Identification of Candidate Genes in Early-Stage Invasive Ductal Carcinoma Patients with High-Risk Mortality using 32 Genes Commonly Involved in Breast Cancer: A Retrospective Study

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Primary research

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Abstract

Background: Invasive ductal carcinoma (IDC) breast cancer is a heterogeneous disease characterized by multiple subtypes. Breast cancer survival is highly impacted by tumor burden, molecular subtypes, and gene profiles. Gene mutation is a type of genomic instability regarded as having a considerable effect on breast cancer prognosis. Using integrated survival analysis, this study identified candidate genes and a high-risk group of patients with early-stage IDC breast cancer to provide further understanding of the genetic characteristics associated with poor survival.

Methods: The gene mutation profiles, baseline demographics, clinicopathological variables, and treatment characteristics of the early-stage breast cancer subpopulation were downloaded from an open access data platform. These data were analyzed for a total of 444 patients. In total, 32 genes commonly involved in breast cancer were listed, and the genes exhibiting significant differences (as estimated using the log-rank test) were selected as the candidate genes.

Results: The patients were divided into control, low-risk, and high-risk groups according to their gene mutation profiles. The 5-year overall survival rates were 97.6%, 96.0%, and 68.2%, respectively. The high-risk group had a significantly higher risk of poor overall survival (adjusted hazard ratio = 9.94, 95% confidence interval = 2.24–44.20, $P = 0.003$) than the other groups and the low-risk group did not have a significantly higher risk of poor overall survival compared with controls.

Conclusions: This study proposed an integrative approach for the identification of candidate genes for risk assessment of overall survival in these patients through typical survival analysis methods. The 10 candidate genes selected are particularly involved in cell-cycle processes, DNA repair, and drug resistance; their mutations were found to be generally associated with disease progression or therapeutic resistance, which is commonly associated with poor survival outcomes.

Background

Invasive ductal carcinoma (IDC), which accounts for approximately 65–85% of breast cancer cases, is a heterogeneous disease characterized by multiple subtypes [1]. Studies have demonstrated that the treatment options, prognosis, and survival outcomes of breast cancer are highly affected by tumor burden, molecular subtypes, and gene profiles [2–5]. Gene mutations also play a crucial role in characterizing the tumor burden and molecular subtypes. For instance, the human epidermal growth factor receptor 2 (HER2)-enriched subtype of breast cancer is characterized by HER2/ERBB2 mutations, and circulating tumor deoxyribonucleic acid (DNA) can be used to monitor tumor burden in breast cancer [6, 7].

Gene mutations are a type of genomic instability considered to have a considerable effect on breast cancer prognosis [8]. Because gene mutations in breast cancer are very broad in scope, identifying all of the related genes is difficult [9]. Therefore, the genes previously reported to be involved in IDC breast cancer were considered appropriate target genes in evaluating the effects of gene mutation on overall

survival. For instance, common genes involved in breast cancer including *TP53*, *HER2/ERBB2*, *CCND1*, *EMSY*, *FGFR1*, *BRCA1*, *BRCA2*, *BRCA3*, *PTEN*, *ATM*, *MSH2*, *MLH1*, *PMS1*, *MSH3*, *CDH1*, *HRAS*, *NRAS*, and *KRAS* have often been discussed in the literature [10, 11]. Moreover, *BIRC5*, *EGFR*, *ESR1*, *HIF1A*, *IGF1R*, *MYC*, *NOTCH1*, *PIK3CA*, *PTEN*, *RARB*, *RASSF1*, *SRC*, *TOP2A*, and *TWIST1* have been noted to be involved in breast cancer progression [8, 12–15].

Overall survival is considered a suitable endpoint for the evaluation of risk characteristics in patients with cancer [16]. The Kaplan–Meier estimator with the log-rank test and the Cox proportional hazard regression model testing are methods typically used for analyzing overall survival. They are based on a time-to-event = model and have been widely used in cancer research [17, 18]. The Kaplan–Meier estimator and log-rank test methods provide reliable results for single-variable risk assessment of overall survival [18]. The Cox proportional hazard regression model can singly or partially assess the effect of both single and multiple variables on overall survival [17].

Most research thus far has focused on a single gene or the effect of a specific gene function on breast cancer survival outcomes. An integration method for identification of candidate genes associated with poor survival is required to better understand the effects of gene mutation on overall survival. Typically, clinicians are more familiar with survival analysis methods than complex machine learning methods. Although multiple machine learning algorithms have provided precise and efficient analyses, using these algorithms is not necessarily worthwhile due to the long running time and the computational complexity—particularly for cases with only slight improvement. Therefore, in this study, we used an integrative approach involving typical survival analysis methods to identify candidate genes in patients with early-stage IDC breast cancer. This study aimed to achieve further understanding of the genetic characteristics associated with poor survival outcomes in breast cancer.

Methods And Materials

Dataset

The gene mutation profiles (i.e., the mutation data for breast cancer from the MuTect2 pipeline) and clinical information were downloaded from the Genomic Data Commons data portal using the TCGAbiolinks package, an R/Bioconductor package for integrative analysis of the Cancer Genome Atlas data. The patient inclusion criteria were as follows: (1) women; (2) diagnosis of pathologic stage I, II or III breast cancer, (3) specifically IDC; and (4) estimable survival status and follow-up interval. A total of 444 patients were included for analysis. Age at diagnosis, race and ethnicity were included as baseline demographics. Age at diagnosis was categorized as < 35, 35–50, and > 50 years. Others prognostic variables, including breast cancer subtypes, pathologic stage, and treatment characteristics, were also included in later analysis.

The 32 genes commonly involved in breast cancer and gene mutation risk groups

In total, 32 genes commonly involved in breast cancer, namely *TP53*, *HER2/ERBB2*, *CCND1*, *FGFR1*, *BRCA1*, *BRCA2*, *BRCA3*, *PTEN*, *ATM*, *MSH2*, *MLH1*, *PMS1*, *MSH3*, *CDH1*, *HRAS*, *NRAS*, *KRAS*, *BIRC5*, *EGFR*, *EMSY*, *ESR1*, *HIF1A*, *IGF1R*, *MYC*, *NOTCH1*, *PIK3CA*, *PTEN*, *RARB*, *RASSF1*, *SRC*, *TOP2A*, and *TWIST1*, were compiled into a list [10]. The gene mutation profile of each patient was matched with the genes on the list. The gene mutation status of each patient was determined based on matching results between the gene list and patient's gene mutation profile. Afterward, survival analysis was used to evaluate the effect of the gene mutation statuses on overall survival. Ten involved genes that displayed significant survival differences between patients who alive and dead were selected as the candidate genes. The patients who had no mutations in any of the 32 genes on the list were defined as controls. According to gene mutation status, the remaining patients were divided into low-and high-risk groups. Patients with mutations in the candidate genes were placed into the high-risk group, and the others were assigned to the low-risk group.

Statistical analysis

The baseline demographics, clinicopathological variables, treatment characteristics, and overall survival outcomes of the study population are presented as frequencies and percentages, and the distribution differences in between the control, low-risk, and high-risk groups were estimated using the chi-square test or Fisher's exact test, as appropriate. The mutation rates of the 32 genes in the patients were determined, and the differences in overall survival by mutation rate were estimated using the log-rank test. The genes that displayed significant differences in survival between the patients with and without mutations in the candidate genes were included in the high-risk gene set. The Kaplan–Meier curve was used to illustrate the differences in overall survival by risk category and single gene mutation status. Cox proportional hazard regression analysis was performed to estimate the effect of gene mutation risk score and covariates on overall survival. All factors were included in the multivariate model. All *P* values were two-sided and the statistical significance was set at 0.05. All analyses were conducted using version 4.0.1 of the computing environment R (R Core Team, 2020).

Results

Patient characteristics

Baseline demographics, clinicopathological variables, and treatment characteristics of the study population are summarized in Table 1. At a median follow-up of 2.2 years (range 1.2–23.4), 16 all-cause deaths (3.6%) had occurred. The study population had a higher proportion of patients who were aged 35–50 years (431; 97.1%), were Caucasian (69.1%), and not Hispanic or Latino (77.7%). The study population was more likely to have been diagnosed as having the luminal A or normal-like (191; 43.0%) and luminal B (110; 24.8%) molecular subtypes of breast cancer. The basal-like subtype was observed in 101 (22.7%) patients and HER-2 overexpression was observed in 42 (9.5%) of patients. In treatment characteristics, 243 (54.7%) patients had received radiation treatment and 363 (81.8%) patients had received pharmaceutical treatment.

Table 1
Baseline demographics and clinicopathological
variables of patients with early-stage IDC breast cancer
(N = 444).

Variables	Overall, n = 444
Age (years)	
< 35	10 (2.3%)
35–50	431 (97.1%)
> 50	3 (0.7%)
Race	
American Indian or Alaska native	1 (0.2%)
Asian	36 (8.1%)
Black or African American	56 (12.6%)
White	307 (69.1%)
Not reported	44 (9.9%)
Ethnicity	
Hispanic or Latino	13 (2.9%)
Not Hispanic or Latino	345 (77.7%)
Not reported	86 (19.4%)
Subtypes	
Basal-like	101 (22.7%)
HER2-enriched	42 (9.5%)
Luminal A or Normal-like	191 (43.0%)
Luminal B	110 (24.8%)
Pathological stage	
Stage I	89 (20.0%)
Stage II	273 (61.5%)
Stage III	82 (18.5%)
Treatment characteristics	
Radiation	243 (54.7%)
HER2: human epidermal growth factor receptor 2.	

Variables	Overall, n = 444
Pharmaceutical	363 (81.8%)
All-cause mortality	16 (3.6%)
HER2: human epidermal growth factor receptor 2.	

Gene mutation profiles

Table 2 presents the mutation rates of the 32 involved genes among the study population by survival outcomes. Mutations in 28 of the 32 involved genes were observed. However, no mutations were found for *BRCA3*, *EMSY*, and *RASSF1*. Figure 1 shows the gene mutation profiles and clinical features for the 308 patients with mutations in the aforementioned 28 genes. Overall, the highest mutation rate was noted for *TP53* (0.421) and *PIK3CA* (0.372), followed by *KRAS*, *MLH1*, and *MSH2*. After the study population was divided into subgroups of patients according to survival status (alive and dead), the log-rank test revealed significant differences in survival rate between the patients who had and did not have mutations in the 10 candidate genes: *BIRC5*, *CCND1*, *ESR1*, *HRAS*, *IGF1R*, *KRAS*, *MLH1*, *MSH2*, *NOTCH1*, and *SRC*. These candidate genes had higher mutation rates in the dead patients (Table 2), and the patients with mutations in the candidate genes had a significantly lower survival rate than the patients without mutations in single gene mutations survival analysis (Fig. 2b–k).

Table 2
Mutation rates of 32 genes commonly involved in breast cancer.

Gene	Mutation rate			<i>P</i>
	Overall	Alive	Dead	
<i>TP53</i>	0.421	0.414	0.625	0.152
<i>HER2/ERBB2</i>	0.018	0.019	-	0.662
<i>CCND1</i>	0.002	-	0.063	<0.001
<i>FGFR1</i>	0.014	0.014	-	0.705
<i>BRCA1</i>	0.023	0.023	-	0.656
<i>BRCA2</i>	0.029	0.026	0.125	0.108
<i>BRCA3</i>	-	-	-	-
<i>PTEN</i>	0.043	0.044	-	0.481
<i>ATM</i>	0.043	0.042	0.063	0.578
<i>MSH2</i>	0.009	0.005	0.125	<0.001
<i>MLH1</i>	0.016	0.014	0.063	0.007
<i>PMS1</i>	0.007	0.007	-	0.725
<i>MSH3</i>	0.014	0.012	0.063	0.072
<i>CDH1</i>	0.023	0.021	0.063	0.141
<i>HRAS</i>	0.002	-	0.063	<0.001
<i>NRAS</i>	0.002	0.002	-	0.887
<i>KRAS</i>	0.014	0.009	0.125	<0.001
<i>BIRC5</i>	0.002	-	0.063	<0.001
<i>EGFR</i>	0.027	0.023	0.125	0.232
<i>EMSY</i>	-	-	-	-
<i>ESR1</i>	0.016	0.014	0.063	0.026
<i>HIF1A</i>	0.009	0.009	-	0.711
<i>IGF1R</i>	0.018	0.014	0.125	0.002
<i>MYC</i>	0.002	0.002	-	0.898

P values were estimated using the log-rank test.

Gene	Mutation rate			<i>P</i>
	Overall	Alive	Dead	
<i>NOTCH1</i>	0.014	0.009	0.125	< 0.001
<i>PIK3CA</i>	0.372	0.362	0.625	0.213
<i>PTEN</i>	0.043	0.044	-	0.481
<i>RARB</i>	0.018	0.019	-	0.765
<i>RASSF1</i>	-	-	-	-
<i>SRC</i>	0.009	0.007	0.063	0.002
<i>TOP2A</i>	0.009	0.009	-	0.771
<i>TWIST1</i>	0.002	0.002	-	0.910
<i>P</i> values were estimated using the log-rank test.				

Gene mutation risk groups

The patients who had no mutations in any of the 32 genes were enrolled as controls. Patients with mutations in *BIRC5*, *CCND1*, *ESR1*, *HRAS*, *IGF1R*, *KRAS*, *MLH1*, *MSH2*, *NOTCH1*, or *SRC* constituted the high-risk group. The low-risk group consisted of patients with mutations in genes commonly involved in breast cancer but in none of the 10 candidate genes. The between-group survival differences are shown in Fig. 2a. The 5-year overall survival rates of the high-risk, low-risk, and control groups were 68.2%, 96.0% and 97.6%, respectively. The control and low-risk groups had a similar overall survival rate, and the high-risk group had significantly worse survival compared with the two other groups ($P < 0.001$).

Baseline demographics, clinicopathological variables, and treatment characteristics of the control, low-risk, and high-risk groups are summarized in Table 3. The three groups had similar distributions of age group, race, ethnicity, pathologic stage, and radiation treatment characteristics. With regard to the molecular subtype distribution, the high-risk subgroup had a significantly higher proportion of patients with the luminal B subtype (29.4% in controls, 21.7% in low-risk, 32.3% in high-risk, $P < 0.001$). Moreover, the high-risk (64.5%, $P = 0.014$) group was significantly less likely to receive pharmaceutical treatment than the control (86.8%) or low-risk (81.2%) groups. The high-risk group had a significantly higher mortality rate (5 deaths; 16.1%) than the control (4 deaths; 2.9%) or low-risk (7 deaths; 2.5%) group.

Table 3

Baseline demographics, clinicopathological variables, and treatment characteristics of gene mutation risk groups in patients with early-stage IDC breast cancer.

Variables	Controls, n = 136	Low-risk, n = 277	High-risk, n = 31	P
Age (years)				0.494
< 35	5 (3.7%)	4 (1.4%)	1 (3.2%)	
35–50	130 (95.6%)	271 (97.8%)	30 (96.8%)	
> 50	1 (0.7%)	2 (0.7%)	-	
Race				0.141
American Indian or Alaska native	-	1 (0.4%)	-	
Asian	5 (3.7%)	27 (9.7%)	4 (12.9%)	
Black or African American	24 (17.6%)	30 (10.8%)	2 (6.5%)	
White	92 (67.6%)	192 (69.3%)	23 (74.2%)	
Not reported	15 (11%)	27 (9.7%)	2 (6.5%)	
Ethnicity				0.741
Hispanic or Latino	5 (3.7%)	7 (2.5%)	1 (3.2%)	
Not Hispanic or Latino	101 (74.3%)	220 (79.4%)	24 (77.4%)	
Not reported	30 (22.1%)	50 (18.1%)	6 (19.4%)	
Subtypes				< 0.001
Basal-like	14 (10.3%)	81 (29.2%)	6 (19.4%)	
HER2-enriched	4 (2.9%)	36 (13%)	2 (6.5%)	
Luminal A or Normal like	78 (57.4%)	100 (36.1%)	13 (41.9%)	
Luminal B	40 (29.4%)	60 (21.7%)	10 (32.3%)	
Pathological stage				0.599
Stage I	25 (18.4%)	56 (20.2%)	8 (25.8%)	
Stage II	82 (60.3%)	175 (63.2%)	16 (51.6%)	
Stage III	29 (21.3%)	46 (16.6%)	7 (22.6%)	

P values were estimated using Fisher's exact or the chi-square test.

HER2: human epidermal growth factor receptor 2.

Variables	Controls, n = 136	Low-risk, n = 277	High-risk, n = 31	<i>P</i>
Treatment				
Radiation	75 (55.1%)	151 (54.5%)	17 (54.8%)	0.993
Pharmaceutical	118 (86.8%)	225 (81.2%)	20 (64.5%)	0.014
All-cause mortality	4 (2.9%)	7 (2.5%)	5 (16.1%)	0.006
<i>P</i> values were estimated using Fisher's exact or the chi-square test.				
HER2: human epidermal growth factor receptor 2.				

Table 4 presents the results of the Cox proportional hazard regression analysis for overall survival. The effect of group category (control, low risk, or high risk) and known prognostic factors (i.e., age group, molecular subtypes, pathologic stages, and treatment characteristics) on overall survival were analyzed. Compared with controls, the high-risk group had a significantly higher risk of poor overall survival. The estimated hazard ratios were 9.60 (95% CI = 2.43–37.96, $P = 0.001$) and 9.94 (95% CI = 2.24–44.20, $P = 0.003$) in the univariate and multivariate models, respectively. The low-risk group did not have a significantly higher risk of poor overall survival than did controls.

Table 4
Cox proportional hazard regression analysis for overall survival in patients with early-stage IDC breast cancer.

Variables	Crude-HR (95%CI)	<i>P</i>	Adjusted-HR (95% CI)	<i>P</i>
Gene mutation risk subgroup				
Controls	Ref		Ref	
Low-risk	1.17 (0.34–4.07)	0.801	1.01 (0.25–4.05)	0.993
High-risk	9.60 (2.43–37.96)	0.001	9.94 (2.24–44.20)	0.003
Age group (years)				
< 35	Ref		Ref	
35–50	0.20 (0.02–1.97)	0.166	0.28 (0.02–3.30)	0.313
> 50	0.54 (0.07–4.24)	0.560	0.54 (0.06–5.21)	0.593
Subtypes				
Luminal A or Normal like	Ref		Ref	
Luminal B	2.82 (0.77–10.34)	0.118	1.76 (0.44–7.04)	0.422
HER2-enriched	3.59 (0.83–15.60)	0.088	3.66 (0.70–18.99)	0.123
Basal-like	1.30 (0.31–5.50)	0.722	1.14 (0.24–5.33)	0.872
Pathological stage				
Stage I	Ref		Ref	
Stage II	4.06 (0.52–31.82)	0.183	6.58 (0.67–64.22)	0.105
Stage III	5.56 (0.62–50.28)	0.127	9.04 (0.75–109.58)	0.084
Treatment				
Radiation vs. without radiation	0.75 (0.28–2.03)	0.577	0.58 (0.19–1.76)	0.336
Pharmaceutical vs. without pharmaceutical	0.33 (0.10–1.07)	0.065	0.39 (0.11–1.40)	0.151
HER2: human epidermal growth factor receptor 2, HR: Hazard Ratio, CI: Confidence Interval, Ref: Reference.				

Discussion

This study adopted an integrative approach in the identification of candidate genes for risk assessment of overall survival in patients with early-stage breast cancer. Mutations in the 10 candidate genes were used to identify the patient group with a higher risk of poor overall survival. These 10 genes mostly participate in cell-cycle processes, DNA repair, and drug resistance.

BIRC5 (baculoviral IAP repeat containing 5) is amplified in 15–30% cases of breast cancers. It is involved in the prevention of apoptotic cell death through negatively regulating inhibitor of apoptosis proteins and has been indicated as an independent prognostic factor in breast cancer [19, 20]. *NOTCH1* (Notch homolog 1) is a protein coding gene contributes to multiple cellular processes, including cell differentiation, proliferation, and survival [21]. High expression of *NOTCH1* may induce breast carcinoma and is strongly associated with poor prognosis and survival outcomes in breast cancer [14, 22]. *MLH1* and *MSH2* are mismatch repair genes involved in repairing DNA replication errors. Defects in *MLH1* can contribute to breast cancer progression [23].

CCND1 (B-cell leukemia/lymphoma 1) is amplified in approximately 15% cases of breast cancers and is strongly associated with the expression of estrogen receptors, particularly in node-positive breast cancer [24]. In addition, the coamplification of *CCND1* and *EMSY* (BRCA2-interacting transcriptional repressor) is associated with poor treatment outcome in ER-positive breast cancer [25]. *ESR1* (estrogen receptor 1) encodes ER alpha, an estrogen receptor, and is amplified in 5–10% of breast cancers [12, 26]. The overexpression of ER alpha protein could be used as a prognostic factor for ER-positive breast cancer and is predictive of endocrine therapy responsiveness [27]. *SRC* [V-Src Avian Sarcoma (Schmidt-Ruppin A-2) Viral Oncogene Homolog] encodes the non-receptor protein tyrosine kinase, which plays a role in the regulation of cell proliferation, migration, and survival [28]. The SRC mutation is known to be involved in malignant progression [29, 30]. Increased SRC activation is correlated with trastuzumab resistance and interacts with estrogen receptors. Therefore, the combined use of SRC inhibitors has been suggested to overcome drug resistance in specific breast cancers [31].

HRAS (Harvey rat sarcoma viral oncogene homolog) and *KRAS* (Kirsten rat sarcoma 2 viral oncogene homolog) are oncogenes in the RAS family and are involved in 0–10% of breast cancers. RAS mutations are typically associated with poor survival; one study found that patients with RAS mutations had worse survival [32]. The intracellular actions mediated by *IGF1R* (insulin-like growth factor 1 receptor) are also involved in RAS signaling pathways [33]. *IGF1R* is a receptor with tyrosine kinase activity and its expression is associated with tumorigenesis [33]. Moreover, *IGF1R* overexpression is more common in ER-positive breast cancer. *IGF1R* expression is generally low in the HER2-enriched subtype and is heterogeneous in the basal-like subtype [33]. In addition, *IGF1R* may be used as an alternative therapeutic target for HER2-enriched, trastuzumab-resistant, or basal-like breast cancer [34, 35].

According to the past studies, the candidate genes are generally involved in breast cancer progression. Some are related to drug resistance in specific subtypes. Disease progression and drug resistance are commonly associated with poor overall survival due to the uncontrollable nature of cancer cells [36–38]. This study had several limitations. The retrospective nature of this study prevented the inclusion of

certain covariates that might be associated with overall survival. Despite this, we have included information on the baseline demographic, tumor characteristics, molecular subtypes, and treatment characteristics used in most breast cancer studies. In addition, we included only patients with early-stage IDC breast cancer; therefore, the generalizability of our findings is limited to this subpopulation.

Conclusion

This study used integrated survival analysis to identify candidate genes in a group of patients with early-stage IDC breast cancer at high risk for poor survival outcomes and achieve further understanding of the effects of their gene mutation profiles on overall survival. The candidate genes are particularly involved in cell-cycle processes, DNA repair, and drug resistance, and their mutations are generally associated with disease progression or therapeutic resistance, which are commonly associated with poor survival outcomes. Future studies should investigate the integrative functionality of candidate genes in breast cancer further. Moreover, the combined use of machine learning algorithms should be the focus of further research on the nonlinear or high-order interaction effects between clinicopathological variables and the involved genes.

Abbreviations

IDC

Invasive ductal carcinoma; HER2:human epidermal growth factor receptor 2; DNA:deoxyribonucleic acid; HR:hazard ratio; CI:confidence interval; Ref:Reference.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

<https://portal.gdc.cancer.gov/projects/TCGA-BRCA>

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

C-CH and S-HM and drafted the manuscript. C-CH and H-IH were responsible for the data processing and interpretation. H-IH and S-HM performed statistics analyses. S-HM conceived and designed the study and fully revised the final manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Gene mutation profiles and clinical features of early-stage IDC breast cancer.

Figure 2

Kaplan–Meier plot of overall survival in patients with early-stage IDC breast cancer according to (a) gene mutation risk groups and (b)–(k) single gene mutation status.