody (1:300 for 30 min at room temperature) for MEIS1 and MEIS2. For MEIS2, 1% normal rabbit serum was added to the dilution of the secondary antibody. ABComplex/HRP (DAKO) was applied for 30 min and 3, 3′-diaminobenzidine was used to visualise all antigen–antibody reactions.

Two observers (APGC and KAH) independently scored immunohistochemical stainings at a double-headed microscope without prior knowledge of the clinicopathologic information. The cases with a discrepant score were re-examined with a gynaecologic pathologist (HH) until consensus was reached. At least two of the four core biopsies representing each whole tumour sample had to be available for scoring. Nuclear and cytoplasmic immunoreactivity for the MEIS and PBX antibodies was graded as weak (0–1), moderate (2) or strong (3). Staining intensity was assessed by visual scoring. The stain intensity score was taken as the mean from the 2–4 biopsies that represented each tumour.

2.3. *Statistical analysis*

2.3.1. *Immunohistochemistry data analysis*

Statistical analysis was performed using the SPSS 12.0 software package (SPSS Inc., Chicago, IL). The relationship between nuclear and cytoplasmic expression of MEIS1, MEIS2

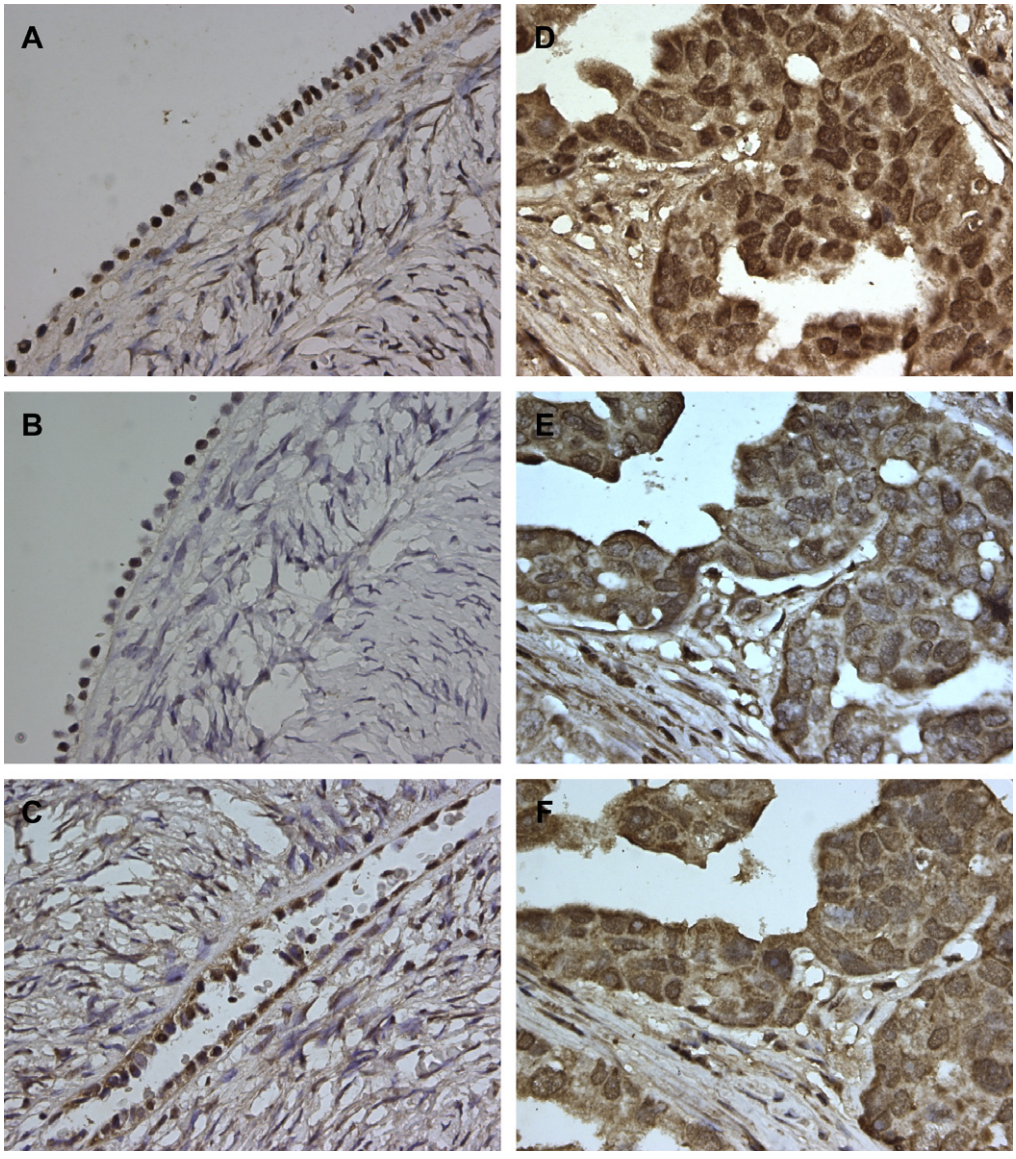

Fig. 1 – MEIS1, MEIS2 and PBX protein expression in ovarian tumour tissue and normal ovarian surface epithelium. (A) Nuclear MEIS1 expression in normal ovarian surface epithelium. (B) Nuclear MEIS2 expression in normal ovarian surface epithelium. (C) Nuclear and cytoplasmic PBX expression in normal ovarian surface epithelium. (D) Nuclear and cytoplasmic MEIS1 expression in ovarian tumour tissue. (E) Nuclear and cytoplasmic MEIS2 expression in ovarian tumour tissue. (F) Nuclear and cytoplasmic PBX expression in ovarian tumour tissue.

and PBX proteins in paired pre- and post-chemotherapy tumour samples was assessed by the Wilcoxon signed rank test. To assess the relation between nuclear MEIS1, MEIS2 and PBX protein expression and clinicopathologic characteristics univariate logistic regression analyses were performed, using MEIS1, MEIS2 and PBX as dependents, respectively. The cut-off-point for nuclear MEIS1 (weak/moderate or strong), MEIS2 (weak or moderate/strong) or PBX (weak/moderate or strong) expression was decided *a priori*. As independent clinicopathologic characteristics were included; age (>59 or ⩽59 years), stage (stage III/IV or stage I/II), histology (serous or non-serous), grade (grade 3/undifferentiated or grade 1/2 and residual disease (>2 cm or ⩽2 cm). For MEIS2 also multivariate logistic regression analysis was performed adjusted for the variables stage, grade and histology. To study whether nuclear MEIS1, MEIS2 and PBX protein expression were predictive for overall survival and progression free survival, survival curves were calculated using Kaplan–Meier analysis with assessment of statistical significance using the log-rank test. Subsequently, to investigate whether MEIS1, MEIS2 and PBX were independent prognostic factors, multivariate overall survival and progression free survival analyses were performed using Cox proportional-hazard regression models adjusted for stage and residual tumour. *P*-values of 0.05 were considered significant.

2.3.2. *Public Affymetrix data set analysis*

Affymetrix data for human normal tissues (N353) and several cancer types (XPO1026 (https://expo.intgen.org/expo/public)) were retrieved from public GEO (Gene Expression Omnibus) data sets on the NCBI website.[19,20] CEL data from the Affymetrix GeneChip Human Genome U133 Plus 2.0 array data sets were downloaded and intensity values and their accompanying *P*-values assigned to *MEIS1*, *MEIS2*, *MEIS3* (*in silico* identified sequence) and *PBX1–4* probe-sets with GCOS software using the MASS5.0 algorithm. Annotations for the tissue samples analysed are available from http://www.ncbi.nlm.nih.gov/geo/query/ through their GEO ID: GSE3526 9 and GSE210 for the N353 and XPO1026 data sets, respectively.

Table 1 – Clinicopathologic characteristics of the ovarian cancer patients

Characteristic	N (%)		
	All stages, N = 232	Stage I/II, N = 64	Stage III/IV, N = 166
Age			
Median	59	54	60
Range	(21–89)	(23–83)	(21–89)
Stage (FIGO)			
I	45 (20)		
II	19 (8)		
III	133 (58)		
IV	33 (14)		
Unknown	2		
Grade			
1	39 (18)	29 (48)	9 (6)
2	52 (25)	22 (37)	29 (20)
3	104 (50)	7 (12)	97 (66)
Undifferentiated	14 (7)	2 (3)	12 (8)
Unknown	23	4	19
Histological subtype			
Serous	128 (55)	13 (20)	115 (69)
Mucinous	27 (12)	18 (28)	8 (5)
Endometrioid	33 (14)	19 (30)	14 (8)
Clear Cell	17 (7)	6 (9)	10 (6)
Other	27 (12)	8 (13)	19 (12)
Debulking status			
Optimal < 2 cm	111 (50)	61 (97)	48 (31)
Suboptimal ⩾ 2 cm	109 (50)	2 (3)	107 (69)
Unknown	12	1	11
First-line chemotherapy			
None	36 (16)	25 (40)	11 (7)
Platinum-based	171 (76)	34 (55)	136 (84)
Non-platinum-based	17 (8)	3 (5)	15 (9)
Unknown	8	2	4
Chemotherapy-response			
CR[a]/PR[b]	82 (71)	3	79 (70)
SD[c]/PD[d]	34 (29)		34 (30)

a CR, complete response.
b PR, partial response.
c SD, stable disease.
d PD, progressive disease.

3. Results

3.1. *MEIS and PBX protein expression in normal ovarian surface epithelium, primary and paired pre- and post-chemotherapy ovarian tumours*

In normal ovarian surface epithelium MEIS and PBX protein expression were clearly visible (Fig. 1). MEIS1 and MEIS2 stained exclusively nuclear, while PBX staining was also cytoplasmic. There were no obvious differences in staining patterns for the three proteins neither in normal ovarian surface epithelium from pre-menopausal women, post-menopausal women or women with familial ovarian cancer.

The clinicopathologic data of the 232 primary cancers present on the TMA are summarised in Table 1. The median follow-up time of the patients was 26 months (range: 0–213 months) and the 5-year overall survival rate was 31% (118 patients died because of ovarian cancer).

MEIS1, MEIS2 and PBX protein expression were identified in ovarian cancers (Fig. 1). Tumours showed nuclear as well as cytoplasmic staining. All tumour sections wholly and homogeneously stained for MEIS 1 and 2 and PBX. The percentage ovarian cancers per staining category for each protein are presented in Table 2. Nuclear MEIS1 and PBX expression were strong in most of the cancers (in 90% and 74%, respectively). Cytoplasmic MEIS1 and PBX expression were moderate in 81% and 66% of the cancers, respectively. Nuclear MEIS2 expression was weak in about half of tumours and moderate/strong in the other half. Cytoplasmic MEIS2 expression was weak in 33% and moderate in 62% of the cancers.

Table 2 – Nuclear and cytoplasmic MEIS and PBX protein expression in ovarian cancer samples

	N	NE[a]	Weak	Moderate	Strong
Nuclear MEIS1					
Primary	232	25 (11%)	2 (1%)	18 (8%)	187 (80%)
After 3/6 cycles chemotherapy	26	6 (23%)	0 (0%)	2 (8%)	18 (69%)
Recurrent disease	20	1 (5%)	0 (0%)	1 (5%)	18 (90%)
Cytoplasmic MEIS1					
Primary	232	25 (11%)	27 (12%)	167 (72%)	13 (5%)
After 3/6 cycles chemotherapy	26	6 (23%)	0 (0%)	19 (73%)	1 (4%)
Recurrent disease	20	1 (5%)	1 (5%)	17 (85%)	1 (5%)
Nuclear MEIS2					
Primary	232	29 (13%)	105 (45%)	88 (38%)	10 (4%)
After 3/6 cycles chemotherapy	26	6 (23%)	16 (62%)	4 (15%)	0 (0%)
Recurrent disease	20	2 (10%)	8 (40%)	10 (50%)	0 (0%)
Cytoplasmic MEIS2					
Primary	232	29 (13%)	66 (28%)	126 (54%)	11 (5%)
After 3/6 cycles chemotherapy	26	6 (23%)	9 (35%)	10 (38%)	1 (4%)
Recurrent disease	20	2 (10%)	5 (25%	13 (65%)	0 (0%)
Nuclear PBX					
Primary	232	25 (11%)	11 (5%)	42 (18%)	154 (66%)
After 3/6 cycles chemotherapy	26	6 (23%)	2 (8%)	8 (31%)	10 (38%)
Recurrent disease	20	2 (10%)	0 (0%)	5 (25%)	13 (65%)
Cytoplasmic PBX					
Primary	232	25 (11%)	47 (20%)	136 (59%)	24 (10%)
After 3/6 cycles chemotherapy	26	6 (23%)	7 (27%)	10 (38%)	3 (12%)
Recurrent disease	20	2 (10%)	4 (20%)	12 (60%)	2 (10%)

All tumour sections wholly and homogeneously stained for MEIS and PBX.
a NE, not-evaluable.

Table 3 – Comparison of MEIS1, MEIS2 or PBX expression between paired ovarian pre-and post-chemotherapy tumour samples

	N	Ties[a]	P[b]
After 3/6 cycles chemotherapy	26		
Nuclear MEIS1	20	17	0.56
Nuclear MEIS2	20	16	1.00
Nuclear PBX	20	12	0.61
Cytoplasmic MEIS1	20	15	0.66
Cytoplasmic MEIS2	20	10	0.78
Cytoplasmic PBX	20	10	0.53
Recurrent disease	20		
Nuclear MEIS1	19	18	0.32
Nuclear MEIS2	17	6	0.76
Nuclear PBX	18	8	0.53
Cytoplasmic MEIS1	19	13	1.00
Cytoplasmic MEIS2	18	9	0.32
Cytoplasmic PBX	18	5	0.32

a Ties: similar expression of MEIS1, MEIS2 or PBX between paired ovarian pre- and post-chemotherapy tumour samples.
b Compared with primary ovarian cancer samples, Wilcoxon paired test.

To study whether chemotherapy influenced MEIS 1 and 2 and PBX expression levels, as observed in the isogenic ovarian cancer cisplatin resistance cell line model, their expression levels were compared between paired pre- and post-chemotherapy samples of 44 patients.[15] Table 3 shows that nuclear and cytoplasmic expression of MEIS1, MEIS2 and PBX were not different between paired pre-chemotherapy samples and samples obtained after three or six courses of first-line chemotherapy, nor between paired pre-chemotherapy samples and samples obtained at surgery for recurrent disease.

From the univariate logistic regression analyses (Table 4) it appeared that moderate/strong nuclear MEIS2 expression was related with early stage (odds ratio 0.46 (0.25–0.87)) and grade 1 or 2 tumours (odds ratio 0.47 (0.26–0.85)). There seemed to be a relation between strong nuclear MEIS1 (odds ratio 0.38 (0.13–1.07)) or moderate/strong MEIS2 expression (odds ratio 0.59 (0.34–1.03)) and non-serous ovarian cancers. The multivariate logistic regression analysis for MEIS2 showed that stage (odds ratio 0.61 (0.26–1.44)), grade (odds ratio 0.65 (0.32–1.33)) and histology (odds ratio 0.90 (0.46–1.79)) were not independently related with MEIS2 expression. Moderate/strong nuclear MEIS2 expression was related with a better overall survival ($p = 0.036$), whereas MEIS1 ($p = 0.12$) and PBX ($p = 0.55$) expression showed no relation with survival. Fig. 2 shows the Kaplan–Meier overall survival curves calculated for MEIS2. The multivariate Cox regression analyses adjusted for stage and residual tumour (Table 5) showed that MEIS 1 and 2 and PBX were not independent prognostic factors for overall survival. The data for progression free survival were comparable to the results for overall survival (not shown).

Table 4 – Results of the univariate logistic regression analysis for nuclear MEIS1, MEIS2 and PBX protein expression and clinicopathologic characteristics in ovarian cancer (odds ratios (OR) and 95% confidence intervals (CI))

Clinicopathologic characteristic	Nuclear MEIS1 (strong or weak/moderate)		Nuclear MEIS2 (moderate/strong or weak)		Nuclear PBX (strong or weak/moderate)	
	OR	95% CI	OR	95% CI	OR	95% CI
Age						
> or ⩽ median age	1.25	0.49–3.20	1.18	0.68–2.06	1.53	0.81–2.89
Stage						
III/IV or I/II	0.42	0.12–1.48	0.46	0.25–0.87[a]	0.67	0.33–1.40
Histologic type						
serous or non-serous	0.38	0.13–1.07	0.59	0.34–1.03	0.85	0.45–1.60
Grade						
3/undifferentiated or 1/2	0.84	0.33–2.16	0.47	0.26–0.85[1]	0.57	2.91–1.12
Residual disease						
>2 cm or ⩽2 cm	0.86	0.33–2.22	0.92	0.52–1.62	0.96	0.50–1.84

a $p < 0.02$.

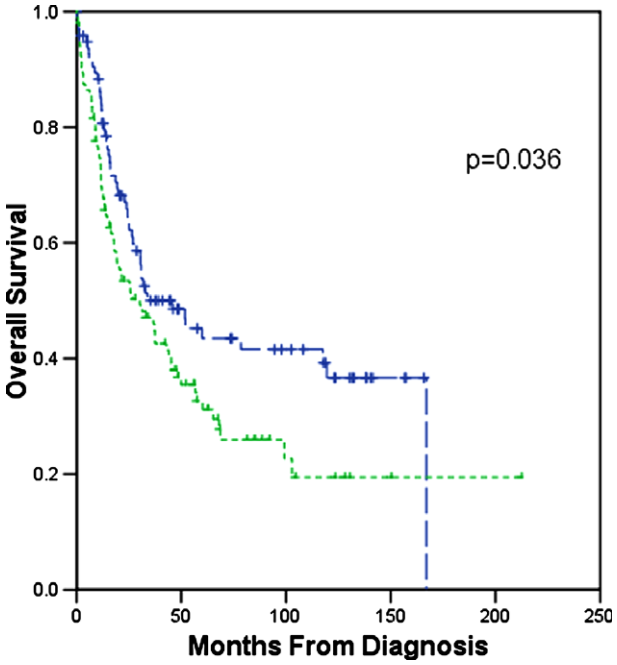

Fig. 2 – The Kaplan–Meier overall survival curves calculated for MEIS2. Moderate/strong nuclear MEIS2 expression (––) was related with a better overall survival ($p = 0.036$) in ovarian cancer patients (all stages). Weak nuclear MEIS2 expression: (- - - -) curve.

Table 5 – Results of multivariate Cox regression overall survival analysis for nuclear MEIS1, MEIS2 and PBX protein expression in ovarian cancer adjusted for stage and residual tumour (hazard ratios (HR) and 95% CI)

TALE protein	Overall survival	
	HR	95% CI
Nuclear MEIS1 (strong or weak/moderate)	1.00	0.54–1.83
Nuclear MEIS2 (moderate/strong or weak)	0.87	0.60–1.26
Nuclear PBX (strong or weak/moderate)	0.89	0.59–1.34

3.2. *MEIS and PBX gene expression in public human Affymetrix data sets of normal (N353) and tumour (XPO1026) tissue of different origins*

The average expression of the *MEIS1*, *MEIS2*, *MEIS3* (*in silico* identified sequence), *PBX1*, *PBX2*, *PBX3* and *PBX4* genes in normal tissue ranges from 53–1249, 60–1792, 15–333, 162–2580, 62–303, 99–774 to 7–364, respectively (see Table 6). In normal ovary average expression of *MEIS1* (559, standard error (SE): 93) and *MEIS2* (489, SE: 72) is comparable. Furthermore, *PBX1* (898, SE: 60) and *PBX3* (747, SE: 183) seem to be well expressed in normal ovarian tissue compared to *PBX2* (248, SE: 34) and *PBX4* (55, SE: 36).

The average expression of *MEIS1*, *MEIS2*, *MEIS3*, *PBX1*, *PBX2*, *PBX3* and *PBX4* in cancer ranges from 86–1018, 178–865, 34–147, 299–899, 64–228, 72–927 to 24–95, respectively (Table 7). In ovarian cancer average *MEIS1* expression (902, SE: 111) is much higher than average *MEIS2* expression (353, SE: 50). Additionally, of the four *PBX* genes *PBX1* has the highest expression (685, SE: 46). Moreover, the average expression of *MEIS1* in ovarian and uterine cancer and in neuroblastoma and medulloblastoma is high compared to the other tumour types (Table 7 and Fig. 3).

4. Discussion

This study shows that in ovarian carcinomas MEIS1, MEIS2 and PBX proteins are extensively expressed, both nuclear and cytoplasmic. In normal ovarian surface epithelium, however, MEIS1 and 2 only stained nuclear. Additionally, MEIS1 RNA is much higher expressed in ovarian cancer compared to other tumour types.

These specific findings in ovarian cancer are of interest as MEIS1 and 2 and PBX could be important in ovarian oncogenesis by potentiating the function of aberrantly expressed HOX proteins.[5,26–28] When a HOX protein forms a complex with a MEIS and a PBX protein, they show powerful downstream target promoter regulation as their DNA-binding affinities and specificities are increased significantly.[29,30] Co-activation of *HOXA9* and *MEIS1* in mouse bone marrow cells has been re-

Table 6 – Average gene expression of MEIS and PBX in various normal tissues (N353 Affymetrix data set)

Tissue type	N[a]	MEIS1		MEIS2		MEIS3[b]		PBX1		PBX2		PBX3		PBX4	
		Exp[c]	SE[d]	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE
Adipose tissue	3	140.7	64.5	241.3	54.9	43.7	18.7	411.1	20.2	121.5	32.0	185.4	30.1	39.0	21.6
Adipose omental tissue	4	467.3	15.6	464.9	21.7	40.1	9.6	873.8	71.1	146.6	10.9	149.7	13.3	19.3	5.3
Adipose subcutaneous tissue	3	96.5	20.1	184.2	37.6	62.5	20.5	485.1	65.9	109.8	16.8	167.6	5.3	42.7	17.1
Adrenal gland cortex	4	804.0	94.3	1161.4	105.4	38.4	12.7	954.08	116.3	120.3	14.7	590.8	65.5	7.1	1.1
Bone marrow	5	89.4	17.8	59.5	3.0	58.5	14.5	201.7	38.7	298.2	48.4	130.2	5.7	16.4	2.0
Bronchus	3	377.6	70.0	268.4	43.3	100.0	19.0	413.9	57.7	155.3	43.6	174.3	10.0	61.3	6.4
Cerebellum	9	408.8	28.7	263.7	13.9	56.3	14.1	416.0	26.3	164.4	16.8	131.7	15.0	25.4	5.4
Cerebral cortex	9	112.0	19.5	410.4	36.9	118.1	19.8	595.2	33.3	171.2	18.4	124.3	19.4	21.1	6.1
Cerebrum	143	134.9	6.4	505.3	41.7	93.7	8.0	514.2	11.7	163.5	4.6	233.5	10.5	26.0	1.6
Cervix	4	1208.5	107.0	749.5	79.7	98.1	11.9	1723.3	149.7	179.9	14.3	449.4	45.9	29.9	8.3
Colon caecum	3	398.2	74.5	392.6	91.5	32.5	8.5	645.8	100.0	149.3	8.6	246.9	56.0	49.5	16.6
Coronary artery	3	110.0	6.6	559.9	48.8	63.3	11.0	546.1	45.0	302.7	121.8	216.6	47.1	30.1	12.7
Dorsal root ganglia	8	71.1	8.3	147.5	15.8	61.5	9.1	293.8	12.3	159.9	13.1	287.7	16.6	27.0	3.2
Endometrium	4	1210.4	166.5	737.7	408.0	209.8	63.0	1424.0	487.0	274.9	61.2	171.4	45.0	43.5	13.8
Oesophagus	4	524.2	68.4	352.6	48.3	50.3	17.0	888.6	113.6	150.3	26.4	264.2	38.8	33.4	5.4
Heart atrium	4	260.0	31.4	436.8	19.1	35.6	7.4	615.7	39.3	208.0	46.9	466.6	72.8	11.9	5.3
Heart ventricle	3	197.2	40.1	605.3	119.8	23.0	5.2	589.1	78.0	175.5	15.4	272.6	41.3	21.2	11.2
Kidney cortex	4	98.7	18.0	372.3	24.0	20.4	1.0	629.0	29.6	132.3	19.1	98.7	12.6	30.2	10.2
Kidney medulla	4	144.3	21.6	509.8	53.2	28.3	8.4	625.3	65.5	120.1	11.7	136.2	13.9	67.8	5.9
Liver	4	81.2	16.5	207.9	26.6	15.0	1.2	210.0	28.2	115.9	11.5	213.3	29.7	14.7	3.5
Lung	3	453.8	20.7	407.5	19.5	39.0	13.9	425.3	37.7	154.2	35.1	230.0	26.3	51.2	23.7
Lymph nodes	4	283.6	184.8	515.7	162.9	48.3	10.3	460.2	167.7	168.0	14.3	272.0	94.5	91.0	30.2
Mammary gland	3	146.4	14.1	358.9	76.1	63.4	8.5	689.0	87.9	167.6	24.2	203.8	40.7	31.1	14.8
Myometrium	5	1249.1	199.8	1792.2	200.5	333.1	69.4	2580.0	272.8	296.9	93.2	252.3	29.2	16.2	5.5
Nipple cross-section	4	194.6	35.8	421.8	45.6	50.8	12.7	1033.9	88.1	215.3	39.0	185.7	12.3	43.4	6.5
Nodose nucleus	8	243.9	17.0	269.4	22.5	32.8	6.4	368.1	19.2	172.6	19.3	368.7	25.9	11.6	2.7
Oral mucosa	4	281.0	44.6	159.4	6.1	39.0	5.1	646.4	44.2	143.6	31.2	214.0	50.1	38.0	11.0
Ovary	4	559.4	92.9	488.6	72.0	79.6	13.7	898.4	60.2	248.4	33.5	774.4	182.6	54.9	35.6
Pharyngeal mucosa	4	463.8	81.8	392.7	51.2	48.8	9.8	379.4	31.1	117.4	10.3	99.1	15.7	44.2	10.0
Pituitary gland	8	105.3	48.1	648.7	85.1	100.2	19.4	745.1	72.5	239.0	27.9	237.2	31.9	50.7	10.0
Prostate gland	3	302.3	63.4	1347.0	70.5	84.6	12.5	893.8	102.6	134.8	22.3	436.5	14.4	39.6	9.7
Salivary gland	4	648.5	55.6	1234.3	106.8	41.2	11.8	877.8	14.7	130.0	20.5	351.0	23.3	14.0	4.6
Saphenous vein	3	106.1	16.1	446.3	113.4	55.4	9.0	652.4	65.3	179.8	26.2	192.0	33.0	12.2	1.5
Skeletal muscle	5	98.4	12.2	90.7	13.2	36.3	9.4	588.1	37.2	128.1	22.5	101.8	15.3	17.4	3.9
Spinal cord	8	191.0	9.8	365.3	14.6	52.0	7.3	493.1	32.5	111.9	14.1	412.0	17.5	26.4	7.3
Spleen	4	245.3	26.6	309.4	60.9	36.1	9.9	338.4	39.8	162.0	23.2	240.9	24.8	60.1	8.0
Stomach cardiac	3	491.9	306.7	370.3	150.2	25.8	7.7	792.0	228.0	197.5	16.2	328.7	88.0	46.8	11.6

(*continued on next page*)

Table 6 – *continued*

Tissue type	N[a]	MEIS1		MEIS2		MEIS3[b]		PBX1		PBX2		PBX3		PBX4	
		Exp[c]	SE[d]	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE
Stomach fundus	4	880.4	343.7	739.0	208.9	50.3	7.8	912.1	250.3	198.8	20.9	389.5	107.4	66.5	15.7
Stomach pyloric	4	446.7	147.0	628.8	168.8	36.6	8.2	592.2	88.7	139.3	15.8	286.7	62.8	95.8	20.3
Testes	3	64.6	4.6	65.0	6.1	80.3	13.0	162.0	21.2	73.4	18.0	143.4	10.9	131.6	8.0
Thyroid gland	4	80.2	8.2	246.6	35.9	53.5	7.1	856.2	58.3	192.9	29.4	408.4	14.0	364.3	41.5
Tongue main corpus	4	186.6	5.5	211.5	19.9	29.8	10.1	491.1	24.2	112.8	6.4	140.0	22.0	24.1	11.5
Tongue superior part w/papillae	4	245.4	90.3	309.2	87.6	42.8	11.2	434.6	80.2	105.7	10.2	147.1	25.5	34.5	22.7
Tonsil	3	148.3	25.3	160.3	28.6	40.2	7.5	275.6	44.1	157.0	27.1	131.1	28.3	104.7	16.6
Trachea	3	477.9	25.9	312.2	25.3	58.3	6.0	546.1	66.3	122.2	19.8	170.4	19.9	39.8	5.6
Trigeminal ganglia	8	53.0	8.8	197.2	20.4	64.6	8.4	299.9	24.7	165.1	16.0	282.5	23.7	26.2	4.4
Urethra	3	469.1	29.3	826.27	54.2	70.4	16.1	858.1	59.5	131.6	26.2	182.8	7.3	34.1	3.6
Vagina	4	905.7	116.1	790.1	199.8	45.4	11.1	1131.6	220.7	180.1	31.9	501.8	50.8	25.6	7.4
Vulva	4	422.4	59.5	377.3	21.2	56.1	8.8	528.3	33.5	244.6	18.0	275.4	30.1	31.0	9.0

a *N*: number of normal tissue samples.
b *In silico* identified *MEIS3* sequence.
c Exp: average expression.
d SE: standard error.

ported to rapidly induce acute myeloid leukaemia, an effect not observed with over-expression of these homeobox genes alone.[31] In ovarian carcinomas the effect of co-activation of HOX, MEIS and PBX has not yet been investigated, although aberrant expression of HOX RNA and proteins has been demonstrated. In ovarian cancer the HOXA9–11 proteins are expressed according to a subtype-specific pattern, whereas they are absent in normal ovarian surface epithelium. The ability of *HOXA9–11* to induce differentiation along their respective pathways was shown to be promoted by *HOXA7*.[26] Additionally, *HOXB7* and *HOXB13* genes were found to be over-expressed in ovarian cancer cell lines and cancers compared to whole normal ovaries and invasive characteristics of the ovarian cancer SKOV3 cells were found to be suppressed by the expression of anti-sense HOXB7 and HOXB13 mRNA.[28] As we have shown that MEIS and PBX proteins are frequently expressed in ovarian carcinomas they may potentiate the effect of these aberrantly expressed *HOX* genes on their target genes.

Moreover, there is evidence that *HOX*, *MEIS* and *PBX* genes are involved in oncogenic processes, such as chromatin binding, cell cycle control, proliferation, apoptosis, angiogenesis and cell–cell communications.[3,7,25,28,32–39] It has been shown that in the normal endometrium MEIS1 protein was expressed in early proliferative glandular epithelium and was absent throughout the rest of the cycle, suggestive of a function in proliferation for MEIS1.[25] Furthermore, after exposure of the ovarian surface epithelium cell line MCV152 to follicle-stimulating hormone, cell proliferation was increased and *MEIS1* expression was up-regulated.[37] Constitutive over-expression of *MEIS1* may thus promote tumour growth in endometrial and ovarian cancer. This is supported by the finding that *MEIS1* RNA is highly expressed in these cancer types.

In Drosophila, MEIS protein is necessary for nuclear localisation of PBX, which is exported to the cytoplasm in the absence of MEIS, and this mechanism was initially confirmed in mammalian cells for both MEIS1 and MEIS2.[40,41] A later report however, indicates that nuclear localisation of PBX1 can also be regulated independently of MEIS proteins.[42] Interestingly, in normal endometrial epithelium cells in the developing female genital tract, PBX1 can be cytoplasmic even in the presence of MEIS, possibly in correlation with the cell cycle.[43] It is therefore difficult to speculate whether our finding that the localisation of MEIS1 and 2 in ovarian cancers is both nuclear and cytoplasmic compared to nuclear in normal ovarian surface epithelium is important for their function as well as the function of PBX. Further research has to elucidate the mechanisms and meaning of MEIS and PBX localisation in both normal and tumour tissues of the female genital tract.

In the present study MEIS1 and PBX RNA and protein were higher expressed than MEIS2, indicating that these are the main HOX cofactors present in ovarian cancers. Univariate analysis showed that moderate/strong nuclear MEIS2 protein expression was related to early stage and non-serous cancers and also associated with better overall survival. An explanation for the lack of relation between nuclear MEIS1 and PBX and clinicopathologic characteristics or survival may be the similar expression pattern in all ovarian cancers.

Analyses of paired samples before and after chemotherapy showed that, the expression of all three proteins was not

Table 7 – Average gene expression of MEIS and PBX in various tumour types (XPO1026 Affymetrix data set)

Tumour type	N[a]	MEIS1		MEIS2		MEIS3[b]		PBX1		PBX2		PBX3		PBX4	
		Exp[c]	SE[d]	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE	Exp	SE
Bladder	8	149.7	21.2	274.0	59.5	40.6	8.4	396.8	85.7	172.9	31.2	201.7	28.9	53.8	9.7
Breast	207	105.1	6.9	197.6	11.6	68.9	2.6	898.7	35.7	167.9	4.8	204.6	8.8	29.8	1.3
Cervix	10	206.6	40.7	288.4	32.0	50.9	13.4	576.0	130.2	183.8	23.2	204.6	24.6	46.4	10.0
Colon	146	177.7	23.3	190.4	9.9	39.7	2.4	349.4	12.4	151.0	4.8	154.8	5.3	48.5	1.8
Corpus uteri	7	517.9	221.0	691.6	270.2	67.4	26.2	792.7	80.8	152.9	48.7	249.7	108.0	37.16	12.7
Endometrium	63	772.9	61.5	457.6	50.9	51.5	5.3	703.8	58.3	179.4	9.4	112.2	19.0	37.2	2.5
Kidney	112	116.5	8.0	349.5	34.1	37.3	3.0	310.7	12.4	196.9	6.1	138.6	5.3	37.4	2.3
Liver	16	142.8	35.1	177.8	46.0	41.9	7.3	342.2	52.7	172.9	17.3	173.5	16.6	46.8	3.6
Lung	74	157.2	9.6	268.0	32.6	46.0	3.9	386.7	29.1	177.1	8.4	212.2	15.2	42.1	2.6
Medulloblastoma	51	384.9	109.5	715.7	74.	130.9	5.8	298.9	55.9	64.2	20.3	123.5	10.3	95.0	3.9
Neuroblastoma	110	965.4	76.4	864.8	40.7	147.0	4.2	452.2	36.6	120.4	10.0	401.4	13.1	40.3	2.6
Omentum	36	1018.2	24.6	429.0	76.0	57.5	5.4	692.2	47.5	227.6	10.3	147.4	33.5	47.9	2.4
Ovary	98	902.0	110.8	353.3	50.4	42.0	7.1	685.1	45.6	193.1	10.4	177.4	19.1	42.7	4.2
Prostate	20	198.9	39.4	671.7	47.9	41.2	8.4	564.7	49.7	173.3	15.8	475.0	33.3	23.5	10.9
Rhabdomyosarcoma	9	282.7	26.0	427.7	49.8	61.4	7.4	464.4	78.7	61.7	28.9	138.0	36.7	140.6	8.4
Rectosigmoid	19	274.6	103.6	224.2	189.5	41.9	12.1	400.8	245.0	182.7	14.9	153.5	61.6	36.4	12.0
Rectum	19	150.2	87.2	188.4	306.0	48.2	9.1	335.9	132.8	158.7	23.4	169.4	58.7	64.4	12.8
Renal pelvis	8	132.6	28.4	327.9	58.9	35.1	5.5	453.7	65.5	194.4	30.3	132.6	353.7	61.8	18.9
Small intestine	10	267.2	43.7	59.23	83.9	57.2	11.1	678.5	98.0	202.7	14.6	280.7	59.9	50.7	6.7
Stomach	6	375.4	82.3	609.9	75.6	34.4	8.3	522.2	74.8	155.6	20.9	252.3	12.4	45.9	3.5
Thyroid	14	86.5	21.2	246.7	59.5	35.9	8.4	588.8	85.7	182.8	31.2	927.2	28.9	81.1	9.7
Urinary bladder	7	200.3	6.9	382.6	11.6	62.8	2.6	746.4	35.7	141.0	4.8	228.3	8.8	49.7	1.3
Uterus	14	679.1	40.7	535.8	32.0	40.2	13.4	615.9	130.2	177.1	23.2	71.5	24.6	28.8	10.0

a N: number of tumour samples.
b *In silico* identified *MEIS3* sequence.
c Exp: average expression.
d SE: standard error.

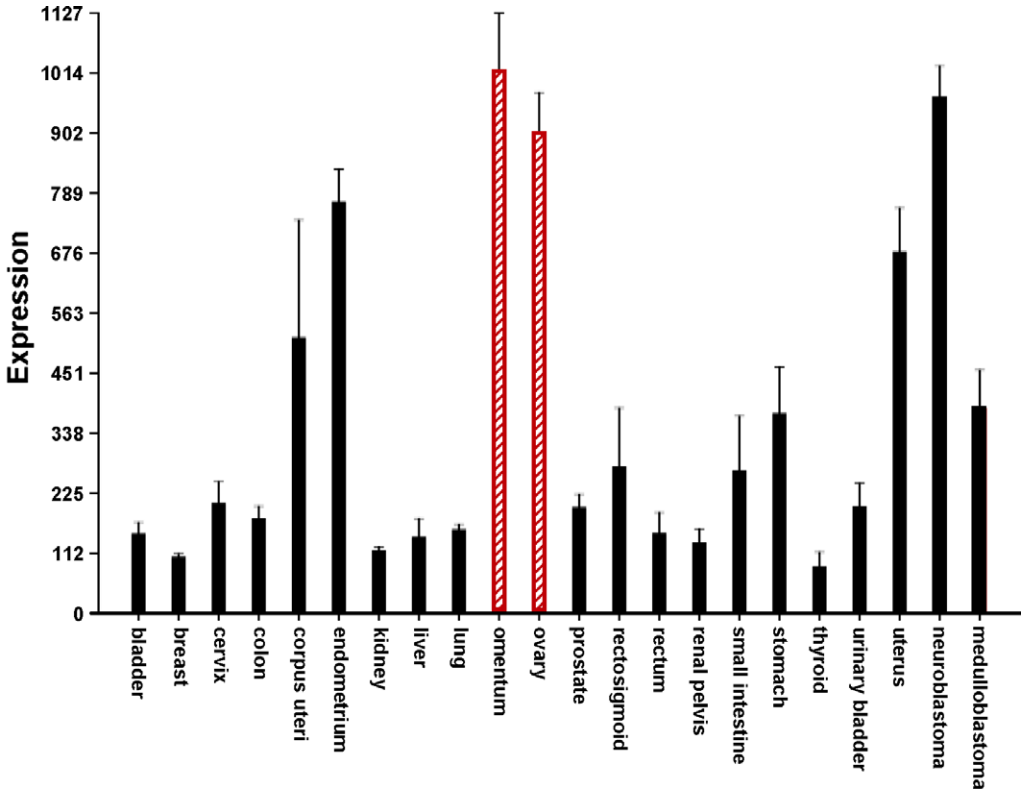

Fig. 3 – Bar diagram showing average MEIS1 RNA expression in ovarian tumours and various other tumour types based on analysis of the public human Affymetrix data set XPO1026. The dashed bars correspond to the average MEIS1 expression in ovarian cancer (omentum and ovary). The error bars represent the standard error of MEIS1 expression. The average expression of *MEIS1* in ovarian cancer is high compared to most other tumour types.

influenced by preceding first-line chemotherapy and not different at the time of recurrence in paired cancers. In our micro-array study of four ovarian cancer cell lines, *MEIS1* and 2 and *PBX3* gene expression were associated with cisplatin resistance.[15] This may be due to the fact that availability of paired patient samples only occurs in the case of residual and resistant disease.

Targeting of MEIS1 or 2 or PBX may impair the oncogenic function of various aberrantly expressed HOX proteins at once. Although targeting of homeobox proteins with drugs is momentarily not possible, targeting MEIS1 or 2 or PBX *in vitro* with siRNA is an option. As *MEIS1* appears to be so highly expressed in ovarian cancers compared to other cancer types especially this gene seems the most interesting candidate for targeted therapy.

It is important in future research to discover aberrantly expressed *HOX* genes in ovarian cancer and how their function is enforced by their cofactors *MEIS1* and 2 and *PBX*. This could lead to insight in how oncogenic HOX function would be abolished by targeting MEIS1 and 2 and PBX.

Conflict of interest statement

There are no financial or personal relationships with other people or organisations that could inappropriately influence the work.

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