ANTICANCER RESEARCH *36*: 4661-4666 (2016)
doi:10.21873/anticanres.11017

Tissue Microarray Is a Reliable Tool for the Evaluation of *HER2* Amplification in Breast Cancer

DANIELA FURRER[1,2,3], SIMON JACOB[4,5,6], CHANTAL CARON[4,5], FRANÇOIS SANSCHAGRIN[2,4], LOUISE PROVENCHER[2,4,7] and CAROLINE DIORIO[1,2,3,4]

[1]Cancer Research Centre at Laval University, [2]Oncology Axis, CHU of Quebec Research Center, and Departments of [3]Social and Preventive Medicine, [6]Molecular Biology, Medical Biochemistry and Pathology, and [7]Surgery, Faculty of Medicine, Laval University, Quebec City, QC, Canada; [4]Deschênes-Fabia Center for Breast Diseases and [5]Pathology Service, Saint-Sacrement Hospital, Quebec City, QC, Canada

Abstract. *Aim: We examined an economical method for evaluating the amplification of the human epidermal growth factor receptor 2 (HER2) gene in breast cancer specimens. Materials and Methods: We compared HER2 amplification determined by fluorescence in situ hybridization (FISH) on whole-tissue (WT) blocks used for diagnostic and on tissue microarray (TMA) sections for a cohort of 521 consecutive patients with breast cancer. In a subset of 116 patients, we examined HER2 concordance from the WT section and a TMA section from a randomly chosen additional block (a proxy of the core biopsy). Results: Overall concordance for HER2 amplification between WT and TMA sections was 98.2%, and between sections from WT and from the additional block was 99.0%. Conclusion: The high concordance rates support the use of TMA for the evaluation of HER2 amplification in breast cancer and suggest that FISH can be used to assess HER2 using core biopsies.*

The *human epidermal growth factor receptor 2* (HER2) gene is located on chromosome 17 and encodes a transmembrane tyrosine kinase receptor protein (1). *HER2* gene amplification and receptor overexpression, which occur in 15 to 20% of patients with breast cancer, are important markers for poor prognosis, including a more aggressive disease and a shorter survival (2). Moreover, HER2-positive status is considered a predictive marker of response to anti-HER2 therapies (3). Given its prognostic, predictive and therefore therapeutic implications, accurate diagnostic assessment of HER2 is essential for reliable identification of patients eligible for HER2-targeted drugs.

Currently, there are several Food and Drug (FDA)-approved methods for evaluating HER2 status, including immunohistochemical (IHC) evaluation of HER2 protein expression and assessment of *HER2* gene amplification by *in situ* hybridization (ISH), most commonly fluorescent ISH (FISH) (4, 5). Since both IHC and FISH present advantages and disadvantages, there is still no consensus on which method is superior for assessing HER2 status in breast cancer specimens (6). In 2013, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) updated the guidelines to clarify the recommendations for HER2 testing in breast cancer specimens published in 2007 (4). In addition to report new scoring criteria, the updated guidelines recommend performing an initial test of a core biopsy. If test results are equivocal, reflex testing on tumor specimen section with an alternative assay should be carried out. In addition, repeat testing should be performed if there is an apparent histopathological discordance with the test result. The 2007 ASCO/CAP guidelines recommended to perform HER2 testing on resection specimens and to retest when results were equivocal (5). Although FISH is a reliable method for evaluation of HER2 status in breast cancer for diagnostic purposes or in the framework of quality control, it is a very expensive technique (7).

Tissue microarray (TMA) allows for simultaneous molecular analysis of large numbers of samples by means of arranging tissue cores from multiple specimens into an empty paraffin block (8, 9). A low degree of representativity

Correspondence to: Associate Professor Caroline Diorio, Ph.D., Département de médecine sociale et préventive , Université Laval, Axe oncologie, Centre de recherche du CHU de Québec, Centre des maladies du sein Deschênes-Fabia, Hôpital du Saint-Sacrement, 1050, chemin Ste-Foy, local J0-16, Québec (Qc) G1S 4L8, Canada. Tel: +1 4186827511, ext. 84726, Fax: +1 4186827949, e-mail: caroline.diorio@crchudequebec.ulaval.ca

Key Words: Breast cancer, trastuzumab, TMA, ASCO/CAP guidelines, HER2 status, FISH.

compared to whole-tissue sections, however, represents the major limitation of this technology. The purpose of this project was to determine the most reliable and economical method for evaluating HER2 status in breast cancer specimens. In a cohort of 521 consecutive patients with breast cancer, we aimed to evaluate if FISH analysis performed on TMA section provides comparable results to those obtained using whole-tissue sections used for diagnostic purposes (one section per slide). Furthermore, we wanted to assess whether FISH analysis carried out on a randomly chosen additional block (a proxy of a random core biopsy) yielded similar results. In a subset of 116 breast cancer specimens, we aimed to analyze the concordance between *HER2* gene amplification status obtained from the same whole-tissue section used for diagnostics and that obtained from the randomly chosen additional block using TMA.

Materials and Methods

Specimen collection and patient population. Specimens were obtained from mastectomies and segmental resections that were performed at the Centre des Maladies du Sein Deschênes-Fabia at the Saint-Sacrement Hospital in Québec, Québec, Canada, between February 2011 and April 2012. Samples were fixed with 10% neutral buffered formalin, embedded in paraffin, cut into 4-μm tissue sections, stained with hematoxylin and eosin (H&E), and used for routine pathological evaluation. The study population consisted of 554 consecutive cases of invasive breast carcinoma, with a tumor size on histological slides of at least 1 cm, which did not receive chemotherapy prior to surgery. All eligible women provided their written informed consent for use of their tissue. Ethical approval of the study was obtained in 2010 from the Research Ethics Committee of the Centre de Recherche du CHU de Québec (#DR-002-1286).

Tissue microarray construction and processing. TMAs were constructed as previously described (10). For the 554 consecutive breast cancer specimens, the most representative tissue block from each case was chosen by the pathologist for HER2 evaluation by IHC and FISH. Subsequently, two tumoral regions showing the strongest IHC staining were delineated on the IHC slide by the pathologist. Within each delimited tumor zone, pathologists indicated where two 0.6 mm tissue cylinders were to be punched. The four tissue cylinders were punched using a manual arraying instrument (Beecher Instruments, Silver Spring, MD, USA) and were inserted into empty recipient paraffin blocks. We call these TMA blocks 'diagnostic TMA'. Among the 554 cases present on the diagnostic TMA, we identified 279 cases that had at least two additional paraffin blocks presenting the same histological type as the paraffin block that was used for the construction of the diagnostic TMA and having enough tumor tissue to extract four tissue cylinders. Among these 279 cases, 100 cases were randomly chosen. For each of these randomly selected cases, one paraffin block was randomly chosen among all eligible paraffin blocks. Suitable blocks were identified using H&E section and two tumoral regions were identified without previous IHC staining on the H&E section by two trained technologists and verified by a pathologist. Four 0.6 mm tissue cylinders were then punched from these regions. Moreover, we performed an oversampling of HER2-positive cases: 16 cases that were scored as HER2-positive on diagnostic TMA section but that had not been selected at random were added to the 100 cases. Therefore, a total of 116 cases were inserted into empty recipient paraffin blocks. We call these TMA blocks 'random TMA' in order to differentiate it from the diagnostic TMA. On each array block, breast cancer cell lines (MCF-7, MDA-231 and SKBR-3) were included in duplicate and served as negative and positive controls. Four-micrometer TMA sections were processed by FISH following the same protocol as for the whole-tissue section used for diagnostics. One section from each TMA block was stained with H&E for reference histology.

Fluorescence in situ hybridization. HER2 gene copy number was evaluated using the FDA-approved PathVysion™ HER2 DNA Probe kit (Abbott Molecular, Des Plaines, IL, USA/Inter Medico, Markham, Canada). Fluorescent signals were analyzed with an epifluorescence microscope Axio Imager M1 (Zeiss, Göttingen, Germany), equipped with a triple-band filter [4',6-diamidino-2-phenylindole (DAPI)/green/orange]. Automated analysis of fluorescence signals was performed using the FDA-approved MetaSystems™ image analysis system, equipped with Metafer software with extended focus/tile sampling (MetaSystems, Altussheim, Germany) (11). After selection of 5 to 10 non-overlapping fields of infiltrating carcinoma, field images were automatically captured and analyzed by the software. *HER2* gene amplification was reported according to the 2007 and 2013 ASCO/CAP guidelines for the evaluation of HER2 status in breast cancer specimens (Table I) (4, 5). For equivocal cases, manual counting was performed from at least 60 nonoverlapping tumor cells from two distinct tumor areas. Equivocal cases were counted by two independent technologists and reviewed by the pathologist of the study. In addition, manual counting was performed in 40 nonoverlapping tumor cells when the average *HER2* copy number per tile was ≥4.0 and ≤6.0 at the automated image analysis. Normal breast epithelial cells, lymphocytes and fibroblasts were used as internal control. Slides were analyzed by trained technologists, and all results were validated by breast pathologists with experience in FISH interpretation.

HER2 evaluation on TMA. A core was considered unsatisfactory for analysis if it was absent, it contained no tumor tissue, or if tumor tissue occupied less than 10% of the total core area. Fluorescent signals on TMA slides were evaluated in the same way as for the whole-tissue section. Fluorescent signals were first analyzed using an FDA-approved automated image analysis system (MetaSystems) (11). Slides were scanned at low magnification (×5) to generate a position list corresponding to each core in order to link the core location to subsequent high-resolution (×40) FISH images. Automated image analysis was followed by manual counting or visual verification using the same criteria as for whole-tissue sections. Each informative core was scored separately in a blind fashion. Average FISH results of informative cores were considered. All results were validated by breast pathologists. *HER2* FISH results were reported using the same scoring guidelines as for whole-tissue section analysis (4, 5).

Statistical analysis. Only cases with at least one informative core were included in the analysis. FISH results on both whole-tissue and

Table I. *Interpretation criteria for fluorescence in situ hybridization according to the 2007 (5) and the 2013 (4) American Society of Clinical Oncology/College of American Pathologists scoring systems.*

Scoring system	Result category	Interpretation criteria
2007	Non-amplified	*HER2/CEP17* ratio <1.8
	Equivocal	*HER2/CEP17* ratio 1.8-2.2
	Amplified	*HER2/CEP17* ratio >2.2
2013	Non-amplified	Average *HER2* gene copy number <4 signals/nucleus or *HER2/CEP17* ratio of <2.0 with an average *HER2* gene copy number <4 signals/nucleus
	Equivocal	Average *HER2* gene copy number ≥4 and < 6 signals/nucleus or a ratio <2.0 with an average *HER2* gene copy number ≥4 and <6 signals/nucleus
	Amplified	Average *HER2* gene copy number ≥6 signals/nucleus or a ratio <2.0 with an average *HER2* gene copy number ≥6 signals/nucleus or a ratio ≥2.0

HER2/CEP17: Human epidermal growth factor receptor 2/chromosome 17 centromere.

Table II. *Concordance of human epidermal growth factor receptor 2 (HER2) gene amplification status between fluorescence in situ hybridization performed on whole tissue sections (reference method) and on diagnostic tissue microarray (TMA) sections according to the 2013 American Society of Clinical Oncology/College of American Pathologists scoring system (4).*

Diagnostic TMA section	Whole-tissue section (reference), n			
	Non-amplified	Equivocal	Amplified	Total
Non-amplified, n	424	19	3	446
Equivocal, n	5	5	0	10
Amplified, n	1	0	64	65
Total	430	24	67	521
Overall agreement				98.2%
Positive agreement (sensitivity)				95.5%
Negative agreement (specificity)				98.6%
k Value				0.81*

*When equivocal cases were excluded: *k* value=0.96.

Table III. *Concordance of human epidermal growth factor receptor 2 (HER2) gene amplification status between fluorescence in situ hybridization performed on whole tissue sections (reference method) and on diagnostic tissue microarray (TMA) sections according to the 2007 American Society of Clinical Oncology/College of American Pathologists scoring system (5).*

Diagnostic TMA section	Whole-tissue section (reference)			
	Non-amplified	Equivocal	Amplified	Total
Non-amplified	451	3	0	454
Equivocal	0	2	1	3
Amplified	0	4	60	64
Total	451	9	61	521
Overall agreement				99.8%
Positive agreement (sensitivity)				98.4%
Negative agreement (specificity)				100.0%
k Value				0.93*

*When equivocal cases were excluded: *k* value=1.00.

TMA sections were available for 521 cases out of the 554 total cases (94.0%). FISH results on both whole-tissue and random TMA sections were available for 103 cases out of the 116 selected cases (88.8%). In order to evaluate the agreement between FISH results obtained by the different methods (whole-tissue section *vs*. diagnostic TMA section and whole-tissue section *vs*. random TMA section), positive, negative and overall concordance were calculated. Positive concordance (sensitivity) was calculated as the number of samples positive for both methods divided by the number of samples positive by the reference FISH assay. Negative agreement (specificity) was calculated similarly. Overall concordance was defined as the combination of sensitivity and specificity, as recommended by the ASCO/CAP guidelines (4, 5). In addition, the level of concordance was measured using Cohen's kappa test. All analyses were performed using SAS software (version 9.1.3; SAS Institute Inc., Cary, NC, USA).

Results

Concordance of the FISH *HER2* results between whole-tissue and diagnostic TMA sections are summarized in Table II. Using the 2013 ASCO/CAP scoring system, the overall agreement was 98.2% (kappa value=0.81). The positive agreement and the negative agreements were 95.5%, and 98.6%, respectively. The one case that was evaluated as non-amplified on the whole-tissue section but amplified on diagnostic TMA section showed borderline amplification (mean *HER2/CEP17* ratio 2.27 on diagnostic TMA section and *HER2/CEP17* ratio of 1.80 on whole-tissue section). Similarly, the three cases that were scored as amplified on whole-tissue sections but non-amplified on diagnostic TMA

Table IV. *Concordance of human epidermal growth factor receptor 2 (HER2) gene amplification status between fluorescence in situ hybridization performed on whole tissue sections (reference method) and on diagnostic tissue microarray (TMA) sections according to the number of informative cores using the 2007 American Society of Clinical Oncology/College of American Pathologists scoring system (n=521) (5).*

Number of assessable cores	Number of cases	Overall agreement		Positive agreement		Negative agreement	
		n/N	%	n/N	%	n/N	%
1 or 2	162	156/157	99.4	21/22	95.4	135/135	100.0
3 or 4	359	355/355	100.0	39/39	100.0	316/316	100.0

n/N: Number of cases on TMA section/number of cases on whole-tissue section.

sections showed borderline amplification (*HER2/CEP17* ratios ranging from 2.11 to 2.31 on whole-tissue sections, mean *HER2/CEP17* ratios ranging from 1.37 to 1.85 on diagnostic TMA sections).

We also assessed the concordance rates between *HER2* gene amplification status determined by FISH on whole-tissue sections and on TMA sections according to the number of informative cores per case (one or two *vs*. three or four cores). The overall, positive, and negative agreements for cases with one or two informative cores were 98.6%, 95.8% and 99.2%, respectively. For cases with three or four evaluable cores, the overall, positive and negative agreements were 97.9%, 95.3% and 98.4%, respectively.

Similar concordance rates between the two methods were observed when the 2007 ASCO/CAP guidelines were applied (Tables III and IV).

Concordance of *HER2* status determined by FISH on whole-tissue sections and random TMA sections is summarized in Table V. The overall agreement was 99.0% (kappa value=0.94). The positive agreement and the negative agreements were 97.3%, and 100.0%, respectively. The case that was considered amplified on the whole-tissue section and equivocal on random TMA section had a *HER2/CEP17* ratio of 2.50 on the whole-tissue section, while on random TMA section it had a mean *HER2/CEP17* ratio of 1.67 and an average *HER2* gene copy number of 4.53.

Table V. *Concordance of human epidermal growth factor receptor 2 (HER2) status determined by fluorescence in situ hybridization on whole-tissue section (reference method) and on random TMA section according to the 2013 ASCO/CAP scoring system (4).*

Random TMA section	Whole-tissue section (reference)			
	Non-amplified	Equivocal	Amplified	Total
Non-amplified	63	2	0	65
Equivocal	0	1	1	2
Amplified	0	0	36	36
Total	63	3	37	103
Overall agreement				99.0%
Positive agreement (sensitivity)				97.3%
Negative agreement (specificity)				100.0%
k Value				0.94*

*When equivocal cases were excluded: *k* value=1.00.

Discussion

We observed a high concordance rate between the *HER2* gene amplification status assessed by FISH on whole-tissue sections and on diagnostic TMA section, independently form the ASCO/CAP guideline used for the classification of cases. Using the 2013 ASCO/CAP scoring criteria, 98.2% of cases were correctly classified, whereas overall concordance was 99.8% when the oldest scoring criteria were applied. Similar studies performed on breast cancer specimens reported concordance rates ranging from 91% to 97% between the *HER2* gene amplification status obtained by FISH on whole-tissue sections and TMA sections using 2007 ASCO/CAP scoring criteria (12-15). In our hands, no case considered amplified on whole-tissue section was evaluated as non-amplified on diagnostic TMA section, when *HER2* gene amplification status was scored according to the 2007 ASCO/CAP scoring guidelines (4.5% using the newest scoring guidelines). Gancberg and collaborators reported similar results (12), whereas another study observed 13% of non-amplified cases on TMA section among those considered amplified on whole-tissue section (15).

In analogy to others (14, 16, 17), we noticed that the overall agreement rate between the two methods was higher with three- or four-core analysis compared to one- or two-core analysis when 2007 ASCO/CAP scoring criteria were used (100.0% *vs*. 99.4%). However, when the 2013 ASCO/CAP guidelines were used, we obtained opposite results (three or four cores: 98.0%, one or two cores: 98.6%).

The overall concordance between *HER2* gene amplification status between the two methods was also high when *HER2* status determined on TMA section was considered the reference method (95.6% using the newest guidelines and 98.6% using the 2007 ASCO/CAP scoring guidelines), further confirming the reliability of this method in the determination of *HER2* gene amplification in breast cancer specimens.

The purpose of our project was also to compare *HER2* gene amplification status obtained from the same whole-tissue section used for diagnostics to those obtained from the randomly chosen additional block using TMA. The updated ASCO/CAP guidelines recommend performing an initial test (IHC or ISH) in core biopsy and to retest specimens using an alternative assay when results are equivocal. Since FISH was not routinely performed in core biopsy for the 554 consecutive breast cancer specimens, we performed FISH for 116 selected breast cancer specimens on an additional paraffin block, randomly chosen among all paraffin blocks of the same specimen that presented the same histological type as the routine diagnostic paraffin block, *i.e.* the paraffin block that was used to make the diagnosis. Since the additional block and the additional cores were randomly chosen, we consider this block a proxy of the core biopsy performed by the radiologist under ultrasound examination. We observed an excellent overall concordance (99.0%, kappa value of 0.94) between the *HER2* gene amplification status observed on whole-tissue and on random TMA sections. These results highlight that reliable *HER2* gene amplification status can be achieved even when the tumor area is randomly chosen.

Similar studies have analyzed concordance of *HER2* status (determined on whole-tissue section) between different blocks from different tumor foci (18, 19). Compared to our results, two studies have reported slightly lower concordance rates in HER2 gene amplification determined on different blocks, ranging from 90.3% to 94%. These studies, however, analyzed their results using the 2007 ASCO/CAP guidelines (19) or other criteria for *HER2* gene amplification (18). Analogous studies that analyzed the concordance between *HER2* gene amplification determined on the needle core biopsy and subsequent excisional biopsy (whole-tissue section) of the same tumor observed comparatively to our study lower agreement rates ranging between 86% and 92% (20, 21).

TMA technology has several advantages over the traditional method. Variability between batches is considerably reduced, since specimens can be simultaneously processed using identical conditions (8, 9). Furthermore, the TMA technique significantly reduces the reagents and technical time required for staining (22, 23). This is particularly attractive for expensive and time-consuming techniques such as FISH. The amount of tissue needed is also reduced (23).

Although TMA represents a useful tool for rapid and efficient examination of large numbers of tumor tissues, this technique presents some weaknesses, including the need for training of highly qualified technicians and core losses (8, 24). Moreover, it has been criticized that TMA may not accurately represent histopathological characteristics of the whole-tissue section. Nonetheless, it has been shown that punching of multiple cores from different representative tumor regions reduces sampling error (15) and provides reliable results even for heterogeneous tumors (25, 26).

In conclusion, our results suggest that FISH is a robust technique and that it can be used for the determination of *HER2* status in core biopsies. The high concordance rates between *HER2* gene amplification status determined on whole-tissue and on random TMA sections (99.0%) suggest that reliable results can be achieved even when tumor area is randomly chosen. Moreover, the observed agreement rates fulfill the ASCO/CAP recommendation of concordance greater than 95% for clearly amplified and non-amplified cases (4, 5). Furthermore, the high concordance rates between whole-tissue and diagnostic TMA sections justify the implementation of TMA for the determination of *HER2* amplification on surgical specimens in the framework of quality control.

Conflicts of Interests

None to declare.

Acknowledgements

The Authors express special thanks to Drs. Anne Choquette, Michel Beauchemin, Mohamed Amin Hashem, Sophie Laberge, Mohib Morcos, Nathalie Mourad, Alexandre Odashiro, and Ion Popa. We are grateful to the personnel of the Service de Pathologie, especially to Céline Plourde, for their precious technical support.

DF received doctoral fellowships from the Fonds de recherche du Québec - Santé (FRQS) and the Laval University Cancer Research Center. CD is a recipient of the Canadian Breast Cancer Foundation-Canadian Cancer Society Capacity Development award (award #703003) and the FRQS Research Scholar.

This study was supported by the Fondation des Hôpitaux Enfant-Jésus – St-Sacrement. Clinical specimens were provided by the Fondation du cancer du sein du Québec and the Banque de tissus et de données of the Réseau de recherche sur le cancer of the FRQS, which is affiliated with the Canadian Tumour Repository Network.

The Authors also acknowledge Hoffmann-La Roche Limited for its support.

References

1 Yarden Y and Sliwkowski MX: Untangling the ERBB signalling network. Nat Rev Mol Cell Biol *2(2)*: 127-137, 2001.

2 Soerjomataram I, Louwman MW, Ribot JG, Roukema JA and Coebergh JW: An overview of prognostic factors for long-term survivors of breast cancer. Breast Cancer Res Treat *107(3)*: 309-330, 2008.

3 Esteva FJ, Yu D, Hung MC and Hortobagyi GN: Molecular predictors of response to trastuzumab and lapatinib in breast cancer. Nat Rev Clin Oncol *7(2)*: 98-107, 2010.

4 Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF, American Society of Clinical Oncology and College of American Pathologists: Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol *31(31)*: 3997-4013, 2013.

5 Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF and American Society of Clinical Oncology/College of American P: American Society of Clinical Oncology and College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med *131(1)*: 18-43, 2007.

6 Furrer D, Sanschagrin F, Jacob S and Diorio C: Advantages and disadvantages of technologies for HER2 testing in breast cancer specimens. Am J Clin Pathol *144(5)*: 686-703, 2015.

7 Ross JS: Point: Fluorescence *in situ* hybridization is the preferred approach over immunohistochemistry for determining HER2 status. Clin Chem *57(7)*: 980-982, 2011.

8 Camp RL, Charette LA and Rimm DL: Validation of tissue microarray technology in breast carcinoma. Lab Invest *80(12)*: 1943-1949, 2000.

9 Gillett CE, Springall RJ, Barnes DM and Hanby AM: Multiple tissue core arrays in histopathology research: A validation study. J Pathol *192(4)*: 549-553, 2000.

10 Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G and Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med *4(7)*: 844-847, 1998.

11 Tubbs RR, Pettay JD, Swain E, Roche PC, Powell W, Hicks DG and Grogan T: Automation of manual components and image quantification of direct dual label fluorescence *in situ* hybridization (FISH) for *HER2* gene amplification: A feasibility study. Appl Immunohistochem Mol Morphol *14(4)*: 436-440, 2006.

12 Gancberg D, Di Leo A, Rouas G, Jarvinen T, Verhest A, Isola J, Piccart MJ and Larsimont D: Reliability of the tissue microarray based FISH for evaluation of the *HER2* oncogene in breast carcinoma. J Clin Pathol *55(4)*: 315-317, 2002.

13 Graham AD, Faratian D, Rae F and Thomas JS: Tissue microarray technology in the routine assessment of *HER2* status in invasive breast cancer: A prospective study of the use of immunohistochemistry and fluorescence *in situ* hybridization. Histopathology *52(7)*: 847-855, 2008.

14 O'Grady A, Flahavan CM, Kay EW, Barrett HL and Leader MB. HER2 analysis in tissue microarrays of archival human breast cancer: Comparison of immunohistochemistry and fluorescence *in situ* hybridization. Appl Immunohistochem Mol Morphol *11(2)*: 177-182, 2003.

15 Zhang D, Salto-Tellez M, Putti TC, Do E and Koay ES: Reliability of tissue microarrays in detecting protein expression and gene amplification in breast cancer. Mod Pathol *16(1)*: 79-84, 2003.

16 Fons G, Hasibuan SM, van der Velden J and ten Kate FJ: Validation of tissue microarray technology in endometrioid cancer of the endometrium. J Clin Pathol *60(5)*: 500-503, 2007.

17 Hoos A, Urist MJ, Stojadinovic A, Mastorides S, Dudas ME, Leung DH, Kuo D, Brennan MF, Lewis JJ and Cordon-Cardo C: Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. Am J Pathol *158(4)*: 1245-1251, 2001.

18 Buggi F, Folli S, Curcio A, Casadei-Giunchi D, Rocca A, Pietri E, Medri L and Serra L: Multicentric/multifocal breast cancer with a single histotype: Is the biological characterization of all individual foci justified? Ann Oncol *23(8)*: 2042-2046, 2012.

19 Choi Y, Kim EJ, Seol H, Lee HE, Jang MJ, Kim SM, Kim JH, Kim SW, Choe G and Park SY: The hormone receptor, human epidermal growth factor receptor 2, and molecular subtype status of individual tumor foci in multifocal/multicentric invasive ductal carcinoma of breast. Hum Pathol *43(1)*: 48-55, 2012.

20 Apple SK, Lowe AC, Rao PN, Shintaku IP and Moatamed NA: Comparison of fluorescent *in situ* hybridization *HER-2/neu* results on core needle biopsy and excisional biopsy in primary breast cancer. Mod Pathol *22(9)*: 1151-1159, 2009.

21 D'Alfonso T, Liu YF, Monni S, Rosen PP and Shin SJ: Accurately assessing *HER-2/neu* status in needle core biopsies of breast cancer patients in the era of neoadjuvant therapy: Emerging questions and considerations addressed. Am J Surg Pathol *34(4)*: 575-581, 2010.

22 Jourdan F, Sebbagh N, Comperat E, Mourra N, Flahault A, Olschwang S, Duval A, Hamelin R and Flejou JF: Tissue microarray technology: Validation in colorectal carcinoma and analysis of p53, hMLH1, and hMSH2 immunohistochemical expression. Virchows Arch *443(2)*: 115-121, 2003.

23 Khouja MH, Baekelandt M, Sarab A, Nesland JM and Holm R: Limitations of tissue microarrays compared with whole-tissue sections in survival analysis. Oncol Lett *1(5)*: 827-831, 2010.

24 Conway C, Dobson L, O'Grady A, Kay E, Costello S and O'Shea D: Virtual microscopy as an enabler of automated/quantitative assessment of protein expression in TMAs. Histochem Cell Biol *130(3)*: 447-463, 2008.

25 Giltnane JM and Rimm DL: Technology insight: Identification of biomarkers with tissue microarray technology. Nat Clin Pract Oncol *1(2)*: 104-111, 2004.

26 Gomaa W, Ke Y, Fujii H and Helliwell T: Tissue microarray of head and neck squamous carcinoma: Validation of the methodology for the study of cutaneous fatty acid-binding protein, vascular endothelial growth factor, involucrin and Ki-67. Virchows Arch *447(4)*: 701-709, 2005.

Received July 4, 2016
Revised July 19, 2016
Accepted July 21, 2016