Antimetastasis gene expression and numerical chromosomal abnormalities of chromosomes 1 & 17 in serous tumours of the ovary

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Summary

Objective(s): The aim of this study was to examine the expression of the antimetastasis gene nm23 and numerical changes on chromosome 1 and 17 in ovarian tumours.

Methods: In this study 20 serous cystadenocarcinomas, ten borderline and five benign tumours were analysed for expression of the nm23 antimetastasis gene by immunohistochemistry and for numerical chromosomal abnormalities of chromosomes 1 and 17 by interphase cytogenetics.

Results: Strong intracytoplasmic immunoreactivity with the antimetastasis gene was observed in late stage carcinomas but not in benign or borderline tumours or in lymph node metastases. Numerical abnormalities were only observed in carcinomas.

Conclusion(s): These sets of data are consistent with the majority of benign and borderline tumours lacking invasive potential. Odds Ratio (OR) assessment indicates that the presence of numerical aberrations correlates with immunopositivity.

Key words: nm23 expression; Interphase cytogenetics; Ovarian tumours.

Introduction

Ovarian cancer is the most frequently fatal form of female genitourinary tumour. The majority of ovarian tumours arise from the surface epithelium and are classed histologically as benign, borderline (low malignant potential) or as invasive carcinoma. The molecular cell biology and interrelationship of these tumours is poorly understood.

The major cause of death for patients with solid tumours is due to metastasis. A decreased expression of the antimetastasis gene nm23 has been identified as characteristic of highly metastatic melanoma cells lines [1]. Varieties of genetic disruption including numerical chromosomal abnormalities also characterise the metastatic condition. Amongst ovarian carcinomas numerical abnormalities involving chromosome 1 are the most common [2]. Chromosomes 3, 6, 7, 11, 17 and X are also frequently numerically altered [2]. Chromosome 17 is of interest being the site of nm23 (17q22) and p53 (17p) and BRCA1 (17q21). This study set out to examine nm23 expression and numerical chromosomal abnormalities across the spectrum of serous ovarian tumours and to look at any correlation between the two types of data. Twenty serous cystadenomas (including one with lymph node metastases), ten serous borderline tumours and five serous benign tumours were analysed for nm23 expression by immunohistochemistry and by interphase cytogenetics for numerical chromosomal aberrations of chromosomes 1 and 17.

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Materials and Methods

Cases: Twenty cases of cystadenocarcinomas, ten borderline tumours and five benign tumours were selected from the diagnostic files of the Marmara University Hospital in Turkey. Slides were classified and staged according to WHO and FIGO classifications. Parallel 6μm paraffin sections were used both for immunohistochemistry and interphase cytogenetics. Normal ovarian epithelial tissues were also selected and breast carcinoma tissue was used as a positive control.

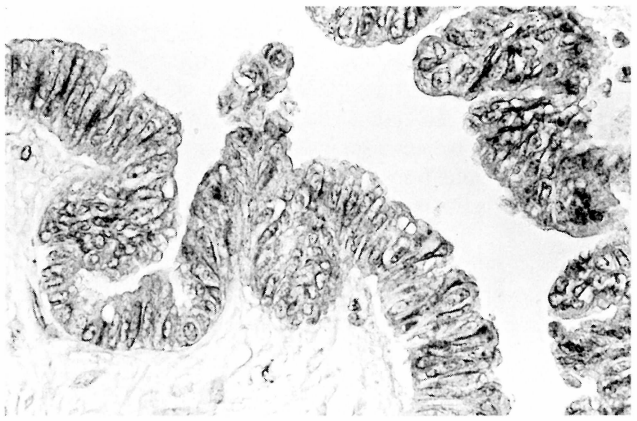
Immunohistochemistry: A nm23 mouse monoclonal antibody (Bio Genex) against the nucleoside diphosphate (NDP) kinase which is theoretically homologous to the nm23-H1 gene product was used to demonstrate expression. The immunohistochemical procedure employed a biotin-streptavidin amplification system. Fast red (Bio Genex) was the chromogene . Sections were counterstained with Mayer's haematoxylin and 1,000 cells were assessed for immunostaining.

Interphase cytogenetics: Interphase cytogenetic analysis was performed on 6 μm sections and assessed as previously detailed [3]. Biotinylated pericentromeric probes pUC1.77, (courtesy of Dr. A. N. Hopman, University of Limburg, The Netherlands) for chromosome 1 and D171 (Oncor) for chromosome 17 were employed. Hybridisation signal was detected using 3,3'-diaminobenzidine tetrahydrochloride (DAB).

Statistics: Immunohistochemistry: Immunoreactivity differences between groups were analysed using the Friedman Nonparametric Repeated Measures Test and Dunn's Multiple Comparisons Test. The comparison between borderline and benign lesions was performed using the Kruskall-Wallis Anova Test.

Interphase cytogenetics: Data was analysed using the Mann-Whitney U Test, Fisher's Exact Test and Odds ratio (OR) assessment.

Fig. 1



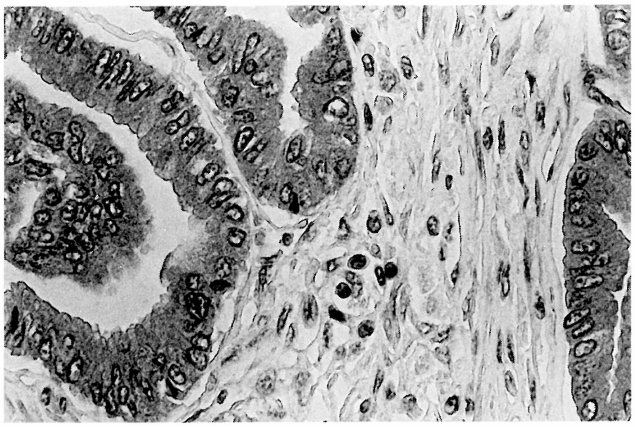


Fig. 2

Figure 1. — Intracytoplasmic immunoexpression of nm23 in borderline serous tumours of the ovary (x200).
Figure 2. — Intense intracytoplasmic staining with nm23 in carcinomas (x200).

Results

Immunohistochemistry: The nm23 immmunohistochemical results are detailed in Table 1. Normal ovarian surface epithelium demonstrated no immunoreactivity.

Table 1. — *Histological grade, stage, metastasis of lymph nodes, follow-up periods and the results of immunohistochemistry*

Cases	Grade	Stage	Lymph node metastasis	Follow-up	nm23 A	B	C	D
1	G 3	I c	N0	1 year	0	844	0	156
2	G 1	I a	N0	2 years	397	122	88	393
3	G 1	I c	Nx	2 years	32	965	0	3
4	G 2	I a	N0	3 years	0	1000	0	0
5	G 1	I b	Nx	5 years	0	500	0	500
6	G 2	II a	N0	1 year	0	997	0	3
7	G 2	II a	Nx	9 months	9	952	0	39
8	G 3	II a	Nx	2 years	0	1000	0	0
9	G 2	III	Nx	7 years	0	732	0	268
10	G 2	III	Nx	3 years	76	924	0	0
11	G 2	III	Nx	postop ex	10	975	15	0
12	G 3	III	Nx	2 years	14	986	0	0
13	G 2	III	Nx	2 years	22	570	18	390
14	G 2	III	Nx	2 years	7	902	0	91
15	G 2	III	Nx	5 years	0	1000	0	0
16	G 3	III	N0	2 years	0	953	0	47
17	G 3	III	N0	1 year	0	991	0	9
18	G 2	III	Nx	7 months	148	485	19	348
19	G 2	III	Nx	2 years	90	600	23	287
20	G 3	III	N1	3 years	0	10	0	990

A: Intracytoplasmic + intranuclear immunoreactivity; B: Intracytoplasmic immunoreactivity; C: Intranuclear immunoreactivity; D: No immunoreactivity.

Table 2. — *Immunohistochemical results of borderline lesions*

Cases	Histological type	nm23 A	B	C	D
1	Serous	1	180	0	819
2	Serous + Mucinous	0	599	0	401
3	Mucinous	0	309	0	691
4	Serous + Mucinous	0	100	0	900
5	Serous	0	121	196	683
6	Serous	16	500	4	480
7	Mucinous	0	265	0	735
8	Serous	0	359	0	641
9	Serous	0	400	0	600
10	Serous	318	318	20	344

Borderline and benign tumours demonstrated faint intracytoplasmic staining (Tables 2 & 3, Figure 1). There was no significant difference in staining patterns between these categories. More intense intracytoplasmic staining was evident for the carcinomas (Table 1, Figure 2). Nm23 staining was significantly greater amongst the carcinomas than the benign and borderline tumours ($p<0.05$). However the only lymph node positive tumour had no immunoreactivity with nm23.

Table 3. — *Immunohistochemical results of benign lesions*

Cases	nm23 A	B	C	D
1	0	286	0	714
2	0	322	0	678
3	0	95	0	905
4	0	253	0	747
5	0	384	0	616

Table 4. — *Signals counted on chromosome 17 for each case*

Cases	Signal 1	Signal 2	Signal 3	Signal 4	Signal 5
1	138	22	40	–	–
2	70	–	130	–	–
3	200	–	–	–	–
4	70	20	130	–	–
5	55	105	25	15	–
6	140	50	10	–	–
7	200	–	–	–	–
8	50	150	–	–	–
9	35	165	–	–	–

Table 5. — *Signals counted on chromosome 1 for each case*

Cases	Signal 1	Signal 2	Signal 3	Signal 4	Signal 5
1	120	50	20	–	10
2	130	–	70	–	–
3	200	–	–	–	–
4	70	30	100	–	–
5	160	20	15	5	–
6	130	68	2	–	–
7	200	–	–	–	–
8	40	160	–	–	–
9	35	165	–	–	–

The first seven cases represent carcinomas and the last two cases are borderline lesions.

Interphase Cytogenetics: The interphase cytogenetic results are summarised in Tables 4 & 5. The majority of tumours proved too fragile for the interphase cytogenetic methodology. Data was obtained for seven carcinomas and two borderline tumours. All seven carcinomas demonstrated chromosome 1 numerical abnormalities and six were also abnormal with chromosome 17 probe. The two borderline tumours showed disomy with each probe. The presence of numerical abnormalities in the carcinomas could not be shown to correlate with immunoreactivity (Fisher's exact test). The Odds Ratio test indicated that when immunoreactivity was present a signal aberration was four times more likely than when there is no immunoreactivity.

Discussion

The main findings of this study are two-fold. Firstly, serous ovarian cystadenocarcinomas were strongly immunoreactive with nm23 not only compared to benign and borderline tumours but also compared to the one metastatic tumour. Secondly, numerical abnormalities were only found in the carcinomas. These data parallel previous studies. High levels of expression with nm23 immunohistochemistry in more advanced carcinomas relative to borderline ones and also higher levels of nm23H1 and H2 mRNA in carcinomas rather than benign tumours have been reported [4]. It has also been reported that mRNA levels of nm23-H1 and H2 were higher in carcinoma tissues compared with benign tumours of the ovary [5]. The studies in gastric cancer and melonomas have demonstrated nm23 expression correlation with either late stage of the tumour or lymph node metastasis [6, 7]. It has also been shown that ovarian carcinomas with detectable metastases at the time of surgery were more frequently negative for nm23-H1 protein than those with no detectable metastases [8]. Schneider *et al.*, have demonstrated nm23 expression is associated with a wörse prognosis in early stage epithelial carcinomas [9]. These data paradoxically suggest that overexpression of nm23H1 may have a positive role in carcinogenesis but not in metastasis. Alternatively the nm23 gene may function as a metastasis suppressor in certain tumour systems whilst its expression in other cell contexts may be related to cell proliferative activity. It is known that in human breast carcinoma cell lines overexpression leads to formation of basement membrane and growth arrest [10]. Another possibility is that serine phosphorylation of nm23 is more important in the suppression of metastasis than the nucleoside diphosphate kinase activity measured in this study [11].

Conclusion

In keeping with metaphase and interphase cytogenetic studies of ovarian tumours, numerical aberrations were common only amongst the carcinomas. Since nm23 expression was also associated with carcinomas the idea that benign and borderline tumours rarely progress to become invasive is supported.

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