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Original Article

Changes in intrinsic subtype of breast cancer during tumor progression in the same patient

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Abstract: Hormone receptor (HR), human epidermal growth factor receptor 2 (HER2) and Ki67 are important prognostic factors and key variables in classification of the intrinsic subtype, which is essential for choice of adjuvant therapy in breast cancer management. There has been earlier reports that instability of hormonal and HER2 status during progression of tumor. However, breast cancer treatment guidelines recently recommended using the intrinsic subtype that is determined by four immunohistochemical (IHC) assays, estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki67. The purpose of study was to investigate whether the intrinsic subtype changes during the tumor progression from ductal carcinoma in situ (DCIS) to lymph node metastasis. The study included 90 patients with breast cancer in Korea University Guro Hospital, between 1992 and 2008. All individuals had DCIS, invasive carcinoma and lymph node metastasis lesion. IHC staining for ER, PR, HER2 and Ki67 as well as SISH assay for HER2 gene amplification was done with following standard method. Overall 25% of breast cancer changed their intrinsic phenotype during progression. Study demonstrated that a subset of breast cancers can change their intrinsic subtype during cancer progression. These changes have an impact on patient prognosis and management, because each breast cancer subtype has their own differently optimized treatment options according to St. Gallen and NCCN guideline.

Keywords: Breast cancer, intrinsic subtype, tumor progression

Introduction

As many previous studies have shown that ER status, PR status, HER2 overexpression or gene amplification and Ki67 proliferation index are definite biomarkers to predict long-term prognosis and their target treatment benefit in breast cancer [1-3]. Assay for these markers are well-developed as an IHC assay with standardized experimental and analysis protocol [4, 5]. Several studies revealed evidences of instability of the hormonal and/or HER2 status during tumor progression, especially between primary tumor and metastatic tumors [6-9]. Unfortunately, current metastatic breast cancer management depends on primary tumor phenotype itself. There are many chances to have inappropriate treatment for metastatic cancer because of discordance between primary tumor and metastatic tumor phenotype.

Since Perou etc. proposed the intrinsic subtype classified by gene expression profiles, there has been a paradigm shift in classification of breast cancer [10]. From their proposed molecular traits of breast cancer and the other following studies identified five main intrinsic subtypes: luminal A, luminal B, basal-like, HER2-related, and normal-like, in which each type has different prognosis and chemotherapy treatment response [11-13]. However, there was no consensus in evaluation for clinically useful biomarkers. 2011 St. Gallen guideline recommend adjuvant and neo-adjuvant chemotherapy treatment option according to pathologic determination of ER, PR, HER2, and Ki67 as in defining intrinsic subtypes. The luminal A subtype is defined as ER+ and/or PR+, HER2-, Ki67 low (<14%); luminal B subtype is ER+ and/or PR+, HER2-, Ki67 high or ER+ and/or PR+, HER2+, Ki67 any; HER2 overexpression subtype is ER-, PR-, HER2+; and Basal-like subtype is ER-, PR-, and HER2-[14].

There is no earlier data showing in changes in the intrinsic subtypes during progressing of

Table 1. Distribution of 90 cases according to clinicopatthologic parameters

Clinicopathologic parameters	No. (%) of cases
Histological grade	
1	20 (22.2)
2	46 (51.1)
3	24 (26.7)
Nuclear grade	
1	3 (3.3)
2	64 (71.1)
3	23 (25.6)
Tumor size (mm)	
≤ 20	36 (40.0)
20~50	52 (57.8)
> 50	2 (2.2)
Nodal status	
N1	54 (60.0)
N2	27 (30.0)
N3	9 (10.0)
Age (yr)	
≤ 40	19 (21.1)
40~50	31 (34.5)
50~60	21 (23.3)
> 60	19 (21.1)

Table 2. Expression of Hormonal receptors, HER2 and Ki67 throughout tumor progression

Hormonal, HER2, Ki67 status	No. (%) of cases
Hormonal receptor positive	
DCIS	63 (70.0)
IDC	57 (63.3)
Metastasis	57 (63.3)
HER2 positive	
DCIS	37 (41.1)
IDC	37 (41.1)
Metastasis	35 (38.9)
Ki 67 status high	
DCIS	31 (34.4)
IDC	42 (46.7)
Metastasis	47 (52.2)

HER2 = human epidermal growth factor receptor 2; DCIS = ductal carcinoma in situ; IDC = invasive ductalcarcinoma.

DCIS to invasive carcinoma and then to nodal metastasis in a same patient at the same point. The purpose of study was to prove how much portion of tumors has shown the phenotypic alterations in molecular subtypes, also what kind of phenotype has more tendencies to change their phenotype in breast cancer during cancer progression.

Material and methods

Cases and clinicopathologic information

Patients who were diagnosed with breast cancer at Korea University Guro Hospital, Seoul, between 1992 and 2008 were enrolled into this study. The patients received surgical treatment and standard chemotherapy. Clinicopathologic data included tumor size, lymph node status, pathological type and histological grade. Histological grade was evaluated according to the Nottingham combined histological grading system with the method described by Elston and Ellis [15]. This study was ethically approved by the Institutional Review Board (IRB) of the Guro Hospital, and the all cases enrolled after 2005 had informed consent from the patients for using their medical information and tumor tissues. Total 94 cases were constructed into tissue microarrays (TMA) for IHC assay. Each case included in situ, invasive carcinoma and lymph node metastasis.

Immunohistochemical study

94 cases in TMA were constructed in 2-mm core sizes and 4um sections of the TMA mounted on electrostatic slides for IHC assay. They were heat-dried at 56°C for 30 minutes, deparaffinized in xylene, then rehydrated with graded ethanol. All IHC assay procedures including antigen retrieval and blocking of endogenous peroxidase activity were performed automatically by the BenchMark XT (Ventana) system, and used primary antibodies, Ventana monoclonal rabbit anti-ERα, clone SP1, ready-to-use (CONFIRM); Ventana monoclonal rabbit anti-PR, clone 1E2, ready-to-use (CONFIRM); Ventana monoclonal rabbit anti-HER-2/neu, clone 4B5, ready-to-use (CONFIRM); Ventana monoclonal rabbit anti-Ki67, clone 30-9, ready-to-use (CONFIRM). The tissue sections were incubated with primary antibody for 32 min at 42°, and then colorized by UltraView DAB kit.

Silver-enhanced in situ hybridization (SISH)

SISH was performed on an automated instrument, Ventana Benchmark (Ven-tana Me-

Table 3. Clinicopathologic characters of each intrinsic subtypes

Clinico-pathologic characters	Intrinsic subtype(invasive carcinoma)				
	Luminal A	Luminal B	HER2	Basal	Total
Age (yr)					
≤ 40	6	5	7	1	19
40-50	13	9	5	4	31
50-60	5	7	7	2	21
> 60	7	5	4	3	19
Tumor size (mm)					
≤ 20	17	9	8	2	36
20-50	14	15	15	8	52
< 50	0	2	0	0	2
Nodal status					
N1	20	13	14	7	54
N2	11	8	6	2	27
N3	0	5	3	1	9
Histologic grade					
1	16	4	0	0	20
2	15	15	11	5	46
3	0	7	12	5	24
Nuclear grade					
1	2	1	0	0	3
2	29	18	12	5	64
3	0	7	11	5	23

Table 4. Intrinsic subtypes and tumor progression

Molecular subtypes	No. (%) of cases
DCIS	
Luminal A	39 (43.3)
Luminal B	24 (26.7)
HER2	19 (21.1)
Basal like	8 (8.9)
IDC	
Luminal A	31 (34.4)
Luminal B	26 (28.9)
HER2	23 (25.6)
Basal like	10 (11.1)
METASTASIS	
Luminal A	28 (31.1)
Luminal B	29 (32.2)
HER2	21 (23.3)
Basal like	12 (13.3)

HER2 = human epidermal growth factor receptor 2; DCIS = ductal carcinoma in situ; IDC = invasive ductal carcinoma.

dical System,Tucson, AZ), as per the manufacturer's protocols for the INFORM HER2DNA probe and chromosome 17 probes. The probes were labelled with dinitrophenol (DNP) and visualized using rabbit anti-DNP primary antibody and Ultraview SISH Detection Kit. In brief, the HER2 DNA probe was denatured at 95°C for 4 minute, and hybridization performed at 52°C for 2 hour. Also, the chromosome 17 probe was denatured at 95°C for 4 minute and hybridization performed 44°C for 2 hours. The final reaction production was metallic silver, which was driven by the sequential addition of silver acetate, hydroquinone and hydrogen peroxide to the peroxidase to the peroxidase-conjugated goat anti-rabbit antibody of the detection kit. The metallic silver was deposited in the HER2 gene, and red centromeric signals in chromosome 17 were seen as a red dot.

Assessment of IHC staining

Cancer cells with nuclear staining of ER and PR were considered to be immunoreactive and scored. Evaluation HR expression was based on the Allred scoring method [6]. For HER2, membranous staining was evaluated according to the guidelines of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) [5]. The cases with a score of 3 were considered to be HER2-positive, and the ones with a score of 2 were evaluated for HER2 gene amplification status according to the ASCO/CAP guidelines [4]. Ki67 analysis was done by Aperio image analysis software for quantitative analysis. According to the results of the IHC analyses, tumors were classified into the following four subtypes. The luminal A subtype is defined as ER+ and/or PR+, HER2-, Ki67

Table 5. Change intrinsic subtype throughout tumor progression

Subtype in DCIS	Subtype in IDC				Subtype in Metastasis			
	Luminal A	Luminal B	HER2	Basal like	Luminal A	Luminal B	HER2	Basal like
Luminal A	30	7	0	2	25	5	0	1
Luminal B	1	19	4	0	3	22	1	0
HER2	0	0	18	1	0	2	20	1
Basal like	0	0	1	7	0	0	0	10

HER2 = human epidermal growth factor receptor 2; DCIS = ductal carcinoma in situ; IDC = invasive ductal carcinoma.

Table 6. Summary of change intrinsic subtype throughout tumor progression

Tumor progression	No. (%) of cases
DCIS to IDC	
Luminal A to Luminal B	7 (46.7)
Luminal A to Basal like	2 (13.3)
Luminal B to HER2	4 (26.7)
HER2 to Basal like	1 (6.7)
Basal like to HER2	1 (6.7)
IDC to Metastasis	
Luminal A to Basal like	1 (12.5)
Luminal B to Luminal A	3 (37.5)
Luminal B to HER2	1 (12.5)
HER2 to Luminal B	2 (25.0)
HER2 to Basal like	1 (12.5)

HER2 = human epidermal growth factor receptor 2; DCIS = ductal carcinoma in situ; IDC = invasive ductalcarcinoma.

low (<14%); luminal B subtype is ER+ and/or PR+, HER2-, Ki67 high or ER+ and/or PR+, HER2+, Ki67 any; HER2 overexpression subtype is ER-, PR-, HER2+; and Basal-like subtype is ER-, PR-, and HER2-.

Results

Tumor cohort characteristics

The total 94 cases are evaluated into four IHC assay by using TMA slides. Each case has three different regions for in situ, invasive and metastatic. Only 90 cases have all three different regions from the data. The clinicopathologic parameters of 90 cases are listed in **Table 1**. Overall, 23 cases had a discordance result between either DCIS and invasive or invasive and metastasis. We repeated IHC assay for these cases using whole section to exclude experimental bias in using a TMA core slide. This cohort showed an unusual distribution of hormone receptor and HER2 positivity as in a bit low hormone receptor positive rate and high HER2 positive rate.

Clinicopathologic characteristics depending on intrinsic subtypes of tumor

The expression levels of four markers were examined, and the results according to stage are demonstrated in **Table 2**. Significantly, hormone receptor expressions were decreased, whereas Ki67 labeling index were increased throughout progression. We could indicate distinct distribution of clinico-pathologic characteristics in the **Table 3**. Luminal A is a well-known good prognosis type, so their nodal stage, tumor size, histologic grade and nuclear grade represent a low risk category. Luminal B type shows an intermediate risk category. However, Basal and HER2 type has a high risk category characteristic, in which are high in histologic and nuclear grade. A nodal stage and tumor size were not differently distributed according to each intrinsic type.

Tumor progression and changes intrinsic subtypes

According to tumor progression, intrinsic subtypes differently take a portion. Luminal A type gradually decreased from DCIS to metastasis, whereas luminal B type increased. HER2 positive rate is usually higher in DCIS than invasive, but our result showed HER2 intrinsic type increased during progressing from DCIS to invasive. We could see a reason that HER2 positive rate itself is same between DCIS and IDC, but the HER2 type is different, because HER2 positive and ER positive tumor were defined as luminal B type. HER2 type only has HER2 positive and ER negative tumor. Interestingly, there is a different trend in progression between DCIS to invasive and invasive to metastasis. During progression from DCIS to invasive, increased aggressive type like luminal B and

HER2 type were found, whereas progression from invasive to nodal metastasis, decreased aggressive type. Finding indirectly supports the occurrence of epithelial to mesenchymal transition for metastasis and mesenchymal to epithelial transition after metastasis [16, 17]. Additionally, basal type is the most conserved phenotype during tumor progression. The **Tables 4-6** summarized results of analysis. Overall of the intrinsic subtype changes during progression, in which luminal A subtype, luminal B, HER2 type and basal type indicated 21.4%, 18%, 9.5% and 5% respectively.

Discussion

Breast cancer is a well-known heterogeneous tumor that included inter-tumoral and intra-tumoral heterogeneity [18-21], affecting clinical prognosis and treatment response [22]. After Perou etc. proposed intrinsic subtypes using molecular gene expression many following studies had supported heterogeneous tumor phenotypes of breast cancer. Also, intrinsic subtypes are biologically conserved distinct phenotype, because selected genes for intrinsic classification were not affected by tumor progression and chemotherapy effect [10]. As well as each subtype has unique clinical behavior. For instance, each subtype has different prognosis and unambiguous treatment response for the hormone therapy and chemotherapy [23-25].

Because a molecular assay for intrinsic subtyping has been expensive and not practical in usual pathology lab, the St. Gallen breast cancer treatment guideline recently adopted intrinsic molecular subtype for a proper choice of adjuvant, neo-adjuvant and hormone treatment [14]. We used the clinicopathologic definition for intrinsic subtypes, which is recommended in the St. Gallen conference in 2011 [14]. The St. Gallen recommendation is used in an IHC assay's result of four markers for intrinsic subtype classification; therefore, it is possible that any qualified pathology lab can do this subtyping in routine pathology works without additional complicated and expensive molecular assay.

The result of study shows changes in intrinsic subtype from primary tumor in situ lesion to invasive carcinoma and nodal metastasis. 25% of patients with breast cancer have a chance to change their molecular phenotypes of tumor during the progression. Most important change in status of marker was hormone receptor and Ki67 status, but not HER2 status. Especially, high status of Ki67 gradually increased during each step of progression. Also, the luminal A type decreased, whereas luminal B and Basal like type increased their portions throughout tumor progression, indicating that Luminal A type mainly changed to luminal B and basal like type. Thus, intrinsic subtypes eventually change to worse prognostic subtype throughout progress. Clinically, these changes in intrinsic subtypes are important, because loss of ER and HER2 phenotype associate with increasing possibility of resistance to hormone and HER2 targeted therapy. Furthermore, patients would have a chance to improve response to the treatment and survival with additional treatment choice by acquired ER and HER2 status.

We can suggest several mechanisms for our findings, 1) de novo heterogeneity and specific selection for invasion or metastasis [26, 27]; 2) formation of different sub-clones which can invade and metastasize [28]; 3) autocrine or paracrine biologic factors may also be involved in the specific clonal expansion during tumor progression [29]. Former studies also demonstrated that each intrinsic subtype has preferred chemotherapy regimen. For example, HER2 type is expected to a sensitive response to anthracycline-based chemotherapy regimens [30], and basal like type is a response to platinum drugs [31]. Up to date, a treatment strategy of breast cancer has been determined according to only result of the IHC marker assay of the primary invasive lesion on the assumption that the molecular characteristics of tumor cells are same throughout tumor progression. Even though, some studies have argued that intrinsic subtype in metastatic tumor is important for better treatment options [32, 33], we strongly suggest that the consideration of intrinsic subtype in metastasis lesions is critical to improve patient's survival.

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Disclosure of conflict of interest

None.

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