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CORRESPONDENCE

Relationship between morphological and cytogenetic heterogeneity in invasive micropapillary carcinoma of the breast: a report of one case

INTRODUCTION

Invasive micropapillary carcinoma (IMPC) is a rare (up to 2%) and aggressive form of breast cancer.[1 2] IMPC shows high intratumoral morphological diversity, which represents the degree of cell differentiation, as well as the architectural and invasive growth patterns of tumour cells. Morphologically, these tumours are characterised by the presence of hollow-like (tubular) and morula-like (alveolar) structures of cuboidal-to-columnar neoplastic cells, which are surrounded by empty spaces (retraction clefts) and display an inversion of cell polarity, detected by aberrant localisation of glycoprotein MUC-1 at the stromal–basal surface.[1 3] In addition, micropapillary tumour clusters can be represented by tumour cells arranged in solid patterns (structures), trabecular structures and discrete (small) groups.[3–6] It has been suggested that morphological diversity of IMPC is related to chemotherapy resistance,[7] whereas the presence of retraction clefts around tumour clusters is associated with increased lymphangiogenesis and lymph node metastasis.[8]

Considerable intratumour morphological heterogeneity in breast cancer most likely results from genetic and epigenetic instability of the tumour cells.[9 10] Previously, the relationships between morphologically distinct components and specific chromosome aberrations have been found in metaplastic and invasive ductal breast carcinomas,[11 12] the latter is now classified as invasive carcinoma of no special type (IC NST), and is the most common histological type of breast cancer.[1]

IMPC demonstrates a heterogeneous pattern of chromosome aberrations, and tends to be genetically a more complex disease than IC NST.[11 12] IMPC more often harboured gains of chromosomes 1q, 8q, 17q and 20q, and losses of 1p, 8p, 13q, 16q and 22q,[13 14] which were emphasised by Marchio and coauthors[13] as previously associated with breast tumours of high histological grade. In contrast, concurrent gain of 1q and 16p and deletion of 16q, related to low tumour grade according to the literature data, were less found in IMPC.[13] In addition, the morphological specific pattern of IMPC was suggested to be maintained

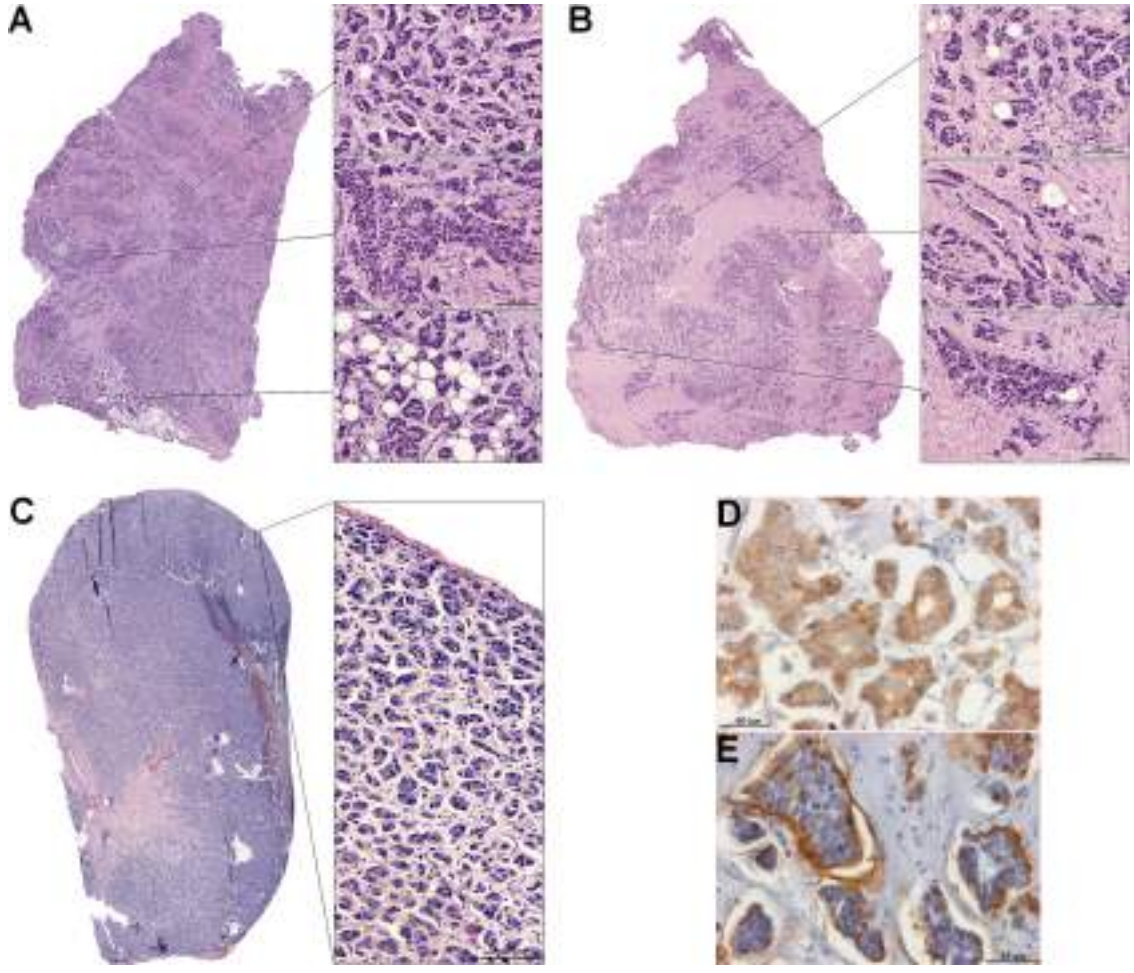

Figure 1 Microphotographs of two regions of the invasive micropapillary carcinoma (IMPC) specimen and lymph node metastasis. (A) Primary tumour region 1. (B) Primary tumour region 2. (C) Lymph node metastasis. Sections have been prepared from frozen surgery samples and stained by H&E. (D and E) Immunohistochemical staining for E-cadherin and epithelial membrane antigen (EMA) (glycoprotein MUC-1), respectively, in different morphological structures of IMPC. E-cadherin expression at the cell surface, EMA expression at the stromal–basal surface and an inversion of cell polarity are detected in hollow-like, morula-like, solid structures and discrete groups of tumour cells, some of whom are surrounded by empty stromal spaces (retraction clefts).

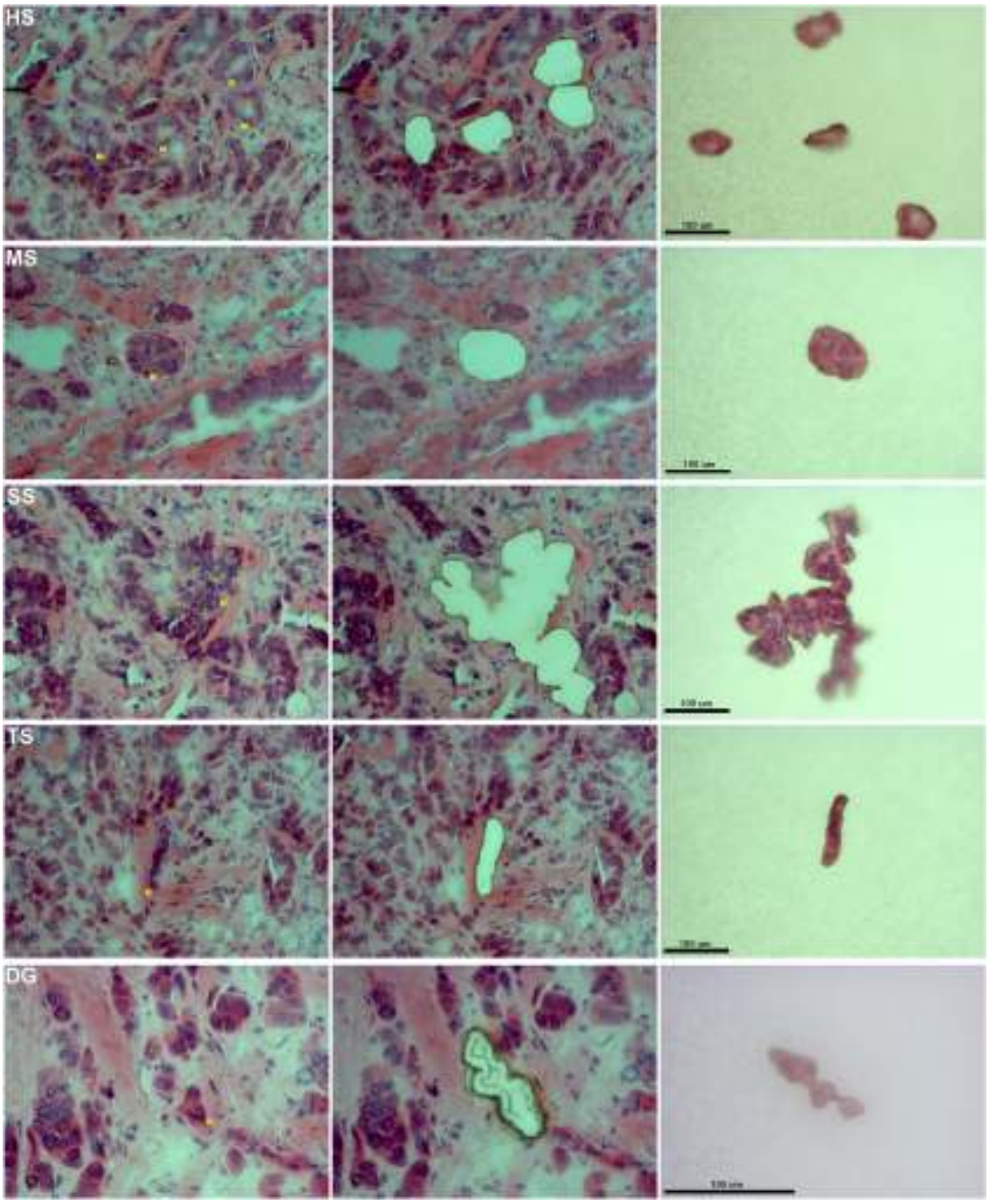

Figure 2 Laser microdissection of different morphological structures of invasive micropapillary carcinoma (IMPC). Hollow-like (HS), morula-like (MS), solid (SS), trabecular (TS) structures and discrete groups of tumour cells (DG) were isolated from H&E-stained sections of the IMPC specimen. Left column: sections with outlined structures. Centre column: remaining sections after cutting and catapulting of the structures. Right column: the structures on adhesive caps. ×200 magnification (HS, MS, SS and TS), ×400 magnification (DG).

by mutations in genes involved in polarity, ciliogenesis and cell shape.[15] However, in spite of all mentioned above, it is not clear whether different morphological structures of IMPC can be associated with specific chromosome aberrations.

The aim of this study was to identify the association of intratumour morphological heterogeneity of IMPC with chromosome aberrations. To reach this aim, array comparative genomic hybridisation (aCGH) was used to analyse chromosome abnormalities in different morphological structures (hollow-like, morula-like, solid, trabecular structures and discrete groups of tumour cells) of two primary tumour regions (R1 and R2) of the IMPC specimen. In addition, lymph node metastasis was studied to understand whether the origin of metastasis is related to certain morphological structure of IMPC.

MATERIALS AND METHODS

Patients and specimens

The analysed tumour was obtained from a 56-year-old woman with IMPC diagnosed in the Tomsk Cancer Research Institute. The tumour had a size of 3 cm, and was classified as luminal B subtype (estrogen and progesterone receptor positive, human epidermal receptor-2 (HER-2)-positive, Ki-67 ≥38%), T2N1M0, grade 2. Immunohistochemical detection of

Table 1 Number of chromosome aberrations in different morphological structures of two tumour regions of the invasive micropapillary carcinoma specimen and in lymph node metastasis

Morphological structures/metastasis	Tumour region	Amplifications	Deletions	Total
Hollow-like	1	60	0	60
	2	5	24	29
Morula-like	1	75	0	75
	2	69	15	84
Solid	1	64	8	72
	2	169	107	276
Trabecular	1	74	2	76
	2	49	5	54
Discrete groups of tumour cells	1	12	23	35
	2	57	1	58
Lymph node metastasis		108	12	120

epithelial membrane antigen (glycoprotein MUC-1) was used in the diagnosis of IMPC (figure 1E). Additionally, analysis of E-cadherin expression was carried out to differentiate IMPC from invasive lobular carcinoma (figure 1D). The case was without any preoperative therapy. The surgery samples (n=2) from primary tumour and lymph node specimens (figure 1A–C) were first frozen by placing in liquid nitrogen and then stored at −80°C until macrodissection and laser microdissection.

Macrodissection and laser microdissection

Five-micrometre-thick sections were obtained by cutting frozen tumour and lymph node samples using Microm HM 525 Cryostat (Thermo Fisher Scientific, USA), and were stained by H&E (Dako, Denmark). Metastasis cells were isolated from lymph node sections using macrodissection as previously described.[16] Hollow-like, morula-like, solid, trabecular structures (50–70 samples of each morphological variant) and discrete groups of tumour cells (150–200 samples) were isolated from tumour sections using PALM MicroBeam laser capture microdissection (Carl Zeiss, Germany; figure 2). Hollow-like structures were represented as rows of tiny tube-shaped cell aggregations. Morula-like structures were identified as tumour spheroids with up to 50 cells. Trabecular structures were formed by two or more rows of cells. Solid structures represented groups with different sizes and shapes consisting of many tens and hundreds of tumour cells. Discrete groups of tumour cells were detected as single cells or as groups of up to five cells. The microdissected material was collected in adhesive caps (Carl Zeiss, Germany).

DNA isolation and whole genome amplification

DNA was isolated from the macrodissected sample of lymph node metastasis using QIAamp DNA Micro Kit (Qiagen, USA). Concentration of DNA measured using NanoDrop 2000 (Thermo Scientific, USA) was 50 ng/μL. The microdissected material was used to perform whole genome amplification by REPLI-g Mini Kit according to the manufacturer's instructions (Qiagen, USA). After the lysis step, adhesive caps were checked under the microscope to ensure the cells were lysed. Amplified DNA was purified from reaction mixtures using QIAquick PCR Purification Kit (Qiagen, USA), and had concentration ranging from 20 to 30 ng/μL. DNA quality was assessed using 2200 TapeStation Instrument and Genomic DNA Screen Tape (Agilent Technologies, USA). Human Female DNA (Agilent Technologies, USA) was used as the reference DNA, and was processed by whole genome amplification (REPLI-g Mini Kit).

Array comparative genomic hybridisation

Analysis of chromosome aberrations was performed using high-resolution 4×180K microarrays (Agilent Technologies, USA). Sample preparation was carried out using SureTag Complete DNA Labeling Kit (Agilent Technologies, USA) according to the protocol of the manufacturer. Analysis was performed using a SureScan Microarray Scanner (Agilent Technologies, USA). Data analysis was performed using CytoGenomics Software V.2.0.6.0 (Agilent Technologies, USA).

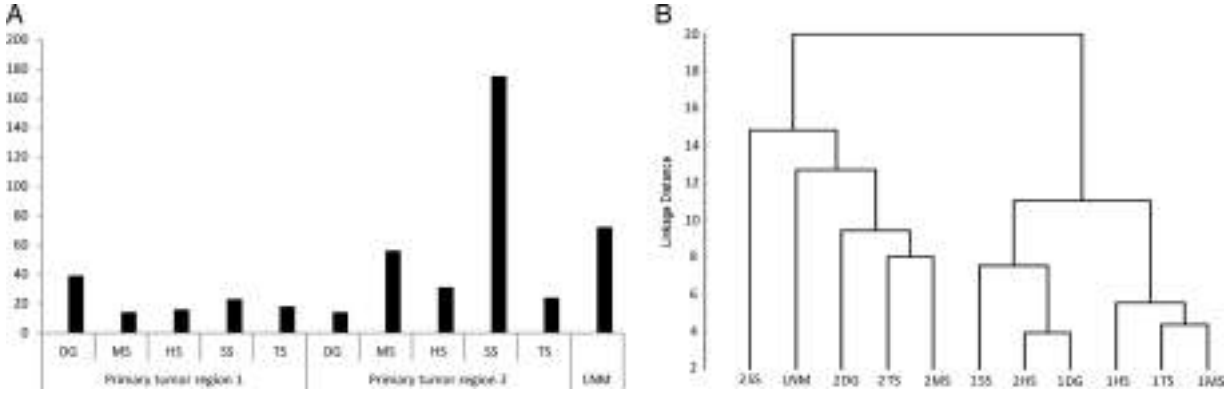

Figure 3 Number of unique chromosome regions with aberrations and cluster analysis on the spectrum of affected regions in different morphological structures of two regions of the IMPC specimen and in lymph node metastasis. Figure (A) summarises the number of chromosome regions with aberrations, which were detected only in certain morphological structures. (B) Cluster analysis is based on the measurement of similarity of different structures of two tumour regions with each other and lymph node metastasis in the number of common chromosome regions containing aberrations. The measure is the Euclidean distance with complete linkage. Numbers indicate the tumour region. DG, discrete groups of tumour cells; HS, hollow-like structures; IMPC, invasive micropapillary carcinoma; LNM, lymph node metastasis; MS, morula-like structures; SS, solid structures; TS, trabecular structures.

Statistical analysis

For the assessment of the association of intratumour morphological heterogeneity of IMPC with chromosome aberrations, cluster analysis was performed based on comparison of different morphological structures with each other and with lymph node metastasis in common chromosome regions with aberrations. A hierarchical cluster analysis was carried out by calculating Euclidean distance metric with complete linkage using the STATISTICA V.8.0 software (StatSoft).

RESULTS AND DISCUSSION

All chromosome aberrations detected in different morphological structures from two tumour regions (R1 and R2) of the IMPC specimen and in lymph node metastasis are given in online supplementary figure S1. The frequent chromosomal gains involved chromosome arms 1q, 8p, 8q 17q, and so on, whereas recurring losses were observed at chromosomal arms 1q, 9q, 21q, Xp (see online supplementary table S1) that is partially consistent with previous results for IMPC.[11 13 15] We also found frequent trisomy of chromosomes 12, 14 and 20. The number of identified unbalanced chromosome aberrations in various structures and metastasis ranged from 29 to 276. The least number of abnormalities was found in the hollow-like structures, and the most in solid patterns and lymph node metastasis (table 1). Moreover, solid structures from R2 and lymph node metastasis had the greatest number of unique chromosome regions with aberrations (figure 3A). It has been suggested to be a result of the high mutation rate ('mutator' phenotype) in tumour cells of these structures that lead to a chromosome instability.[17] In addition, 19 chromosome regions with mutations were common for solid structures from R2 and for lymph node metastasis. Among them, the most remarkable abnormality was the amplification of 17p13 that partially involves the *TP53* gene. Breakpoint was located at the border of exon 8 of *TP53*, suggesting that the gene product was probably abnormal. Moreover, amplification of 12q14.1-q21.33 containing *MDM2* gene (p53 ubiquitin ligase) was detected in five different structures, including solid structures from R2 and lymph node metastasis. It is most likely that amplification of the *MDM2* gene and of its regulatory sequences results in the increased expression of the Mdm2 protein. Combination of these two 'mutator' amplifications in tumour cells may lead to p53 inhibition and defective checkpoint activation, resulting in a high mutation rate.

Significant predominance of amplifications versus deletions was observed in tumour cells of all morphological structures (table 1) (average ratio 3.76). Similar results were shown by Lv *et al*[18] who found the prevalence of amplifications in breast tumours (114 amplifications vs 35 deletions, ratio 3.2), whereas the prevalence of deletions (30 amplifications vs 37 deletions, ratio 0.8) was observed in benign tumours.[18]

No chromosome abnormalities common for all samples were observed. However, several chromosome aberrations were common for most samples. Four amplifications involving chromosomes 8, 14 and 17 were common for 8 of 10 specimens (80%), and 15 amplifications on chromosomes 8, 12, 16, 17, 20 and X occurred in 7 of 10 samples (70%, see online supplementary table S1). It has been suggested that these chromosome aberrations originated in common ancestor tumour clone at the early stages of tumourigenesis. The prevalence of amplifications versus deletions was even higher among common chromosome aberrations (24 vs 4, respectively, ratio 6.0) that may indicate a late origin of deletions in breast tumour development or greater rate of elimination of cells with deletions. It should be pointed that high number of common chromosome aberrations was detected in the same types of morphological structures. However, such genomic abnormalities seem to be unrelated to formation of morphologically similar tumour clusters of IMPC because some of them were also identified in another types of structures.

To analyse the similarity of the investigated morphological structures in the spectrum of chromosome abnormalities, we conducted a cluster analysis (figure 3B). Different types of structures within the same region of the tumour had more similar sets of chromosome abnormalities than the same structures in different parts of the tumour. This indicates that all structures within one tumour region probably had common ancestor. In other words, the same types of structures could be formed on the basis of clones with different origin and karyotype. And interestingly enough that morula-like and trabecular structures both from R1 and R2 demonstrated a high similarity, whereas solid patterns were most different from all others (figure 3B). In addition, cluster analysis showed that lymph node metastasis was close to the morphological structures (solid and discrete structures) from R2 (figure 3B).

Thus, using laser microdissection-based aCGH, we determined that intratumour morphological heterogeneity in IMPC is not associated with chromosome aberrations, but similarity of chromosomal abnormalities suggests that some morphological structures (namely, solid and discrete) can be involved in the development of lymph node metastases. Probably, the formation of the similar structures is the result of same gene point mutations or similar changes in epigenetic profile, regulation of gene expression and biochemical pathways of tumour cells, wherein the affected chromosome regions and type of mutations may be completely different. In particular, our previous study demonstrated that intratumour morphological diversity in breast cancer correlates with expression of cell adhesion genes, and thus, can reflect different patterns of invasive growth.[3] In addition, intratumour morphological heterogeneity representing, for example, the altered epithelial architecture and differentiation may be determined by tumour microenvironment.[19 20] Further studies are needed to confirm our findings in a larger series of patients with IMPC and to investigate other genetic and/cellular factors, which could be involved in the development of intratumour morphological heterogeneity in IMPC.

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Contributors EVD and NAS designed the study. MVZ and VMP collected the tumour samples. EVD and TSG performed laser microdissection and whole genome amplification. NAS and SAV performed array comparative genomic hybridisation and analysed data. EVD, NAS and SAV wrote the paper. INL, NVC and VMP assisted in critiquing, editing and refining the paper. All authors reviewed the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The procedures followed in this study were in accordance with the Declaration of Helsinki (1964, amended in 1975 and 1983). This study was approved by the institutional (Cancer Research Institute, SB RAMS) review board, the patient signed an informed consent for voluntary participation, and the number of ethical approval was 10 (29 September 2011).

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Notes