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ARTICLE

Homologous recombination repair pathway alteration and its association with survival of breast cancer patients

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Breast cancer is a highly heterogeneous neoplasm with different response to chemotherapy. In this study, we investigated if homologous recombination repair (HRR), one of the important pathways of DNA damage repair, could serve as biomarkers for breast cancer. Breast cancer patients were selected from the Cancer Genome Atlas (TCGA) database. Data of RNA-seq or mutation alteration of HRR pathway-related genes were extracted and analyzed. Correlations between HRR pathway mutation and clinicopathological features of breast cancer were analyzed using chi-square test. Based on the Kaplan-Meier method and log-rank test, survival analysis was done to identify the correlation between each HRR gene and survival rates. Using data retrieved from TCGA database, 1108 cases were identified of breast cancer with full data on RNA-seq and 986 cases with full data on mutation. We demonstrated that high expression of HRR gene RAD50, RAD51, RAD51C, RAD54L and XRCC2 were associated with favorable prognosis (Log-rank P=0.02686, 0.03734, 0.00664, 0.01147 and 0.01818, respectively). Moreover, mutation in the HRR pathway was present in 15.0% of cases. RIM1, PPP4R2, PPP4R4, RAD50 and RAD51D gene mutation were associated with unfavorable outcome (Log-rank P=0.0346, 0.0051, 0.0326, 0.0213 and 0.0007, respectively). The N stage and estrogen receptor (ER) status were significantly related to HRR pathway mutation (all factors P<0.05). Additionally, basal-like breast cancer subtype took up more percentage in HRR pathway mutation patients. Low expression or mutation in HRR pathway were associated with unfavorable prognosis in breast cancer. HRR pathway could serve as potential predictor, emphasizing the significance of more research on HRR pathway genes to facilitate more profound clinical implications in breast cancer molecular treatment.

Keywords: breast cancer; homologous recombination repair; expression; mutation

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Breast cancer is an important public health threat to women worldwide and is the second leading cause of cancer-related morality, accounting for an estimated 268,600 new cases and 41,760 deaths in 2019 [1]. Multiple studies have confirmed that breast cancer is a highly heterogeneous malignant tumor which is constitutive of different subtypes according to estrogen receptor (ER) status, progesterone receptor (PR) status and HER2 status, with diverse clinical performance, biological characteristics and prognosis [2]. Despite great improvement have been achieved in breast cancer treatment,

which includes chemotherapy, radiation therapy, endocrine therapy, molecular targeted therapy and the emerging immunotherapies, response to therapies varied widely [3]. Therefore, identification of novel biomarkers for therapeutic targets and efficacy prediction of breast cancer is urgently required.

Alterations in DNA damage response, especially in homologous recombination repair (HRR), are discovered in various cancers and have been targeted effectively. For example, the poly(ADP-ribose) polymerase (PARP) inhibitor has exhibited remarkable benefit in BRCA1/2 mutated ovarian and breast cancer [4-6]. The intrinsic mechanism underlying this novel molecular inhibitor could be the effect of synthetic lethality. In normal cells, DNA damage breaks (DSBs) are immediately repaired with a proficient HRR mechanism. However, in tumors lacking HRR, the left un-repaired DNA damage could not be effectively restored, which ultimately accumulates and lead to cell death [7]. Moreover, Guo et al indicated that certain biomarkers in HRR could predict response to chemotherapy and select potential patients in early-phase treatment [8]. However, as far as we known, most of HRR genes, except BRCA1/2 [9], have not yet been fully understand to date.

On the other hand, the mainstream studies of BRCA1/2 mainly focused on triple-negative breast cancer, which was characterized with ER negative, PR negative and HER2 negative and associated with elevated gene mutation [10, 11]. Nevertheless, Timms et al established that HRR showed close association with BRCA1/2 mutation regardless of breast cancer subtype [12]. Hence, more expanded studies are needed to facilitate the precise clinical applications of HRR in all breast cancer subtypes.

In this study, The Cancer Genome Atlas (TCGA) was investigated to examine the prevalence and the clinical significance of HRR pathway alteration in breast cancer cases. HRR pathway alterations were elaborated from two aspects of mRNA expression and mutation respectively. We demonstrated that low expression or mutation in HRR pathway were associated with unfavorable prognosis in breast cancer, indicating the importance of more research on HRR pathway in breast cancer molecular treatment.

Materials and Methods

Patients and Samples

Breast cancer tissue samples with RNA-seq data, mutation data and clinicopathological information were downloaded from the cancer genome atlas (TCGA) database released on October 31, 2019. The HRR-related genes included in the study were BLM, BRCA1, BRCA2, DMC1, EME1, EME2, GEN1, HFM1, MRE11, MUS81, NBN, PPP4C, PPP4R1, PPP4R2, PPP4R4, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54B, RAD54L, RAD54L2, RDM1, RECQL, RECQL4, RECQL5, RMI1, RMI2, RPA1, RPA2, RPA3, SEM1, SLX1A, SLX4, PPP4R3A, PPP4R3B, SPO11, TOP3A, TOP3B, WRN, XRCC2 and XRCC3. All patients' clinicopathological information including age, menopause status, T stage, N stage, M stage, tumor-node-metastasis (TNM) stage, ER status, PR status, HER2 status and OS data were comprised. OS was measured from the date of diagnosis to the date of death due to all causes or the last follow-up.

Statistical analysis

The gene expression data were expressed as mean standard variation. Samples with full data on mutation alteration of HRR pathway were included in the study and were categorized into two groups, the first being wild (no alteration) and the second being mutation (any gene mutation in the HRR pathway). Correlations between HRR gene mutation status and clinicopathological features of breast cancer were analyzed through using chi-square test. For survival analysis based on expression of a single gene, patients were dichotomized as having low or high expression using a gene-specific cut-off value which was generated from the lowest log-rank P value of a series of 10 cut-offs spaced evenly between the maximum and minimum expression value. Survival analysis was performed using the Kaplan-Meier method and the log-rank test. All P values were two sided, and $P<0.05$ was considered statistically significant. All the statistical analysis was performed using SPSS version 22.0 software (IBM Corp.) and R 3.4.2.

Results

Homologous recombination repair pathway gene expression level in breast cancer patients

Analysis of gene mRNA expression and overall survival was conducted in 1108 tumor samples with complete RNA-seq data retrieved from TCGA. The HRR pathway gene list was mainly based on a published article that summarized the genes related to homologous recombination repair, which including 44 genes (Figure 1A). Most of the HRR genes has not been fully studied to date. Therefore, the association of each HRR gene and OS was analyzed. We reported that that high expression of five HRR gene RAD50, RAD51, RAD51C, RAD54L and XRCC2 were significantly associated with favorable prognosis (Log-rank P=0.02686, 0.03734, 0.00664, 0.01147 and 0.01818, respectively, Figure2A-E) in breast cancer samples.

Homologous recombination repair pathway gene mutation in breast cancer patients

Analysis of HRR gene mutation and OS was conducted in

A

Pathway	Genes
HRR (n = 44)	BLM, BRCA1, BRCA2, DMC1, EME1, EME2, GEN1, HFM1, MRE11, MUS81, NBN, PPP4C, PPP4R1, PPP4R2, PPP4R4, RAD50, RAD51, RAD51B, RAD51C, RAD51D, RAD52, RAD54B, RAD54L, RAD54L2, RDM1, RECQL, RECQL4, RECQL5, RMI1, RMI2, RPA1, RPA2, RPA3, SEM1, SLX1A, SLX4, PPP4R3A, PPP4R3B, SPO11, TOP3A, TOP3B, WRN, XRCC2, XRCC3

B

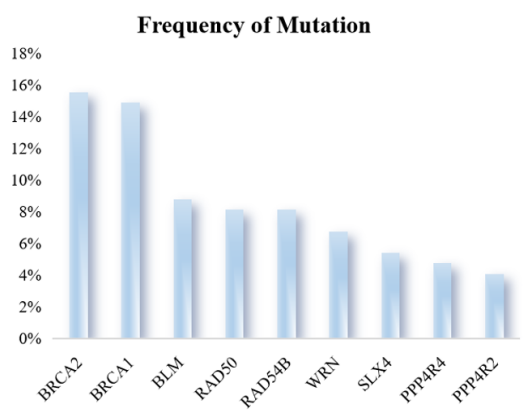

Figure 1. A: Gene list of HRR pathway. B: TOP 10 mutated genes in HRR pathway.

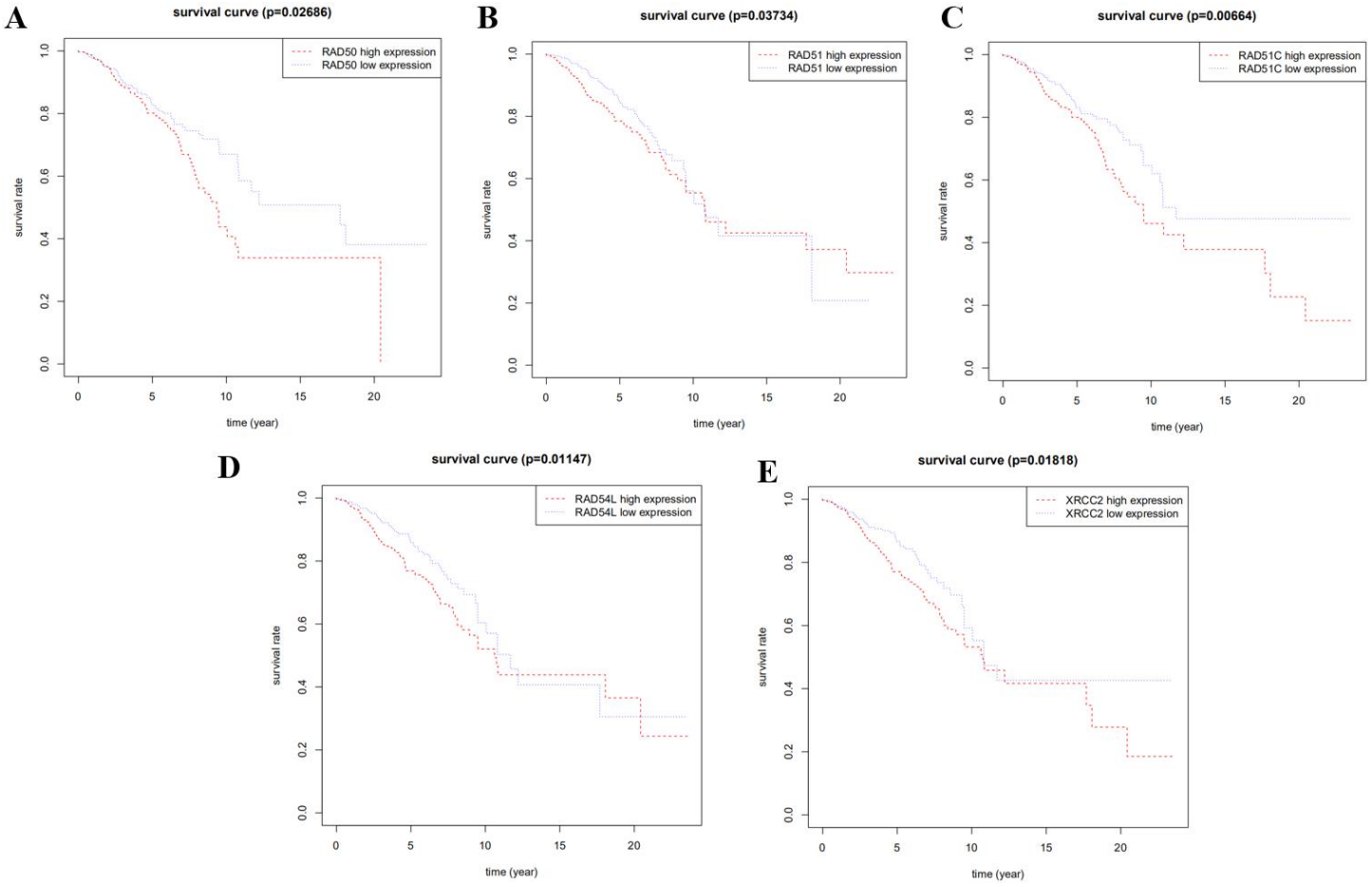

Figure 2. Analysis of homologous recombination repair pathway gene expression and Kaplan-Meier estimates of overall survival in breast cancer patients. A: RAD50. B: RAD51. C: RAD51C. D: RAD54L. E: XRCC2.

968 tumor samples. The overall incidence of HRR pathway mutation of breast cancer in the TCGA database was 15.0%. Among all the altered genes, the most frequently involved genes were BRCA1, followed by BRCA2, BLM1, RAD50 and RAD54B (15.5%, 14.9%, 8.8%, and 8.1%, respectively, Figure1B). We analyzed each gene mutation and its relation with overall survival. RIM1, PPP4R2, PPP4R4, RAD50 and RAD51D gene mutation were significantly associated with unfavorable outcome in breast cancer patients (Log-rank P=0.0346, 0.0051, 0.0326, 0.0213 and 0.0007, respectively,

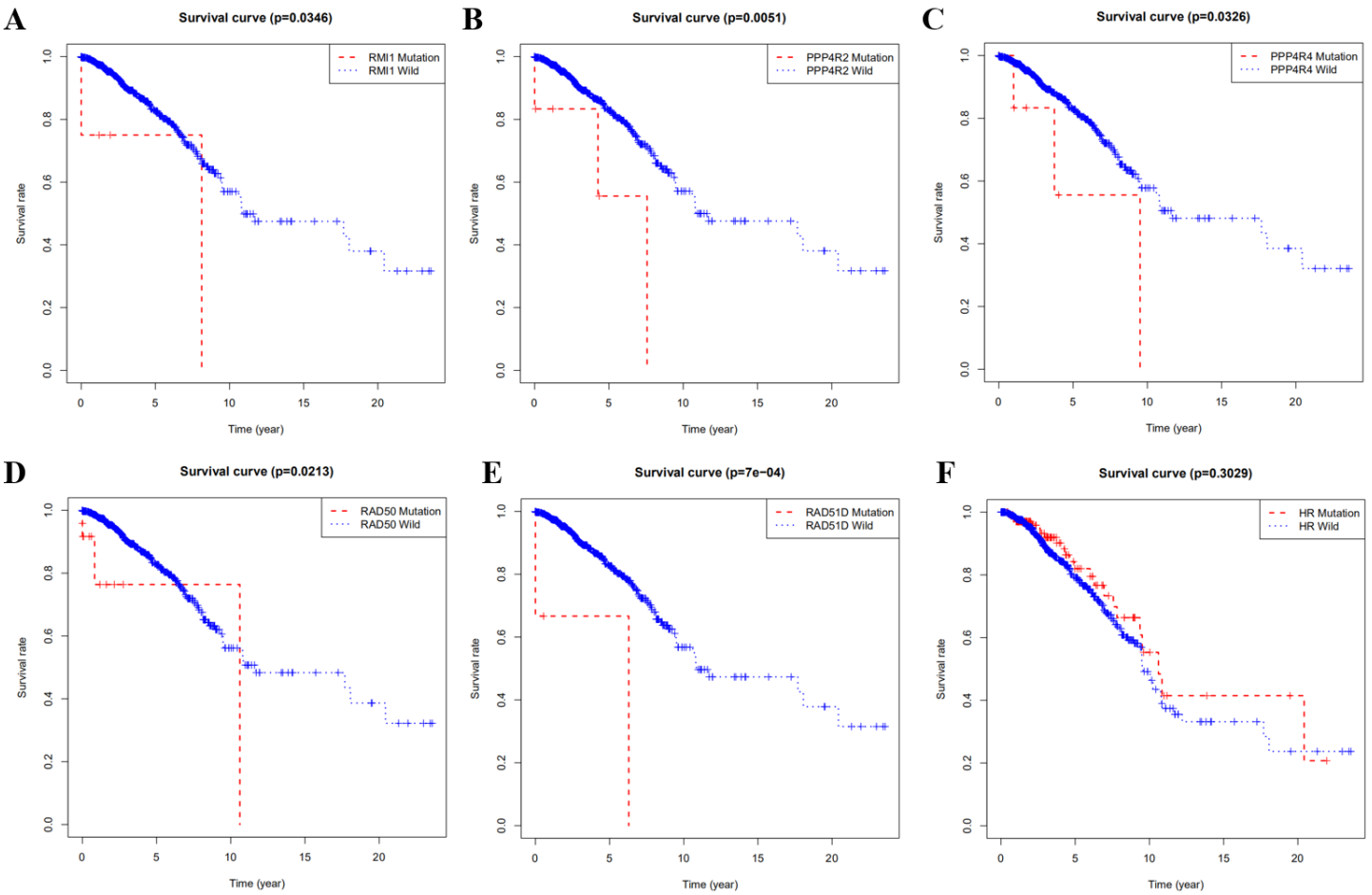

Figure 3. Analysis of homologous recombination repair pathway gene mutation status and survival in breast cancer patients. A: RIM1. B: PPP4R2. C: PPP4R4. D: RAD50. E: RAD51D.

Figure3A-E). Furthermore, we merged each HRR gene mutation to define the HRR pathway mutation and demonstrated that the mutation of HRR pathway was not significantly associated with overall survival in breast cancer (P=0.3029, Figure3F).

Relationship between homologous recombination repair pathway gene mutation status and clinicopathological features

We further evaluated whether HRR pathway gene mutation was associated with the clinical development and progression of breast cancer. The clinicopathological features of HRR pathway mutation or wild patients were summarized in table1. The N stage (c2=6.677, P=0.010) and estrogen receptor (ER) status (c2=4.280, P=0.039) were significantly related to HRR pathway mutation status (Table 1). However, the age, menopause status, T stage, M stage, PR status and HER2 status had no significant correlation with HRR pathway mutation (Table 1). Furthermore, we evaluated if the pathological types of breast cancer were related to HRR mutation status. In HRR pathway mutation samples, basal-like type breast cancer ranked 3rd and accounted for 23.58% of all breast cancer types, compared with 4th and 13.59% in HRR pathway wild breast cancer patients (Figure4A-B).

Discussion

The hereditary breast cancer is associated with the presence of germline mutations in BRCA1 or BRCA2 genes, which both taking part in the HRR pathway to repair DNA damage and maintaining genomic stability. Biomarkers of HRR have been investigated in breast cancer to determine if they might be able to select patients for therapies targeting tumors with defective DNA repair, including platinum and PARP inhibitors [13].

In the present study, we investigated the clinical implications of the alteration of HRR pathway in breast cancer using the TCGA database. We illustrated that alterations of HRR gene RAD50 and RAD51 were significantly associated with prognosis in breast cancer. As one of the important com-

Table 1. Relationship between homologous recombination repair pathway mutation and clinicopathologic characteristics of breast cancer patients

Variables	Pathway mutation (n=148)		Pathway wild (n=838)		χ^2	*P*-value
	No.	%	No.	%		
Age					1.273	0.259
≤59 years	74	50.0	461	55.0		
>59 years	74	50.0	377	45.0		
Menopause status					1.497	0.473
pre	29	19.6	184	22.0		
post	96	64.9	520	62.1		
peri	3	2.0	31	3.7		
Missing	20		103			
T stage					0.040	0.842
T1-T2	124	83.8	705	84.1		
T3-T4	24	16.2	130	15.5		
Missing	-		3			
N stage					6.677	**0.010**
N0	83	56.1	375	44.7		
N1-3	62	41.9	447	53.3		
Missing	3		16			
M stage					0.421	0.516
M0	122	82.4	704	84.0		
M1	4	2.7	16	1.9		
Missing	22		118			
TNM stage [b]					2.617	0.106
I-II	118	79.7	613	73.2		
III-IV	28	18.9	209	25.0		
Missing	2		16			
ER status [c]					4.280	**0.039**
negative	36	24.3	111	13.2		
positive	80	54.1	394	47.0		
Missing	32		333			
PR status [d]					2.641	0.104
negative	46	31.1	158	18.9		
positive	71	48.0	344	41.1		
Missing	31		336			
HER-2 status [e]					1.666	0.197
negative	90	60.8	427	51.0		
positive	21	14.2	70	8.4		
Missing	37		341			

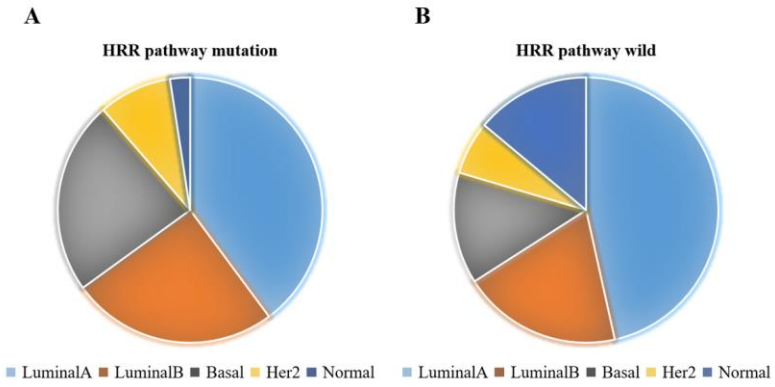

Figure 4. Percentages of different subtypes of breast cancer in HRR mutation (A) and wild patients (B).

ponents of multi-protein complex MRE11-RAD50-NBS1 (MRN), Rad50 is a DNA damage repair protein, acting as the main sensor of DSB. MRN involved in two major pathways after DNA damage repair (NHEJ, non-homologous recombination repair and HRR, homologous recombination repair), and plays an important role in telomerase maintenance, normal mitosis and cell cycle regulation [14]. In addition, Rad51, which has many homologs of XRCC2, XRCC3, Rad51B, Rad51C, Rad51D, Rad52 and Rad54, contributed to the HRR process. Previous studies have shown that Rad51 protein and its homologs played an important role in catalyzing homologous pairing in vivo. The formation of Rad51 nuclear foci after DNA damage was particularly necessary for homologous recombination repair [15]. Therefore, taken together, our results implicated that RAD50 and RAD51 showed promising potential to serve as clinical predictors in breast cancer treatment of DNA damage agents, which warrants future prospective investigation.

Interestingly, low expression and mutation of HRR genes was associated unfavorable outcome in breast cancer. However, some studies shown that Rad51 gene mutation play a weak role in the evolution of tumors, so the potential relationship between them is probably resulting from transcription and protein level. The specific mechanism of them still remained controversial [16]. It might be speculated that multiple signaling pathways and physiological processes of cells will be activated due to DNA damage caused by various carcinogenic factors. Tumors occur because the DNA repair system cannot maintain a relatively balanced state with the physiological processes of the cell for a long time.

Moreover, clinicopathological analysis identified that N stage and ER status were significantly related to HRR pathway mutation status, with basal-like type breast cancer taking up more percentage in HRR pathway mutation patients. Basal-like breast cancer was particularly aggressive in biological characteristics. Chandler et al reported that basal-like breast cancer could impair HRR and repair efficiency through a small molecular inhibitor, and decreased the formation of Rad51 foci [17]. Therefore, this type of breast cancer might benefit more from HRR pathway.

Additionally, our study had some deficiencies in some aspects. On one hand, samples of HRR mutations are not large enough, which would lead to study bias. On the other hand, homologous recombination deficiency has some standard measurement, which consists of HRD-loss of heterozygosity score (LOH), HRD-telomeric allelic imbalance score (TAI), and HRD-large-scale state transition score (LST) [18]. Therefore, our statistical analysis has many limitations, which required more experimental and clinical researches in the future.

As HRR might be the ideal candidate to select patients who may benefit from treatment of PARP inhibitors and other DNA damage molecular therapies, Some studies indicated that tumors harboring HRR are supposed to have a higher number of mutations, hence a higher tumor mutational burden, which could potentially make them more sensitive to immunotherapy [19]. Therefore, further studies are needed to validate novel functional biomarkers in the clinic. Our data suggest that these biomarkers should potentially be considered independently in future clinical trials.

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Conflicting interests

The authors have declared that no conflict of interests exist.

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