의학석사 학위논문

Comprehensive somatic genetic alteration profiling for triple-negative breast cancer patients by targeted kinome sequencing

표적 키놈 염기서열 분석을 이용한 삼중음성 유방암 환자에서의 포괄적 유전자 분석

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A thesis of the Master's Degree

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February 2015

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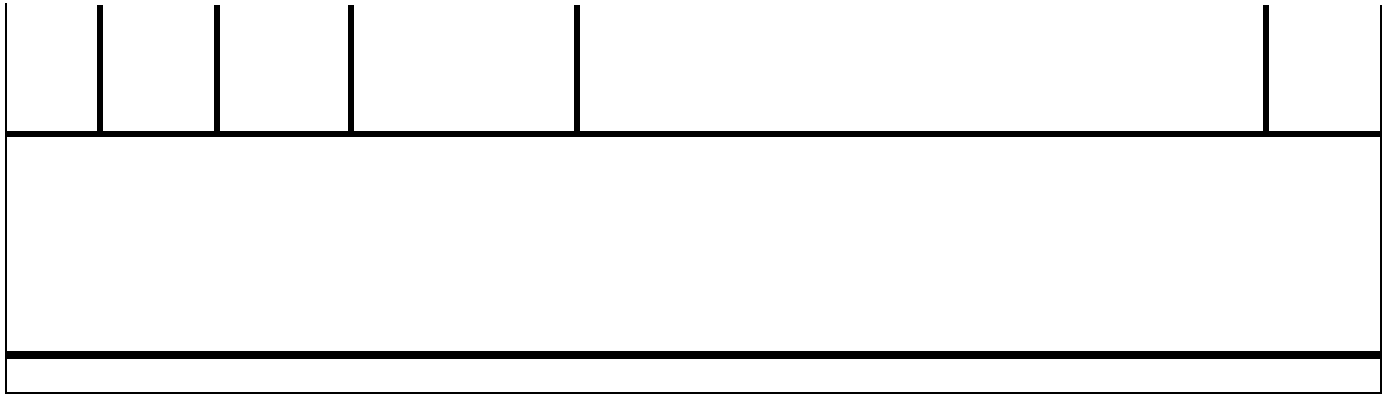

표적 키놈 염기서열 분석을 이용한

삼중음성 유방암 환자에서의

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위 원 임 석 아 (인)

Comprehensive somatic genetic alteration profiling for triple-negative breast cancer patients by targeted kinome sequencing

By

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(Directed by Wonshik Han, M.D., Ph.D.)

A thesis submitted to the Department of Surgery in partial fulfillment of the requirements for the Master's Degree in Medicine (Surgery) at Seoul National University College of Medicine

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Approved by Thesis Committee:

Professor _____ **Chairman**

Professor _____ **Vice chairman**

Professor _____

Abstract

Introduction: Triple-negative breast cancer (TNBC) is a heterogeneous, complex disease and due to its aggressiveness, targeted therapy is an unmet need continuously being challenged. Protein kinases are one of the main therapeutic targets with innumerable targeted agents being developed without profound success in TNBC patients. Along with development of targeted therapy predictors of these kinase inhibitors is also an obstacle needed to be overcome. In this study, we investigated targetable or potentially targetable somatic genetic alterations in TNBC, by target kinome sequencing of TNBC cancer tissues.

Methods: A total of 247 breast cancer tissues, 59 fresh frozen samples and 188 formalin-fixed paraffin embedded (FFPE) samples, each with a matched normal sample were collected. Targeted sequencing was done by using Agilent SureSelect Human Kinome Panel (612 genes including over 500 kinases) by next-generation sequencing technology. An average of 250x depth coverage was acquired in all sequenced samples. SNP microarray was also done for 46 pairs of frozen tissue samples.

Results: A final total of 200 tumor-normal matched tissues were used for targeted sequencing and 170 of these were analyzed as TNBC samples. At least one genetic alteration was identified in 148 cases (87.1%) with a median of four (range 0-137) alterations detected per case. A sum of 719 somatic mutations was found in 256 genes. 412 nonsynonymous single nucleotide variations (SNV) were detected, 279 of these being novel mutations. Copy number variations (CNV) were found in 115 genes from 87 patients. The most frequently altered genes were TP53 in 92 (54.1%) patients, followed by MYC in 20 (11.8%)

patients and OBSCN, PIK3CA, each in 16 (9.4%) patients. 83 potentially actionable target genes were identified in 128 (75.3%) patients with more than half of these patients having more than 1 potentially actionable gene aberration. A median of 8 potential genetic alterations were identified in each patient. These target genes were classified into 6 functionally relevant pathways, showing receptor tyrosine kinase/growth factor pathway and PI3K/MTOR pathway being the most frequently dysregulated pathways.

Conclusion: Our strategy of targeted kinome sequencing of TNBC cancer tissues to detect cases with alterations in potentially targetable genes is a feasible approach as a cancer panel.

Keywords: Triple-negative breast cancer, Next-generation sequencing, Targeted kinome sequencing, single nucleotide variations, copy number variation

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Lists of Tables and Figures

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List of Abbreviations

CNV	Copy number variation
FFPE	Formalin-fixed paraffin embedded
FISH	Fluorescence in situ hybridization
GF	Growth factor
HER2	Human epidermal growth factor receptor-2
IHC	Immunohistochemistry
Indel	Insertions/deletions
MAPK	Mitogen-activated protein kinase
MTOR	Mammalian target of rapamycin
NGS	Next-generation sequencing
PI3K	Phosphatidylinositol-3-kinase
RTK	Receptor tyrosine kinase
SNUH	Seoul National University Hospital
SNV	Single nucleotide variation
TNBC	Triple-negative breast cancer

Contents

Introduction

Triple-negative breast cancer (TNBC) is a subtype defined by lack of expression of estrogen receptor, progesterone receptor and HER2 gene overexpression. TNBC accounts for 10-15% of breast cancer and is known for its aggressiveness and higher mortality compared to other subtypes. Also with the absence of any targeted therapies, the only pharmalogical tool in treating TNBC is conventional chemotherapy. Although treated as a single disease entity, response to these agents is not uniform, presenting with remarkable heterogeneity in therapeutics response and prognosis. Heterogeneity of TNBC has been a large obstacle in developing targeted therapies, which is one of the major unmet needs in breast cancer treatment.

Protein kinases constitute about 1.7% of the human gene (1), encoding approximately 518 kinases. Dysregulation of kinase activities has emerged as a major mechanism on human cancer progression (2) and is currently an attractive therapeutic target. Cancer target therapy has benefited significantly on kinase inhibitors with over 130 kinase-specific inhibitors currently in phase 1-3 clinical trials (3). Several representative potential therapeutic targets, such as dysregulated EGFR, PI3K pathway, and mTOR signaling, have been identified to be associated with TNBC. Although many are ongoing, current results of clinical trials using agents targeting these pathways have shown disappointing results (4-7). Considering the heterogeneity of TNBC and disappointing clinical trial results, identifying predictors of kinase inhibitor sensitivity is a challenge that is invaluably needed to be overcome.

Next-generation technology generates results quickly with a small amount of

DNA. It also has the advantage of sequencing all genes needed, according to the objective of the sequencing process. Targeted sequencing enables to produce reliable results with sufficient sequencing depth and customized panels using this platform are being developed to sequence genes that are more commonly mutated to offer focused multi-gene testing (8, 9). Through this approach potentially effective therapeutics can be identified, and under this notion many papers are being published reporting its application in clinical practice in various cancers (9-12).

In this study, comprehensive somatic genetic profiling for TNBC patients was performed by target kinome sequencing using NGS technology. Our final objective was to describe targetable or potentially targetable somatic genetic alterations in TNBC.

Materials and Methods

A total of 247 breast cancer tissues each with a matched normal breast tissue or peripheral blood sample were collected at Seoul National University Hospital (SNUH). 59 fresh frozen tumor samples were collected from surgical specimens between 1995 and 2010 and 188 formalin-fixed paraffin embedded (FFPE) tumor samples were collected from surgical specimens operated between 2003 and 2013. Each tumor sample had matching normal samples derived from fresh frozen normal breast tissues or peripheral blood samples collected at the time of surgery or normal breast samples from FFPE tissues. FFPE slides were reviewed by an expert pathologist who marked paraffin blocks for tumor and normal FFPE tissue extraction and were provided from the SNUH Tumor Bank. Frozen tissues and peripheral blood samples were derived from Seoul National University Laboratory of Breast Cancer Biology Biorepository. Clinico-pathological data was acquired from SNUH Breast Care Center web-based database.

Genomic DNA was extracted from samples and 1μg of genomic DNA was fragmented by nebulization. The fragmented DNA was repaired by ligating an 'A' to the 3' end and Illumina adapters were ligated to the fragments. Each sample was size selected aiming for a 350-400 base pair product. The size selected product was PCR amplified and the final product was validated by Agilent Bioanalyzer. Target enrichment was done by using Agilent SureSelect Human Kinome Panel. This panel targets a broad set of kinases and kinase related genes for enrichment, targeting 612 genes including over 500 kinases.

The paired-end libraries were constructed and sequenced on Illumina HiSeq 2000 instrument, with an average 250x depth coverage. Generated sequence reads were aligned to human genome hg19 with the Burrows-Wheeler Aligner (BWA) mapping algorithm. Picard-tools (ver 1.59) was used to sort reads by mapping-coordinate and remove PCR duplicates, and BEDtools (ver 2.15.0) was used to include only reads that uniquely mapped to the reference genome. Somatic single nucleotide variation (SNV) and copy number variation (CNV) were identified using VarScan2 algorithm. For frozen tissue samples, SNP microarray experiment (Illumina Human Omni5 Exome microarray) was also done to confirm the existence of identified somatic SNV and CNV. There were 18,720 SNP probes in the SNP microarray covering target regions of the kinome panel. If the same SNV and CNV were detected in both NGS and microarray data, we considered them validated.

A customized algorithm to identify CNV was also used with SK Telecom in-house CNV calling algorithm (SKT algorithm). This algorithm calls CNV with higher sensitivity by reducing noise caused by targeted sequencing. The amount of amplification is reported in this algorithm, divided into high/medium/low.

Somatic point mutations, indels (small insertions and deletions) and copy number variations were detected by comparing tumor DNA sequences to its matching normal DNA sequences. Only somatic missense, nonsense and splicing SNVs or insertions/deletions were considered valid mutations. SNVs and germline alterations were identified and filtered using the Single Nucleotide Polymorphism Database (dbSNP; version 142; http://www.ncbi.nlm.nig.gov/SNP), Catalogue Of Somatic Mutations In

Cancer (COSMIC) database (version 70; http://cancer.sanger.ac.uk/cancergenome/projects/cosmic) and cBioPortal (http://www.cbioportal.org/public-portal).

This study was approved by Institutional Review Board of Seoul National University Hospital and the committee waived for informed consent.

Results

Patient characteristics

A total of 200 paired breast cancer tissues and normal breast tissues or blood samples were used for targeted sequencing. Forty seven samples were excluded from targeted sequencing for reasons described in Figure 1.

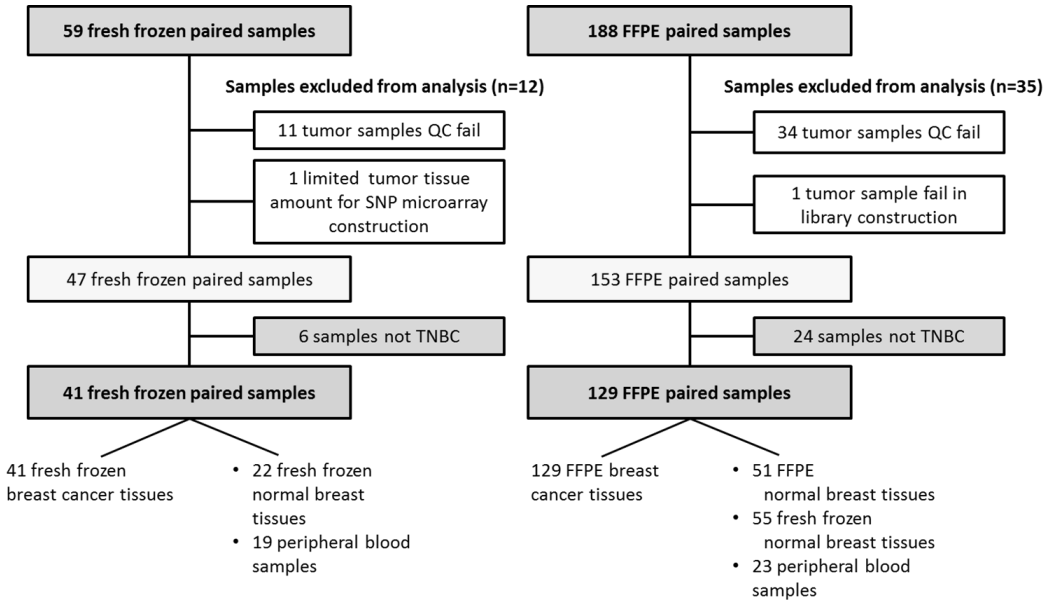

Figure 1. Flowchart of patient samples that were analyzed.

Abbreviations: QC, quality control; SNP, single nucleotide polymorphism; FFPE, formalin-fixed paraffin embedded

Patient baseline characteristics are described in Table 1. Median age was 50 years old and the majority of patients had stage I or II malignancy (85%). 178 patients were classified into triple negative breast cancer (TNBC) when subtype was defined by immunohistochemistry results. But 8 patients showed

Human epidermal growth factor receptor-2 (HER2) amplification after NGS sequencing and re-classification was done for analysis. Final somatic genetic alteration profiling was done with 170 TNBC samples.

Characteristics		**n**	**% (range)**	**n (TNBC)**	**% (range) (TNBC)**
Age	Median	50	(28-83)	50	(28-83)
Stage (Clinical stage in neoadjuvant chemotherapy)	1	45	22.5	40	23.5
	2	125	62.5	106	62.4
	3	30	15.0	24	14.1
Neoadjuvant chemotherapy		6	3.0	4	2.4
Subtype by Immunohistochemistry	Luminal	19	19.5		
	HER2	3	1.5		
	TNBC	178	89.0		
Subtype by NGS results	Luminal	19	9.5		
	HER2	11	5.5		
	TNBC	170	85.0		

Table 1. Baseline characteristics

Abbreviations: NGS, next generation sequencing; HER2, Human Epidermal growth factor Receptor-2; TNBC, triple-negative breast cancer

Somatic genetic alteration profiling

At least one genetic alteration was identified in 148 cases (87.1%) with a median of four (range 0-137) alterations detected per case. Distribution of number of genetic alteration by case is shown in Figure 2. Number and type of somatic mutation was diverse by case. 34(20.0%) patients had 10 and over somatic mutations and only 3(1.8%) cases had over 50.

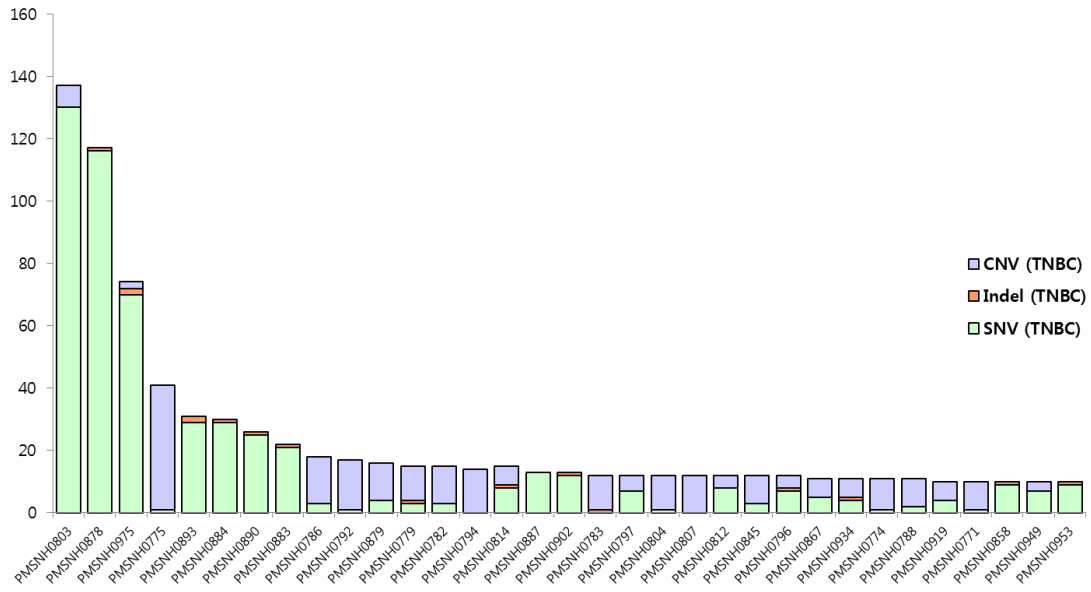

Figure 1. Figure 2. Distribution of number of genetic alteration by case.

Abbreviations: CNV, copy number variation; Indel, insertion/deletions; SNV, single nucleotide variation; TNBC, triple-negative breast cancer

Of the 612 genes that were sequenced, 395(64.5%) had somatic alterations.

A total of 719 somatic mutations were found in 256 genes. 412

nonsynonymous SNVs were detected in 247 genes from 123 patients. Point mutations were composed of 383 missense mutations and 29 nonsense mutations. 18 splicing SNVs were detected in 17 genes from 16 patients. 32 indels were observed in 16 genes from 33 patients. 133(32.3%) point mutations were known mutations from dbSNP, COSMIC and cBioportal and 279 were novel mutations. All indels and splicings were novel mutations, not known in dbSNP or COSMIC.

Copy number variations (CNV) were found in 115 genes from 87 patients. 385 CNVs were amplifications and 40 were heterogeneous deletions.

The most frequently altered gene was TP53, having 92(54.1%) patients with somatic mutation or copy number change. Following TP53 was MYC, with 20(11.8%) patients having genetic alterations, and OBSCN and PIK3CA, genetic alterations of each gene found in 16(9.4%) patients respectively. Number of genetic alterations in each specific gene is shown in Figure 3. Point mutations were most frequently observed in TP53(50 mutations in 75 patients), followed by PIK3CA (5 mutations in 15 patients) and TTN (13 mutations in 13 patients). Genes showing frequent copy number changes were MYC(20 patients, 11.8%), PTK2(12, 7.1%) and PKHD1L1(11, 6.5%).

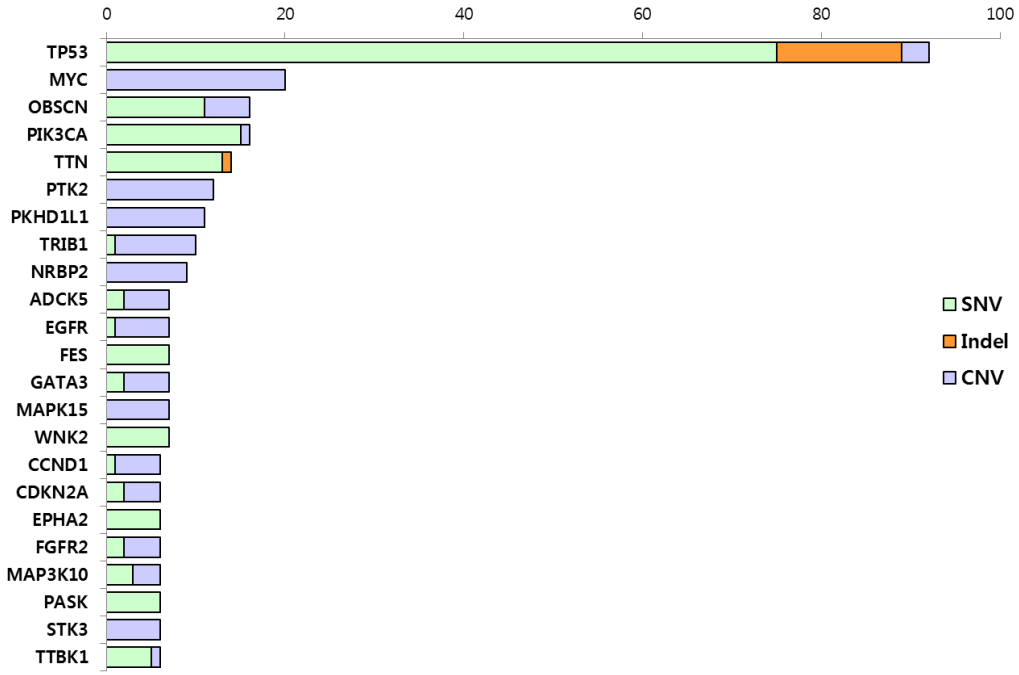

Figure 2. Number of genetic alterations in each specific gene

Abbreviations: SNV, single nucleotide variation; Indel, insertion/deletions; CNV, copy number variation

Comparison of genetic alteration detect rate between FFPE and fresh frozen samples is presented in Figure 4. SNVs and indels had similar detect rates between FFPE and fresh frozen tissues but CNVs had significantly low detection rate in FFPE samples. But when sensitivity was reduced in calling CNVs, the in-house SKT algorithm, CNV detection rate became similar.

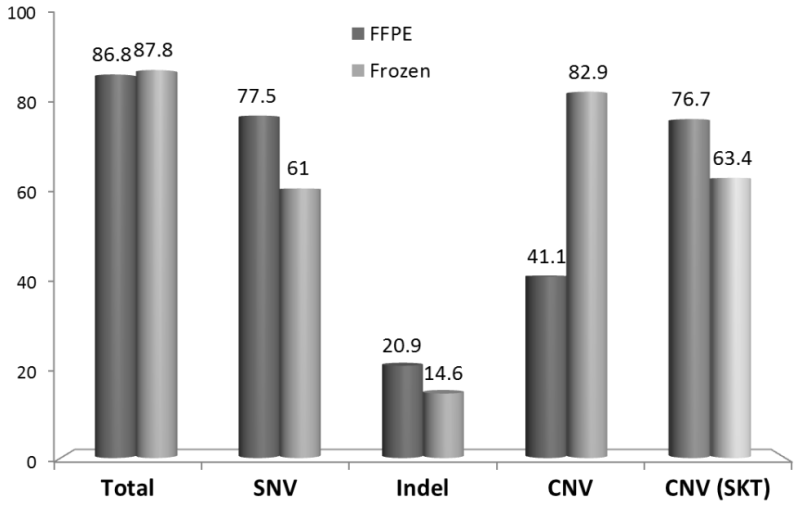

Figure 4. Comparison of genetic alteration detect rate between FFPE and fresh frozen samples

Abbreviations: FFPE, formalin-fixed paraffin embedded tissue samples; Frozen, fresh frozen tissue samples; SNV, single nucleotide variation; Indel, insertion/deletions; CNV, copy number variation

Recurrent novel mutations

Several interesting recurrent novel SNVs and CNVs were found. PASK c.3665A>T (p.H1222P) and FES c.1742A>G (p.E581G) were each observed in 6 patients, separately. STK38 c.648C>A (p.D216E) was observed in 10 patients. All three mutations are novel mutations not reported in TCGA(13) or Sanger database(14). No novel recurrent CNVs were observed when analyzed by VarScan2 algorithm. But SKT algorithm resulted with more than 4 times more CNVs compared to VarScan2. Observed CNVs from Varscan2 algorithm

were not always included in the results from the SKT algorithm. Using the SKT algorithm two relatively highlyl recurrent copy number gains were observed in PIM1 (10 cases, 5.9%) and JAK3 (12 cases, 7.1%). PIM1 and JAK3 amplification were reported in TCGA but with low incidence rate (1.5%, 1.7%, respectively). Samples showing high amplification of PIM1 on the SKT algorithm have been validated by fluorescence in situ hybridization (FISH) (Figure 5).

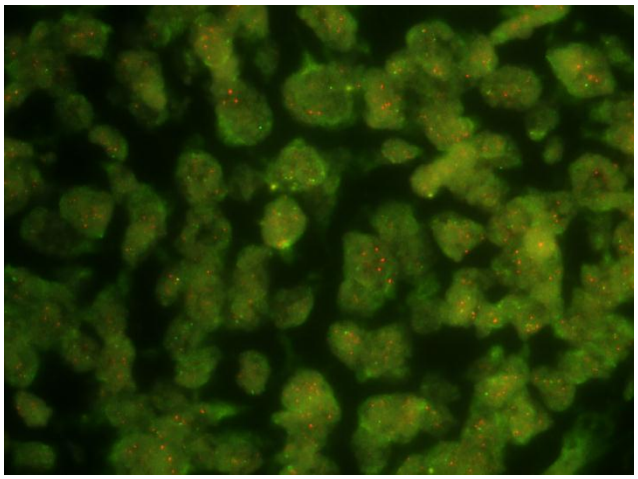

Figure 5. Validation of copy number variation. PMSNH0985 sample showing amplification of PIM1 (red).

HER2 amplification

According to immunohistochemistry (IHC) and FISH results at initial pathology report, only 3 cases had HER2 overexpression and 1 case was equivocal (HER2 2+). But NGS results showed differently with VarScan2 algorithm showing 6 extra cases with HER2 amplification and SKT algorithm

showing 3 extra cases which were not observed by Varscan2. These cases were excluded from somatic genetic alteration profiling presented above.

Cases with discrepancy between pathology report and NGS results were validated with HER2 FISH at SNUH Department of Pathology. HER2 high amplification by SKT algorithm was fully validated but medium amplification showed discordant results. All low amplification cases were not validated. Except for 1 case, HER2 FISH validation results corresponded with VarScan2 result. This case (PMNSH0868) had low amplification on SKT algorithm, suggesting false positive on NGS or tumor heterogeneity of HER2 amplification.

Results are summarized in Table 2.

Case	IHC result (grade)	FISH result	VarScan2	SKT	FISH validation
PMSNH0784	Negative	Unknown	Amplification	Amplification / High	
PMSNH0789	1+	Unknown	Amplification	None	
PMSNH0846	2+	Positive	None	Amplification / High	-
PMSNH0868	2+	Negative	Amplification	Amplification / Low	Negative
PMSNH0886	2+	Unknown	Amplification	Amplification / High	Amplification
PMSNH0892	1+	Unknown	Amplification	Amplification / High	
PMSNH0914	Negative	Unknown	None	Amplification / Low	Negative
PMSNH0917	Negative	Unknown	Amplification	Amplification / Medium	Amplification
PMSNH0923	Negative	Unknown	None	Amplification / Medium	Negative
PMSNH0929	Negative	Unknown	None	Amplification / Low	Negative
PMSNH0973	3+	Unknown	None	None	Negative
PMSNH0998	2+	Positive	Amplification	Amplification / Medium	-

Table 2. Summary of cases with HER2 amplification.

Abbreviations: IHC, Immunohistochemistry; FISH, Fluorescence in situ hybridization

Germline alterations

Germline alteration analysis was done for all 200 cases, including cases that were not TNBC. Known pathogenic mutations were found in BRCA1 gene in 2 cases and in 3 cases in BRCA2. Although not previously reported, 2 nonsense mutations in BRCA1 and 3 frameshift substitutions in BRCA1 and 1 frameshift substitution in BRCA2 were observed also. Other risk factor mutations or polymorphisms are summarized in Table 3.

BRCA1,2 mutations are the most well-known germline alterations with high penetrance for breast cancer. But other cancer related genes have also been

known to have breast cancer penetrance which were analyzed in this study. One patient had a known nonsense mutation in CHEK2 and 2 other patients had an unknown nonsense mutation and frameshift substitution, each in PALB2 and PTEN.

Observed germline alterations are summarized in Table 3.

		BRCA1	BRCA2	ATM	CDH1	CHEK2	PALB2	PTEN	STK11	TP53
Pathogenic mutation	**Missense**									
	Nonsense	1 (1)	3(3)			1 (1)				
	Frameshift	1 (1)								
Suspicious pathogenic mutation	**Missense**									
	Nonsense	2 (2)					1 (1)			
	Frameshift	3 (4)	1 (1)					1 (1)		
Mutation as risk factor	**Missense**		1 (18)		1 (2)				1 (5)	
	Nonsense									
	Frameshift									
Polymorphism or unclassified variant	**Missense**	8 (172)	11 (60)	4 (10)	3 (6)		3 (3)		1 (1)	1 (43)
	Nonsense									
	Frameshift									

Table 3. Germline alterations. Number of altered positions in each gene and number of patients in brackets are shown. Mutation as risk factor meaning mutations that have been reported conflictingly as pathogenic, benign or uncertain significance.

Potentially actionable genetic alterations

83 potentially actionable target genes were classified into 6 pathways shown in Figure 6. 128(75.3%) patients had altered genes that were able to be

classified into functionally relevant pathways shown in Figure 5. 70 (41.2%) patients had more than 1 potentially actionable gene alteration, with a median of 8 potential genetic alterations (range, 0-18) in each patient.

Mutations of TP53 was the most frequently altered single gene, and receptor tyrosine kinase(RTK)/growth factor(GF) pathway and PI3K/MTOR pathway were the most frequently dysregulated pathways.

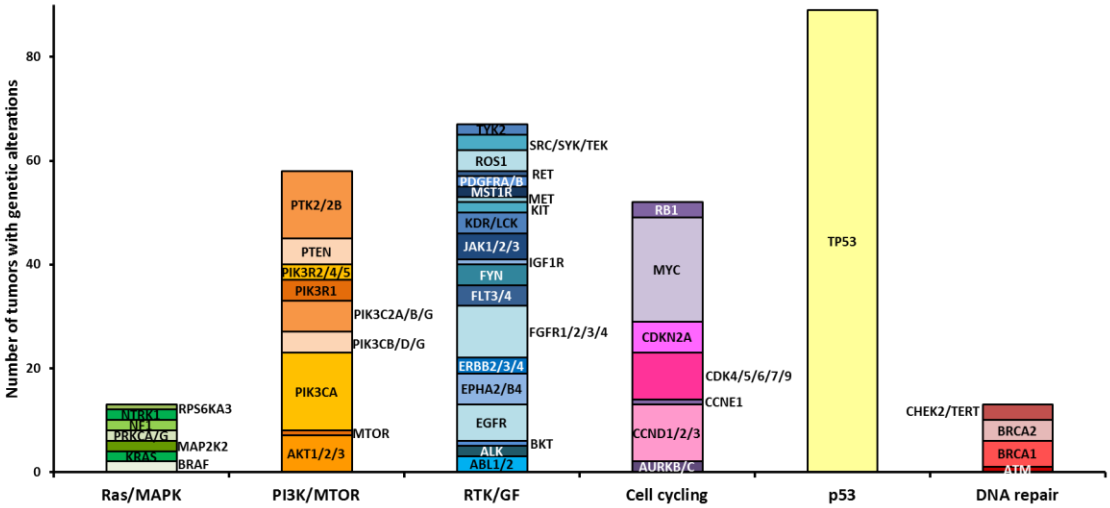

Figure 6. Spectrum of potentially actionable genetic alterations classified by functionally relevant pathways.

Abbreviations: MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3-kinase; MTOR, mammalian target of rapamycin; RTK, receptor tyrosine kinase; GF, growth factor.

Discussion

TNBC is a very heterogeneous disease not to be treated uniformly (15-17). Clinical trials on targeted therapy for TNBC have repetitively reported disappointing results, but it is a jump to conclusion to assume failure of these agents as most of the targeted therapies being developed have no clinical methods to determine which patients will benefit to these novel agents (15). In effort to understand and stratify TNBC, Lehmann et al analyzed gene expression profiles from 21 breast cancer datasets and identified 6 different TNBC subtypes with different survival patterns (18). But diverse response to several therapeutic agents from the same molecular subtype has been described in other papers, demonstrating that gene expression signatures alone are unlikely to be effective predictors of targeted therapies (19, 20).

TNBC is a complex disease, not only presenting with inter-individual heterogeneity, but also inter-tumor and intra-tumor heterogeneity within the same patient (21). Also kinase pathways are interrelated and inhibition of one pathway can often be bypassed by activation of other kinases, leading to failure of treatment or emergence of resistant mutants (21, 22). Considering the complexity of the disease and rapid development of resistant mutants to kinase inhibitors, planned multi-targeted inhibitors or combinations in advance of clinical application might be the answer in effectively targeting TNBC.

Identification of several target genes from an extensive profile of genes will be needed in order to apply combination targeted therapy. Comprehensive sequencing through NGS technology will be a cost and time-efficient

approach to detect several target genes at a time. From the perspective of this notion, we performed targeted kinome sequencing of 170 TNBC samples and investigated targetable or potentially targetable somatic genetic alterations. 128 (75.3%) patients had potentially actionable gene aberrations and more than half of these patients had more than one. Through these results we could demonstrate the possibility of our strategy of target sequencing kinome genes to detect genetic alterations with potential drug targets. This approach has been introduced in other papers, showing the feasibility of this approach in metastatic settings and after neoadjuvant chemotherapy (9, 23, 24)

Several novel recurrent somatic genetic aberrations were identified and among these PIM1 amplification was validated. PIM1 (Provirus integration site for Moloney murine leukemia virus 1) is a proto-oncogene that belongs to the PIM serine/threonine kinase family. The three-dimensional structure indicates that it is constitutively active (25, 26) and has been explored in mainly hematologic malignancies and prostate cancer (27). Small molecular inhibitors and antibodies for PIM1 have shown positive results in inhibiting cancer cell growth in both in vitro and in vivo studies (28, 29). The role of PIM1 in breast cancer is less known, only with a recent report by Malinen et al, suggesting PIM1 as a novel estrogen receptor target adding a new potential mechanism by which estrogens assist breast cancer cell proliferation and carcinogenesis (26). Recurrent PIM1 amplification in TNBC, an estrogen receptor negative breast cancer, is an interesting finding worthwhile of functional studies in the future.

FFPE tissue samples are an invaluable source of cancer tissues but unfortunately it is challenging to sample for molecular assays such as NGS.

Due to the fixation process in FFPE tissues, paraffin removal and counteraction of covalent protein-DNA interactions are needed, which is not an easy process. Fixation process also brings along fragmentation, cross-linking and chemical modification of the DNA/RNAs derived from FFPE samples (30). Despite these pitfalls, many attempts have been done on applying NGS to FFPE material, resulting with comparable results to conventional sequencing methods or paired fresh frozen tissues (30-33). FFPE tissue samples are robust, excessive source of cancer tissues and NGS-based analysis of these samples will bring invaluable data. In this study, direct comparison between FFPE and fresh frozen tissues is not possible, as paired samples were not used. But presenting with similar detect rates in SNVs and indels is a result worth paying attention to. On the other hand, CNVs had significantly lower detection rate in FFPE samples, showing the limitation of targeted sequencing of FFPE tissues in our study. DNA fragmentation of FFPE tissues is assumed to be one of the reasons of this low detection rate and would need to be overcome by deeper sequencing or more efficient DNA preparation method.

Another limitation of this study is the use of commercialized gene panel. Although easy to use this panel was not customized for breast cancer, leading to many genes (45.5%) having no observed alterations. Breast cancer-specific customized panel will be needed to increase coverage and better represent druggable pathways. Currently we are designing and validating a customized gene panel on the basis of previous studies including this one.

In conclusion, our strategy of targeted sequencing of kinomes genes using NGS technology resulted in comprehensive profiling of somatic genetic

alterations in TNBC FFPE cancer tissues. 75.3% patients were identified of potentially targetable gene aberrations demonstrating the feasibility of this strategy as a cancer panel.

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국문 초록

서론: 삼중음성유방암은 이질적이고 복잡한 질환이며, 나쁜 예후를 보이는 공격성으로 인해, 이에 대한 표적 치료는 끊임없이 도전하고 있는 unmet need이다. 단백질 키놈은 주요 치료 표적 중의 하나로, 이를 바탕으로 개발한 여러 삼중음성유방암 표적 치료가 있으나 아직 확실한 성공 사례가 없다. 표적 치료의 개발과 함께 키놈 억제제에 대한 예측 인자 개발이 또한 필요하다. 이 연구에서는 삼중음성유방암 조직에서의 표적 또는 잠재적인 표적이 가능한 체성 유전자 변이를, 표적 키놈 염기서열분석을 통해서 찾고자 하였다.

방법: 총 247개의 유방암 조직, 59 개의 동결조직과 188개의 포르말린고정 파라핀포매 조직, 및 그에 상응하는 정상 조직을 수집하였다. 표적 염기서열 분석은 차세대염기서열분석 기술을 이용하여 Agilent SureSelect Human Kinome Panel(500개의 키나제를 포함한 612개의 유전자)으로 진행하였다. 모든 조직에서 평균 250x의 염기서열 분석 깊이를 획득하였다. 46개의 동결 조직 쌍에 대해서는 단일염기변형(single nucleotide variation, SNV) 마이크로어레이(microarray)도 수행하였다

결과: 최종 분석시 200개의 종양-정상의 대응조직이 표적 염기서열 분석되었으며, 이 중 170개의 삼중음성유방암 조직에 대해 분석하였다. 148명(87.1%)의 환자에서 적어도 한 개의 유전자 변이가 관찰되었으며, 각 환자별 중앙값 4개(범위 0-137)의 변이가 관찰되었다. 총 256개의 유전자에서 719개의 체성 유전자 변이가 발견되었다. 412개의 체성 SNV가 발견되었으며, 이 중 279개가 새롭게 발견한 유전자 변이이었다. 유전자 복제 수 변이(copy number variation, CNV)는 87명의 환자에서 115개의 유전자에서 관찰되었다. 가장 흔하게 변이가 나타나는 유전자는 TP53으로 92명(54.1%)

의 환자에서 관찰되었으며, 그 뒤로 MYC이 20명(11.8%), OBSCN, PIK3CA는 각각 16명(9.4%)의 환자에서 관찰되었다. 83개의 잠재적인 표적 유전자가 128명(75.3%)의 환자에서 발견되었고, 과반수의 환자에서 2개 이상의 잠재적인 표적 유전자 변이가 발견되었다. 각 환자별 중앙값 8개의 잠재적 표적 유전자 변이를 관찰하였다. 잠재적 표적 유전자를 6개의 기능적 연관 pathway로 분류하였으며, receptor tyrosine kinase/growth factor pathway와 PI3K/MTOR pathway에서 가장 많은 유전자 변이를 관찰하였다.

결론: 잠재적인 표적 유전자를 가진 삼중음성유방암 환자를 찾기 위해 표적 키놈 염기서열 분석을 진행한 전략은 cancer panel로서 적용 가능한 접근 방법이다.

주요어: 삼중음성유방암, 차세대 염기서열 분석, 표적 키놈 염기서열 분석, 단일염기변이, 유전자 복제 수 변이

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