Feasibility of fine-needle aspiration biopsies

for the detection of somatic mutations using

next-generation sequencing in breast cancer

차세대염기서열분석법을 이용하여

유방암에서 체세포돌연변이를 발견하기

위한 세침흡인생검술의 유용성

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February 2015

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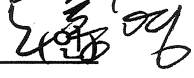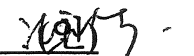
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Feasibility of fine-needle aspiration biopsies

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next-generation sequencing in breast cancer

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Abstract

Introduction: Next-generation sequencing (NGS) is being incorporated rapidly into clinical practice. Fine-needle aspiration biopsy (FNAB) specimens have been used feasibly in molecular analysis including microarray gene expression profiling and genomic processing. They are readily available and enriched in malignant cells, thus providing opportunities for genomic analysis for more clinical samples. In this study, we assessed the feasibility and sensitivity of FNAB compared to gross surgical sampling (GSS) for the detection of somatic mutations using NGS in breast cancer.

Methods: GSS tissue and FNAB was sampled via skin superficial to the palpable tumor from surgically resected breast cancer specimen. DNA was extracted from the GSS tissues and FNAB samples obtained from twelve patients. Somatic mutations detected from whole exome sequencing (WES) by NGS were analyzed for corresponding pairs of GSS tissue and FNAB. Validation of somatic mutations detected exclusively from FNAB was carried out by Sanger sequencing. Invasive tumor percentages of GSS tissues were evaluated using hematoxylin and eosin-stained sections. Tumor purities were calculated in each samples using the sequencing data.

Results: There was no difference in the total amount of DNA extracted from GSS tissue and FNAB (2.11 μg vs. 2.39 μg; p = 0.44). Samples were sequenced to a mean coverage depth of 158.8x (range 138.6x – 180.5x) for GSS tissue and 158.3x (range 135.2x – 185.1x) for FNAB. Median number of somatic mutations identified in individual samples was higher in FNAB than GSS (39.5

vs. 18.5, p=0.036). When GSS tissues had high tumor content by H&E staining, somatic mutation profiles showed high correlation between matched samples by the two sampling methods. Nineteen selected mutations identified exclusively in FNAB underwent Sanger sequencing and 13 (68.4%) were validated. The mean estimated tumor purity was higher in FNAB than GSS tissue (55.87% vs. 25.76%, $p < 0.0001$). All FNAB samples were estimated to have consistently higher proportion of malignant cells compared to GSS tissues.

Conclusion: WES was successfully carried out in all pairs of GSS tissue and FNAB from twelve breast cancer patients. In general, FNAB detected more somatic SNVs. When the GSS tissue had high tumor content by H&E staining, somatic mutation profiles showed high correlation between matched samples by the two sampling methods. FNAB samples were estimated to have consistently high proportion of malignant cells. This study suggests that FNAB is a feasible method, and furthermore, provides a reliable specimen for NGS analysis that identify somatic mutations with potential prognostic or therapeutic implication.

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List of Abbreviations

NGS   next-generation sequencing

FFPE   formalin-fixed, paraffin-embedded

CNB   core needle biopsy

FNAB   fine-needle aspiration biopsy

GSS   gross surgical sampling

PBS   phosphate buffered saline

WES   whole exome sequencing

BWA   Burrows-Wheeler Aligner

hg19   Human Genome Build 19

VAF   variant allele frequency

GATK   Genome Analysis Toolkit

Contents

Introduction

All cancers arise as a result of changes that have occurred in the DNA sequence of the genomes of cancer cells (1). Molecular profiling of the tumors in addition to traditional diagnostic methods is essential to fully understand the characteristics of an individualized tumor. Following the completion of the Human Genome Project in 2004, the focus has been shifted to cancers and characterizing them on the molecular level. Large-scale projects such as The Cancer Genome Atlas and International Cancer Genome Consortium was possible with the introduction of next-generation sequencing (NGS) (2). NGS is a high throughput technology being incorporated rapidly into clinical practice due to its cost-effectiveness and various applications in genome, exome, and transcriptome sequencing, as well as epigenetic profiling. With the evolution of personalized cancer treatment using molecularly targeted diagnostic, therapeutic and prognostic methods, NGS is expected to have a significant role in cancer treatment in general.

The availability of biospecimens is critical for use in advanced genomic analysis and sequencing technologies. Fresh tissue samples or formalin-fixed, paraffin-embedded (FFPE) blocks obtained from surgically resected cancer specimen is being used extensively for DNA or RNA extraction. However, breast cancer samples from core needle biopsy (CNB) or fine-needle aspiration biopsy (FNAB) have also been demonstrated in many studies to be useful in microarray gene expression profiling and genomic processing (3–7). Moreover, it has been demonstrated that FNAB specimens are enriched significantly with

regard to the percentage of tumor cells compared with surgical resection and CNB specimens (7,8). Biospecimens obtained from FNAB may provide a more appropriate sample for use in NGS, minimizing the disturbance caused by normal cellular components including normal breast epithelium, fibroblasts, lymphocytes, and adipocytes.

Breast cancer is initially diagnosed pathologically with specimen obtained by CNB or FNAB. Although CNB is more widely used because it can provide a more definitive diagnosis of borderline lesions and can be used to distinguish between ductal and lobular carcinoma (9), FNAB is being applied steadily in clinic as well because of its minimal invasiveness, simplicity, rapidness, and cost-effectiveness (10). The timeliness of FNAB, which can be performed before tumor is surgically resected, and the practical characteristics which allow serial sampling when necessary (i.e. during neoadjuvant chemotherapy) may be key factors when integrating genomic information into therapeutic planning.

The objective of this study was to evaluate the feasibility and sensitivity of FNAB compared to gross surgical sampling (GSS) for the detection of somatic mutations using NGS in breast cancer.

Materials and Methods

Patient specimens

This study was approved by the Institutional Review Board of Seoul National University Hospital and all patients signed an informed consent for the collection of specimens.

Twelve surgically resected specimens from patients who underwent mastectomy or lumpectomy for breast cancer at Seoul National University Hospital were evaluated in this study (Figure 1). Immediately after resection, FNAB was performed via the skin superficial to the palpable tumor using a 5 ml plastic disposable syringe with 22 gauge needle for approximately 5 seconds to yield 0.1 - 0.2ml of tissue. The contents inside the needle and syringe were blown into a tube containing phosphate buffered saline (PBS). The needle was then detached from the syringe and immersed in the same PBS in order to preserve as much cells as possible. Following FNAB, the tumor was approached through an incision made on the skin superficial to the palpable tumor. Bulk tissue of about 0.5 to 1cm^3 was sampled with blade under gross visualization (GSS) from the core of the area judged to be cancer tissue. Blocks of fresh tissue were immediately frozen and stored in a -70℃ deep freezer. Whole blood was drawn from the patients just prior to surgery.

DNA extraction

After blood cells included in the FNAB samples had been spun down and removed, genomic DNA was extracted from GSS, FNAB, and whole blood

samples using QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). DNA integrity was verified by 0.8% agarose gel electrophoresis. Quality and quantity of DNA was measured using NanoDrop Spectrophotometer and Quant-iT PicoGreen dsDNA Reagent and Kits (Thermo Fisher Scientific, Waltham, MA, USA), respectively.

Whole exome sequencing

An amplicon library was generated using SureSelect[XT] whole exome v4.0 (Agilent Technologies, Santa Clara, CA, USA). Whole blood samples were used as normal controls for each patient. Whole exome sequencing (WES) was performed for GSS tissues and FNAB samples using the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA, USA).

Data analysis

For WES, fastq files were aligned with Burrows-Wheeler Aligner (BWA) 0.7.5a with reference to Human Genome Build 19 (hg19). BAM files were processed using Genome Analysis Toolkit (GATK)-2.4-8. MuTect-1.1.4 was used for identification of somatic point mutations. Variants were annotated using Annovar v. 21-Feb-2013.

In addition, tumor purity of GSS and FNAB samples were calculated using the software package called PyLOH, which integrates somatic copy number alterations and loss of heterozygosity (11).

Validation of somatic mutations

Validation of somatic mutations with a variant allele frequency (VAF) of >20% and an alteration copy number of >10 underwent validation for both FNAB and GSS tissue samples by Sanger sequencing. Validation was performed on GSS tissue to confirm the absence of mutations in those samples. Primers were designed with NCBI Primer-BLAST using refseq ID and location of the mutation.

Invasive tumor percentage

The percentage of invasive tumor present in GSS tissues were evaluated by a pathologist using hematoxylin and eosin (H&E)-stained sections developed from the GSS tissue samples that had been processed for DNA extraction. The pathologist did not have information on the WES result.

Statistical analysis

All statistical analyses were performed using SPSS 21.0 software (SPSS Inc, Chicago, IL, USA) and a p-value of <0.05 was considered statistically significant.
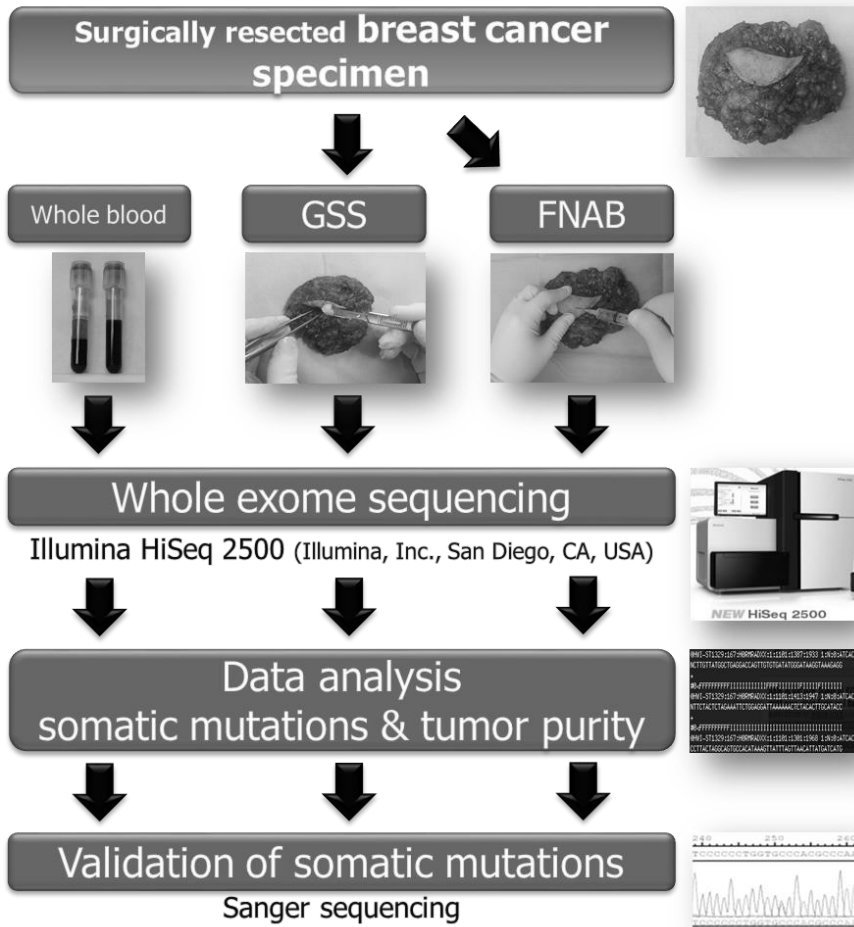
Figure 1. Summary of materials and methods

Results

Specimen characteristics

The clinical and pathological information of the patients from whom specimen was obtained are summarized in Table 1. All but one patient was diagnosed with invasive ductal carcinoma. Five patients had estrogen receptor positive disease and four patients were positive for human epidermal growth factor receptor 2. The pathologic size of the tumors had a range of 2.2 to 8.3cm. Three patients had received neoadjuvant chemotherapy. Invasive tumor percentage was judged to be ≥30% in seven GSS tissues by H&# stain and pathologic examination. Four GSS tissues had only 0% to 3% invasive tumor.

There was no difference in the total amount of DNA extracted from GSS tissue and FNAB (2.11 μg vs. 2.39 μg; $p = 0.44$). WES was successfully performed with GSS, FNAB, and whole blood samples from all twelve patients.

Performance of WES using GSS tissue and FNAB

Mean coverages of sequencing depth were equivalent between GSS tissue and FNAB (158.8x vs. 158.3x, $p = 0.898$) (Figure 2). The number of somatic mutations detected from GSS tissue and FNAB was 330 and 946, respectively, for a total of 1276. Median number of somatic mutations in individual samples was higher in FNAB than GSS tissue (39.5 vs. 18.5, $p= 0.036$). The number of correlated mutations in each paired specimen ranged from 0 to 147 (median 16). The mean number of mutations unique to GSS tissue was 3.67 compared to 55.00 in FNAB. The number of detected mutations was equal or higher in GSS

tissue than in FNAB sample in only two specimens with highest tumor percentage on H&E staining.

Allele fractions plotting of corresponding pairs of GSS tissue and FNAB showed good, intermediate, and poor correlation in five, two, and five specimens, respectively (Figure 3). Invasive tumor percentage by H&E staining was higher in the five samples with good correlation than that in five with poor correlation (range from 45% to 98% vs. 0% to 25%, $p = 0.002$) (Figure 4). In the seven samples with good or intermediate correlation, the mean overall VAF was higher in FNABs than in GSS tissues (31.79 vs. 16.97, $p < 0.0001$).

Specimens with good or intermediate correlation showed different features compared to specimens with poor correlation. Those specimens were sequenced to a deeper depth of coverage (164.7x vs. 150.0x; $p = 0.011$), had a larger median number of correlated mutations (24 vs. 16), and had more mutations unique to GSS tissue (5.14 vs. 1.60; $p = 0.011$) as well as a tendency to have less mutations unique to FNAB (15.57 vs. 110.20; $p = 0.072$).

Estimated tumor purity of GSS and FNAB samples calculated using PyLOH are summarized in Table 2. Tumor purity of GSS tissue was well correlated with tumor percentage in H&E staining (Figure 5). The mean estimated tumor purity was higher in FNAB than GSS tissue (55.87% vs. 25.76%, $p < 0.0001$). All FNAB samples were estimated to have consistently higher proportion of malignant cells compared to GSS tissues (Figure 6). GSS tissues showed high variation in the tumor cell proportion.

Validation of somatic mutations detected exclusively in

FNAB

Among 660 mutations detected exclusively from FNAB, seventeen different genes from nineteen foci were evaluated for both FNAB and GSS tissue samples by Sanger sequencing. Fourteen genes that had a VAF >20 and alteration copy number >10 were selected from specimens with good or intermediate correlation, and five cancer relevant genes from specimens with poor correlation were selected for validation testing. Mutations in 13 of 19 (68.4%) foci were validated and heterozygous peaks were identified on sequencing chromatograms (Table 3). Absence of mutation in genes from GSS tissues was confirmed for all samples.

The VAF in mutations that were validated ranged from 20.7% to 61.0% and those failed to be validated ranged from 20.3% to 45.8%, and mean values showed no statistical difference (41.8% vs. 32.2%, $p = 0.169$).

Table 1. Clinicopathologic data of patients

Case no.	Diagnosis	Estrogen receptor	Progesterone recptor	HER-2	Pathologic size (cm)	USG size (cm)	Bulk tissue invasive tumor %	Remarks
1	Invasive ductal carcinoma	negative	negative	negative	2.3 x 2.1 x 2.0	1.8 x 1.4 x 2.2	45	
2	Invasive ductal carcinoma	positive in 70%	positive in 2%	negative	3.6 x 1.8 x 4.0	3.7 x 3.0	98	
3	Invasive lobular carcinoma	positive in 70%	positive in 80%	negative	3.5 x 1.3 x 3.5	3 x 2.1	55	
4	Invasive ductal carcinoma	positive in 95%	positive in 15%	negative	2.2 x 1.3 x 2.0	2.0 x 1.4	30	
5	Invasive ductal carcinoma with micropapillary feature	positive in 30%	positive in 1%	negative	3.3 x 3.0 x 2.5	3 x 2.4	0	
6	Invasive ductal carcinoma	negative	negative	positive (2+/3) FISH(+)	4.5 x 2.7 x 4.0	4.8 x 7.1 x 3.4	75	NAC
7	Invasive ductal carcinoma with lymphoplasmacytic infiltration	negative	negative	negative	2.8 x 1.8 x 1.5	3.7 x 2.4	3	
8	Invasive ductal carcinoma	negative	negative	positive (2+/3) FISH(+)	2.7 x 1.3 x 1.5	3.0 x 3.0 x 1.5	0	
9	Invasive ductal carcinoma	negative	negative	positive (2+/3) FISH(+)	2.2 x 1.8 x 1.5	5.6 x 2.9 x 4.6	20	
10	Invasive ductal carcinoma	negative	negative	negative	3.4 x 3.3 x 3.5	4.9 x 3.7	25	NAC
11	Invasive ductal carcinoma	positive in 50%	negative	negative	8.3 x 2.1 x 4.5	7.4 x 5.0	45	IBTR
12	Invasive ductal carcinoma with micropapillary feature	negative	negative	positive (3+/3)	7.0 x 2.5 x 6.0	5.8 x 8.8 x 4.0	0	NAC

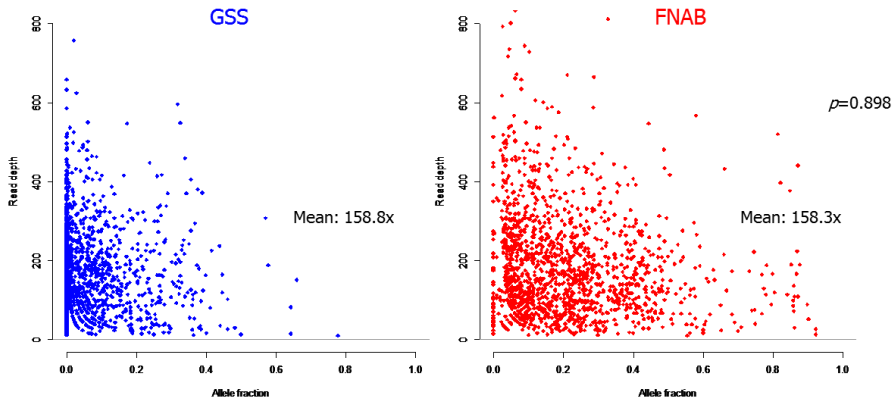USG: ultrasonography; NAC: neoadjuvant chemotherapy; IBTR: ipsilateral breast tumore recurrence

Figure 2. Distribution of the read depth of allele fractions
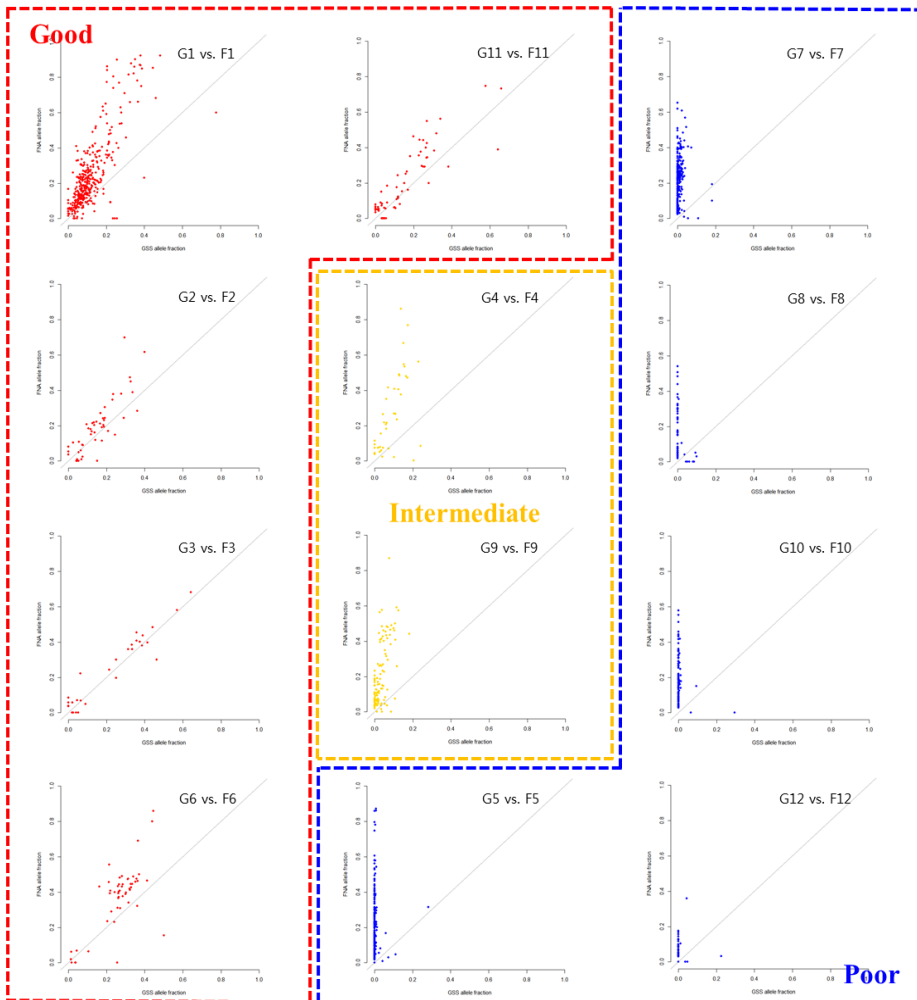

Figure 3. Allele fraction plotting - correlation between bulk and FNAB
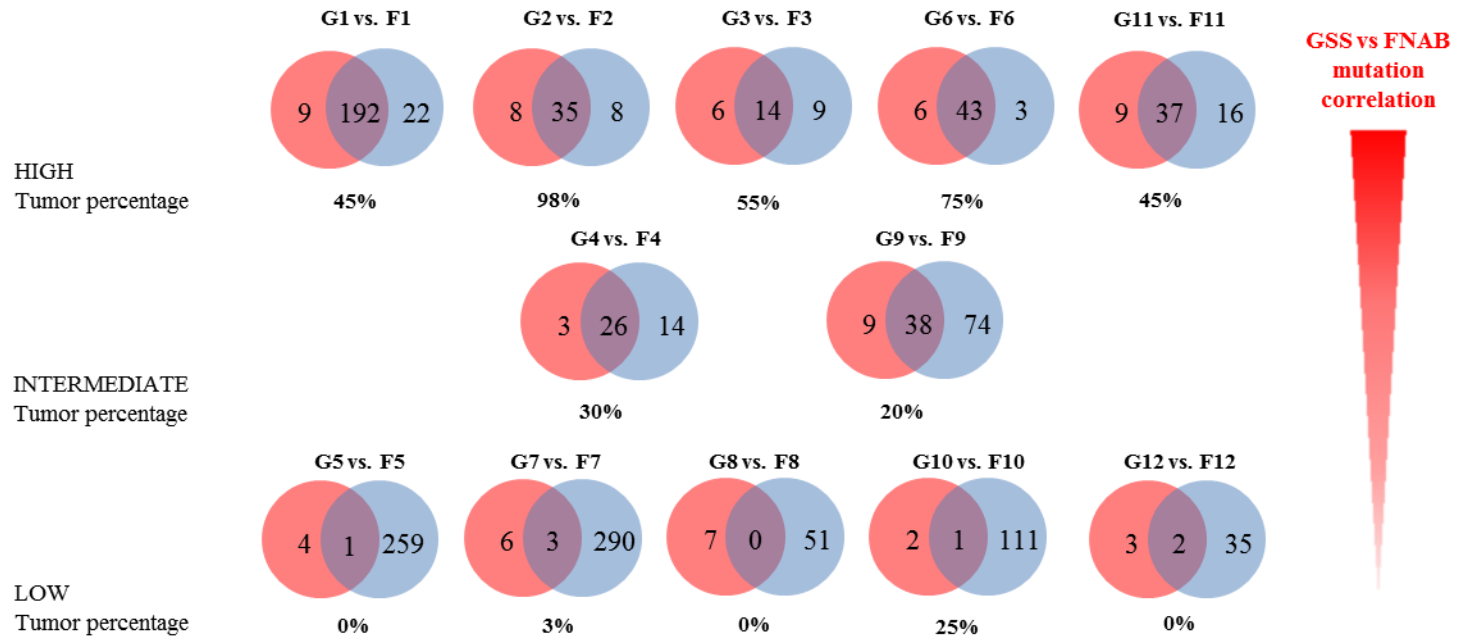
Figure 4. Overlap of somatic mutations according to invasive tumor percentage

Table 2. Estimated tumor purity by PyLOH

sample		estimated tumor purity (%)	pathologic invasive tumor %	sample		estimated tumor purity (%)	
GSS	G1	38.3	45	FNAB	F1	60.7	
	G2	29.4	98		F2	36.3	
	G3	51.5	55		F3	82.4	
	G4	20.9	30		F4	81	
	G5	12.5	0		F5	42.9	
	G6	63.5	75		F6	87.6	
	G7	15.5	3		F7	47.4	
	G8	11.5	0		F8	42.7	
	G9	16.1	20		F9	40.7	
	G10	10.9	25		F10	49.7	
	G11	30	45		F11	44.4	
	G12	9	0		F12	54.6	
	mean	25.76			mean	55.87	$p < 0.0001$

GSS: gross surgical specimen; FNAB: fine-needle aspiration biopsy
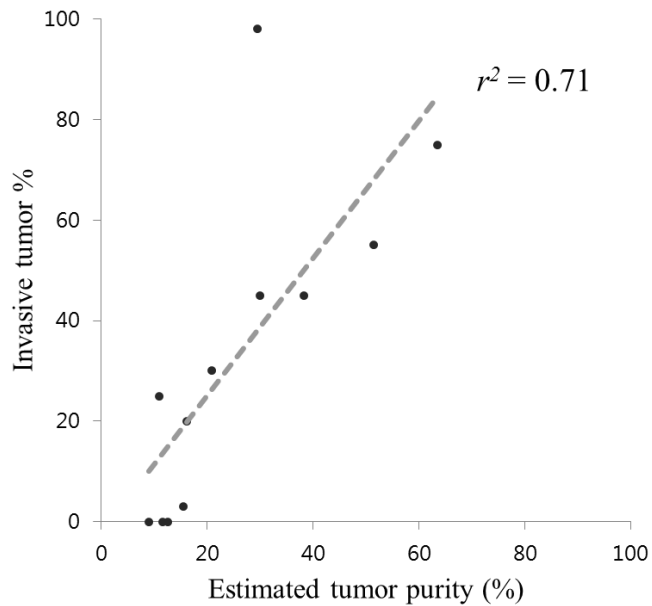
Figure 5. Correlation of invasive tumor percentage and estimated tumor purity in GSS tissue

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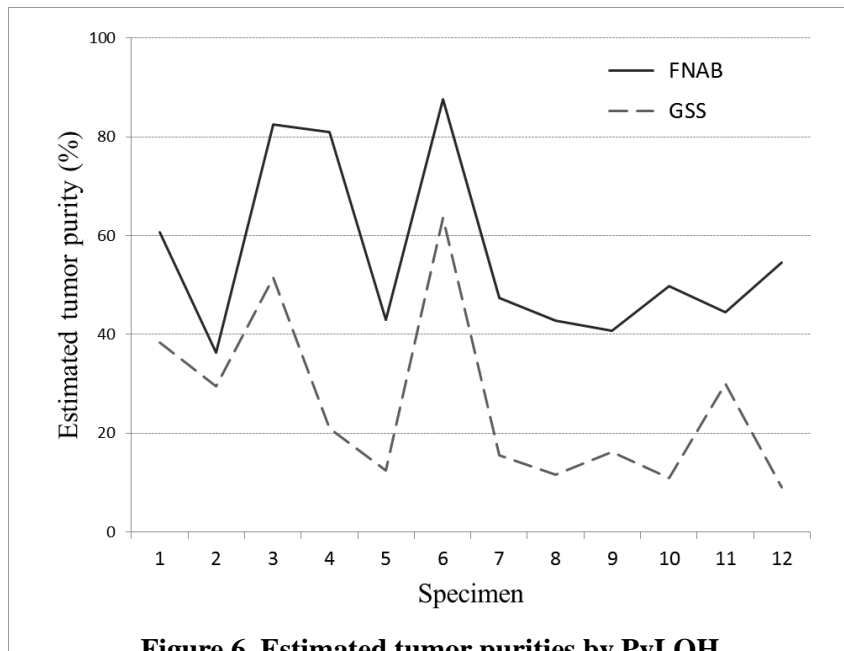
Figure 6. Estimated tumor purities by PyLOH

Table 3. Validation of mutations detected exclusively in FNAB

Sample	Gene	Reference	Alteration	Reference copy no.	Alteration copy no.	VAF	Validation
F1	FSCB	C	T	46	12	20.7	yes
F1	CCSER1	G	A	51	26	33.8	yes
F2	ERN2	T	G	47	23	32.9	no
F5	MET	C	T	97	45	31.7	yes
F5	PIK3CA	A	T	85	110	56.4	yes
F7	TET2	C	G	142	57	28.6	yes
F7	TP53	T	C	17	24	58.5	yes
F8	TP53	C	A	60	62	50.8	yes
F9	CCDC120	G	A	102	26	20.3	no
F9	MLL2	G	T	52	14	21.2	no
F9	KCNQ1	G	A	56	19	25.3	no
F9	RAG1	G	T	137	51	27.1	yes
F9	WDR7	T	G	57	25	30.5	yes
F9	CCAR2	A	G	52	37	41.6	yes
F9	AACS	C	T	21	17	44.7	yes
F9	TMEM62	C	T	45	38	45.8	no
F9	ZNF330	A	G	50	69	58.0	yes
F9	RTL1	G	A	87	136	61.0	yes
F10	PIK3CA	G	A	64	59	48.0	no

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Discussion

We successfully carried out WES in all pairs of GSS tissue and FNAB from twelve breast cancer patients. It has been demonstrated that samples obtained from FNAB can be used feasibly to perform WES and detect somatic mutations more sensitively compared to those from GSS tissue. To our knowledge, the feasibility of FNAB on breast cancer for use as biospecimens in NGS has never been demonstrated.

The total amount of DNA extracted from FNAB was similar to the amount obtained from GSS tissue and was sufficient for use on Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA, USA). In a previous study which used fine needle aspiration smears of solid tumors, only 51% of samples derived from cell blocks and aspirates yielded the 10ng of DNA required for successful NGS analysis using the Ion PGM (Thermo Fisher Scientific, Waltham, MA, USA) platform (12). Obtaining fresh tissue instead of fixed or smeared aspirates and applying enough cutting or scraping motion of the needle through the tumor during FNAB (13) may be crucial for FNAB performed for the purpose of retaining biospecimens for NGS.

A retrospective analysis of invasive tumor percentages of GSS tumor samples showed that samples with high tumor content correlated well with FNAB samples in terms of somatic mutation profiles, whereas those with low tumor content failed to show correlation. This failure was mostly due to the scarcity of tumor portions in the GSS tissues, indicating that FNAB more

reliably retained malignant tumor portion. . This difference in tumor purity between FNAB and GSS tissue was confirmed with the estimation calculated by PyLOH (11), which integrates somatic copy number alterations and loss of heterozygosity. Furthermore, this indicates that FNAB samples sufficiently reflect the mutations detected from surgical specimen and it may be used as an alternative method for tissue sampling. WES performed without evaluation of invasive tumor percentage provided a similar environment to when genomic testing may be performed preoperatively.

When we evaluated mutations detected in both GSS tissue and FNAB samples that showed good or intermediate correlation, mean overall VAF was greater for those detected with FNAB samples. This suggests that for a given mutation in identical genes, FNAB sample results in a more sensitive detection of variations compared to GSS tissue. This is due to the fact that GSS tissue is more likely to be contaminated with genetically normal tissue that disturbs variant calling in NGS. When utilizing GSS tissue, close collaboration with histopathologists is necessary to ensure that the specimen is representative of the tumor and to assess the degree of contamination by normal tissue (2). The ability of FNAB to sample purer cancer cells makes it a candidate to provide specimens with better quality for use in cancer NGS. Abrogation of adhesion which is common in malignancy, along with the mechanophysical properties of the FNAB procedure are two explanations suggesting that FNAB specimens should be rich in malignant cells (8,14). Furthermore, the fan shaped, radial motion of the needle during FNAB allows thorough access to all portions of the

tumor. Accordingly, FNAB samples may be more representative of the tumor and may reflect different mutations in separate cancer cells arising from tumor heterogeneity.

The modern need to both diagnose the disease and obtain a sample adequate for biomarker testing has led to developments in genomic procedures to yield more detailed therapy-directing information on tumor samples (15). Detection of critical biomarkers such as *EGFR* sequencing and *EML4:ALK* fusion testing for patients with non-small-cell lung cancer (16,17) and selected genomic alterations such as *HER2* gene amplification in pancreatic cancer (18) has led to necessities in simple sampling techniques such as FNAB. It has been demonstrated to be a feasible method for genomic analysis in various solid tumors (19–23). Similar methods should be applied to breast cancer for a more accurate diagnosis and therapeutic planning. A recent trial showed that personalization of medicine for metastatic breast cancer according to rare genomic alterations by comparative genomic hybridization array and DNA sequencing is feasible (24). For the clinical use of these approaches, FNAB might be the most optimal sampling method currently available.

Approximately 70% of mutations that underwent Sanger sequencing were validated in this study. Although only a small proportion of 660 mutations detected exclusively in FNAB samples were selected using VAF and alteration copy number, over 30% of the mutations could not be confirmed. Furthermore, there was no difference in VAF between mutations that were validated and those that failed validation. The sensitivity and specificity of NGS compared to

conventional sequencing methods needs to be evaluated in order to identify factors and values for developing criteria regarding selection of significant mutations detected by NGS.

One of the limitations of this study is that FNAB was performed on an already surgically resected specimen. It may not directly reflect the practical application of FNAB in clinic where the patient will be susceptible to pain caused by needle passages through the breast and tumor. Furthermore, palpation of the tumor may be more difficult compared to a surgically resected specimen and FNAB might require ultrasound guidance in real clinical situations.

Conclusion

WES was successfully carried out in all pairs of GSS tissue and FNAB from twelve breast cancer patients. In general, FNAB detected more somatic SNVs. When the GSS tissue had high tumor content by H&E staining, somatic mutation profiles showed high correlation between matched samples by the two sampling methods. FNAB samples were estimated to have consistently higher proportion of malignant cells compared to GSS tissues. This study suggests that FNAB is a feasible method, and furthermore, provides a reliable specimen for NGS analysis that identify somatic mutations with potential prognostic or therapeutic implication.

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국문초록

서론: 차세대염기서열분석법 (next-generation sequencing, NGS)은 임상에 빠르게 적용되고 있다. 세침흡인생검술 (fine-needle aspiration biopsy, FNAB) 조직은 마이크로어레이 (microarray) 유전자 발현 자료수집과 유전체가공 등의 분자생물학적 분석에 유용하게 사용되어 왔다. 이들 조직은 손쉽게 구할 수 있고 악성 세포 비율이 높아 더 많은 임상 조직에 대한 유전체 분석을 가능하게 해준다. 본 연구에서는 차세대염기서열분석법을 이용하여 유방암에서 체세포돌연변이를 발견하기 위한 방법으로 세침흡인생검술의 유용성 및 예민성을 평가하고자 하였다.

방법: 수술을 통해 절제된 유방암 조직에서 만져지는 종양의 표면에 위치한 피부를 통해 육안 수술 시료 (gross surgical specimen, GSS) 조직과 FNAB 조직을 채취하였다. 12명의 환자에서 얻은 GSS 조직과 FNAB 조직에서 DNA를 추출하여 NGS을 통해 전엑솜염기서열분석 (whole exome sequencing, WES)을 시행하였다. 이를 통해 발견된 체세포 돌연변이들을 서로 대응되는 GSS 조직과 FNAB 조직에 대해 분석하였다. FNAB 조직에서만 발견된 체세포 돌연변이에 대해 생거염기서열분석을 하여 검증하였다. GSS 조직의 H&E 염색 절편을 관찰하여 침윤성종양비율을 판독하였다. 염기서열분석 데이터를 이용하여 각 조직의 종양 순도를 계산하였다.

결과: GSS 조직과 FNAB 조직에서 추출한 DNA 양의 차이는 없었다 (2.11 μg vs. 2.39 μg; p = 0.44). 염기서열분석 depth of

coverage는 **GSS** 조직과 FNAB 조직에서 각각 158.8x (range 138.6x – 180.5x)와 158.3x (range 135.2x – 185.1x)였다. 각 조직에서 발견된 체세포 돌연변이의 중앙값은 **GSS** 조직보다 FNAB 조직에서 컸다 (39.5 vs. 18.5, *p*=0.036) H&E 염색을 통해 판독한 GSS의 침윤성종양비율이 높았던 경우 두 채취 방법으로 얻은 조직 간에 체세포 돌연변이 프로필의 상관관계가 좋았다. FNAB에서만 발견된 19개의 돌연변이에 대해서 생거염기서열분석을 시행하여 13개 (68.4%)가 입증되었다. 추축 종양 순도의 평균값은 FNAB에서 더 컸다(55.87% vs. 25.76%, *p* < 0.0001). 모든 FNAB 조직은 GSS 조직에 비해 지속적으로 높은 악성세포 비율을 보였다.

결론: 유방암 환자 12명으로부터 각각 얻은 GSS 조직과 FNAB 조직을 이용하여 성공적으로 WES을 시행하였다. 일반적으로 FNAB 조직으로 통해 더 많은 체세포 돌연변이를 발견할 수 있었다. 종양 비중이 높았던 조직에서는 체세포 돌연변이 분석에서 **GSS** 와 FNAB 두 조직 간에 좋은 상관관계가 관찰되었으나 종양비중이 낮았던 조직에서는 상관관계가 없었다. FNAB 조직은 GSS 조직에 비해 지속적으로 높은 악성세포 비율을 보였다. 이 연구를 통해 FNAB가 진단 및 치료에 영향을 주는 체세포돌연변이를 발견하기 위한 NGS 분석에 유용하게 사용될 수 있는 방법임을 보였다.