

Genomic Imbalance and Onco-protein Expression of Ovarian Endometrioid Adenocarcinoma Arisen in an Endometriotic Cyst

F. NOACK¹, H. SCHMIDT², O. BUCHWEITZ³, E. MALIK⁴ and H.-P. HORNY¹

¹Department of Pathology, University Hospital Lübeck;

²Department of Pathology, University of Halle;

³Department of Obstetrics & Gynaecology, University of Münster;

⁴Department of Obstetrics & Gynaecology, University of Aachen, Germany

Abstract. *Background:* Malignant transformation in endometriosis is a rare but well known complication. However, detailed mechanisms of malignant transition and tumourigenesis in endometriosis remain unknown. We here describe the case of an endometrioid carcinoma arisen in endometriotic ovarian cyst fulfilling Sampson's criteria for malignant transformation of endometriosis. We compared genetic alterations and onco-protein expression in the endometriotic ovarian cyst and the associated endometrioid adenocarcinoma. *Materials and Methods:* Genomic instability was evaluated by comparative genomic hybridization (CGH). Onco-protein expression was analysed by immunohistochemistry with antibodies against bcl-2, c-MYC, cyclin D1, p53, HER-2 and KIT protein. *Results:* CGH revealed a gain of 8q, including the locus of the oncogene c-MYC at 8q24. Immunohistochemistry disclosed a differing protein expression profile between the epithelia of the pre-existing cyst and adenocarcinoma. Apart from HER-2, all onco-proteins were more strongly expressed in epithelial cells of the adenocarcinoma than of the endometriotic cyst. *Conclusion:* The solitary genomic imbalance of chromosome 8 possibly reflects the importance for initiation and / or progression of endometrioid carcinoma. Overexpression of onco-proteins then may occur subsequently in the malignant transformation of ovarian endometriosis. However, the exact mechanisms of malignant transition in ovarian endometriosis remain to be elucidated in future studies with a higher number of cases.

Endometriosis is defined as dissemination of endometrial tissues outside the lining epithelium of the cavum uteri and

is found in 7%-20% of women (1). Malignant transformation is an infrequent but well known complication of endometriosis and may occur in the epithelial and, rarely, in the stromal cells of the endometriotic tissue. About three-quarters of endometriosis-associated malignant tumours arise in the ovaries (2). Histological aspects of ovarian carcinomas arisen in endometriosis have been well described in literature, but mechanisms of malignant transition and tumourigenesis in endometriosis remain poorly understood.

Materials and Methods

Case report. A 34-year-old female patient presented with a tumour of the right ovary. The past medical history was unremarkable. Preoperative ultrasound scan showed a partly cystic partly solid tumour of the right ovary measuring 16 x 12 x 7 cm. Tumour marker CA 125 was moderately elevated at 41.6 U/ml (norm: below 35 U/ml). Laparotomy with removal of the right adnexa, pelvic and paraaortal lymphadenectomy, omentectomy and peritoneal biopsies was performed. Histological examination revealed an endometrioid adenocarcinoma arisen in an endometriotic ovarian cyst. Cytology of the peritoneal washing yielded no tumour cells. The ovarian carcinoma was staged pT1a, G2 or FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) Ia. Postoperative recovery was uneventful. Chemotherapy with carboplatin was commenced.

Cytogenetic analysis. Comparative genomic hybridization (CGH) was performed according to the protocol described by Kallioniemi (3) modified by Schmidt and co-workers (4). In brief, tumour DNA was labelled with biotin 16-dUTP and reference DNA from peripheral blood of healthy probands with digoxin-11-dUTP (Boehringer Mannheim, Germany), using nick-translation. After hybridization (3 days, 37°C), biotinylated sequences were detected with avidin-fluorescein isothiocyanate (FITC) (Boehringer Mannheim) and probe sequences were visualized with anti-digoxigenin rhodamine (Boehringer Mannheim). After chromosome counterstaining with 4,6-diamino-2-phenylindole (DAPI) (Serva, Heidelberg, Germany), hybridization was analyzed with the ISIS digital image analysis system (MetaSystems, Altlußheim, Germany). Chromosomal regions with a green-to-red ratio above 1.15 were considered as "gains", whereas regions with a ratio below 0.85 were considered as

Correspondence to: Dr. F. Noack, Department of Pathology, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. Tel: + 49-451-500 2700, Fax: + 49-451-500 4851, e-mail: noack@patho.mu-luebeck.de

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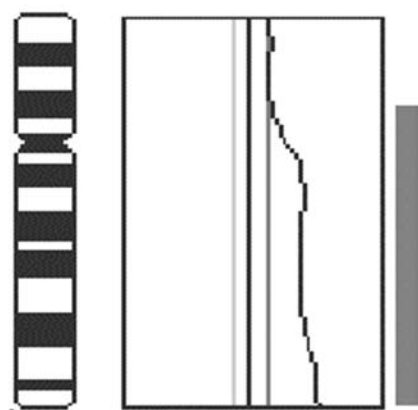


Figure 1. CGH analysis of the endometrioid carcinoma-mid profile of chromosome 8. The beam on the right marks the region of gain in 8q.

"losses". Results were confirmed by calculating the double standard deviation of the individual ratio profiles of each chromosome within the same experiment. Heterochromatic regions were excluded from the analysis (4).

Histology and immunohistochemistry. After fixation of the tumour specimen with 10% buffered formalin (24h), representative sections of the cyst and the tumour were selected and processed for histological examination. Five- μ m sections were cut from paraffin-embedded material and stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Immunohistochemistry (IHC) with antibodies against bcl-2 (clone 100/D5, Biocarta Europe, Hamburg, Germany), c-MYC (clone 9E10, Santa Cruz Biotechnology, California, USA), cyclin D1 (clone P2D11F11, Santa Cruz Biotechnology), HER-2 (pAb, DAKO, A/S, Glostrup, Denmark), p53 (clone Pab1801, Novocastra, Newcastle upon Tyne, UK), and KIT protein (clone 104D2, DAKO A/S) was performed. The avidin-biotin complex (ABC)-method was applied. Two observers (F. N. and H.-P. H.) evaluated immunoreactivity using a double-headed microscope according to a semiquantitative score: no immunostaining (=0); weak and/or focal staining (=+); moderate and/or diffuse staining (==+); and strong positivity (===+). The HercepTest score was used for evaluating HER-2 expression.

Results

Cytogenetic analysis. We detected a gain of 8q as sole chromosomal imbalance in the endometrioid carcinoma with CGH (Figure 1). No chromosomal aberrations were detected in the endometriotic cyst.

Histology and immunohistochemistry. On histological examination, the lining of the cyst consisted of columnar epithelial cells with basal slightly pleomorphic nuclei. The subepithelial connective tissue contained foci of spindle cells and scattered macrophages (siderophages). The solid parts revealed features of an intracystic endometrioid adeno-

carcinoma with neoplastic tubular and cribriform glands lined by stratified nonmucin-containing tumour cells. The diagnosis of an endometrioid carcinoma arisen in an endometriotic ovarian cyst was established (Figure 2). The different IHC staining patterns of the epithelium of the endometriotic ovarian cyst and the endometrioid carcinoma are shown in Figure 3 and summarized in Table I.

Discussion

An average of only 6% of all ovarian carcinomas are associated with endometriosis (1). The coexistence of endometriosis and malignant tumours of the ovary was first reported by Sampson as early as 1925. He defined the histological criteria for diagnosing malignant transformation of endometriosis as: 1) close proximity of benign endometriosis to the malignant tumour; 2) no other primary tumour site identified, and 3) tumour histology compatible with an endometrial primary lesion (5). Based on histological observations and clinical documentation, the incidence of malignant transformation in endometriosis of the ovary according to Sampson's criteria is only about 1% (6). The presented case meets all of Sampson's criteria for malignant transformation.

We performed CGH on the endometriotic cyst and the endometrioid adenocarcinoma, because this method allows the examination of the entire genome of a tumour for chromosomal imbalances without the need of cell culture. Previous CGH studies in ovarian carcinoma displayed a variety of genetic alterations. Chromosomal gains in 6p, 12p, 1q, 2q, 3q, 8q, 20q and 19q, and losses of chromosomes 4, 13q, 16q and 18q are the most frequent genetic alterations (7,8). We observed a gain of the long arm of chromosome 8. This region harbours c-MYC, an oncogene encoding a DNA-binding protein that acts as transcription factor and plays a significant role in cell proliferation. Copy number increases of 8q24.1 equivalent to c-MYC amplifications have been repeatedly observed in up to 38% of ovarian carcinomas (7-9). In accordance with our results, Mhawech and colleagues reported gains in the long arm of chromosome 8 in an endometrioid adenocarcinoma arisen in endometriosis (10). Our finding of a sole genomic imbalance in chromosome 8 possibly reflects the importance of this early chromosomal event for initiation and/or progression of endometrioid carcinoma.

IHC disclosed a different protein expression profile between the epithelium of the pre-existing cyst and the adenocarcinoma. Cyclin D1 and bcl-2 were significantly more strongly expressed in the adenocarcinoma than in the endometriotic cyst, whereas c-myc, KIT protein and p53 were only slightly more overexpressed in tumour cells. However, there was no discrepancy in staining intensity of HER-2 in tumour cells and epithelial cells of the

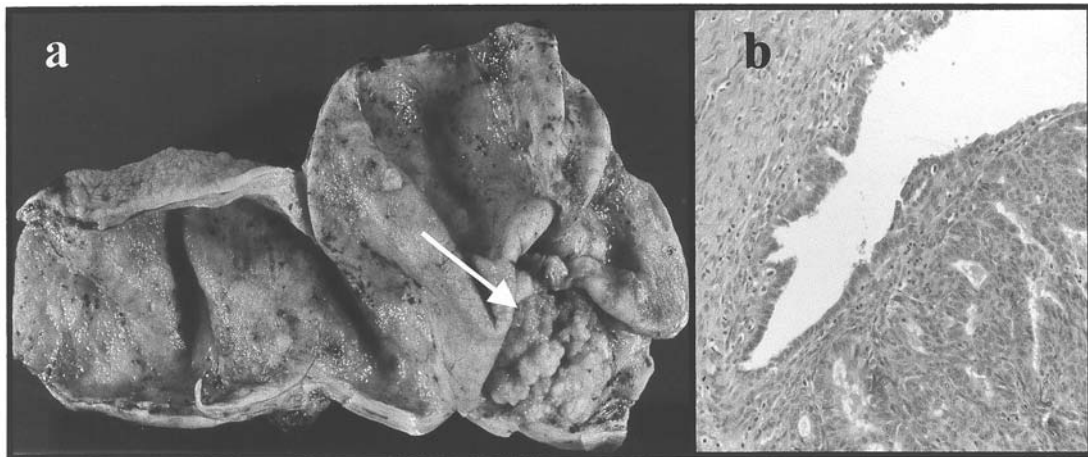


Figure 2. (a) The right cystic ovarian mass was 16 cm in diameter. The cyst was filled with hemorrhagic fluid and admixed a solitary firm mass (arrow). Transition of endometriotic cyst to endometrioid adenocarcinoma is shown in (b) (H&E staining, x 200).

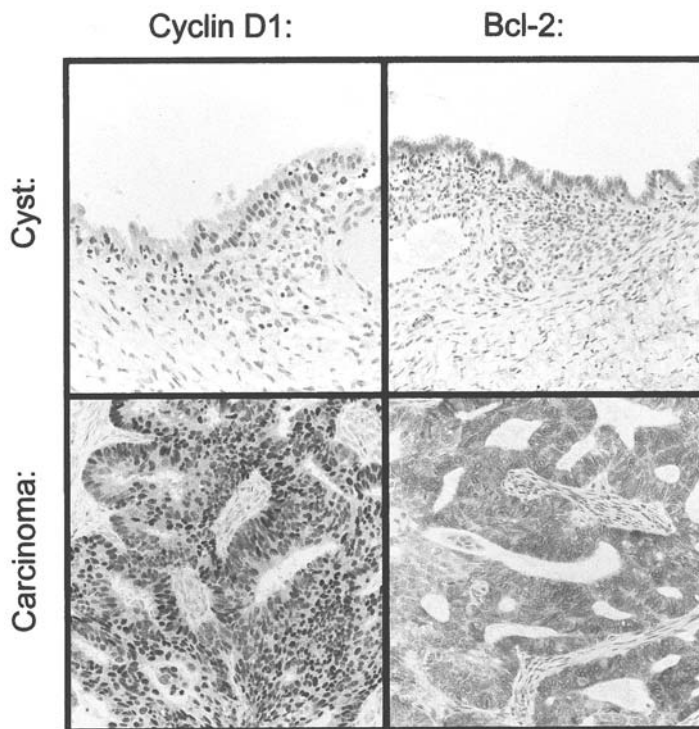


Figure 3. Over-expression of cyclin D1 and bcl-2 in the endometrioid adenocarcinoma versus the endometriotic cyst (x200).

endometriotic cyst. Overexpression of cyclin D1 has been repeatedly reported in ovarian carcinoma (11,12). In accordance with our observations, Han and co-workers demonstrated cyclin D1 overexpression in two of their three cases of adenocarcinoma arisen in extragonadal endometriosis (13). The apoptosis inhibitor bcl-2 has also been shown to be overexpressed in ovarian carcinoma (14). Expression of transmembrane type III tyrosine kinase receptor KIT protein has been found in 71% of malignant

ovarian tumours (15). Hitherto, no data about KIT protein expression in malignant transition of endometriosis exist. Overexpression of p53 has been shown recently in carcinoma arising in endometriosis (10,13,16). Yet, we observed only weak overexpression of p53 in tumour cell nuclei of the endometrioid carcinoma.

However, discrepancies between CGH result, *i.e.* the gain in chromosome 8 and IHC results, in particular the lack of marked overexpression of c-MYC, remain difficult

Table I. IHC staining pattern of the endometriotic ovarian cyst versus the endometrioid carcinoma.

Antibody	Endometriotic cyst	Endometrioid adenocarcinoma
bcl-2	0	++, cytoplasmatic
c-MYC	+, nuclear and cytoplasmatic	++, nuclear and cytoplasmatic
cyclin D1	+, nuclear	+++ , nuclear
HER-2	HercepTest score : 1+	HercepTest score : 1+
p53	0, nuclear	+, nuclear
KIT protein	+, cytoplasmatic	++, cytoplasmatic

to explain. An analogous discrepancy of c-MYC gene amplification and protein overexpression has been reported previously in urinary bladder, colonic and pancreatic cancer (17-19). It has been proposed that c-MYC protein expression might underlie epigenetic events independent of intragenetic alterations (20). Alternatively, amplified genes may still be under the control of transcriptional regulation, such as promoter activation or attenuation (21). The significance of higher protein expression of bcl-2, cyclin D1, p53 and KIT protein in the endometrioid adenocarcinoma remains unclear. One could speculate that overexpression of these onco-proteins has occurred after malignant transformation. However, the exact mechanisms of malignant transition in ovarian endometriosis remain to be elucidated. More studies with a greater number of cases are needed.

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