Exploring the true immunotherapy candidates to acquire the promising immunological response in breast carcinomas

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Research

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Abstract

Background: Immune checkpoint blockade of immune therapy has been applied in multiple systemic malignancies, and have improved the overall survival to a greater extent, whether it will apply to breast cancer remains unknown.

Methods: We endeavored to explore the possible factors that may influence the breast carcinoma associated immune therapy outcomes with using several public databases.

Results: The possible treating target of TNF superfamily member 4 (TNFSF4), was selected from massive candidates for its abnormal expression profile, survival associated status, immune system reactions predication. For the first time, we revealed the oncogenic features of TNFSF4 in breast carcinoma, and TNFSF4 was further revealed to be closely related to anti-tumor immunity treatment, including multiple immune effector molecules and T-cell signatures, which was independent on endocrine status, and has not ever been referred to. Moreover, the immune treatment of TNFSF4 blockade also indicated the underling effects on stem cells' expansion, which more strongly and specifically demonstrated the powerful effects of applying the TNFSF4 blockade based immune therapies in breast carcinomas.

Conclusions: For the first time, we aim to dig out the embedding stars that may contribute to breast cancer therapies, and provide one potential but crucial potent for treating breast carcinoma indistinguishably, and long-term effective.

1. Introduction

Breast carcinoma treatments have been evolving for years accompanying with emergence of kinds of reagents referring to endocrine therapy, targeted therapy, and also with the chemotherapeutics improvement. However, the therapying mode seems to encounter with the bottleneck, what road should it be to further prolong the life expectance is in the mist [1–3]. Could we draw lessons from the experience of immune therapy?

Improving the cancer related immune resistance is mainly the immune checkpoint blockade therapy, and multiple immune checkpoints agents have been identified and clinically applied in treating lung cancer, esophageal cancer, melanoma, et al. The therapy targets included but not limited to PD-L1(CD274), PD-L2 (PDCD1LG2), CD80, CD86, CD70, et al, and the novelty applied therapy has put a prospect to lung cancer and melanoma treatments [4–7]. However, how did the immune blockade therapy acted in breast cancer treatment process, and what check point may bring the benefits were totally unknown. Recently, CCR9 was demonstrated to exert strong immune-regulatory effects on T cell responses in multiple tumors, and through inhibiting the TCR signaling, CCR9 regulates STAT signaling in T cells, resulting in reduced T-helper-1 cytokine secretion. Unlike PD-L1, inhibition of CCR9 expression on tumor cells facilitated immunotherapy by tumor-specific T-cells in vivo [8]. However, whether and how such immune checkpoints are involved in the prognosis and therapeutic efficacy of breast cancer remain largely unclear.

Therefore, this study aimed to clarify the clinical significance of immune therapies, especially the uncertain checkpoint blockade efficacy in breast cancer patients, and to probably propose more suitable strategies of improving breast cancer treatments of applying immunity.

2. Methods And Materials

To analyze the immune checkpoint-related prognosis in breast cancer, breast cancer genomics related datasets in TCGA (http://cancergenome.nih.gov), International Cancer Genome Consortium (ICGC, https://icgc.org), were individually collected, and subsequently subjected to a bioinformatics analysis by web servers, Gene Expression Profiling Interactive Analysis 2 (GEPIA2, http://gepia2.cancer-pku.cn)[9, 10], cBioPortal for Cancer Genomics (http://www.cbioportal.org)[11, 12], and Tumor and Immune System Interaction Database (TISIDB, http://cis.hku.hk/TISIDB)[13, 14], respectively. In detail, GEPIA2 was used to calculate the prognostic indexes, including the differential expression, pathological stage, gene correlation, and patient survival; cBioPortal was used to conduct visualization and comparison of gene alterations; TISIDB was used to explore the correlation between abundance of immunomodulators and expression of inquired genes. OS and DFS analyses were performed using the Kaplan-Meier method with 50% cut-off for both low- and high-expression groups. Log-rank test, also known as the Mantel-Cox test, was used for hypothesis test. The Cox proportional hazard ratio (HR) and the 95% confidence interval information were also included in the survival plots. A P-value < 0.05 was considered to be statistically significant. Spearman method was used to analyze the pair-wise gene expression correlations, and a P-value < 0.05 was considered statistically significant. The correlated degree was identified by the absolute value of the correlation co-efficient, detailed classification: ≤0.4, weak; 0.41–0.60, moderate; 0.61–0.80, strong; and 0.81-1.0, very strong. The co-occurrence and mutual exclusivity of genetic alteration between inquired gene and each immune checkpoint was determined by log2 odds ratio, P-value, and Q-value. A Q-value < 0.05 was statistically significant. The investigated immune-inhibitors were collected according to Charoentong's study, and each Spearman correlation between inquired gene and a distinct immune-inhibitor in an individual cancer type was integrated into the indicated heatmap.

3. Results

3.1 Screening the possible immunity functions related anti-cancer treatment.

Nearly all implicated immune checkpoints were browsed and screened for possible immunotherapies values, and the representative immunity therapeutics of ADORA2A, BTLA, Nectin-2 (CD112), CD160, CD244, PD-L1 (CD274), CD96, CSF1R, CTLA4, HAVCR2, IDO1, IL10, IL10RB, KDR, KIR2DL1, KIR2DL3, LAG3, LGALS9, PDCD1, PDCD1LG2, PVRL2, TGFB1, TGFBR1, TIGIT, VTCN1, TNF Receptor Superfamily Member 14 (TNFRSF14), TNF superfamily member 4 (TNFSF4), TNF superfamily member 18 (TNFSF18), were all input for possible effects predication in the integrated repository portal for tumor-immune system interactions systems at TISIDB [15]. Primarily, the PD-L1, CD112, TNFRSF14, TNFSF4, TNFSF18, CD48, and LGALS9 were selected for their significantly and differently expressed patterns, and the illustrating

body-map was shown in Fig. S1, the paired red and green color indicated the expression patterns in multiple organs and systems.

Further, the PD-L1 (Fig. 1A), CD112 (Fig. 1B), TNFRSF14 (Fig. 1C), TNFSF4 (Fig. 1D), TNFSF18 (Fig. 1E), CD48 (Fig. 1F), and LGALS9 (Fig. 1G) were analyzed for their abnormally expressed styles in breast carcinomas. Specifically, CD112, TNFSF4, TNFSF18, and LGALS9 were significantly overexpressed in breast carcinomas, strongly suggested the potent and valuable effects.

3.2 Immunotherapeutic targets participated in multiple breast carcinogenesis through interacting with the key cancer stimulating factors

The heterogenetic features of breast cancer determined that breast carcinomas of different hormone receptors status may benefit from different and specific treating strategies. As a double-edged sword, one specific agent will not function in another kind of breast carcinoma, and to dig out the potentials of immune blockades therapies, the native expression signatures and correlations were studied through using the CBIOPORTAL and the GEPIA2 (GENE EXPRESSION PROFILING INTERACTIVE ANALYSIS). The expression heatmap indicated the expression patterns of the candidates in red bar, and the selected representative genes described in section 3.1 were labeled with RED STAR (Fig. 2A), and the analyzing chart flow was shown in Fig. 2B. All the enrolled factors were input and studied for potential functional correlations (Fig. S2). Specifically, the main carcinogens of ERBB2, KRAS, TP53 were analyze for their intrinsic connection in breast carcinomas, and the promising correlations between TNFSF4 and the ERBB2, KRAS, TP53 were all confirmed (Fig. S3, Table 1).

In general, nearly all the immunotherapeutic targets showed expanded expression intervals referring to ESR1 and PGR, two of which tended to react well to formal and anti-hormone treatments, indicating that immunotherapy may compensate the current deficiencies. More importantly and interestingly, TNFSF4, TNFSF18, CD48, all showed aggregated expressions in breast carcinomas bearing no ERBB2, ESR1, PGR expression, strongly suggested that the immune therapy may cover all types of breast cancer, and the partial enlarged drawing was shown in Fig. 2C. In detail, TNFSF4, TNFSF18 and CD48 showed exotic expression to all kinds of breast cancer carcinogens, including those without KRAS (Fig. 2D), TP53 (Fig. 2E), ERBB2 (Fig. 2F) expressing and activating, the three of which dominated the survival expectance and drawn the dark survival expectance.

3.3 Evaluation of the cluster of TNFSF4, TNFSF18, CD48 and LGALS9 as the immune therapeutics targets.

The clinical significance of the cluster of TNFSF18 (Fig. 3A), LGALS9 (Fig. 3B), TNFSF4 (Fig. 3C) and CD48 (Fig. 3D) were also analyzed for their values referring to immune therapeutics targets, and the TNFSF4, TNFSF18 showed greater significances in predicating the disease-specific survival and the overall survival. KRAS stimulated the carcinogenesis greatly and determined the anti-cancer treatment response, we further explored its correlation with TNFSF18 (Fig. 3E), LGALS9 (Fig. 3F), TNFSF4 (Fig. 3G)

and CD48 (Fig. 3H), and the positive correlation between TNFSF4 and KRAS strongly suggested the prospect therapy effects of using TNFSF4 as the manipulating or the blocking targets.

Stem cells groups were considered as the root for cancer recurrence for their steady status and super renewal ability. We previously identified the stem-like ALDH1A1+ cells in breast cancer groups [16-18], and in this study, we noticed the close correlation between TNFSF4 and ALDH1A1 expressions (Fig. 3I), the latter of which indicated the poorer survival length (Fig. 3J) and progression free length (Fig. 3K). These results strongly proved that the oncogenic TNFSF4 may be one effective immunotherapeutic target, and the proposed survival benefits may also function through repressing the stem cells expansion, fully assure the anti-TNFSF4 treatments' results. The other candidates of CD112, TNFRSF14, PD-L1 failed to be involved in further analysis, as their negative survival indicating roles (Fig. S4).

3.4 The mechanistic procedure that TNFSF4 was assumed to function through

The immune system was locked when carcinoma is getting aggressive, and when the immune-function inhibitors were unlocked (immune blockades), the active immune cells begin to infiltrate and execute the cellular order (Fig. 4A). The whole cancer groups, including the heterogenetic subtype cells of cancer stem cells, will be perished under the immune system attack. To undermine the crucial role of the selective immune target, the lymphocytes infiltrating functions and the connective functional factors were analyzed respectively for underlining the mechanisms. Both the ALDH1A1 (Fig. 4B) and TNFSF4 (Fig. 4C) overexpression correlated with more active lymphocytes. However, the guardian soldiers were disabled with the highly expressed immune-inhibitors in cells with increased ALDH1A1 (Fig. 4D) and TNFSF4 (Fig. 4E). These results indicated that TNFSF4 will potentially reactive the immune response, and will partially function through precisely demilitarize the stem cells.

3.6 Promising immune therapies response are the stem cells signatures associated predicating mode

The immune therapies response and effects were believed to be correlated with lymphocytes activation and infiltration, and we first assessed the immune therapies effects in multiple systems, treatments targeted at TNFSF4 tended to implicit the better outcomes (Fig. 5A). Also, the TNFSF4 associated TP53 (Fig. 5B), KRAS (Fig. 5C), and ERBB2 (Fig. 5D) all indicated better immune therapy response, which further consolidated the crucial position of TNFSF4.

TNFSF4 and ALDH1A1 were confirmed for their participants in immune activation therapies, and TNFSF4 treatment predicated the prospect therapy response. Immune therapy consistently repressed the tumor growth once the immune system was activated, and in above results we further found that TNFSF4 associated therapy may also influence the stem cells expansion. Increased ALDH1A1 expression indicated shorter disease specific survival (Fig. 5E) and overall survival (Fig. 5F), and in clinical assessing, and the ALDH1A1 surprisingly correlated with higher therapy responds ratios (Fig. 5G), which has not ever been reported and analyzed.

Associations between ALDH1A1 expression and immune subtypes across human cancers were all included for analysis, and ALDH1A1 expression dominated in all the subtypes (Fig. 5H), participating in multiple immune reaction process (Fig. 5I). Associations between ALDH1A1 expression and molecular subtypes across human cancers were also identified (Fig. 5J), and the signature of increasing ALDH1A1 tended to express in all kinds of breast carcinomas (Fig. 5K), indicating the universal therapeutic responsive role of ALDH1A1 referring to better outcomes.

4. Discussion

The treatment modes of breast carcinoma were evolving consistently, and were also in accordance with the newly confirmed molecular and biological findings, which refigured the treating manners. Shifting treating strategies may spread the benefits in various carcinomas, however, the immune therapy did not cover most malignancies yet. Several specific contributions were identified for breast carcinogenesis, and the related therapies referring to targeting or relieving the malignant process have greatly rescue the lives. For now, the endocrine therapy, the anti-Her-2 therapy, the improved chemotherapy have all prolonged the survival outcomes. We have to confront the fact that the basal like breast carcinoma, which are always identified as triple-negative carcinoma, were treated without any endocrine factors and precise targets. As an example, immune therapy was used across the squamous, adenocarcinoma, and small cells of lung cancers, indicating the universal inhibitions. To explore the potential and effective immune targets, we first input all the related immune therapy related functional factors, and after screening in all kinds of breast carcinomas, several candidates were selected, and their correlation with aggressive breast carcinogens were analyzed for evaluating the possible effects of targeting therapy. To finally confer the most prospect immune therapy candidate, the selective was assessed for clinical significance (Fig. 2B).

The TNF superfamily member 4 (TNFSF4) belongs to the tumor necrosis factor ligands family, and functions in T cell antigen-presenting cell (APC) interactions, mediating the adhesion of activated T cells to targeted cells. There were only limited studies exploring the possible functions of TNFSF4 [19, 20], and its roles in breast carcinoma were totally empty. We found that TNFSF4 was highly expressed in all kinds of breast carcinomas, and its aberrant overexpression was associated with shorter overall survival, disease-free survival, and importantly, TNFSF4 was further revealed to be closely related to anti-tumor immunity, including multiple immune effector molecules and T-cell signatures, as were presented and illustrated.

5. Conclusions

To be concluded, TNFSF4 is one potential immune therapy target, for its aberrant expression pattern in breast carcinoma, and was even overexpressed in carcinomas without ERBB2, ESR1, PGR1 amplification, either without KRAS, TP53 mutation and amplification. TNFSF4, together with other candidates, were all included for evaluation, and the positive correlation with immune-functional inhibitions and lymphocytes draw a lot attention. In clinical cases analysis, the TNFSF4 targeting therapy may show the best therapy response, and interestingly, TNFSF4 also perturb the stem cells expansion, the signature of which was

critical for long-term recurrence and therapy response. Therefore for the first time, we aim to dig out the embedding stars that may contribute to breast cancer therapies, and provide one potential but crucial potent for treating breast carcinoma indistinguishably, and long-term effective.

Abbreviations

TNF Receptor Superfamily Member 14 (TNFRSF14); TNF superfamily member 4 (TNFSF4); TNF superfamily member 18 (TNFSF18); PD-L1(CD274); PD-L2 (PDCD1LG2),

Declarations

7.1 Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For retrospective studies.

7.2 Consent for publication

Authors declare each has approved this article to be published.

7.3 Competing interests

This research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

7.4 Funding

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7.5 Authors' contributions

XL: Paper drafting; Study designation; Statistical analysis; Data base screening; Figures preparation; References cross checking. LM: Bioscientific experiments; Cells culturing. YS: Experimental tests; RNA/Protein tests. KL: Bioscientific experiments; Study designation. HR: Paper drafting; Statistical analysis. ND: RNA/Protein tests; Figures preparation. XS: Study designation; Statistical analysis. ST: Paper drafting; Study designation; Statistical analysis; Images quality control.

7.6 Acknowledgements

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7.7 Availability of data and material

The data and relative supporting materials relating to the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1 The analysis tested in 276 pairs between the 24 tracks in the OncoPrint to reveal the connections among immunotherapies related genes

A	B	A Not B	B Not A	Both	Log2	p-Value	q-Value
TNFSF4	TNFSF18	5	4	70	>3	<0.001	<0.001
TNFSF18	CD48	11	40	63	>3	<0.001	<0.001
TNFSF4	CD48	14	42	61	>3	<0.001	<0.001
CD274	PDCD1LG2	3	1	20	>3	<0.001	<0.001
ERBB2	TP53	369	1345	441	1.409	<0.001	<0.001
PVR	NECTIN2	3	9	12	>3	<0.001	<0.001
ERBB2	LGALS9	120	9	27	>3	<0.001	<0.001
TNFSF9	CD70	1	3	7	>3	<0.001	<0.001
HHLA2	CD200	6	3	6	>3	<0.001	<0.001
KRAS	TP53	47	1717	69	1.501	<0.001	<0.001
CD80	CD200	2	5	4	>3	<0.001	<0.001
CD86	CD200	7	5	4	>3	<0.001	<0.001
CD274	TP53	10	1013	27	2.355	<0.001	<0.001
CD80	CD86	3	8	3	>3	<0.001	<0.001
CD80	HHLA2	3	9	3	>3	<0.001	<0.001
NECTIN2	CD48	12	94	9	2.902	<0.001	0.001
CD86	HHLA2	8	9	3	>3	<0.001	0.003
PVR	CD48	8	96	7	>3	<0.001	0.004
CD44	TP53	10	332	19	1.984	<0.001	0.005
VSIR	TP53	5	338	13	2.421	<0.001	0.011
CD200	TP53	1	343	8	>3	<0.001	0.012
PDCD1LG2	TP53	7	484	14	2.138	<0.001	0.012
VTCN1	TNFRSF14	27	19	3	>3	0.001	0.015
CD274	PVR	18	12	3	>3	0.003	0.031
HHLA2	TP53	3	342	9	2.614	0.004	0.041
ALDH1A1	CD44	10	26	3	>3	0.004	0.048

Figures

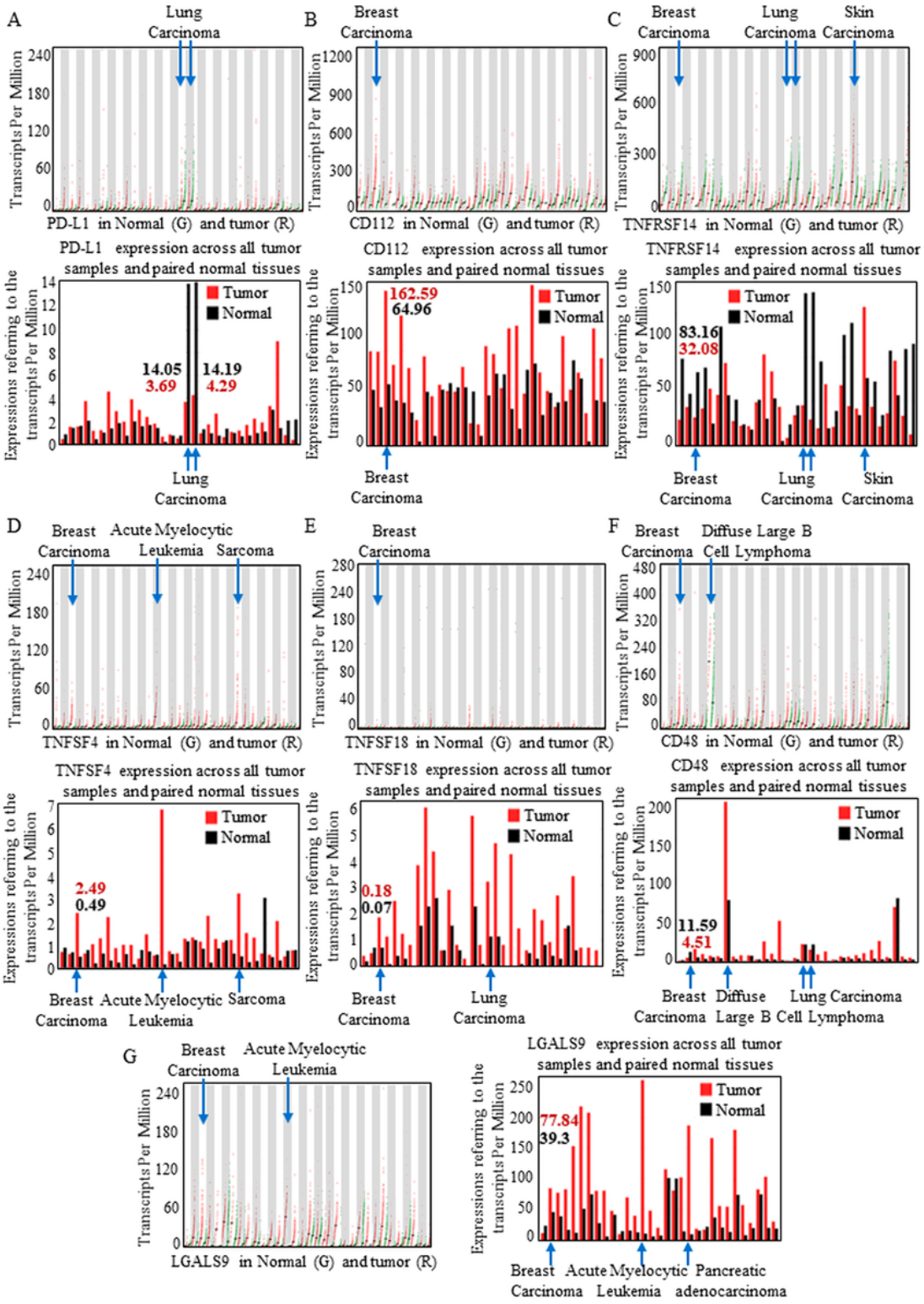

Figure 1

Analyzing the candidate immune checkpoints blockades in carcinomas The whole-body carcinomas were enrolled for analyzing, and multiple systems showed various immune checkpoints patterns. The PD-L1 (A), CD112 (B), TNFRSF14 (C), TNFSF4 (D), TNFSF18 (E), CD48 (F), and LGALS9 (G) were analyzed for their abnormally expressed styles in breast carcinomas. Specifically, CD112, TNFSF4, TNFSF18, and LGALS9 were relatively overexpressed in breast carcinomas.

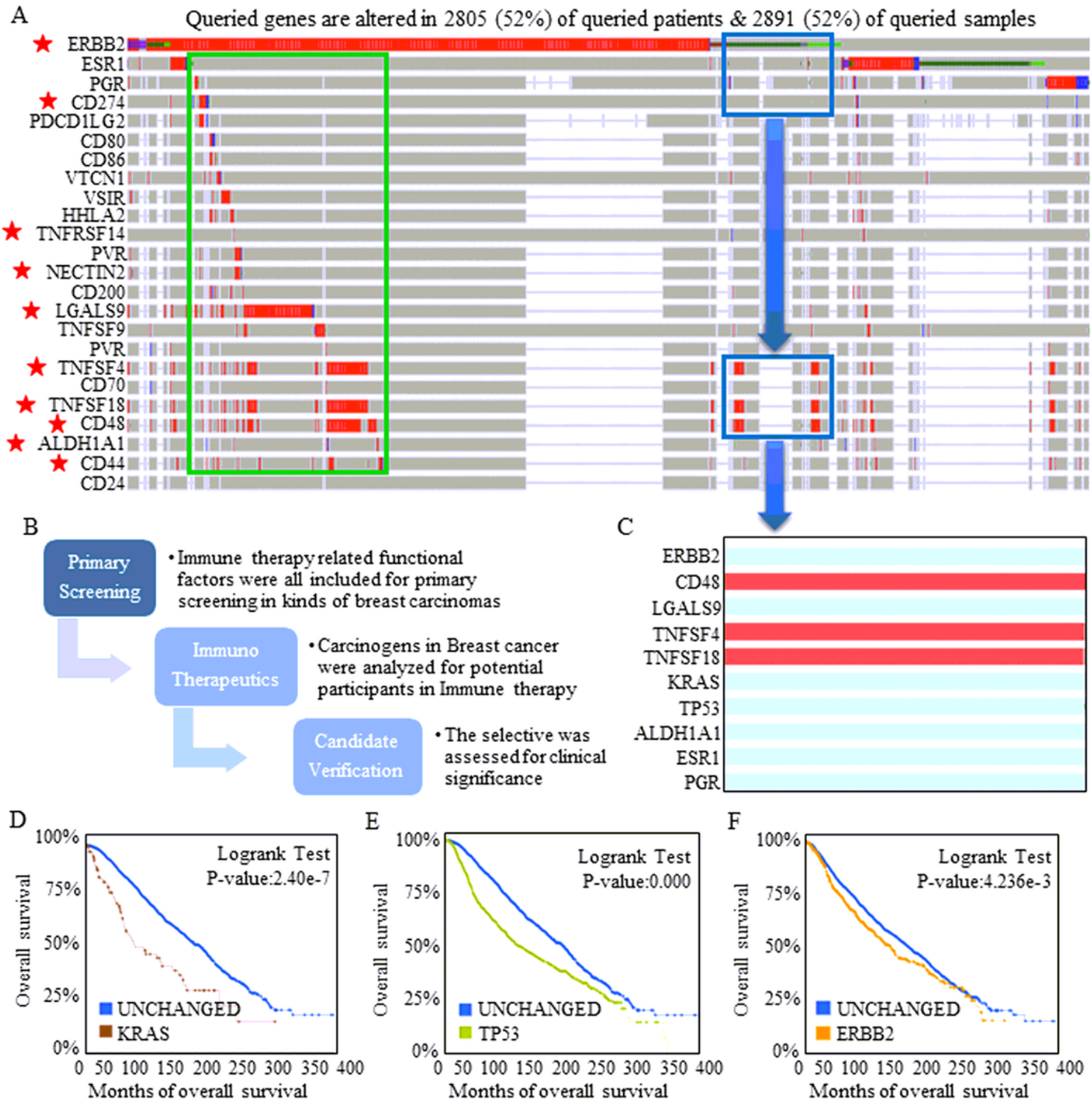

Figure 2

Selection of the most representative immune checkpoints blockade The expression signatures and correlations were studied through using the CBIOPORTAL and the GEPIA2. A. The expression heatmap showed the expression of each candidate in red bar, and the most representative genes were labeled with RED. B. The analyzing chart flow was exhibited for better understanding. C. Nearly all the immunotherapeutic targets showed expanded expression intervals referring to ESR1 and PGR, and the

TNFSF4, TNFSF18, CD48, all showed aggregated expressions in breast carcinomas bearing no ERBB2, ESR1, PGR expression. The amplification or mutation of either KRAS (D), TP53 (E), or ERBB2 (F), pointed to poorer survival expectance.

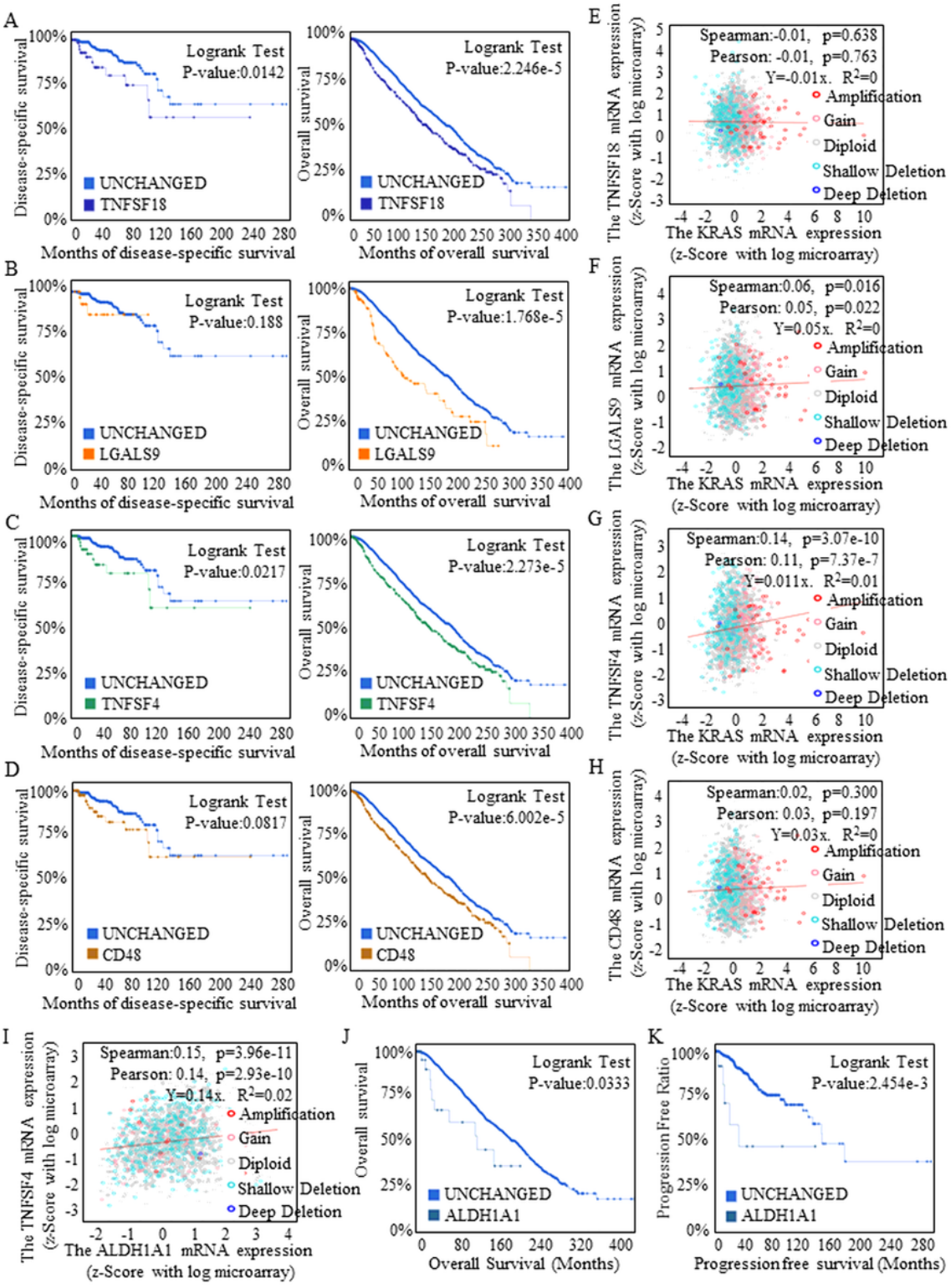

Figure 3

Clinical evaluation of the potential therapeutic candidates Data are acquired from combined study cohorts of BREAST (METABRIC 2016), BREAST CANCER (MSK 2018), BREAST INVASIVE CARCINOMA

BREAST (TCGA PANCAN 2018). The clinical significance of the cluster of TNFSF18 (A), LGALS9 (B), TNFSF4 (C) and CD48 (D) were analyzed for their values referring to immune therapeutic targets. The correlation between KRAS and TNFSF18 (E), LGALS9 (F), TNFSF4 (G) and CD48 (H) were analyzed, and the positive correlation between TNFSF4 and KRAS strongly suggested the prospect therapy effects of using TNFSF4 as the manipulating or the blocking targets. I. The close correlation between TNFSF4 and ALDH1A1 expressions was identified. Stem cells with positive ALDH1A1 phenotype indicated shorter survival length (J) and shorter progression free length (K).

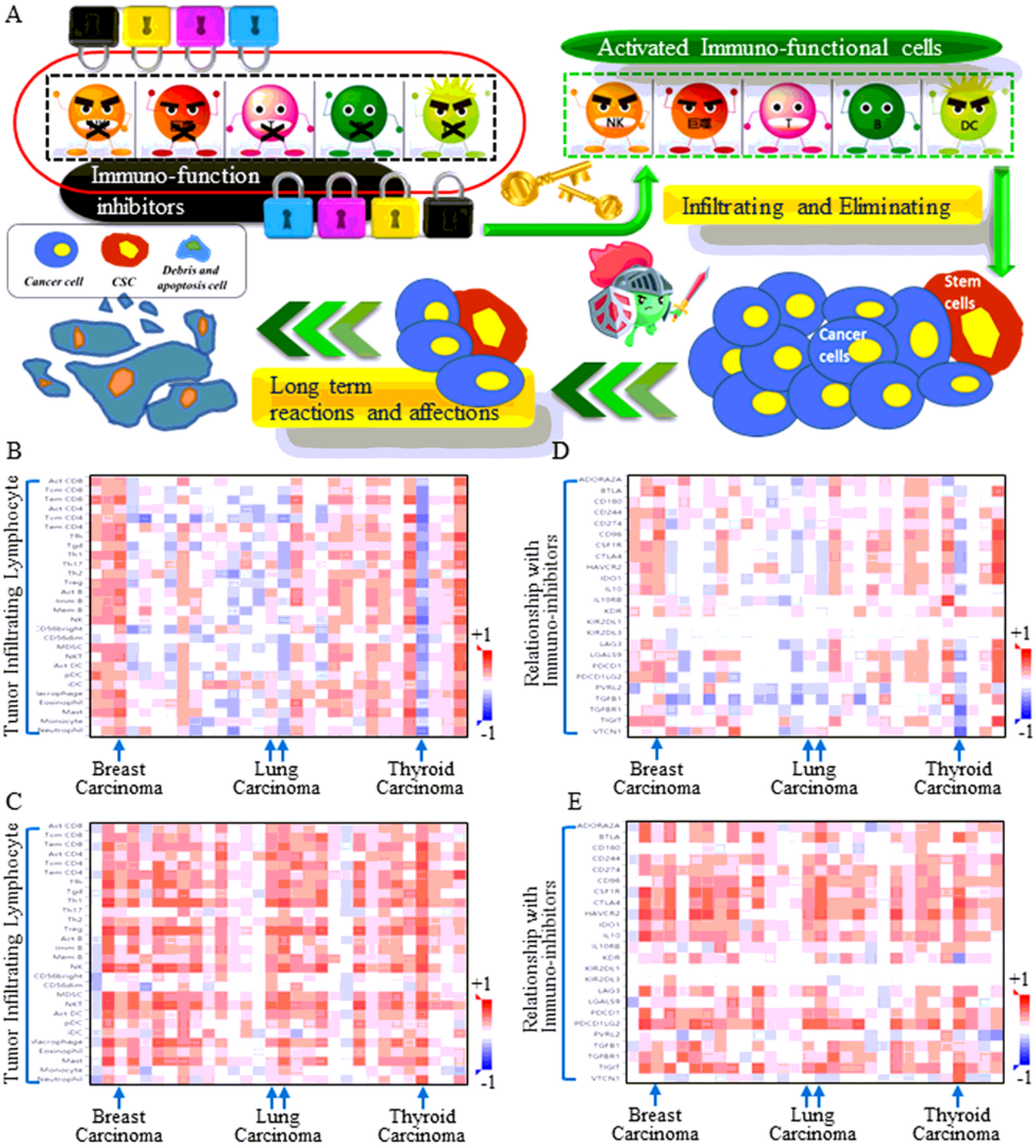

Figure 4

TNFSF4 based immune therapy maybe intersect with cancer stem cells signatures' repression A. The schematic figure was drafted to illustrate the immune response, and the system was locked when carcinoma is getting aggressive, later when the immune-function inhibitors were unlocked, the active immune cells begin to infiltrate and execute the cellular order to perished the whole cancer group. Spearman correlations between TNFSF4 and Immuno-inhibitive factors (Y axis) across human cancers (X axis). The items in column was listed in sequence as: ADORA2A, BTLA, CD160, CD244, CD274, CD96, CSF1R, CTLA4, HAVCR2, IDO1, IL10, IL10RB, KDR, KIR2DL1, KIR2DL3, LAG3, LGALS9, PDCD1, PDCD1LG2, PVRL2, TGFB1, TGFBR1, TIGIT, VTCN1. The lymphocytes infiltrating functions and the connective functional factors were analyzed respectively, and both the overexpressed ALDH1A1 (B) and overexpressed TNFSF4 (C) were correlated with more lymphocytes infiltrating. Spearman correlations between TNFSF4 and kinds of lymphocytes (Y axis) across human cancers (X axis). The items in column was listed in sequence as ADORA2A, BTLA, CD160, CD244, CD274, CD96, CSF1R, CTLA4, HAVCR2, IDO1, IL10, IL10RB, KDR, KIR2DL1, KIR2DL3, LAG3, LGALS9, PDCD1, PDCD1LG2, PVRL2, TGFB1, TGFBR1, TIGIT, VTCN1. However, the guardian soldiers were disabled with the highly expressed immune-inhibitors in cells with increased ALDH1A1 (D) and TNFSF4 (E). These results indicated that TNFSF4 blockade treatment will potentially reactivate the immune response, and will partially function through precisely demilitarize the stem cells.

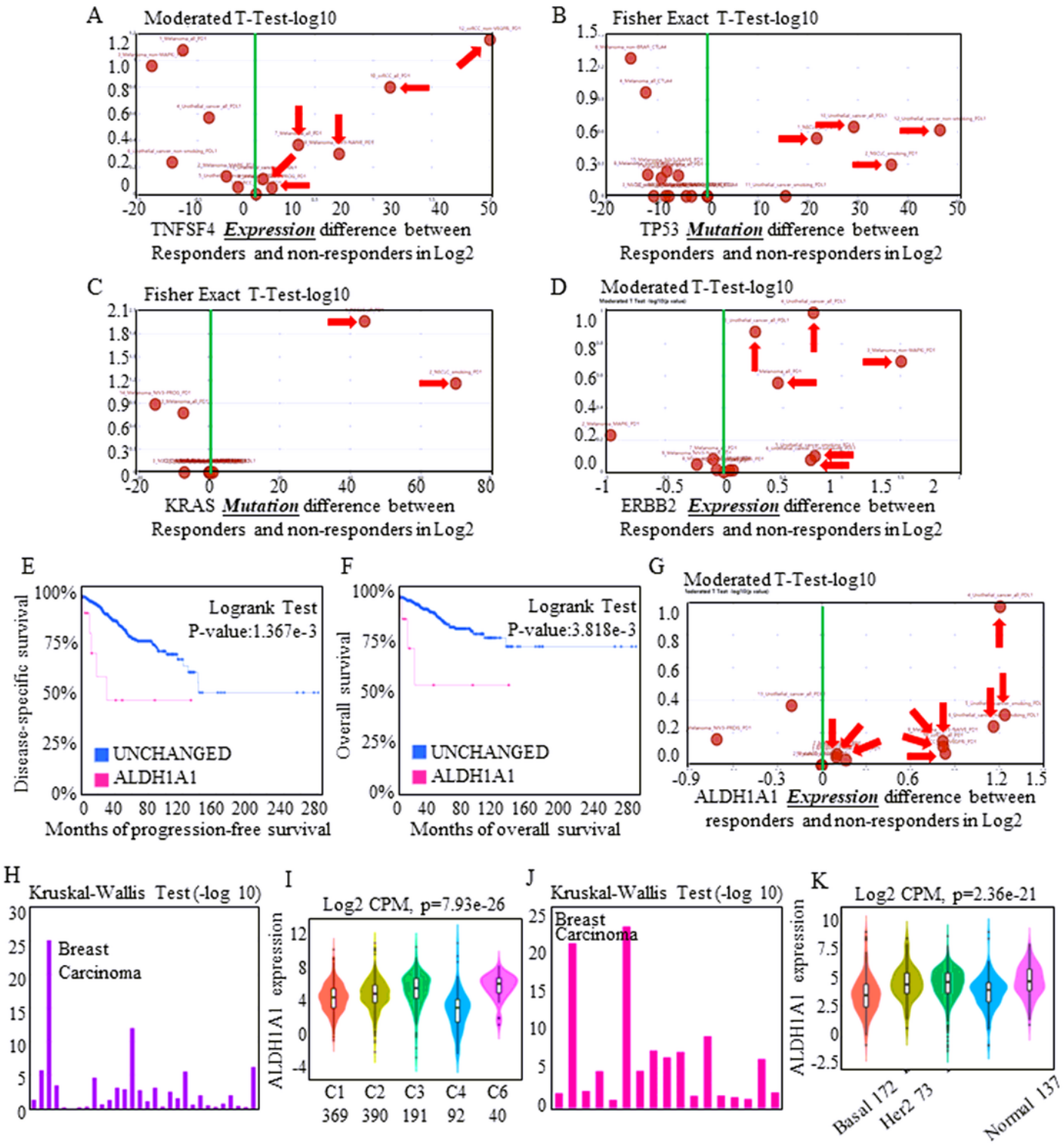

Figure 5

TNFSF4 blockade therapies could be assessed in the stem cells signatures associated mode Immune therapeutic effects assessment from the real world referring to TNFSF4 (A) tended to implicit the better outcomes, and the TNFSF4 associated TP53 (B), KRAS (C), and ERBB2 (D) all indicated better immune therapy response. Increased ALDH1A1 expression indicated shorter disease specific survival (E) and overall survival (F), and the ALDH1A1 surprisingly correlated with higher therapy responds ratios (G)

through clinical data assessing. H-I. ALDH1A1 was analyzing for its roles in implicating the immunotherapy response predication, and totally 5 subgroups of C1 (N=369), C2 (N=390), C3 (N=191), C4 (N=92), C6 (N=40) were involved for assessing functional aspects, and ALDH1A1 expression dominated in all the subtypes, participating in multiple immune reaction process. J-K. Associations between ALDH1A1 expression and molecular subtypes across human cancers were also identified, and the signature of increasing ALDH1A1 tended to express in all kinds of breast carcinomas. Specifically, C1 equals wound healing, C2 equals IFN-gamma dominant, C3 equals inflammatory, C4 equals lymphocyte depleted, C5 (immunologically quiet and was not showed, C6 equals TGF-b dominant.

Supplementary Files

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