Two Progressive Pathways of Microinvasive Carcinoma:

Low Grade-Luminal Pathway and

High Grade-HER2 Pathway basing on High Tumor Infiltrating Lymphocytes

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Abstract

While cancer immunity is involved in tumor progression from the very early stage of tumorigenesis, no detailed study has been reported on the relationship between “early-stage” breast cancer and tumor infiltrating lymphocytes (TIL).

Of 2593 resected breast carcinomas, 46 microinvasive carcinomas (1.8%) were included in this study. The relationships between tumor characteristics (invasive form, grade, comedo, subtype) and immune characteristics (TIL, healing) were examined. The invasive form was divided into "cluster-like" (i.e.; invasive foci consisted of a small number of cancer cells) and "non-cluster-like" (i.e.; nested and classifiable into particular histological type).

Among all microinvasive carcinomas, 34.8% were Grade1. ER^+HER2^-, ER^+HER2^+, ER^-HER2^+, and ER^-HER2^- accounted for 58.7%, 8.7%, 28.3%, and 4.3%, respectively. Compared to ER^+HER2^- microcarcinomas, ER^-HER2^+ cases had a significantly stronger association with Grade3 (92.3% vs. 0%), comedo necrosis (100% vs. 55.6%), high TIL (100% vs 29.3%), high $CD8^+$TIL (92.3% vs. 33.3%), and healing (76.9% vs. 14.8%) ($P < 0.001$). Compared to “non-cluster-like”, “cluster-like” carcinomas showed significantly higher rates of HER2 positivity (69.2% vs.24.2%), high TIL (92.3% vs.42.4%) and $CD8^+$ TIL (76.9% vs. 39.4%) ($P < 0.01$).

The present study revealed microinvasive carcinoma has two progressive pathways; “low-grade-luminal pathway” and “high-grade-HER2 pathway”. HER2-positive

microinvasive carcinoma showed the following unique characteristics: high-grade; comedo, high TIL and $CD8^{+}$TIL; healing; cluster-like invasion. These results suggest the cluster-like invasion might occur because of tumor immunity that leads to disruption of the duct and formation of microinvasive carcinoma, and tumor immunity and HER2 expression together play a role in the step of invasion.

Introduction

The historic progression model of breast cancer proposes that the normal ductal epithelium gradually becomes atypical, and after going through phases of atypical ductal hyperplasia and low-grade noninvasive carcinoma, the lesion increases in malignancy and becomes high-grade noninvasive carcinoma and eventually invasive carcinoma [1]. In recent years, however, genetic analyses have revealed that there are two pathways of development of invasive carcinoma: the low-grade pathway and the high-grade pathway, with low-grade noninvasive carcinoma progresses to low-grade invasive carcinoma and a high-grade noninvasive carcinoma to high-grade invasive carcinoma [2]. In this model, microinvasive carcinoma is positioned as the first step of development to invasive carcinoma. Microinvasive carcinoma is defined as a tumor in which the invasive component is no greater than 1mm in its largest dimension, and in cases with multiple foci, the largest invasive component measures no greater than 1mm. Most reports suggest that microinvasive carcinoma develops from high-grade comedo-type ductal carcinoma in situ (DCIS) [3-7]. According to the reports by Yang et al. [4] and Mori et al. [8], the HER2-positive type was found frequently in microinvasive carcinoma, suggesting the possible involvement of the HER2 pathway in the invasive process.

In recent years, the amount of tumor infiltrating lymphocytes (TIL) has attracted increasing attention from the point of view of tumor immunity as prognostic and predictive factors of

invasive carcinoma, in particular of HER2-positive breast cancer and triple negative (TN) breast cancer [9, 10]. We also reported that the degree of TIL in needle biopsy samples of HER2-positive and TN breast cancer is predictive of the response to neoadjuvant chemotherapy [11]. Furthermore, it was reported that in invasive carcinoma, HER2-positive and TN cases show higher TIL compared to ER-positive cases [9].

In 1934, Muir et al. reported the phenomenon of "healing" as follows: "cancer cells undergo retrogressive change and disappear, this being accompanied by fibrous thickening of the intra-duct walls" [12]. Rosen et al. also mentioned "healing" as "marked periductal fibrosis can, on occasion, be associated with extensive obliteration of DCIS" [7]. Chivukula et al. discussed regressive changes as accessory findings of high-grade DCIS and reported that fibrosis occurs after marked infiltration of immune cells around high-grade DCIS [13]. This observation appears to be similar to the "healing" phenomenon and can be understood as a step in the tumor immune response of the host.

Such tumor immunity is involved in tumor progression and suppression not only in invasive cancer but also from the very early stage of breast cancer development; it is thought that progression or regression of tumor results from the interrelation of tumor immunity with tumor malignancy and proliferative capacity. However, no detailed study has reported on the relationship between TIL and tumor factors in early-stage breast cancer.

In this study, we focus our attention on microinvasive carcinoma, which is the first step in the

development to invasive carcinoma, and investigate the relationship between tumors and immunity in "early" breast cancer through comprehensive observation of characteristics relating to tumor factors (histologic architecture of the underlying DCIS, histologic type of the invasive component, grade, subtype, comedo, etc.) and characteristics relating to tumor immunity (TIL, healing).

Materials and Methods

Cases

Out of 2593 resected breast carcinomas treated at Japan Community Healthcare Organization Kurume General Hospital between 2007 and 2014, this study cohort included 46 microinvasive carcinoma patients diagnosed as having microinvasive carcinoma by core biopsy and with subsequent postoperative histologic examinations. All core biopsies and resected materials were formalin fixed and paraffin embedded. Four sections were cut and stained with hematoxylin and eosin (HE). All slides were reviewed and the diagnosis of microinvasive carcinoma was confirmed by two pathologists (M.M. and R.Y.).

The diagnosis of microinvasive carcinoma is defined as an invasive tumor no greater than 1 mm in its greatest dimensions according to AJCC/TNM criteria [14].

This retrospective study was approved by JCHO Kurume General Hospital Ethical Committee (No. 143).

Definition

Histologic data

For the intraductal component, we evaluated the architecture of *in situ* carcinoma, Grade, Van Nuys classification, the presence and size of comedo necrosis. For the microinvasive component, we noted the histologic subtype and number of microinvasive foci.

TIL, healing, positivity of estrogen receptors (ER), progesterone receptors (PgR) and HER2 and immunohistochemistry (IHC)-based subtype were assessed for both intraductal and microinvasive components, except for 7 cases in which microinvasive components had disappeared through thin sectioning for immunohistochemistry.

Histologic subtyping

The microinvasive component was divided into two categories; cluster-like invasion and non-cluster-like invasion (Figure 1). Cluster-like invasion was used for invasive foci consisted of small number of cancer cells and could not be classified into any histologic subtype described later. “Non-cluster-like invasion” was used for microinvasive foci that had some cancer cells and could be classified any histologic subtype according to the WHO classification of tumors of the breast 4th edition [15] (Figure 2).

TIL

TIL were assessed according to the recommendation of the international TIL working group [16]. TIL were defined as high when 50-100% of the stroma surrounding microinvasive and

intraductal components was occupied by lymphocytes (Figure 3a).

CD8 positive TIL

As for high TIL cases, we manually counted the number of CD8 positive lymphocytes within 0.0625mm^2 at four fields using square micrometer (Olympus). Cutoff value of high CD8 positive lymphocytes was determined from the median values of all cases (Figure 3b).

Healing

Healing was defined as a phenomenon with the presence of thick fibrosis and/or phagocytes surrounding intraductal and microinvasive components as modified from definitions in previous studies [7, 13, 17, 18] (Figure 4).

Comedo size

The maximal length of the minor axis of comedo necrosis was measured manually using an eyepiece micrometer (square, 10 mm/10 units, Olympus).

Immunohistochemistry

Immunohistochemistry for ER, PgR and HER2 were performed using BenchMarkXT (VENTANA, Tucson, AZ, USA) according to the manufacturer's protocols. The primary antibodies are as follows:

ER (clone SP1, VENTANA, Tucson, AZ, USA), PgR (clone IE2, VENTANA, Tucson, AZ, USA), HER2 (C-erbB-2) (clone 4B5, VENTANA, Tucson, AZ, USA).

ER/PgR positivity

ER and PgR expression were defined as positive when 1% or more of tumor cell nuclei were immunoreactive based on ASCO/CAP guidelines [19].

HER2 positivity

HER2 IHC expression was assessed according to ASCO/CAP guidelines [20] (Figure 5). In cases in which the IHC score was 2+, the specimens were retested by HER2 dual color *in situ* hybridization and cases in which the HER2/ CEP17 rate was > 2.0 or HER2 copy number >6.0 were determined as positive.

IHC based subtype

IHC based subtype was classified as follows based on modified St. Gallen consensus [21]: Luminal; HR+ (ER+ and/or PR+)/ HER2+, Luminal–HER2; HR+/ HER2+, HER2; HR- (ER- and PR-)/ HER2+, Triple negative (TN); HR-/ HER2-.

Statistical analysis

For statistical analysis, Pearson Chi-square test or Fisher's test were performed. Relationship between IHC-based subtype and other histological findings was analysed by Fisher's test. Other analysis was performed by Pearson Chi-square test. It was determined as significant at a *P* value of 0.05 or less.

Results

Clinicopathological findings of microinvasive carcinoma

Of 2593 resected breast carcinomas between 2007 and 2014, 46 microinvasive carcinomas (1.8%) were found. 25 partial mastectomies and 21 total mastectomies were included in this cohort. The median number of blocks were 17 (range, 4-39) for breast-conserving surgeries and 4 (range, 2-12) for mastectomies. The clinicopathological findings of these 46 microinvasive carcinomas are shown in Table 1.

Table 1. Clinicopathological characteristics of microinvasive carcinoma

	Number of cases	
	(n=46)	**(% of patients)**
Age		
< 50	14	30.4
> 50	32	69.6
Architecture of *in situ* carcinoma		
Cribriform	18	39.1
Solid	4	8.7
Micropapillary	1	2.2
Clinging	0	0
solid-papillary	2	4.3
lobular carcinoma *in situ*	1	2.2
Mixed	20	43.5
Van Nuys classification		
group1	11	23.9
group2	20	43.5
group3	15	32.6
Nuclear grade		
grade1	16	34.8
grade2	15	32.6
grade3	15	32.6
Comedo		
Present	32	69.6

Absent	14	30.4
Lymphovascular invasion		
Present	4	8.7
Absent	42	91.3
Histological subtyping		
cluster like appearance	14	30.4
non-cluster like invasion		
Invasive carcinoma no special type	17	37
Invasive micropapillary carcinoma	2	4.3
Mucinous carcinoma	5	10.9
tubular/ cribriform	7	15.2
Invasive lobular carcinoma	1	2.2
Number of invasion site		
Single	34	73.9
Multiple	12	26.1
Tumor infiltrating lymphocyte		
High	26	56.5
Low	20	43.5
Healing		
Present	18	39.10%
Absent	28	60.90%
Estrogen receptor		
Positive	31	67.4
Negative	15	32.6
Progesterone receptor		
Positive	26	56.5
Negative	20	43.5
HER2		
Positive	17	37
Negative	29	63
Immunohistochemistry based subtype		
Luminal	27	58.7
Luminal-HER2	4	8.7
HER2	13	28.3
Triple Negative	2	4.3

The mean age of this cohort was 57 years (range, 34-77). As for the architecture of the

intraductal component, the combined type was most frequently seen (20 cases, 43.5%). The most common pure type was cribriform and seen in 18 cases (39.1%) otherwise clinging pattern did not exist as pure type. Microinvasion from lobular carcinoma *in situ* was rare and only seen in one case. According to Van Nuys classification and grade, microinvasive carcinomas occurred in low grade intraductal neoplasms; 11 cases were Van Nuys classification group 1 which were without comedo necrosis (23.9%) and 16 cases were grade 1 (34.8%). Only 32.6% of microinvasive carcinoma occurred in high grade intraductal carcinoma. Comedo necrosis was present in 32 cases (69.6%).

From the viewpoint of histologic subtyping, cluster-like and non-cluster-like invasion occurred in 30.4% (14/46) and 69.6% (32/46) of cases, respectively. Among all non-cluster-like invasions, invasive carcinoma of no special type was most common and seen in 17 cases, tubular or cribriform carcinoma, mucinous carcinoma, invasive micropapillary carcinoma and invasive lobular carcinoma were 7, 5, 2 and 1 case, respectively. Thirty-four cases (73.9%) had single focus of invasion and 12 cases (26.1%) had multiple foci of invasion. Lymphovascular invasion was present in 4 cases (8.7%).

Regarding TIL and healing, 26 cases (56.5%) had high TIL and 18 cases (39.1%) had healing. According to positive rates of ER, PgR, and HER2 were 67.4% (31/46), 56.5% (26/46) and 37% (17/46), respectively. As for IHC-based subtype, luminal, luminal-HER2, HER2 and TN accounted for 58.7%, 8.7%, 28.3% and 4.3% of all cases respectively.

Relationship between IHC based subtyping and histologic findings

The relationships between IHC-based subtype and other histologic findings are shown in Table 2.

Table 2. Relationship between IHC based subtyping and histological findings

	Luminal (n = 27)	**Luminal-HER2 (n = 4)**	**HER2 (n = 13)**	**Triple Negative (n = 2)**	***P* value**
Architecture of *in situ* carcinoma					0.76
Cribriform	11	1	4	2	
Solid	0	0	4	0	
Micropapillary	0	0	1	0	
Clinging	0	0	0	0	
solid-papillary	2	0	0	0	
lobular carcinoma *in situ*	0	1	0	0	
Mixed	14	2	4	0	
Van Nuys classification					<0.001
group1	10	1	0	0	
group2	17	0	1	2	
group3	0	3	12	0	
Nuclear grade					<0.001
grade1	14	1	0	1	
grade2	13	0	1	1	
grade3	0	3	12	0	
Comedo					*0.008
Present	15	2	13	2	
Absent	12	2	0	0	
Lymphovascular invasion					0.306
Present	3	0	0	1	
Absent	24	4	13	1	
Histological subtyping of invasive area					0.45
cluster like appearance	3	3	6	1	
non-cluster like invasion					
Invasive carcinoma no special type	12	0	6	0	
Invasive micropapillary carcinoma	2	0	0	0	

Mucinous carcinoma	5	0	0	0	
tubular/ cribriform	5	0	1	1	
Invasive lobular carcinoma	0	1	0	0	
Number of invasion					0.29
Single	22	3	7	2	
Multiple	5	1	6	0	
Tumor infiltrating lymphocyte					<0.001
High	8	3	13	2	
Low	19	1	0	0	
CD8 positive lymphocytes					*0.007
High (≥210)	9	2	12	1	
Low (< 210)	18	2	1	1	
Healing					<0.001
Present	4	2	10	2	
Absent	23	2	3	0	

* represents statistically significant results.

Notably, HER2 subtype had a significantly stronger association with grade3 (92.3%; 12/13 cases vs. 0%; 0/27), comedo necrosis (100%; 13/13 vs. 55.6%; 15/27), high TIL (100%; 13/13 vs. 29.6%; 8/27), high CD8 positive TIL (92.3%; 12/13 vs. 33.3%; 9/27) and healing (76.9%; 10/13 vs. 14.8%; 4/27) than luminal subtype ($P < 0.001$). (Table 3.)

Table 3. Histological findings in Luminal and HER2 microinvasive carcinoma.

	Luminal (n = 27)	**HER2 (n = 13)**	***P* value**
Architecture of *in situ* carcinoma			0.066
Cribriform	11	4	
Solid	0	4	
Micropapillary	0	1	
Clinging	0	0	
solid-papillary	2	0	

lobular carcinoma *in situ*	0	0	
Mixed	14	4	
Van Nuys classification			<0.001
group1	10	0	
group2	17	1	
group3	0	12	
Nuclear grade			<0.001
grade1	14	0	
grade2	13	1	
grade3	0	12	
Comedo			<0.001
Present	15	13	
Absent	12	0	
Lymphovascular invasion			0.151
Present	3	0	
Absent	24	13	
Histological subtyping of invasive area			0.090
cluster like appearance	3	6	
non-cluster like invasion			
Invasive carcinoma no special type	12	6	
Invasive micropapillary carcinoma	2	0	
Mucinous carcinoma	5	0	
tubular/ cribriform	5	1	
Invasive lobular carcinoma	0	0	
Number of invasion			0.081
Single	22	7	
Multiple	5	6	
Tumor infiltrating lymphocyte			<0.001
High	8	13	
Low	19	0	
CD8 positive lymphocytes			<0.001
High (≥210)	9	12	
Low (< 210)	18	1	
Healing			<0.001
Present	4	10	
Absent	23	3	

Relationships between Cluster-like microinvasion and other histological findings

We investigated the relationships between cluster-like microinvasion and other histological findings (Table 4).

Table 4. Relationship between cluster like microinvasion and histological findings

	cluster like (n = 13)	**non cluster like (n = 33)**	***p* value**
Estrogen Receptor			0.053
Positive	6	25	
Negative	7	8	
HER2			*0.004
Positive	9	8	
Negative	4	25	
Immunohistochemistry-based subtype			*0.013
Luminal	3	24	
Luminal-HER2	3	1	
HER2	6	7	
Triple negative	1	1	
Tumor infiltrating lymphocyte			*0.021
High	12	14	
Low	1	19	
CD8 positive lymphocyte			*0.022
High (≥210)	10	13	
Low (<210)	3	20	
Healing			0.2
Present	7	11	
Absent	6	22	
Lymphovascular invasion			0.189
Present	0	4	
Absent	13	29	
Number of invasion			0.052
Single	7	27	
multiple	6	6	

* represents statistically significant results.

Non-cluster-like invasion cases had a higher ER positive rate (75.8%) than cluster-like invasion (46.2%) (P = 0.053). HER2 positive rate was significantly higher in cluster-like invasion cases (69.2%) than in non-cluster-like cases (24.2%) (P = 0.004). In the site of IHC-based subtype, luminal type was most common in non-cluster-like invasion (72.7%; 24/33 cases) and HER2 in cluster-like invasion (46.1%; 6/13) (P = 0.013). Cluster-like invasion had significantly higher relationships with high TIL (92.3%; 12/13 vs. 42.4%; 14/33, P = 0.021) and high CD8 positive TIL (76.9%; 10/13 vs. 39.4%; 13/33, P = 0.022) and it tended to have more healing cases (53.8%; 7/13 vs. 33.3%; 11/33, P = 0.343) than non-cluster like invasion.

Relationships between Comedo size and other histological findings

Among 32 comedo necrosis present cases, the median comedo size was 450 μm (range; 75 – 1425 μm). We divided all 46 microinvasive cases into three groups; large comedo group (comedo size was 450 μm or more), small comedo group (less than 450 μm) and non-comedo group. The relationships between comedo necrosis and other histological findings are shown in Table 5.

Table 5. Relationships between Comedo size and other histological findings

	large comedo (short axis ≥ 450) (n = 18)	small comedo (short axis < 450) (n = 14)	non-comedo (n = 14)	*p* value
Estrogen Receptor				<0.001
Positive	6	11	14	
Negative	12	3	0	
HER2				*0.003
Positive	12	3	2	
Negative	6	11	12	
IHC-based subtype				*0.005
Luminal	5	10	12	
Luminal-HER2	1	1	2	
HER2	11	2	0	
Triple negative	1	1	0	
Nuclear Grade				*0.003
Grade 1 or 2	7	11	13	
Grade3	11	3	1	

* represents statistically significant results.

ER positive rate was significantly lower in the large comedo group than in the small and non-comedo groups (33.3%; 6/18 cases vs. 78.5%; 11/14 vs. 100%; 14/14, $P < 0.001$), otherwise, HER2 positive rate was significantly higher in the large comedo group than in the small or non-comedo groups (66.7%; 12/18 vs. 21.4%; 3/14 vs. 14.3%; 2/14, $P = 0.003$). As for the IHC-based subtype, the HER2 subtype was most common in the large comedo group (61.1%; 11/18) and the luminal subtype was most common in the small comedo (71.4%; 10/14) and non-comedo (85.7%; 12/14) groups ($P = 0.005$). From the viewpoint of grade, grade 3 was significantly more frequent in the large comedo group (61.1%; 11/18) than in the

small (21.5%; 3/14) and non-comedo (7.1%; 1/14) groups ($P = 0.003$).

Clinical follow up data

We investigated metastasized and recurred cases in this cohort. The median follow up time was 4.4 years (range; 0.5-8.4 years). Among all 46 microinvasive carcinomas, 2 cases (4.3%) had lymph node metastasis at the point of operation. Both cases were given appropriate adjuvant therapy and they showed no evidence of recurrence. Only 1 case (grade 2, Van Nuys classification group 2, luminal subtype) showed recurrence. In spite of endocrine therapy, the patient died from multiple lung and brain metastases 4 years and 7 months after operation subsequently to local recurrence 2 years and 8 months after the operation.

Discussion

Microinvasive carcinoma is the first step in the development of invasive carcinoma and the lesion is positioned between *in situ* cancer and invasive cancer. However, the present study revealed a number of histological features of microinvasive carcinoma that differ from both *in situ* cancer as well as invasive cancer.

Most microinvasive carcinomas are reported to develop from high-grade cancer [15]. However, about 30% of the cases of microinvasive carcinoma examined in the present study were found to be associated with low-grade *in situ* cancer, and that the remaining cases were

associated with medium-grade and high-grade cancers at comparable ratios. We confirmed the presence of low-grade and high-grade pathways proposed by Lopez et al. based on genomic analysis [2] and demonstrated that low-grade cancers *in situ* develop microinvasive carcinoma at a certain rate.

The rate of HER2-positivity of 37% found in microinvasive carcinoma in the present study was higher compared to the rates of earlier reports on invasive breast cancer in Japanese (12% - 15%) [22, 23]. The characteristics found for HER2-positive microinvasive breast cancer can be summarized as follows: 1) high-grade; 2) large comedo (> 450 μm); 3) high TIL and CD8 positive TIL; 4) healing phenomenon; 5) high rate of cluster-like invasion. In their comparative study of DCIS, microinvasive carcinomas and T1a cancers Mori et al. [8] demonstrated a higher rate of HER2 positivity in microinvasive carcinoma similar to the present study and formulated the hypothesis that "HER2 plays an important role in making the first invasion." Based on the five characteristics mentioned above, we propose two possible explanations for the higher HER2 positivity rate in microinvasive carcinoma: firstly, that HER2-positive cancers are likely to be detected at breast cancer screenings using mammography because they have large comedo; secondly, that due to its strong immunogenicity, HER2 causes increased accumulation of TIL especially CD8 positive cytotoxic T lymphocytes already in the *in situ* stage, and that a disruption of the myoepithelium and basement membrane occurs during the stage of healing, so that cancer

cells are exposed in the stroma as a cluster-like invasion. In a study on colorectal cancer, Jiang et al. reported that TIL is involved in the focal disruption of tumor [24]. Chivukula et al. reported that the presence of myoepithelium was confirmed in only 91% of cases of high-grade DCIS with regressive changes [13], which suggests that TIL may be involved in not only elimination of tumor but also in the disruption of the epithelium and the resulting formation of microinvasive foci in early breast cancer.

In the present study, TN breast cancer accounted for only 4.3% of the cases studied. Studies on microinvasive carcinoma such as that conducted by Mori et al. [8] and Zhang et al. [25] in Asian patients demonstrated low incidences of TN in microinvasive carcinoma (approximately 4%) similar to the current study. In contrast, studies conducted by Orzalesi et al. [26] and Castaneda et al. [27] in Europe showed that the TN subtype comprises 11% to 15% of microinvasive carcinomas, which was not much different from the results found in invasive carcinomas. Therefore, it seems that Asians and Caucasians have different mechanisms of breast cancer development. It is also possible that Asians might have a different pathway of TN breast cancer development in which DCIS does not play a central role.

At this time, albeit with a short period of observation, 1 case of local recurrence occurred in the luminal subtype, whereas no recurrence occurred in HER2-positive and TN subtypes. A possible explanation for the paucity of local recurrence may be that although HER2-positive

DCIS itself is of high-grade malignancy, it is eliminated at the early stage of invasion by the host immunity because it is strongly immunogenic and causes a healing phenomenon to take place.

There were some limitations in this cohort study. Although a large number of resected breast cancers was included, the number of microinvasive carcinoma cases was still small because of its rarity. All patients included in this cohort study were Japanese, therefore it may have difficulties to infer other populations from our result.

In conclusion, in the present study, microinvasive carcinoma showed a higher rate of HER2 positivity than ordinary invasive carcinoma whereas there was only a small number of cases of TN breast cancer. HER2-positive carcinoma was found to have a strong association with high CD8 positive TIL and healing suggesting the possible contribution of these forms of tumor immunity to the formation of microinvasion and change in frequency of HER2 positivity during breast cancer progression. Further analyses of TIL subsets and observation of TIL subsets in association with change in proportion of tumor subtype is expected to assist in our understanding of the interaction between a tumor and its microenvironment during the progression of breast cancer.

Key Messages:

1. Microinvasive carcinomas occur not only in high-grade DCIS but also in low-grade

DCIS.

2. HER2 positive microinvasive breast carcinoma shows following unique characteristics: 1) high-grade, 2) large comedo (> 450 μm), 3) high TIL and CD8 positive TIL, 4) healing phenomenon, 5) high rate of cluster-like invasion.

3. The unique characteristics of HER2 positive microinvasive carcinoma suggest that host anti-tumor immunity may lead HER2 positive intraductal carcinoma to microinvasion.

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Figure Legends

Figure 1 Cluster-like invasion and non-cluster-like invasion. a) cluster-like invasion (arrow). Marked tumor infiltrating lymphocytes and microinvasive foci consisting of a small number of cancer cells are seen. b) Non-cluster-like invasion; cribriform/tubular carcinoma. The microinvasive foci have some cancer cells and we can recognize a cribriform structure.

Figure 2 Histological subtyping of non-cluster-like invasion. a) Invasive carcinoma of no special type. A lymphovascular invasion is present near the microinvasive focus (arrow head). b) Invasive micropapillary carcinoma. These microinvasive foci show inside-out growth pattern. c) Mucinous carcinoma. There are several cancer cell nests in a mucinous lake.

Figure 3 High tumor infiltrating lymphocytes and high CD8 positive tumor infiltrating lymphocytes. a) TIL were defined as high when 50-100% of the stroma surrounding microinvasive and intraductal components was occupied by lymphocytes. b) CD8 positive tumor infiltrating lymphocytes surrounded intraductal and microinvasive carcinoma. Some lymphocytes were also seen in intraductal and microinvasive component.

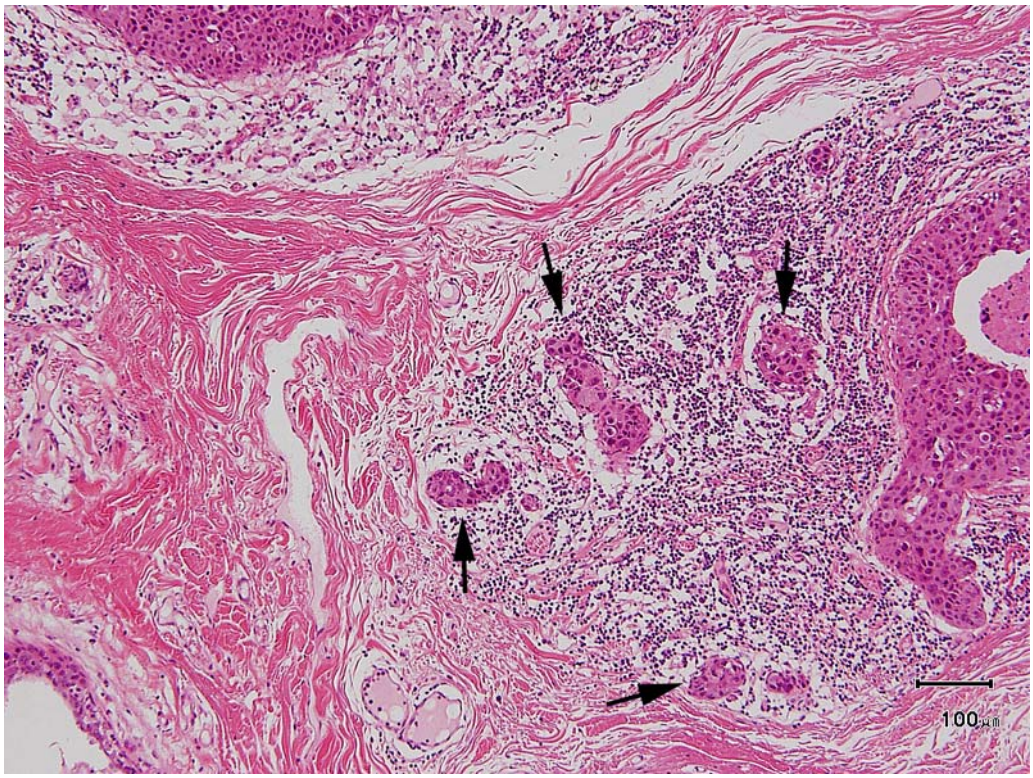
Figure 4 Healing. a) Marked fibrosis surrounding tumor. Fibrosis contains cluster-like invasion (arrow head). b) Phagocytes are infiltrating (arrow). On the upper part, cluster-like

invasion (arrow head) is seen with high TIL.

Figure 5 HER2 positive microinvasive breast cancer. a) H&E stain of HER2 positive microinvasive carcinoma. It shows cluster-like microinvasion (arrow head) and large comedo DCIS with healing. b) HER2 immunohistochemistry of microinvasive carcinoma. Both intraductal component and microinvasive foci (arrow head) are strongly positive for HER2.

Figure 1

a)



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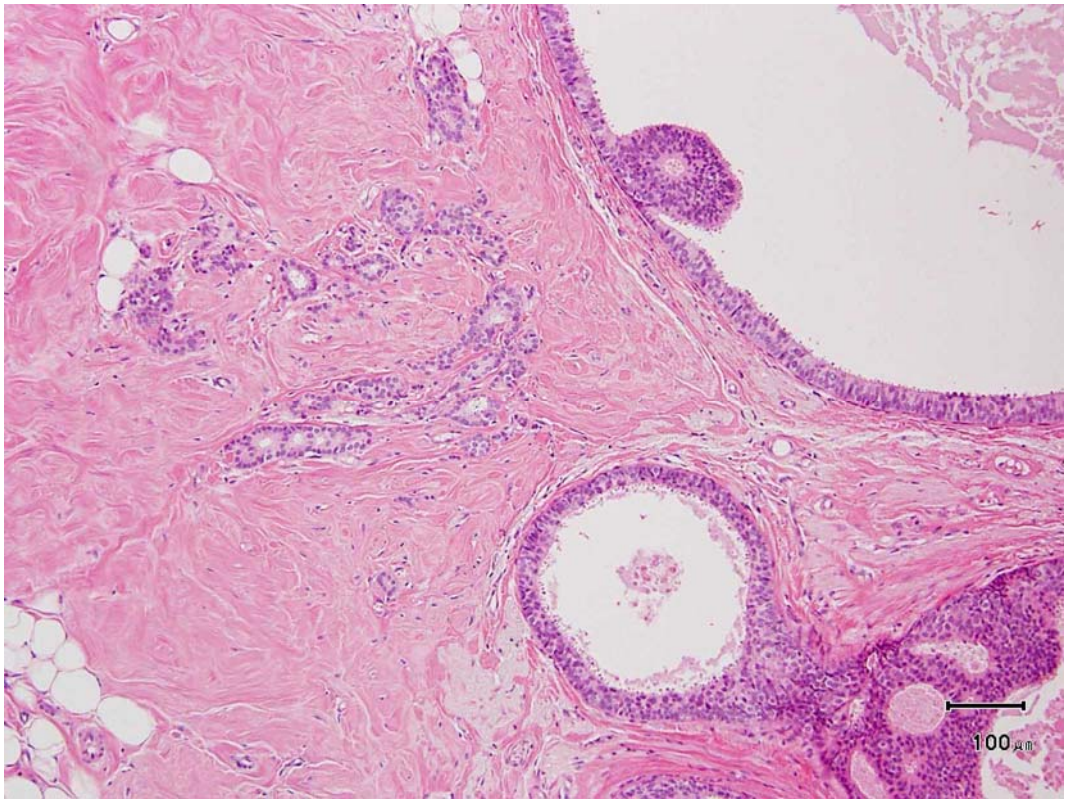

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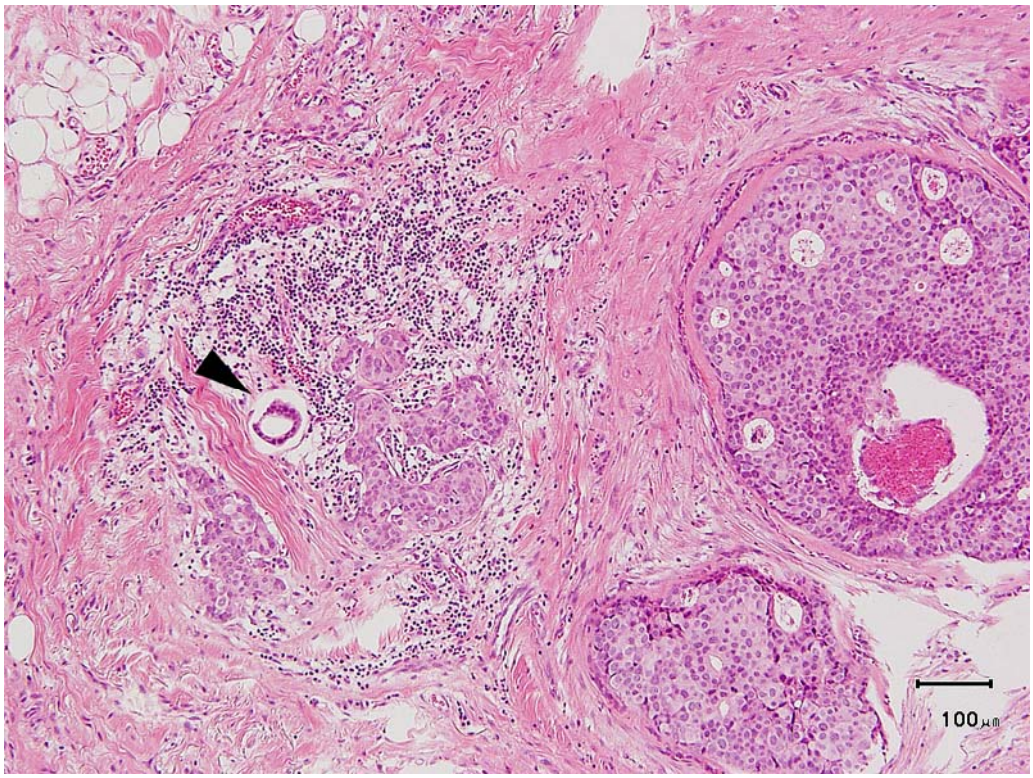

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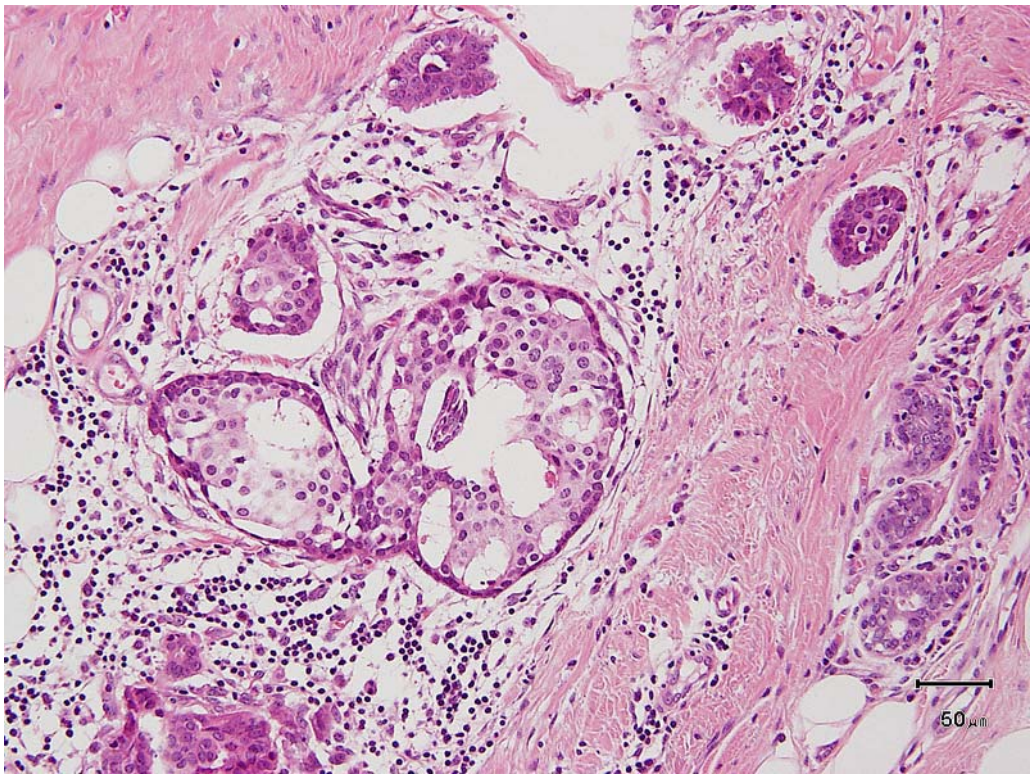

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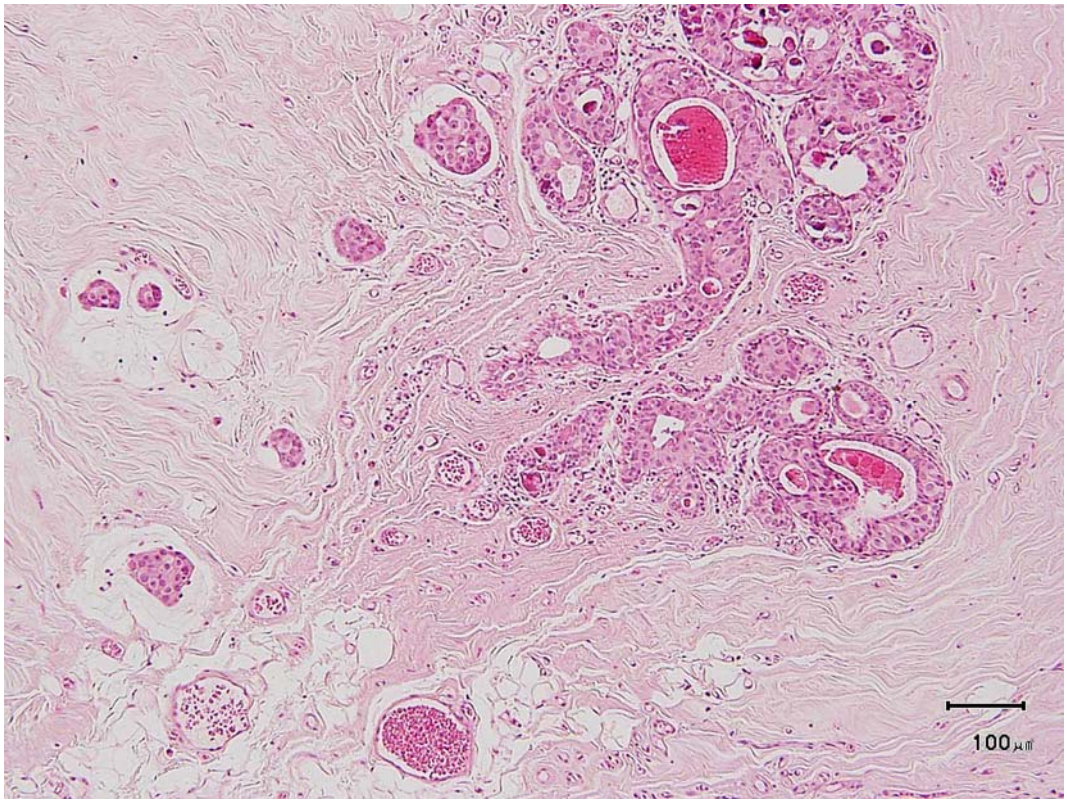

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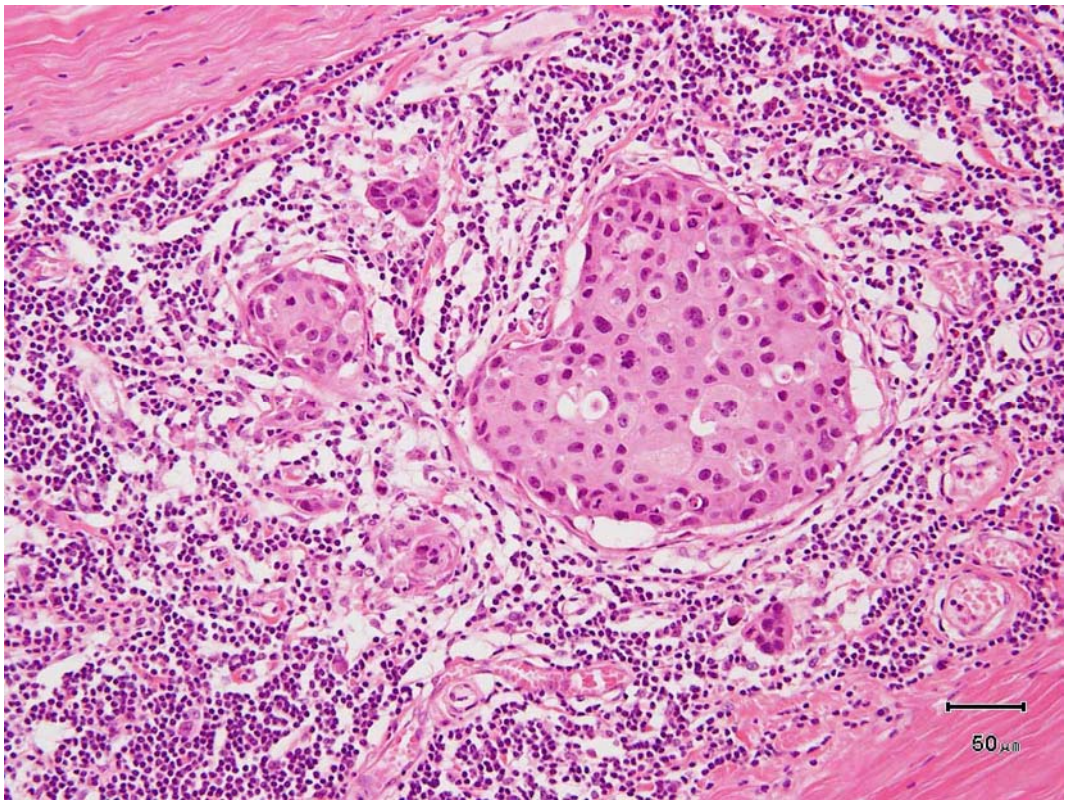

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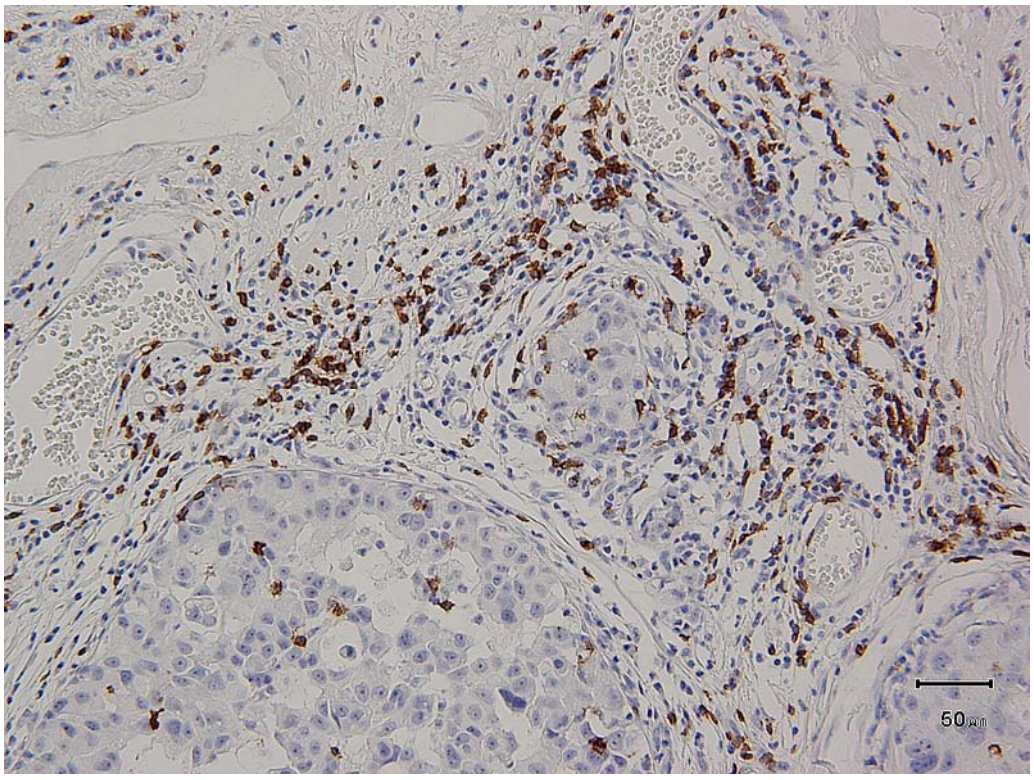

Figure 4

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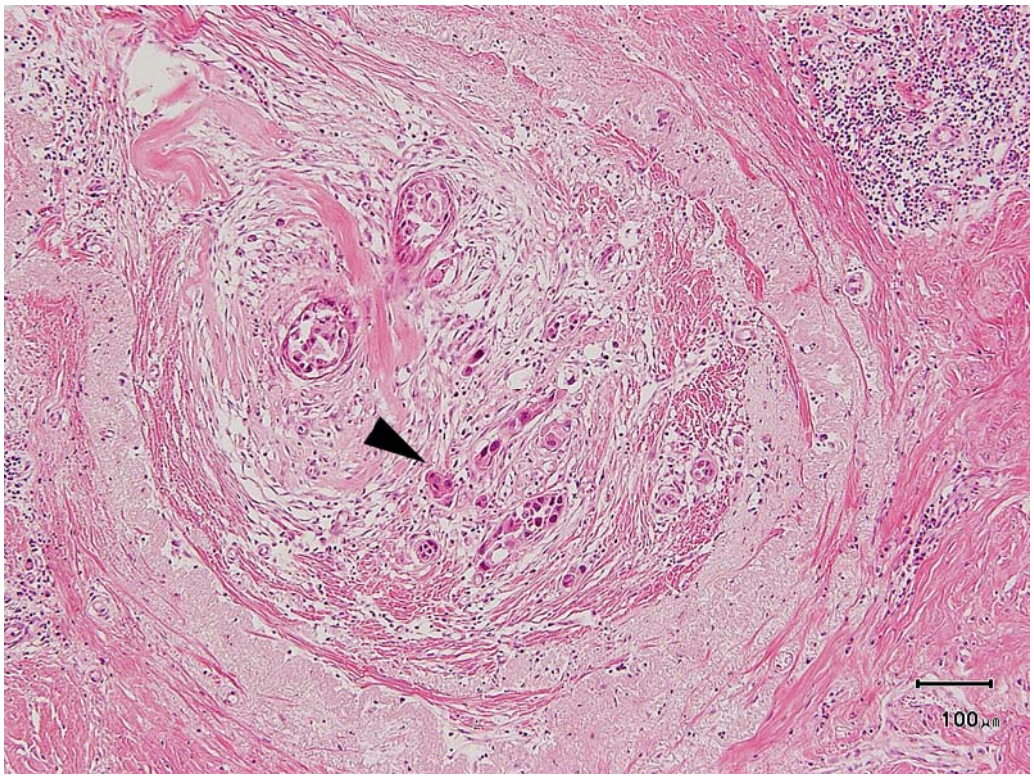

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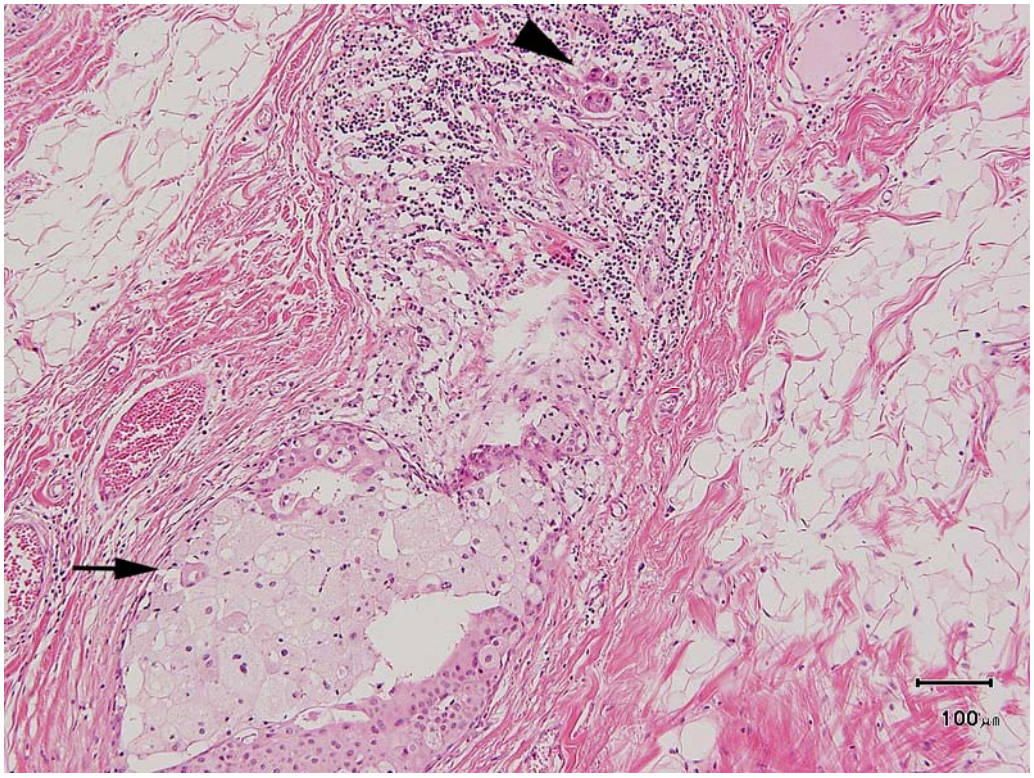
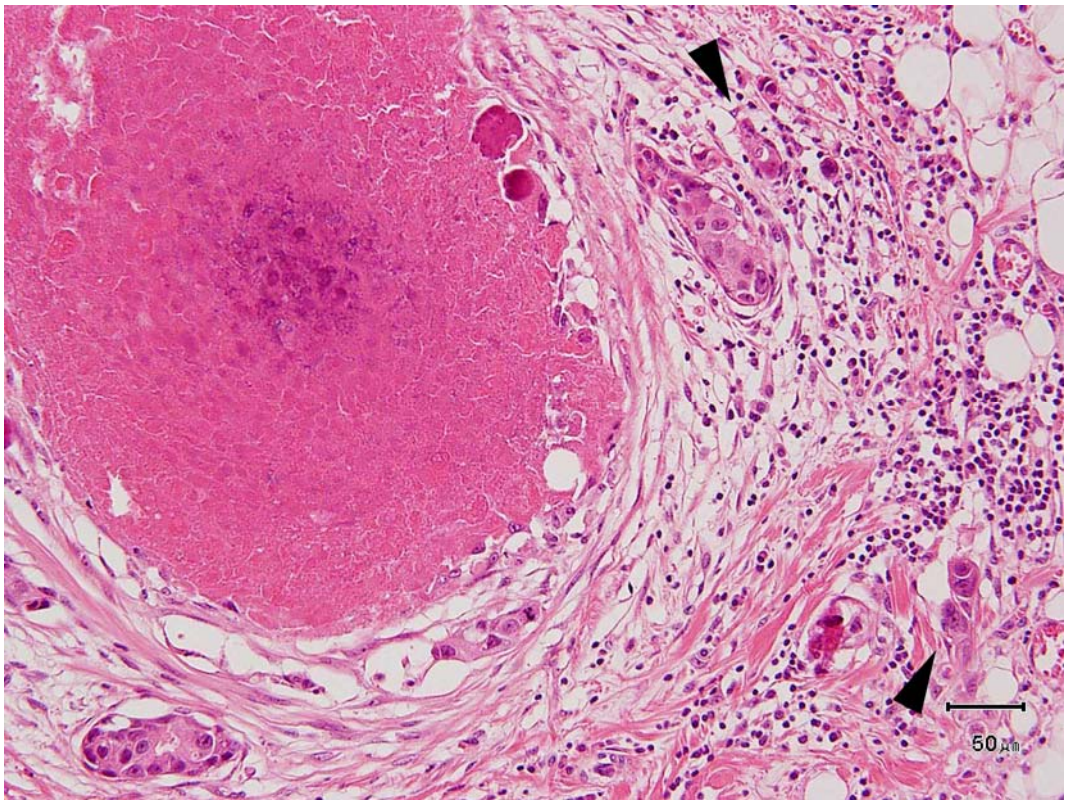


Figure 5

a)



b)