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Original Article

Absence of microsatellite instability in mucinous carcinomas of the breast

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Abstract: Microsatellite instability (MSI) is a form of genetic instability that results from defects in DNA mismatch repair. MSI is reported to be rare in unselected breast cancers, however it is a common feature in subsets of colorectal, ovarian and endometrial cancers. In these anatomical sites, MSI-high carcinomas often display a mucinous histology. The aim of this study was to determine whether mucinous carcinomas of the breast would more frequently display MSI-high than invasive ductal carcinomas of no special type (IDC-NSTs). The expression of four MSI markers (i.e. MSH2, MSH6, MLH1 and PMS2) was immunohistochemically assessed in 35 mucinous breast carcinomas and 35 histological grade- and oestrogen receptor (ER) status-matched IDC-NSTs, and in a series of 245 invasive breast cancers. Cases were considered as potentially MSI-high if tumour cells lacked expression of at least two MSI markers and internal controls displayed nuclear staining. Nine mucinous carcinomas were microdissected and subjected to MSI analysis by PCR using the MSI markers BAT26 and BAT40. No immunohistochemical evidence of MSI-high was found in the 35 mucinous carcinomas and 35 grade- and ER-matched IDC-NSTs, and in the cohort of 245 invasive breast cancers. In addition, no evidence of MSI-high was observed by PCR analysis using the BAT26 and BAT40 markers in the nine mucinous carcinomas tested. Our results demonstrate that MSI-high phenotype is remarkably rare in invasive breast cancer, and that, in contrast to mucinous carcinomas of other anatomical sites, MSI is not a common event in mucinous carcinomas of the breast.

Keywords: Mucinous carcinoma, breast cancer, mismatch repair, microsatellite instability, immunohistochemistry, genetics

Introduction

Mucinous carcinoma is a rare histological special type of invasive breast cancer, accounting for up to 2% of all breast carcinomas. Mucinous carcinoma of the breast occurs preferentially in older women and is associated with good clinical outcome [1-4]. In a way akin to mucinous cancers of other anatomical sites, mucinous carcinomas of the breast are characterised by abundant production of extracellular and/or intracellular mucin. Pure mucinous carcinomas of the breast are defined as tumours with >90% of mucinous component and display bland cytological features, with uniform neoplastic cells showing mild nuclear atypia and low mitotic activity, arranged in clusters floating in large amount of mucin [5]. Therefore, by definition, the vast majority of mucinous breast carcinomas is classified as of low histological grade [1-4,6] when graded using the Nottingham grading system [7].

We and others have shown that mucinous carcinomas of the breast are typically oestrogen receptor (ER)-positive [1,4,6] and classified as of 'luminal' subtype according to the molecular breast cancer taxonomy based on microarray gene expression profiling [8-14]. Furthermore, we previously demonstrated that mucinous carcinomas of the breast are (i) distinct from grade- and ER-matched invasive ductal carcinomas of no special type (IDC-NSTs) at the genomic and transcriptomic levels [6,8], (ii) display a relatively low level of genetic instability [6], and (iii) lack the concurrent loss of 16q and gain of 1q

[6], a hallmark feature of grade I IDC-NSTs and classic lobular carcinomas [15-19]. These data suggest that mucinous carcinomas may evolve through genetic pathways distinct from those altered in tumours from the low-grade breast neoplasia family [20].

Microsatellite instability (MSI) is a form of genetic instability characterised by frequent errors occurring during the replication of short nucleotide repeats, often due to a defective DNA mismatch repair gene such as *hMSH2*, *hMLH1*, *hPMS2* and *hMSH6* [21-25]. In fact, two distinct MSI phenotypes have been described in cancer: MSI-high (MSI-H) cancers, which result from defective mismatch repair, and MSI-low (MSI-L) tumours, which display lower levels of MSI and are not associated with defective mismatch repair [26-28]. However, the definition of the MSI-L phenotype remains controversial [28]. In addition, several independent groups have shown that MSI-H cancers are diploid and harbour fewer chromosomal copy number aberrations than tumours lacking MSI or that are MSI-L [29,30].

Subsets of colorectal [24], gastric [31], pancreatic [31], ovarian [32] and endometrial tumours [22,31,33], and particularly those occurring in the hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome [31], are characterised by microsatellite instability. Interestingly, however, MSI-H appears to be vanishingly rare in breast cancer [21,34]. Likewise, breast cancers displaying an MSI-L status are remarkably rare, whereas in tumours from other anatomical sites, such as colorectal, endometrial or ovarian cancers [27], this phenomenon is not as uncommon. Of note, in some anatomical sites (e.g. colorectal and ovarian), tumours displaying microsatellite instability often display a mucinous histology [32,35,36]. However, the prevalence of MSI in mucinous carcinomas of the breast has not yet been systematically addressed.

Immunohistochemical analysis of DNA mismatch repair proteins, in particular the combination of MLH1/PMS2 and MSH2/MSH6 immunostainings, has been used as a surrogate for the detection of *MLH1* or *MSH2* mutations, the most frequently mutated mismatch repair genes in MSI-H tumours [23,25,26]. This four-marker immunohistochemistry (IHC) panel has a high sensitivity (>90%) in predicting *MLH1/MSH2* gene mutations and has been suggested to be an optimal first-line screening tool [23,37]. Of note, however, mismatch repair protein deficiency as explored by immunohistochemistry may also be underpinned by alternative mechanisms such as methylation. Indeed, a recent multicentric study on colorectal cancers showed that specific *MLH1* promoter polymorphism predisposed to *MLH1* methylation and MLH1 silencing, leading to loss of MLH1 protein as assessed by IHC and MSI [38]. This observation supports the fact that, after excluding germline mutation, mismatch repair gene methylation may be the underlying cause of at least a subset of MSI-H cancers [39].

The aims of this study were to determine whether mucinous carcinomas of the breast would display MSI, and whether mucinous carcinomas would more frequently display an MSI-H phenotype than IDC-NSTs. To address these questions, we analysed a series of pure mucinous carcinomas of the breast and grade- and ER-matched IDC-NSTs for MSI by IHC for MSH2, MLH1, MSH6 and PMS2 expression, a subset of which was subjected to polymerase chain reaction (PCR) amplification of the mononucleotide repeat markers BAT26 and BAT40. As a secondary aim, we investigated the prevalence of immunohistochemical profiles suggestive of MSI-H in a series of 245 invasive breast cancers treated with adjuvant anthracycline-based chemotherapy.

Materials and methods

Cases

Mucinous carcinomas: Consecutive cases of pure mucinous carcinomas of the breast (n=35) were retrieved from the histopathological files of the authors' institutions. Cases were centrally reviewed by at least two pathologists (MLT, FCG and/ or JRF) and detailed histopathological features are described elsewhere [6]. Briefly, nine cases were of grade I, 24 of grade II, and three of grade III as assessed using the Nottingham grading system [7]. All cases were ER-positive [6]. A tissue microarray (TMA) was constructed with triplicate 0.6mm tumour cores.

Control group: A control group of consecutive 245 invasive breast cancers was included in a separate TMA containing three replicate 0.6mm tumour cores. Tumours were graded according to the Nottingham grading system [7] and de-

Table 1. Immunohistochemistry protocols for MSH2, MLH1, MSH6 and PMS2 antibodies

Marker	Clone	Dilution	Antigen retrieval	Detection system	Company
MSH2	FE11	1:20	2 min, PC citrate pH 6.0	EnVision™ system, Dako, Glostrup, Denmark	Calbiochem, EMD Chemicals, Gibbstone, NJ, USA
MLH1	G168-728	1:20	2 min, PC citrate pH 6.0	EnVision™ system, Dako, Glostrup, Denmark	Pharmingen, BD Biosciences, Oxford, UK
MSH6	44/ MSH6	1:15	2 min, PC citrate pH 6.0	EnVision™ system, Dako, Glostrup, Denmark	Transduction Labs, BD Biosciences, Oxford, UK
PMS2	A16-4	1:10	2 min PC, EDTA pH8.0	EnVision™ system, Dako, Glostrup, Denmark	Pharmingen, BD Biosciences, Oxford, UK

MSH: mutS homolog; MLH: mutL homolog; PC: pressure cooker; PMS2: postmeiotic segregation increased 2.

tailed histopathological features are described elsewhere [40-42]. Out of this cohort, 35 consecutive IDC-NSTs were matched according to histological grade and ER-status with the mucinous carcinomas studied. This study was approved by local research ethics committee.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 3μm TMA sections as previously described [43], using antibodies against the mismatch repair proteins MSH2, MLH1, MSH6 and PMS2. Combination of the four makers MLH1, MSH2, MSH6 and PMS2 for MSI screening by IHC stems from observations showing that mutations in *MLH1* or *MSH2* result in concurrent loss of MLH1/PMS2 or MSH2/MSH6 respectively, whereas mutations of *PMS2* or *MSH6* will result in isolated loss of PMS2 or MSH6 only [23,25,26]. Details of the antibodies and IHC protocols used are provided in **Table 1**. Positive (ovarian carcinomas) and negative controls (omission of the primary antibody) were included in each experiment. Slides were interpreted by two pathologists (MLT & FCG), who evaluated the expression of each marker semi-quantitatively, and resolved discordant cases on a multi-headed microscope. Only nuclear expression was considered as specific and the presence of internal positive controls (nuclear staining in either normal epithelial cells, lymphocytes or stromal cells) was assessed for each case. Cases without optimal positive internal controls were deemed inconclusive. Following the IHC interpretation criteria applied in colorectal cancers [25], tumours were recorded as MSH2, MLH1, MSH6 or PMS2 negative when there was a complete absence of nuclear staining in neoplastic cells. If a case was negative for at least two markers or was inconclusive for more than one marker, IHC was repeated on full-face sections.

All breast cancers included in this study were previously characterised for their expression of ER, progesterone receptor (PR), HER2, Ki67, p53, Bcl2, cyclin D1 and basal markers [6,40].

We compared the results of the MSI IHC analysis performed in 35 pure mucinous carcinomas with those from 35 IDC-NSTs matched for histological grade and ER expression, selected from the cohort of 245 invasive breast cases, as previously described [6]. The rationale for matching case and control samples according to grade and ER status stems from several lines of evidence suggesting that grade and ER are strongly associated with the pattern of genomic changes in breast cancer [16,44,45].

Microdissection and DNA extraction

Ten representative 10μm thick sections of the mucinous tumours were stained with nuclear fast red and were microdissected with a sterile needle under a stereomicroscope (Olympus SZ61, Tokyo, Japan) to ensure >70% of purity of cancer cells as previously described [43,46]. DNA was extracted as previously described using an FFPE DNA extraction kit (FFPE DNeasy Kit, Qiagen, Hamburg, Germany) [43], and DNA concentration was determined using the PicoGreen® assay according to the manufacturer's protocol (Invitrogen, Paisley, UK). Sufficient amount of DNA to perform MSI PCR analysis was obtained in nine mucinous carcinomas.

Microsatellite instability analysis

The presence of MSI was evaluated by amplification of the mononucleotide repeat markers

BAT26 and BAT40. DNA was subjected to standard PCR and gel electrophoresis as previously described [25]. Briefly, forward primers with a fluorescent tag at the 5' end to allow microsatellite detection by the ABI Prism were purchased from Applied Biosystems (Carlsbad, CA, USA). PCR conditions were as follows: 5μl of 10x PCR reaction mix buffer (AmpliTaq, Applied Biosystems); 3μl of $MgCl_2$ (AmpliTaq); 5μl of 2.5mM dNTPs; 0.5μl of Taq polymerase (AmpliTaq); 0.5μl of each primer and 50ng of DNA in a final volume of 50μl. After initial denaturation at 94°C for 4min, 35 cycles of PCR steps (94°C for 1min, annealing at 56°C for 1min and 72°C for 3min) were run, followed by a final extension at 72°C for 7min. After amplification, PCR products were mixed with 18μl of Hi-Di formamide (Applied Biosystems) and 0.3μl of size standards (GeneScan™ 500 ROX™, Applied Biosystems), followed by denaturation at 95°C for 3min, before analysis on ABI prism using GeneScan Analysis software (Applied Biosystems). Tumours were considered MSI-H when both BAT26 and BAT40 displayed MSI, defined by multiple extra bands above background [25,27]. As control, a blood DNA sample from a healthy female donor was run in parallel in each experiment. The technique was validated for both markers on two endometrial cancer cell lines, AN3CA and EFE-184, which were MSI-H and MSI-stable, respectively. The microsatellite marker BAT26 has been shown to be particularly useful in determining the MSI-H phenotype without the requirement for matching normal DNA given that it is 'quasi-monomorphic' in DNA from normal individuals or microsatellite stable tumours compared to MSI-H cancers [25,26,47]. Therefore, no normal tissue matching was performed in this study.

Results

All 35 pure mucinous carcinomas of the breast analysed were positive for MLH1 and MSH6 as determined by IHC, and 33 out of 35 (94.2%) and 32 out of 34 cases (94.1%) showed expression of MSH2 and PMS2, respectively (**Table 2** and **Figure 1**). Out of the three mucinous tumours lacking expression of MSH2 and/or PMS2, two cases were considered inconclusive and no case displayed complete lack of expression of two MSI markers. Following the guidelines for the interpretation of MSI IHC markers in colorectal carcinomas [23,25], no case in this cohort of mucinous carcinomas would be considered as potentially MSI-H. Immunohistochemical analysis of full-face sections of the negative and inconclusive cases revealed one case lacking expression of one marker (i.e. MSH2) and no case lacking expression of two or more MSI markers (**Table 2**). Of the 35 grade- and ER-matched IDC-NSTs, 97.1%, 97.1%, 88.6% and 100% showed nuclear expression of MLH1, MSH2, MSH6 and PMS2, respectively. One case lacked MLH1 expression, one case MSH2 expression and three cases MSH6 expression, the remaining case displaying lack of expression of MSH6 was considered inconclusive. No IDC-NST lacked expression of two or more MSI markers (**Table 2**). When the pattern of expression of the four MSI markers in the 35 mucinous carcinomas was compared with that of 35 grade- and ER-matched IDC-NSTs, no significant differences were observed (**Table 2**).

Albeit limited to a small number of cases given the restricted tumour tissue availability and generally low DNA yield of mucinous tumours owing to their histology, PCR amplification of the microsatellite markers BAT26 and BAT40 was successfully performed and informative in nine mucinous carcinomas of the breast. Tumours would have been considered MSI-H if both BAT26 and BAT40 displayed MSI, however none of the nine mucinous cases displayed MSI for any of these two markers (**Figure 2**).

Analysis of the entire cohort of primary breast cancers from patients treated with adjuvant anthracycline-based chemotherapy (n=245) revealed that the vast majority of cases were positive for MLH1 (97.8%), MSH2 (91.7%), MSH6 (93.7%) and PMS2 (98.2%) (**Table 3** and Supplementary Tables 1-4). Similar frequencies of positivity were observed for the subset of IDC-NSTs (n=180) (**Table 3**). When combining the IHC conclusive results of all four MSI markers tested in the 245 invasive breast cancers, no case showed complete lack of expression of two MSI markers on TMA cores (**Table 3**). Reanalysis of negative and inconclusive cases on full face tissue sections revealed focal expression of the MSI markers (**Figure 3**), and, therefore, these cases were considered MSI stable. Two cases that lacked expression of two MSI markers also lacked internal controls and were deemed inconclusive [25]. No significant correlations between the lack of expression of MLH1, MSH2, MSH6 or PMS2 and clinico-pathological features and immunohistochemical markers in this

Table 2. Comparison of MSI marker expression in 35 pure mucinous carcinomas and 35 grade- and ER-matched IDC-NSTs determined by immunohistochemistry on tissue microarrays

	N	Pure mucinous (n=35)	IDC-NSTs (n=35)	p value*
MLH1	70			0.999
Positive		35 (100%)	34 (97.1%)	
Negative		0	1 (2.9%)	
Inconclusive		0	0	
MSH2	70			0.999
Positive		33 (94.2%)	34 (97.1%)	
Negative		1 (2.9%)	1 (2.9%)	
Inconclusive		1 (2.9%)	0	
MSH6	70			0.114
Positive		35 (100%)	31 (88.6%)	
Negative		0	3 (8.5%)	
Inconclusive		0	1 (2.9%)	
PMS2	69			0.239
Positive		32 (94.1%)	35 (100%)	
Negative		0	0	
Inconclusive		2 (5.9%)	0	
Combination analysis of MSI markers#	67			
1 marker negative		1 (3%)	5 (14.7%)	
2 markers negative		0	0	
3 markers negative		0	0	
4 markers negative		0	0	

*3x2 Fisher's test; #: inconclusive cases excluded; ER: oestrogen receptor; IDC-NSTs: invasive ductal carcinomas of no special type; MLH1: mutL homolog 1; MSH: mutS homolog; MSI: microsatellite instability; PMS2: postmeiotic segregation increased 2.

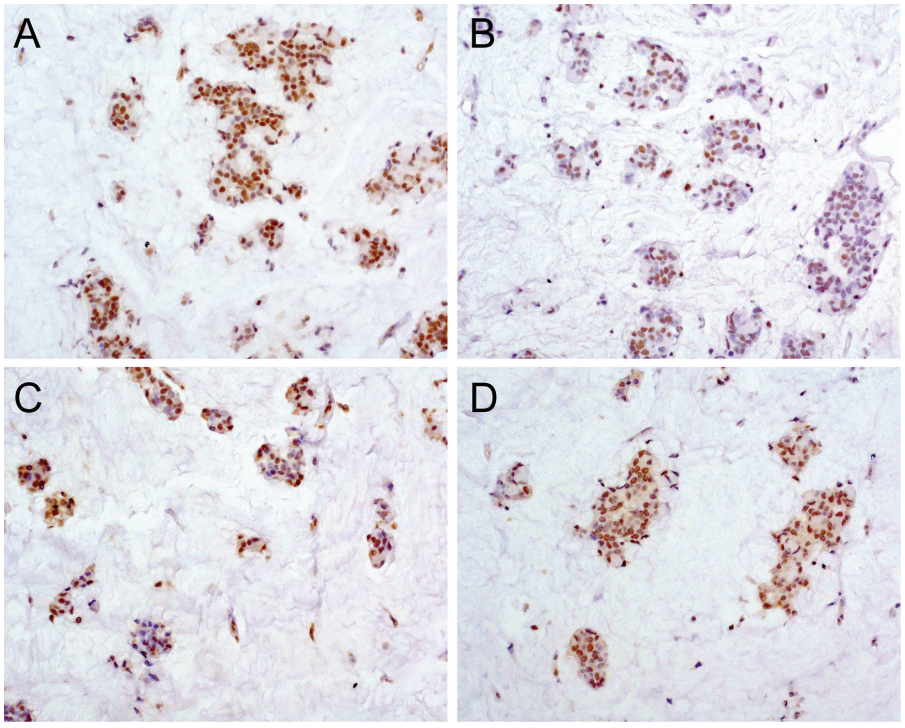

Figure 1. Immunohistochemical pattern of MSI marker expression in mucinous carcinoma of the breast. Representative micrographs of a pure mucinous carcinoma of the breast displaying nuclear expression of (**A**) MLH1, (**B**) MSH2, (**C**) MSH6 and (**D**) PMS2. Note the nuclear expression in isolated stromal cells in the mucin surrounding neoplastic cells, serving as internal positive control. MLH1: mutL homolog 1; MSH: mutS homolog; MSI: microsatellite instability; PMS2: postmeiotic segregation increased 2.

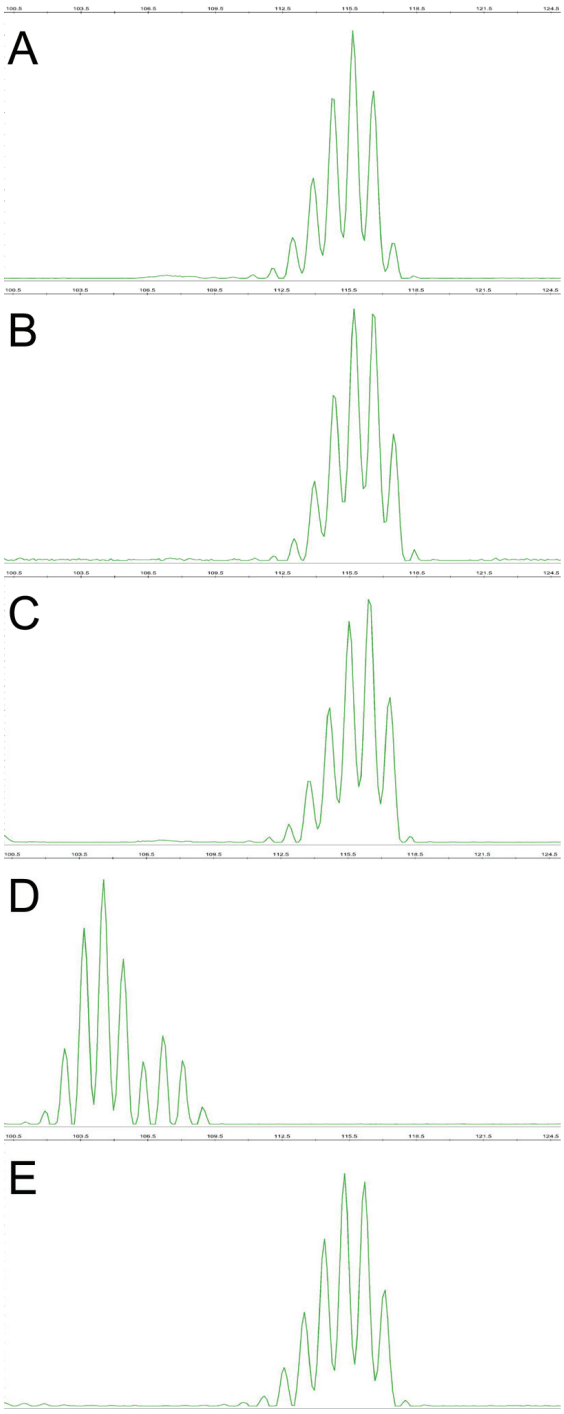

Figure 2. PCR-based MSI analysis of mucinous carcinoma of the breast using the mononucleotide repeat marker BAT26. Electropherograms of (A) mucinous carcinoma 1 (MSI-stable), (B) mucinous carcinoma 2 (MSI-stable), (C) blood DNA from healthy individual (control; MSI-stable), (D) endometrial cell line AN3CA (control; MSI-high), and (E) endometrial cell line EFE-184 (control; MSI-stable). MSI: microsatellite instability; PCR: polymerase chain reaction.

cohort of 245 invasive breast cancers were identified (Supplementary Tables 1-4).

Discussion

Our findings corroborate those of previous studies, which demonstrated the lack or low prevalence (i.e. 2-3%) of MSI in invasive breast cancers [21,27,31,34,48] and breast cancer cell lines [21]. Importantly, we provide direct evidence that unlike mucinous carcinomas of the colon, ovary and endometrium, which display an MSI-H phenotype in about 30% of cases [22,24,49-51], MSI is a remarkably rare phenomenon in mucinous carcinomas of the breast. This finding is entirely consistent with the observation that unlike colorectal or endometrial cancers of mucinous subtype, mucinous carcinoma of the breast is uncommon in HNPCC/ Lynch syndrome, which is characterised by germline mutations in DNA mismatch repair genes [52].

Our results provide evidence to suggest that the assessment of mismatch repair proteins by IHC should preferably be performed on full tissue sections, as their expression may be focal in some tumours resulting in false negative results in TMA analyses. This observation supports previous reports addressing the issue of reliability of MSI IHC on small biopsy specimens and focal expression of MSI markers, in particular with MSH6 immunostaining [23]. Furthermore, there are several lines of evidence to demonstrate that immunohistochemical stainings of MSI markers should be interpreted with caution when no nuclear staining of internal controls (i.e. normal epithelial, lymphocytes or stromal cells) is observed on repeated experiments [23,25].

It should be noted that the presence of an MSI-L phenotype in the mucinous carcinomas and control invasive breast cancers has not been addressed in the present study. MSI-L tumours, which have been described in 26%, 32% and 35% of ovarian, endometrial and colorectal cancers, respectively [27], are believed to harbour distinct molecular aberrations, including *MSH6* germline mutations [26]. However, these tumours did not seem to be associated with distinct clinicopathological features [27,28]. Moreover, it has been reported previously that MSI-L cancers do not display loss of MLH1, MSH2, MSH6 and PMS2 protein expression [25].

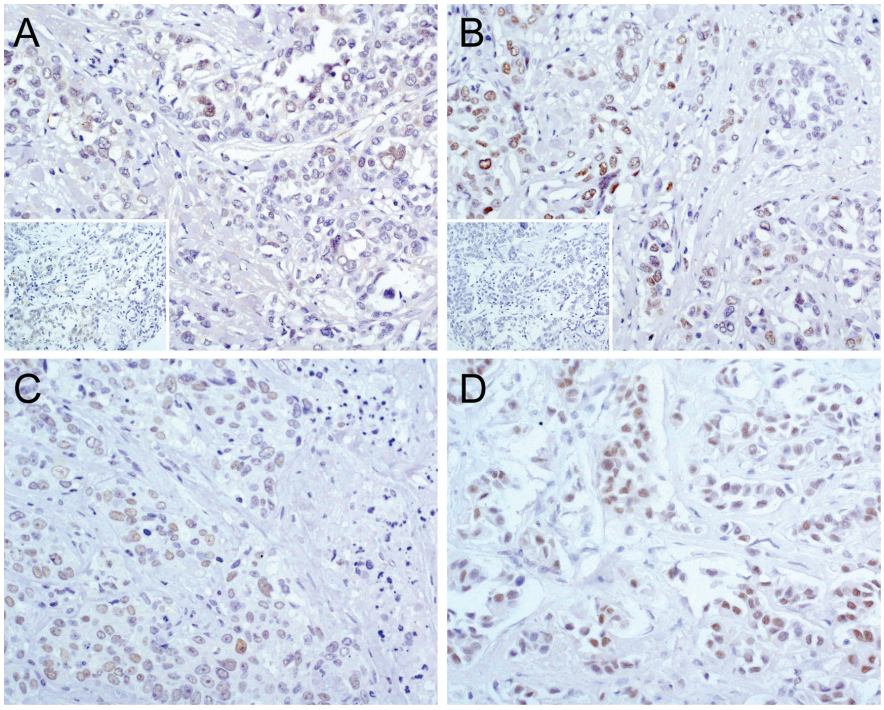

Figure 3. Immunohistochemical pattern of MSI marker expression in invasive ductal carcinoma of the breast. Representative micrograph of an invasive ductal carcinoma of no special type displaying nuclear expression of (A) MLH1, (B) MSH2, (C) MSH6, and (D) PMS2. This case was negative for MLH1 (A, insert) and MSH2 (B, insert) on immunostains performed on TMA sections but displayed focal positivity on full sections. MLH1: mutL homolog 1, MSH: mutS homolog; MSI: microsatellite instability; PMS2: postmeiotic segregation increased 2; TMA: tissue microarray.

Table 3. MSI marker expression in invasive breast carcinomas determined using immunohistochemistry on tissue microarrays

	N	Whole cohort (n=245)	*N*	IDC-NSTs only (n=180)
MLH1	178		133	
Positive		174 (97.8%)		129 (97%)
Negative		4 (2.2%)		4 (3%)
Inconclusive		0		0
MSH2	180		134	
Positive		165 (91.7%)		125 (93.3%)
Negative		14 (7.7%)		8 (6%)
Inconclusive		1 (0.6%)		1 (0.7%)
MSH6	225		164	
Positive		211 (93.7%)		151 (92.1%)
Negative		11 (4.9%)		10 (6.1%)
Inconclusive		3 (1.4%)		3 (1.8%)
PMS2	172		128	
Positive		169 (98.2%)		125 (97.6%)
Negative		2 (1.1%)		2 (1.5%)
Inconclusive		1 (0.7%)		1 (0.9%)
Combination analysis of MSI markers#	171		127	
1 marker negative		26 (15.1%)		18 (14%)
2 markers negative		0		0
3 markers negative		0		0
4 markers negative		0		0

#: inconclusive cases excluded; IDC-NSTs: invasive ductal carcinomas of no special type; MLH1: mutL homolog 1; MSH: mutS homolog; MSI: microsatellite instability; PMS2: postmeiotic segregation increased 2.

Therefore, we cannot rule out that a subset of invasive breast cancers, including mucinous carcinomas, may display a MSI-L phenotype. Despite the controversy regarding the definition of MSI-L, further studies using specific additional markers, such as those proposed by Halford et al [27,28], for the identification of the MSI-L phenotype in different histological subtypes of invasive breast cancer are warranted.

Taken together, here we demonstrate that in contrast to mucinous carcinomas of other anatomical sites, mucinous carcinomas of the breast do not display an MSI-H phenotype as determined by IHC and a PCR-based method. Moreover, no concurrent lack of MLH1/PMS2 or MSH2/MSH6 expression, the IHC surrogate markers of the most frequent mutations (i.e. mutations of *MLH1* or *MSH2*) observed in MSI-H cancers of other anatomical sites, were found in a consecutive series of 245 invasive breast carcinomas (including IDC-NSTs, invasive lobular and mixed carcinomas).

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Supplementary Table 1. Analysis of correlation between MLH1 expression, clinico-pathological features and markers in 245 invasive breast carcinomas

	N	MLH1 negative (n=4)	MLH1 positive (n=174)	p value
Tumour size	176			0.550*
T1		3	83	
T2		1	77	
T3		0	12	
Histological grade	174			0.589*
I		0	19	
II		2	50	
III		2	101	
Lympho-vascular invasion	176			0.587**
present		2	52	
absent		2	120	
Lymph node metastasis	172			0.307**
present		4	113	
absent		0	55	
ER	178			0.499**
positive		3	147	
negative		1	27	
PR	178			0.304**
positive		2	127	
negative		2	47	
HER2	178			0.095**
positive		2	23	
negative		2	151	
Ki67	166			0.674*
low (<10%)		1	73	
intermediate		2	67	
high (>30%)		1	22	
Topoisomerase IIα	163			0.625**
positive		3	85	
negative		1	74	
Cyclin D1	169			0.623*
low (Allred score 0-3)		1	18	
intermediate (Allred score 4-5)		1	33	
high (Allred score 6-8)		2	114	
Bcl2	145			0.545**
positive		1	97	
negative		1	46	
FOXA1	146			1**
positive		3	108	
negative		0	35	
p53	166			1**
positive		1	47	
negative		2	116	
Cytokeratin 5/6	170			0.311**
positive		1	14	
negative		3	152	
Cytokeratin 14	176			1**
positive		0	13	
negative		4	159	
Cytokeratin 17	175			1**
positive		0	19	
negative		4	152	
Basal cytokeratins	176			0.432**
positive		1	22	
negative		3	150	
EGFR	178			0.281**
positive		1	13	
negative		3	161	
Caveolin 1	178			1**
positive		0	14	
negative		4	160	
Caveolin 2	165			1**
positive		0	10	

negative		4	151	
Nestin	146			0.296**
positive		1	15	
negative		2	128	
Any basal marker	176			0.476**
positive		1	25	
negative		3	147	
Molecular phenotype***	173			0.513*
luminal		2	127	
HER2+		1	23	
basal-like		1	29	
E-cadherin	165			0.330*
normal		2	101	
reduced		1	10	
negative		1	50	
HER2 CISH	168			0.433**
amplified		1	21	
not amplified		3	143	
TOP2A CISH	169			0.295**
amplified		1	13	
not amplified		3	152	
CCND1 CISH	176			1**
amplified		0	24	
not amplified		4	148	

* Chi-squared test; ** Fisher's exact test; *** according to criteria by Nielsen *et al* [53]. *CCND1*: cyclin D1 gene; CISH: chromogenic *in situ* hybridisation; MLH1: mutL homolog 1; *TOP2A*: topoisomerase IIα gene.

Supplementary Table 2. Analysis of correlation between MSH2 expression, clinico-pathological features and markers in 245 invasive breast carcinomas

	N	MSH2 negative (n=14)	MSH2 positive (n=165)	MSH2 inconclusive (n=1)	p value*
Tumour size	178				0.528
T1		6	81	0	
T2		8	69	1	
T3		0	13	0	
Histological grade	176				0.882
I		2	17	0	
II		5	49	0	
III		7	95	1	
Lympho-vascular invasion	178				0.781
present		10	112	1	
absent		4	51	0	
Lymph node metastasis	174				0.758
present		9	108	1	
absent		5	51	0	
ER	180				0.545
positive		13	136	1	
negative		1	29	0	
PR	180				0.385
positive		12	116	1	
negative		2	49	0	
HER2	180				0.267
positive		0	25	0	
negative		14	140	1	
Ki67	169				0.560
low (<10%)		8	68	0	
intermediate		4	65	1	
high (>30%)		1	22	0	
Topoisomerase IIα	166				0.094
positive		3	87	0	
negative		8	67	1	
Cyclin D1	172				0.101

low (Allred score 0-3)		3	16	0	
intermediate (Allred score 4-5)		3	32	1	
high (Allred score 6-8)		5	112	0	
Bcl2	148				0.760
positive		7	91	1	
negative		4	45	0	
FOXA1	148				0.149
positive		6	107	0	
negative		3	31	1	
p53	169				0.577
positive		2	47	0	
negative		9	110	1	
Cytokeratin 5/6	172				0.909
positive		1	15	0	
negative		13	142	1	
Cytokeratin 14	178				0.961
positive		1	12	0	
negative		13	151	1	
Cytokeratin 17	177				0.879
positive		2	18	0	
negative		12	144	1	
Basal cytokeratins	178				0.921
positive		2	22	0	
negative		12	141	1	
EGFR	180				0.941
positive		1	14	0	
negative		13	151	1	
Caveolin 1	180				0.941
positive		1	14	0	
negative		13	151	1	
Caveolin 2	167				0.634
positive		0	11	0	
negative		11	144	1	
Nestin	148				0.750
positive		2	14	0	
negative		10	121	1	
Any basal marker	178				0.760
positive		3	25	0	
negative		11	138	1	
Molecular phenotype**	175				0.475
luminal		13	116	1	
HER2+		0	25	0	
basal-like		1	19	0	
E-cadherin	168				0.230
normal		4	100	0	
reduced		1	10	0	
negative		6	46	1	
HER2 CISH	171				0.261
amplified		0	24	0	
not amplified		14	132	1	
TOP2A CISH	172				0.511
amplified		0	15	0	
not amplified		12	144	1	
CCND1 CISH	178				0.306
amplified		0	24	0	
not amplified		13	140	1	

* Chi-squared test; ** according to criteria by Nielsen *et al* [53]. *CCND1*: cyclin D1 gene; CISH: chromogenic *in situ* hybridisation; MSH2: mutS homolog 2; *TOP2A*: topoisomerase IIα gene.

Supplementary Table 3. Analysis of correlation between PMS2 expression, clinico-pathological features and markers in 245 invasive breast carcinomas

	N	PMS2 negative (n=2)	PMS2 positive (n=169)	PMS2 inconclusive (n=1)	p value*
Tumour size	170				0.853
T1		1	82	0	
T2		1	75	1	
T3		0	10	0	
Histological grade	168				0.444
I		1	19	0	
II		0	47	0	
III		1	99	1	
Lympho-vascular invasion	170				0.676
present		1	115	1	
absent		1	52	0	
Lymph node metastasis	166				0.042
present		0	112	0	
absent		2	51	1	
ER	172				0.352
positive		1	144	1	
negative		1	25	0	
PR	172				0.592
positive		2	125	1	
negative		0	44	0	
HER2	172				0.771
positive		0	25	0	
negative		2	144	1	
Ki67	160				0.771
low (<10%)		1	72	0	
intermediate		1	63	1	
high (>30%)		0	22	0	
Topoisomerase IIα	158				0.657
positive		1	85	1	
negative		1	70	0	
Cyclin D1	163				0.003
low (Allred score 0-3)		2	17	0	
intermediate (Allred score 4-5)		0	34	0	
high (Allred score 6-8)		0	109	1	
Bcl2	139				0.615
positive		1	92	1	
negative		0	45	0	
FOXA1	140				0.713
positive		1	103	1	
negative		0	35	0	
P53	160				0.188
positive		0	44	1	
negative		2	113	0	
Cytokeratin 5/6	164				0.148
positive		1	15	0	
negative		1	146	1	
Cytokeratin 14	170				0.093
positive		1	13	0	
negative		1	154	1	
Cytokeratin 17	168				0.208
positive		1	18	0	
negative		1	147	1	
Basal cytokeratins	170				0.294
positive		1	22	0	
negative		1	145	1	
EGFR	172				0.110
positive		1	14	0	
negative		1	155	1	
Caveolin 1	172				0.110
positive		1	14	0	
negative		1	155	1	
Caveolin 2	159				0.052

positive		1	10	0	
negative		1	146	1	
Nestin	140				0.885
positive		0	15	0	
negative		1	123	1	
Any basal marker	170				0.358
positive		1	25	0	
negative		1	142	1	
Molecular phenotype**	169				0.512
luminal		1	123	1	
HER2+		0	24	0	
basal-like		1	19	0	
E-cadherin	159				0.781
normal		2	98	1	
reduced		0	10	0	
negative		0	48	0	
HER2 CISH	162				0.777
amplified		0	23	0	
not amplified		2	136	1	
TOP2A CISH	163				0.857
amplified		0	15	0	
not amplified		2	145	1	
CCND1 CISH	169				0.030
amplified		0	21	1	
not amplified		2	145	0	

* Chi-squared test; ** according to criteria by Nielsen *et al* [53]. *CCND1*: cyclin D1 gene; CISH: chromogenic *in situ* hybridisation; PMS2: postmeiotic segregation increased 2; *TOP2A*: topoisomerase IIα gene.

Supplementary Table 4. Analysis of correlation between MSH6 expression, clinico-pathological features and markers in 245 invasive breast carcinomas

	N	MSH6 negative (n=11)	MSH6 positive (n=211)	MSH6 inconclusive (n=3)	p value*
Tumour size	223				0.284
T1		6	108	0	
T2		5	86	3	
T3		0	15	0	
Histological grade	220				
I		0	22	0	0.765
II		3	61	1	
III		8	123	2	
Lympho-vascular invasion	223				0.074
present		4	145	2	
absent		7	64	1	
Lymph node metastasis	218				0.129
present		5	140	1	
absent		6	64	2	
ER	225				0.726
positive		9	174	3	
negative		2	37	0	
PR	225				0.568
positive		8	153	3	
negative		3	58	0	
HER2	225				0.679
positive		1	31	0	
negative		10	180	3	
Ki67	208				0.828
low (<10%)		4	83	1	
intermediate		6	83	2	
high (>30%)		1	28	0	
Topoisomerase IIα	198				0.319
positive		4	104	1	
negative		7	80	2	
Cyclin D1	207				0.908

low (Allred score 0-3)		1	23	0	
intermediate (Allred score 4-5)		3	38	1	
high (Allred score 6-8)		7	132	2	
Bcl2	169				0.424
positive		6	102	3	
negative		4	54	0	
FOXA1	173				0.584
positive		6	123	1	
negative		3	39	1	
P53	207				0.979
positive		3	55	1	
negative		8	138	2	
Cytokeratin 5/6	216				0.849
positive		1	20	0	
negative		9	183	3	
Cytokeratin 14	223				0.519
positive		0	18	0	
negative		11	191	3	
Cytokeratin 17	221				0.656
positive		2	24	0	
negative		9	183	3	
Basal cytokeratins	223				0.693
positive		1	30	0	
negative		10	179	3	
EGFR	225				0.870
positive		1	17	0	
negative		10	194	3	
Caveolin 1	225				0.867
positive		1	18	0	
negative		10	193	3	
Caveolin 2	196				0.164
positive		2	9	0	
negative		9	173	3	
Nestin	166				0.051
positive		3	16	0	
negative		5	139	3	
Any basal marker	223				0.449
positive		3	33	0	
negative		8	176	3	
Molecular phenotype**	218				0.766
luminal		8	149	3	
HER2+		1	32	0	
basal-like		2	23	0	
E-cadherin	201				0.861
normal		7	120	2	
reduced		0	14	0	
negative		4	53	1	
HER2 CISH	212				0.765
amplified		1	30	0	
not amplified		9	170	2	
TOP2A CISH	213				0.862
amplified		1	18	0	
not amplified		10	181	3	
CCND1 CISH	222				0.551
amplified		1	28	1	
not amplified		10	180	2	

* Chi-squared test; **according to criteria by Nielsen *et al* [53]. *CCND1*: cyclin D1 gene; CISH: chromogenic *in situ* hybridisation; MSH6: mutS homolog 6; *TOP2A*: topoisomerase IIα gene.