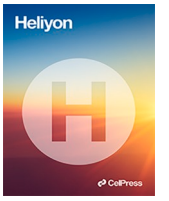
Heliyon 10 (2024) e27294

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Necroptosis-related KLRB1 was a potent tumor suppressor and immunotherapy determinant in breast cancer

Jie Xia [a], Xudong Zhou [b,*]

[a] Department of Respiratory and Critical Care Medicine, National Clinical Research Center of Respiratory Disease, Key Laboratory of Pulmonary Diseases of Health Ministry, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[b] Department of Thyroid and Breast Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

ARTICLE INFO

Keywords:
Breast cancer
Tumor microenvironment
Cancer Immunotherapy
Bioinformatics
Biomarker

ABSTRACT

Breast cancer is a multifaceted and diverse illness that impacts millions of people globally. Identifying the underlying causes of BRCA and creating efficient treatment plans are urgent. Necroptosis is widely involved in cancer development. However, the specific roles of necroptosis in cancer immunotherapy of breast cancer have not been explored. In this study, we aim to establish the connection between necroptosis and immunotherapy in BRCA. TCGA, METABRIC, GSE103091, GSE159956, and GSE96058 were included for bioinformatics analysis. NMF and CoxBoost algorithms were used to develop the necroptosis-related patterns and model, respectively. A necroptosis-related model was developed and determined KLRB1 as a critical tumor suppressor by in vitro validation. The mutation characteristics, immune characteristics, and molecular functions of KLRB1 were explored. We further examined how necroptosis-related KLRB1 functions in BRCA as a powerful tumor suppressor and regulates the activity of macrophages by in vitro validation, including CCK8, EdU, and Transwell assays. KLRB1 was also revealed to be an immunotherapy determinant.

1. Introduction

Breast cancer (BRCA) is a multifaceted and diverse illness that impacts millions of people globally. It is the most prevalent type of cancer in women and is responsible for a large number of cancer-related fatalities. Many genetic, environmental, and lifestyle factors have a role in the onset and spread of BRCA [1]. Much research has been done over the years to identify the underlying causes of BRCA and to create efficient treatment plans [[2,3]].

Using the body's immune system to combat cancer cells, immunotherapy is a state-of-the-art treatment option for BRCA [4]. It entails using various methods and drugs to boost the immune system and kill cancer cells. Immunotherapy has shown much promise in treating BRCA, giving patients fresh hope and possibly leading to better results [5].

A type of planned cell death different from necrosis and apoptosis is called necroptosis [[6,7]]. It is a tightly controlled process important in many clinical and physiological situations. Receptor-interacting protein kinase 1 (RIPK1), receptor-interacting protein kinase 3 (RIPK3), and mixed lineage kinase domain-like protein (MLKL) are among the signaling pathways that are activated during necroptosis. These signaling molecules plan actions that eventually result in the demise of cells. Numerous illnesses, such as cancer,

* Corresponding author.
E-mail address: drzhouxudong@126.com (X. Zhou).

https://doi.org/10.1016/j.heliyon.2024.e27294
Received 21 December 2023; Received in revised form 27 February 2024; Accepted 27 February 2024
Available online 6 March 2024

neurological conditions, and inflammatory diseases, have been linked to necroptosis [[8,9]]. Necroptosis has become a promising therapeutic target and a predictor of immunotherapy success in the setting of BRCA [10]. Knowing the basic processes behind necroptosis and how it contributes to BRCA may help create new treatment approaches and the identification of biomarkers for personalized medicine.

In this study paper, we aim to establish the connection between necroptosis and immunotherapy in BRCA. A necroptosis-related model was developed and determined KLRB1 as a critical tumor suppressor. We further examined how necroptosis-related KLRB1 functions in BRCA as a powerful tumor suppressor and immunotherapy determinant. We will investigate KLRB1 expression patterns in BRCA tissues and assess the importance of these expression patterns for prognosis. Furthermore, we will investigate the functional implications of KLRB1 in animal models and BRCA cell lines, providing insight into its potential utility as a therapeutic target. Our study intends to contribute to the expanding body of information on the genetic mechanisms behind this tragic disease by clarifying the role of necroptosis-related KLRB1 in BRCA. Ultimately, our research could open the door for creating cutting-edge therapeutic approaches that target necroptosis pathways, enhancing patient outcomes and transforming how BRCA is treated.

2. Materials and methods

2.1. Data collection

TCGA [11], METABRIC [12], GSE103091 [13], GSE159956, and GSE96058 [14] datasets were collected for the subsequent analysis. TCGA is a comprehensive and publicly available resource that contains genomic, transcriptomic, and clinical data from various cancer types. METABRIC is a large-scale database that aims to classify breast cancer based on molecular subtypes and provides valuable genomic and clinical information. GSE103091, GSE159956, and GSE96058 were collected from GEO. GEO contains many high-throughput gene expression data sets, including microarray and next-generation sequencing data. Researchers can access and analyze these data sets to gain insights into gene expression patterns and regulatory mechanisms across different biological conditions and diseases. The GEO database is a valuable resource for studying the molecular basis of diseases, identifying potential biomarkers, and discovering new therapeutic targets.

2.2. Development of necroptosis-related patterns

The necroptosis-related genes were collected from the previous publication [15]. NMF algorithm was applied to determine necroptosis-related patterns [16]. The NMF algorithm, short for Non-negative Matrix Factorization, is a popular unsupervised learning technique used for dimensionality reduction and feature extraction [17]. It is particularly useful for analyzing non-negative data, such as images, text, and gene expression data. The NMF algorithm decomposes a non-negative matrix into two lower-rank non-negative matrices, which can be interpreted as basis vectors and coefficients. These basis vectors and coefficients can then be used to reconstruct the original matrix or extract meaningful features. NMF has applications in various fields, including image processing, text mining, and bioinformatics. The R package survival generates the survival curves of necroptosis-related patterns. The R packages pheatmap and ComplexHeatmap generate the expression pattern of necroptosis-related genes in necroptosis-related patterns [18].

2.3. Annotation of the necroptosis-related pattern

The R package EnhancedVolcano generates the volcano plot illustrating DEGs of necroptosis-related patterns. GSEA (Gene Set Enrichment Analysis) was performed on DEGs of necroptosis-related patterns [19,20]. GSEA is a computational method used to determine whether a predefined set of genes shows statistically significant differences between two biological states. GSEA can be used to analyze gene expression data and identify biological pathways or gene sets that are associated with a particular phenotype or condition. It is a powerful tool for understanding the underlying biological mechanisms and pathways involved in a given disease or experimental condition.

2.4. Identification of KLRB1

CoxBoost was performed for the dimension reduction of DEGs [21]. The CoxBoost algorithm is a machine learning algorithm used for survival analysis. It is an extension of the Cox proportional hazards model, which is a widely used statistical model for analyzing survival data. CoxBoost combines boosting, a technique for creating an ensemble of weak learners, with the Cox proportional hazards model to improve its predictive performance. It is particularly useful when dealing with high-dimensional data or when there are complex relationships between the predictors and the survival outcome. The algorithm iteratively fits a series of weak learners to the data, with each learner focusing on the residuals of the previous learners. This allows CoxBoost to capture non-linear relationships and interactions between the predictors and the survival outcome. Overall, CoxBoost is a powerful tool for survival analysis and has been widely used in various fields such as medical research and finance. The R package survival generates the survival curves of KLRB1-related groups.

2.5. Mutation characteristics of KLRB1

Mutation characteristics of KLRB1 were explored using the R package maftools [22]. The maftools is an R package that provides a

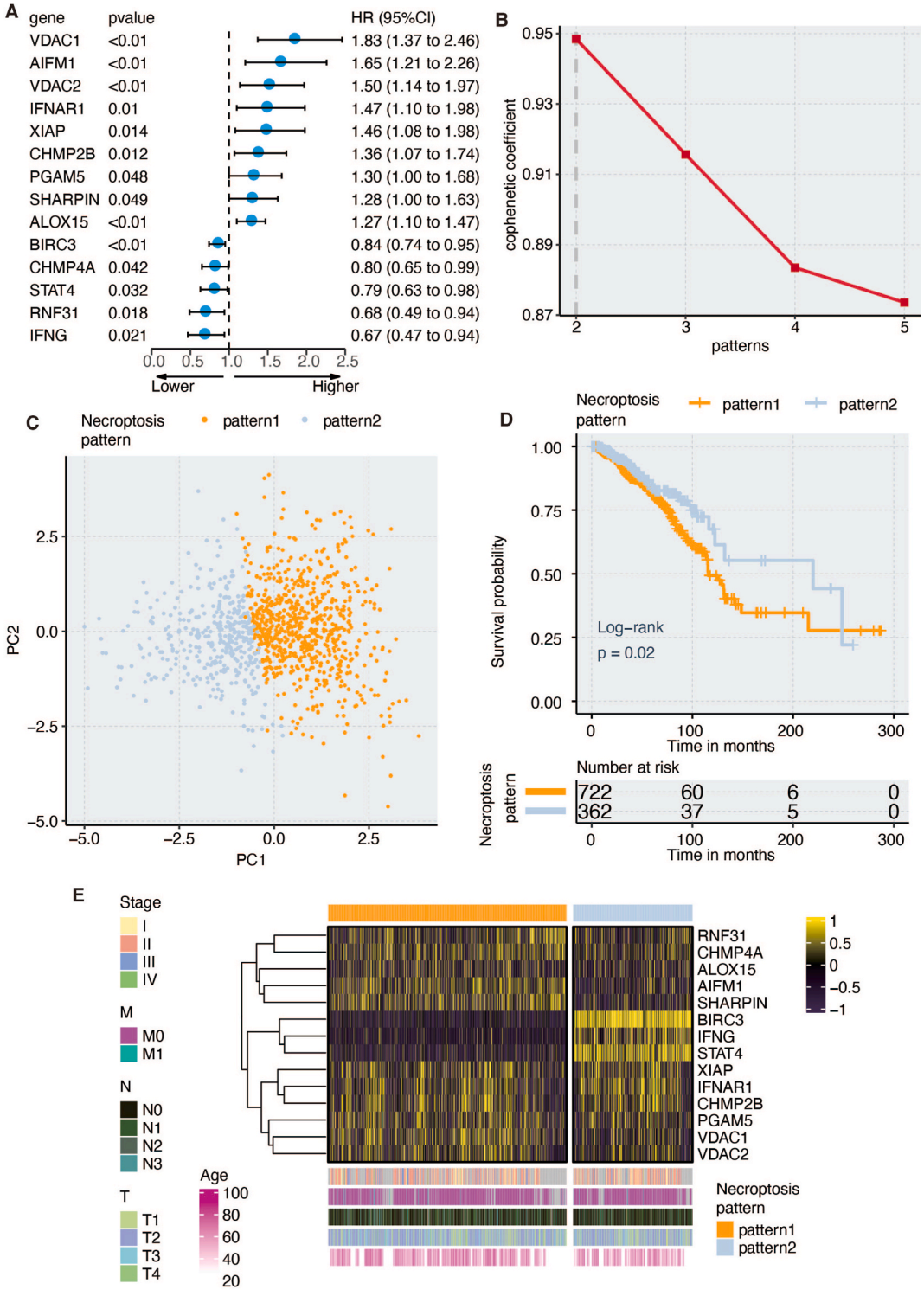


Fig. 1. Development of necroptosis-related patterns. A. HR of necroptosis-related genes. B. Cophenetic coefficient of different patterns. C. PCA of necroptosis-related patterns. D. Survival curves of necroptosis-related patterns. E. The expression pattern of necroptosis-related genes in necroptosis-related patterns.

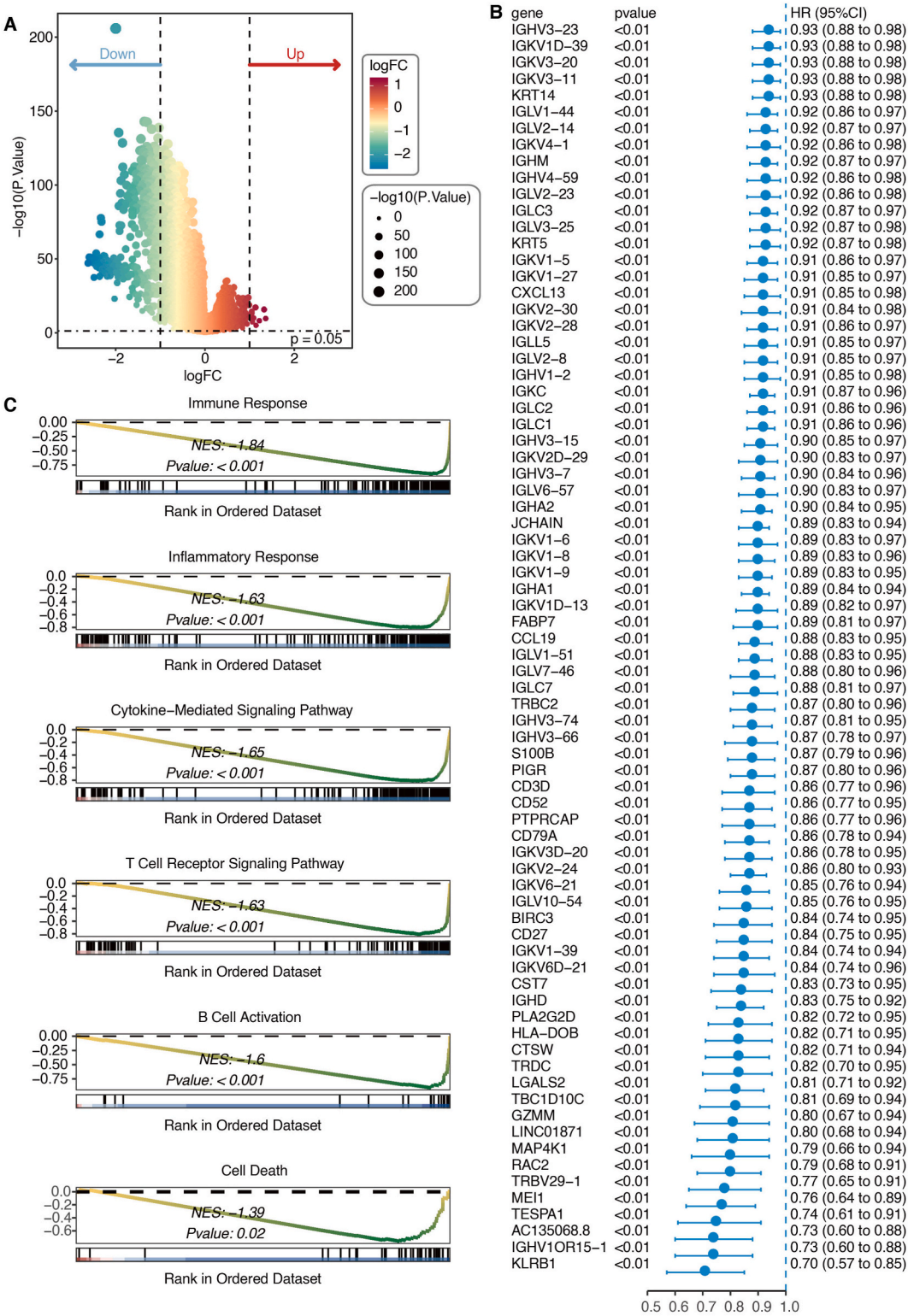


Fig. 2. Annotation of the necroptosis-related pattern. A. DEGs of the necroptosis-related pattern. B. HR of DEGs. C. GSEA of DEGs.

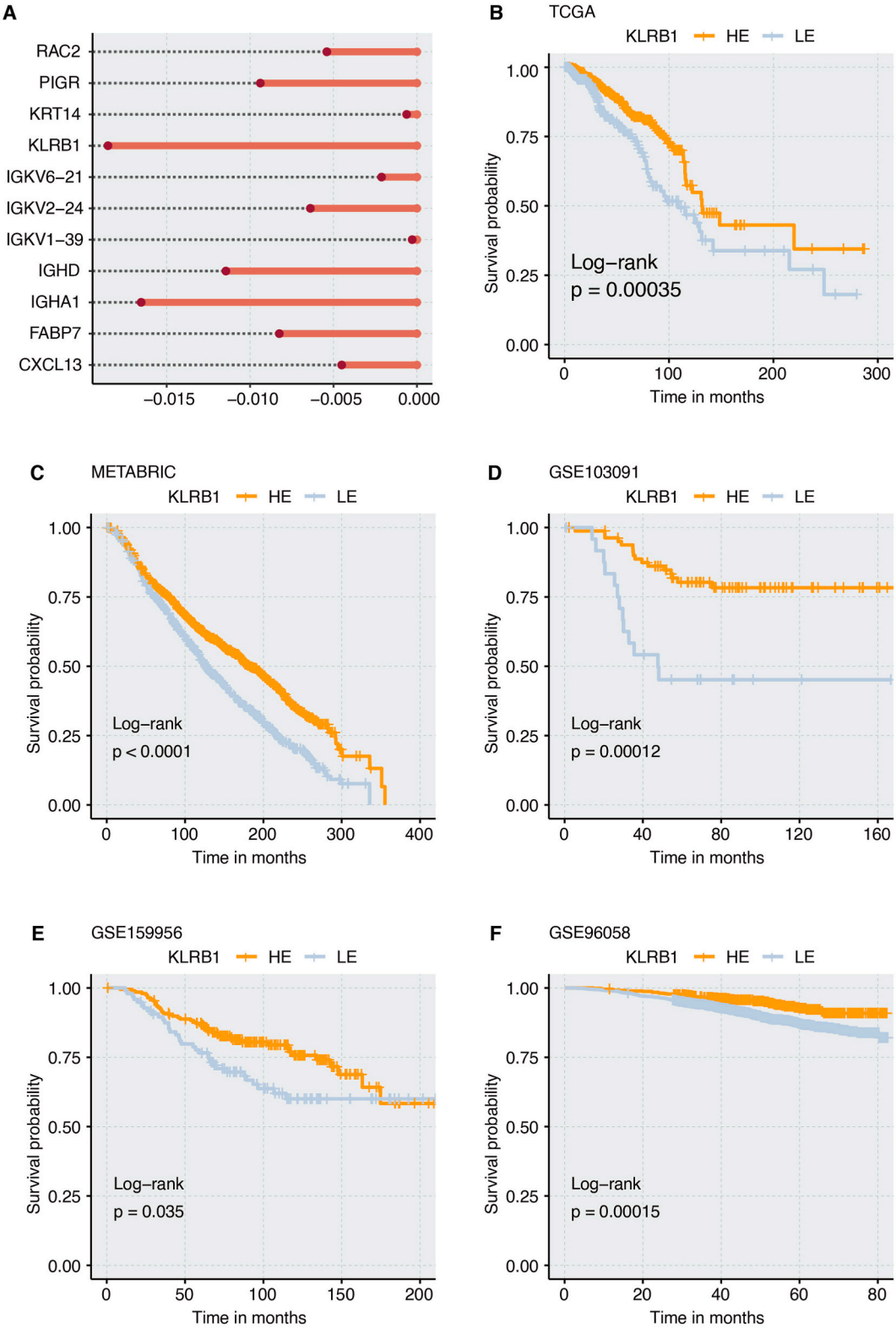


Fig. 3. Identification of KLRB1. A. CoxBoost for dimension reduction of DEGs. B. Survival curves of KLRB1-related groups in TCGA. C. Survival curves of KLRB1-related groups in METABRIC. D. Survival curves of KLRB1-related groups in GSE103091. E. Survival curves of KLRB1-related groups in GSE159956. F. Survival curves of KLRB1-related groups in GSE96058.

set of functions for analyzing and visualizing somatic mutation data from cancer genomic studies. It allows users to perform various tasks such as mutation annotation, visualization of mutation landscapes, identification of significantly mutated genes, and exploration of mutation signatures. The maftools is widely used in cancer genomics research to gain insights into the genetic alterations driving cancer development and progression.

2.6. Immune characteristics of KLRB1

The R packages pheatmap and ComplexHeatmap [23] generate the correlation between KLRB1 and immune modulators [18]. The R package estimate was used to calculate the ESTIMATE scores [24]. The R package “estimate" is a tool used for estimating tumor purity, stromal score, and immune score in tumor samples using gene expression data [25]. It provides a quantitative assessment of the tumor microenvironment and can be used to study the tumor-immune interactions and their impact on cancer progression and response to therapy. The TIMER algorithm was applied to calculate the abundance of immune cells [26]. The TIMER algorithm is a computational tool used for analyzing tumor immune interactions. It provides an estimation of the abundance of immune infiltrates in tumor samples based on gene expression data. The algorithm can be used to study the tumor microenvironment and its impact on cancer progression and response to immunotherapy.

2.7. Annotation of KLRB1

GSEA was performed on DEGs of KLRB1-related groups.

2.8. Experimental validation on KLRB1

The cell lines AU565 and THP-1 were obtained from iCell (http://www.icellbioscience.com/search) in Shanghai, China. They were cultivated in DMEM media and 1640 media with 10% FBS and 1% double-antibody, respectively. Following a 6-h exposure to 320 nM of phorbol 12-myristate 13-acetate (PMA) at 37 °C, THP-1 cells were polarized into M0 macrophages. SiRNA sequences of KLRB1: Forward CCAACAAGCAATATATGCTGAGTTA; Forward ACCTTGGCATCAATTTGCCCTGAAA; Forward TGGCATCAATTTGCCCT-GAAACTTA. The detailed methods are provided in the supplementary materials.

2.9. Statistical analysis

The Pearson correlation test was used to conduct the correlation analysis. Students’ t-tests and wilcoxon rank sum tests were used for continuous variables.

3. Results

3.1. Development of necroptosis-related patterns

Following a univariate Cox regression analysis, the HR of necroptosis-related genes showed that nine genes were hazardous while five were favorable (Fig. 1A). Using the NMF algorithm, BRCA patients were clustered into different patterns (Fig. 1B), with a cophenetic coefficient of 2 reaching the highest clustering ability. PCA of necroptosis-related patterns showed that BRCA patients were separated (Fig. 1C). Survival curves of necroptosis-related patterns showed that BRCA patients in pattern 1 had significantly reduced survival time (Fig. 1D). The expression pattern of necroptosis-related genes in necroptosis-related patterns is shown in Fig. 1E.

3.2. Annotation of the necroptosis-related pattern

The volcano plot in Fig. 2A illustrates DEGs of necroptosis-related patterns. All significantly upregulated DEGs were favorable (Fig. 2B). The GSEA of DEGs revealed that the immune and inflammatory responses were inactivated (Fig. 2C).

3.3. Identification of KLRB1

CoxBoost was performed for the dimension reduction of DEGs (Fig. 3A), which came to 11 potent genes. KLRB1 was the most potent one. Survival curves of KLRB1-related groups in TCGA showed that BRCA patients in the KLRB1-high group had significantly prolonged survival time (Fig. 3B). Survival curves of KLRB1-related groups in METABRIC showed that BRCA patients in the KLRB1-high group had significantly prolonged survival time (Fig. 3C). Survival curves of KLRB1-related groups in GSE103091 showed that BRCA patients in the KLRB1-high group had significantly prolonged survival time (Fig. 3D). Survival curves of KLRB1-related groups in GSE159956 showed that BRCA patients in the KLRB1-high group had significantly prolonged survival time (Fig. 3E). Survival curves of KLRB1-related groups in GSE96058 showed that BRCA patients in the KLRB1-high group had significantly prolonged survival time (Fig. 3F). The correlation between KLRB1 and non-apoptotic cell death showed that KLRB1 was negatively associated with pyroptosis, ferroptosis, autophagy, and necroptosis, while positively associated with cuproptosis and parthanatos (Fig. S1).

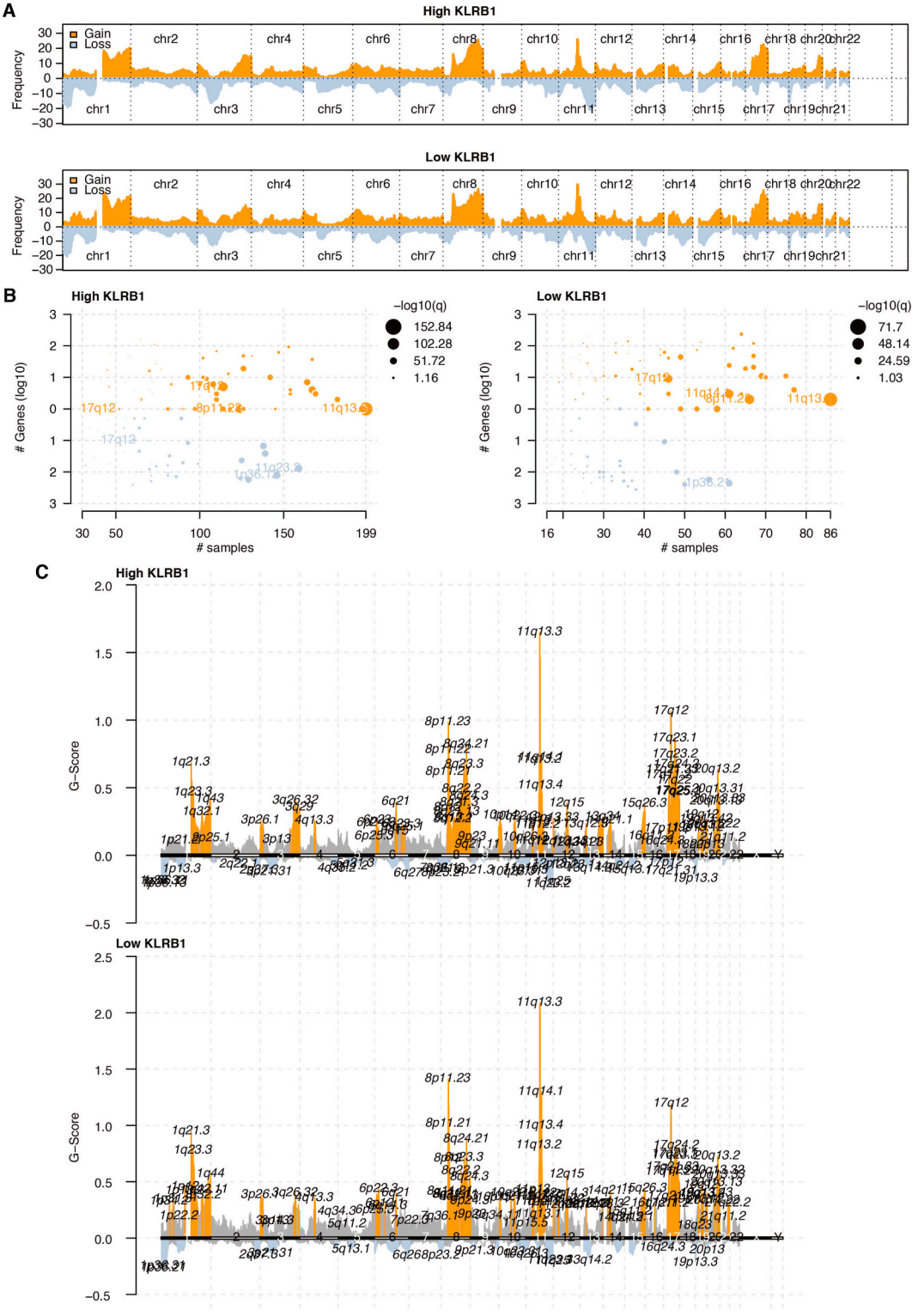


Fig. 4. Mutation characteristics of KLRB1. A. Gain and Loss of chromosomes of KLRB1-related groups. B. Mutation probabilities of mutation sites of KLRB1-related groups. C. G-Scores of mutation sites of KLRB1-related groups.

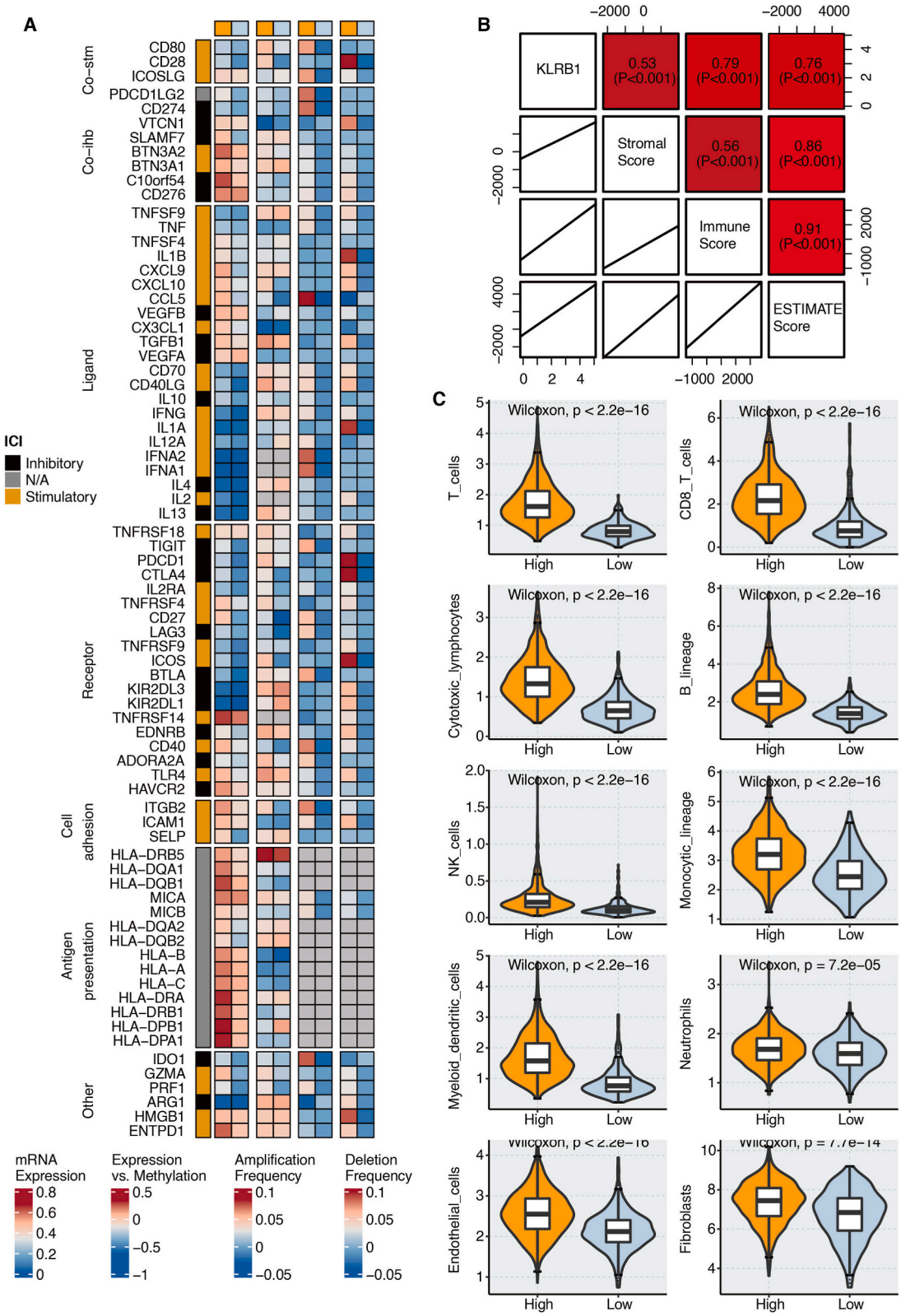


Fig. 5. Immune characteristics of KLRB1. A. The correlation between KLRB1 and immune modulators. B. The correlation between KLRB1 and ESTIMATE scores. C. The relative activities of immune cells of KLRB1-related groups.

3.4. Mutation characteristics of KLRB1

Gain and Loss of chromosomes of KLRB1-related groups are shown in Fig. 4A. We observed the significantly gained cytobands (17q12, 8p11.23, and 11q13.3) and significantly lost cytobands (17q12, 1p36.13, and 11q23.2) in the high KLRB1-related group. Meanwhile, in the low KLRB1-related group, 17q12, 11q14.1, 8p11.23, and 11q13.3 were significantly gained and 1p36.21 was significantly lost. (Fig. 4B). G-Scores of mutation sites of KLRB1-related groups showed the overall alteration patterns (Fig. 4C).

3.5. Immune characteristics of KLRB1

KLRB1 correlated with multiple immune modulators, such as BTN3A2, VTCN1, CD276, VEGFB, and CXCL9 (Fig. 5A). KLRB1 significantly correlated with ESTIMATE scores (Fig. 5B). The relative activities of immune cells of KLRB1-related groups showed that T cells, B cells, NK cells, and neutrophils were more activated in the KLRB1-high group (Fig. 5C).

3.6. Annotation of KLRB1

GSEA of KLRB1 showed that nine immunogenic pathways were more active in the KLRB1-high group (Fig. 6A).

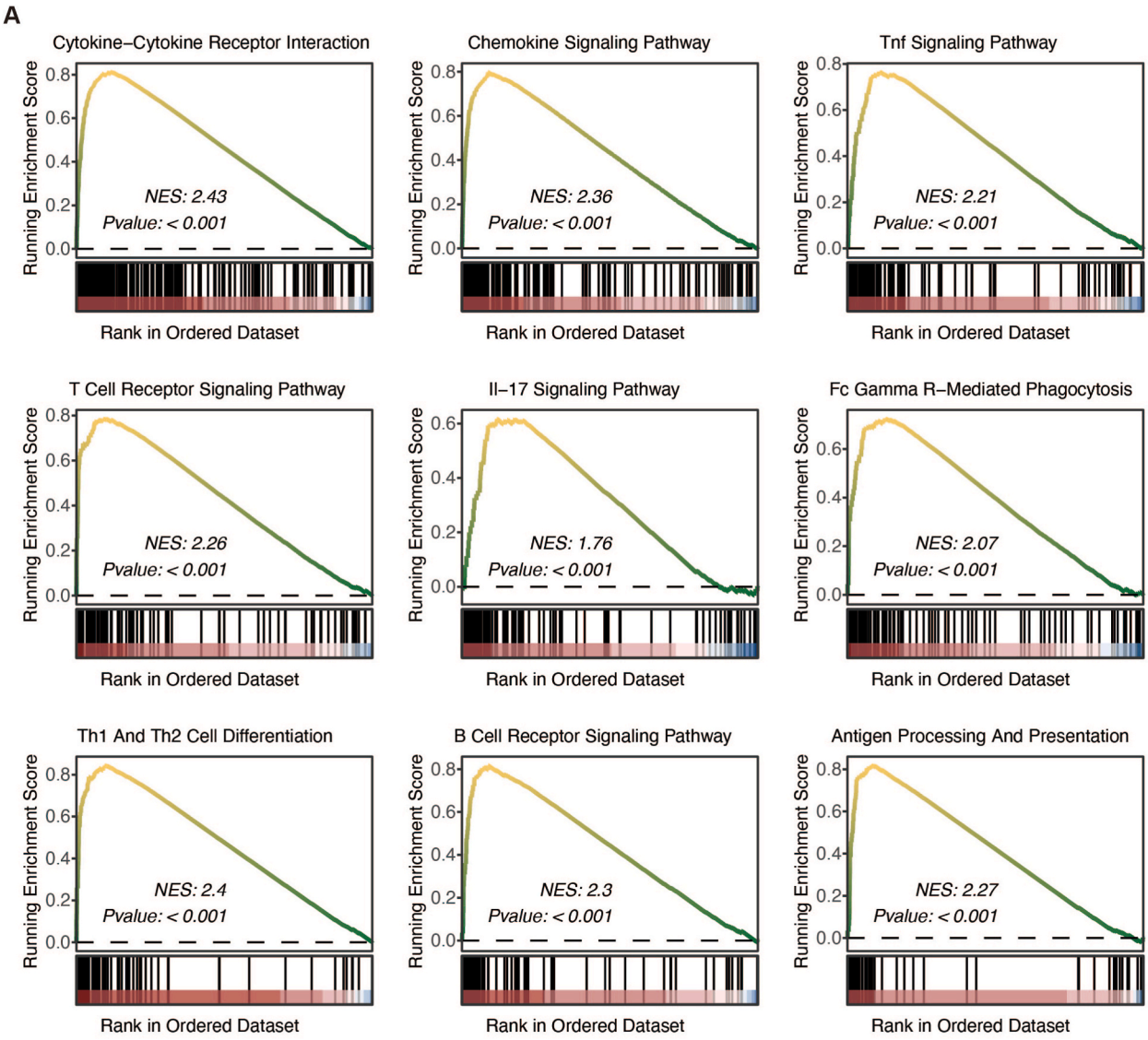


Fig. 6. Annotation of KLRB1. A. GSEA of KLRB1.

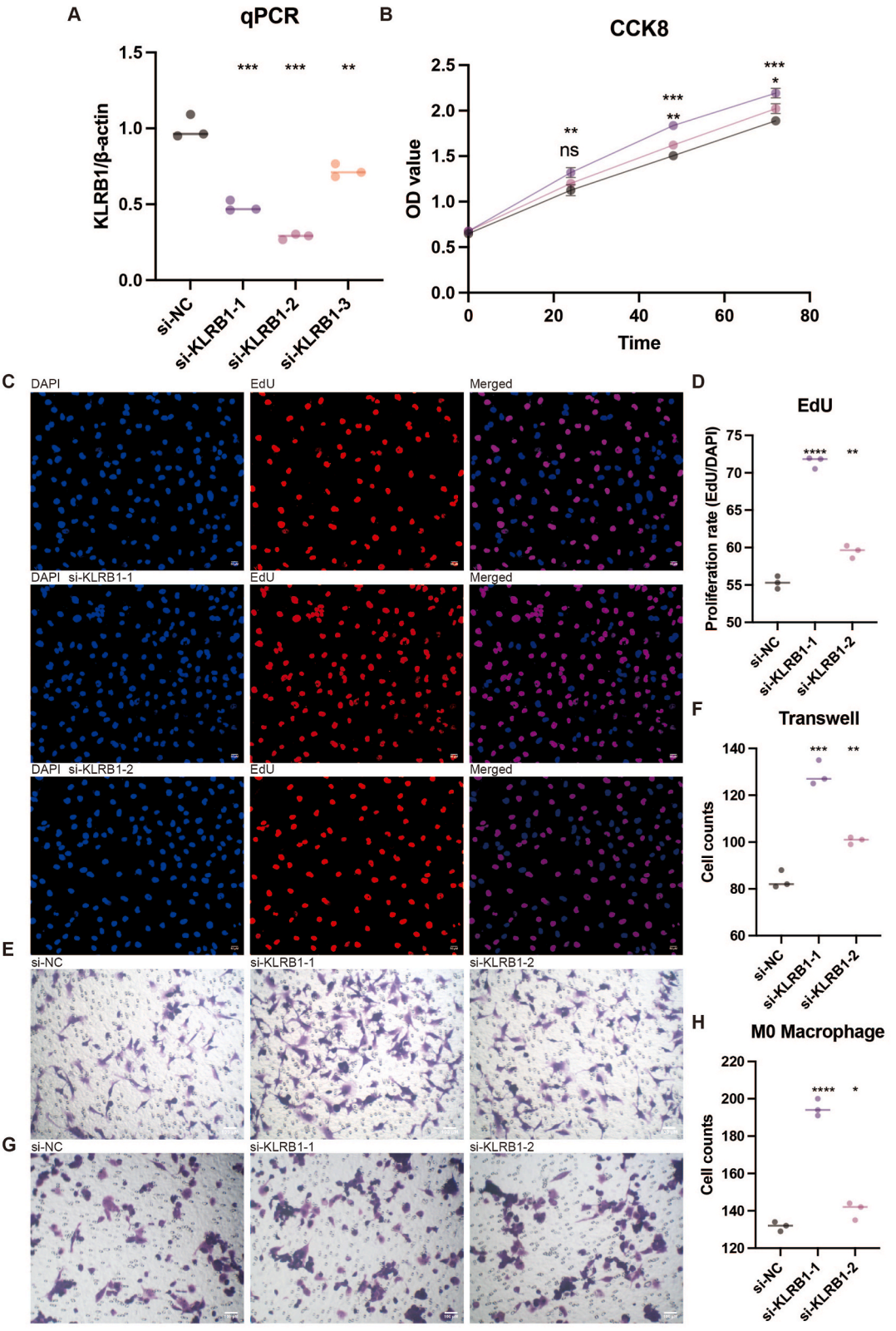


Fig. 7. Experimental validation on KLRB1. A. qPCR assay for optimal siRNA targets. B. CCK8 assay for KLRB1. C. EdU assay for KLRB1. D. Statistical analysis of EdU assay for KLRB1. E. Transwell assay for KLRB1. F. Statistical analysis of Transwell assay for KLRB1. G. Co-culture Transwell assay for KLRB1. H. Statistical analysis of Co-culture Transwell assay for KLRB1.

3.7. Experimental validation on KLRB1

qPCR assay was conducted for optimal siRNA targets (Fig. 7A). CCK8 assay for KLRB1 showed that BRCA cells in two siRNA groups had enhanced proliferation ability (Fig. 7B). EdU assay for KLRB1 showed that BRCA cells in two siRNA groups had enhanced proliferation ability (Fig. 7C and D). Transwell assay for KLRB1 showed that BRCA cells in two siRNA groups had enhanced migration ability (Fig. 7E and F). Co-culture Transwell assay for KLRB1 showed that macrophages in two siRNA groups had enhanced proliferation ability (Fig. 7G and H).

4. Discussion

Necroptosis in breast cancer can be beneficial or detrimental [27,28]. Necroptosis can, on the one hand, aid in removing cancer cells and stopping tumor growth. However, it can also exacerbate inflammation and cause tissue damage, which can aid in the spread and metastasis of tumors. Numerous investigations have demonstrated that breast cancer may result in dysregulation of necroptosis. For instance, RIPK3 expression or activity may be downregulated in some breast cancer cells, hindering necroptosis induction. Furthermore, breast cancer necroptosis may be influenced by the tumor microenvironment [29]. Stem cell invasion, cytokine signaling, and hypoxia are examples of factors that can alter the necroptotic pathway in breast cancer cells. It is critical to comprehend necroptosis's significance in breast cancer to create novel treatment approaches [30]. One intriguing strategy to minimize harm to healthy organs while selectively inducing cell death in breast cancer cells may be to target the necroptotic pathway [31]. To completely understand the intricate interactions between necroptosis and the advancement of breast cancer, more study is necessary.

Machine learning has numerous advantages in cancer research. One major advantage is its ability to analyze large amounts of data quickly and accurately. This is crucial in cancer research, as there is a vast amount of data to be analyzed, including genetic information, medical records, and imaging data. Machine learning algorithms can process this data and identify patterns and correlations that may take time to be apparent to human researchers. In our study, necroptosis-related patterns could effectively discriminate BRCA patients' survival time. Besides, KLRB1, based on machine learning, CoxBoost, was found to be the most powerful gene related to necroptosis-related patterns.

The KLRB1 gene codes for a protein known as KLRB1 or CD161. It is a C-type lectin-like receptor family member and a type II transmembrane protein. Natural killer (NK) cells, T cells, and certain subsets of dendritic cells are the main immune cells that express KLRB1 [32]. It participates in immunological modulation and cell activation by functioning as a receptor for the lectin-like transcript 1 (LLT1) ligand. Numerous immune-related functions, such as controlling T cell activation, NK cell cytotoxicity, and cytokine generation, have been linked to KLRB1 [33]. It has also been linked to the onset and advancement of several illnesses, such as cancer and autoimmune diseases. KLRB1 expression has been seen in a variety of cancerous tumor forms, including breast cancer. Research has demonstrated that KLRB1 expression in NK and T cells within the tumor microenvironment may impact the anti-tumor immune response [34]. According to certain theories, KLRB1 may regulate the ratio of immune evasion to immunological surveillance in cancer [32,35].

In our study, KLRB1 was associated with a better survival time in BRCA patients. KLRB1 could predict the alteration status of 17q12 (DEL17Q12), 8p11.23 (FGFR1), 11q13.3 (EPAS1), 1p36.13 (EPHA2), and 11q23.2 (DRD2). DEL17Q12, FGFR1 [36], EPAS1 [37], EPHA2 [38], and DRD2 [39] are all genes that have been found to be mutated in various types of cancer. The mutation status of these genes can have implications for cancer development, progression, and response to treatment. For example, mutations in FGFR1 have been associated with lung cancer and breast cancer, while mutations in EPAS1 have been linked to renal cell carcinoma. Understanding the specific mutations in these genes can provide valuable insights into the underlying biology of cancer and potentially guide personalized treatment approaches. This means that by analyzing the expression of KLRB1, we can determine whether these specific gene mutations are present in BRCA patients. Besides, KLRB1 was involved in a more immune-active microenvironment. Our findings were in accordance with the previous studies. The close connection between KLRB1 and immune modulators further proved the immunotherapy predictive value of KLRB1.

It has been discovered that necroptosis affects immunotherapy [40]. Certain immunotherapeutic drugs can cause cancer cells to undergo necroptosis, which ultimately results in their death [40]. Eliminating cancer cells that could be resistant to other forms of cell death can help increase the efficacy of immunotherapy. Necroptosis can also trigger an immunological response, further strengthening the anti-tumor immune response by activating immune cells and producing cytokines [41]. Necroptosis's role in immunotherapy underscores its potential as a therapeutic target for cancer management [42]. Our research on KLRB1 may offer fresh perspectives on necroptosis's role in immunotherapy.

Our in vitro validation laid a solid foundation for the tumor suppressive role of KLRB1. KLRB1 could inhibit the proliferation and migration of BRCA. In addition, KLRB1 could inhibit the recruitment of macrophages. It is possible that KLRB1 may interact with other molecules or signaling pathways involved in macrophage activation or function. Further research would be needed to understand how KLRB1 fully regulates macrophage activity [43].

In sum, our study developed an effective clustering system, necroptosis-related patterns. Necroptosis-related KLRB1 was a potent tumor suppressor and indicated an immune-active microenvironment. Our study is expected to provide some guidance on exploring necroptosis and cancer immunotherapy.

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because no patient or animal experiment was

involved. Informed consent was not required for this study because no participants or patients were involved.

Funding

None.

Availability of data and materials

All data generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Jie Xia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xudong Zhou:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27294.

References

[1] P. Xing, S. Wang, Y. Cao, B. Liu, F. Zheng, W. Guo, J. Huang, Z. Zhao, Z. Yang, X. Lin, L. Sang, Z. Liu, Treatment strategies and drug resistance mechanisms in adenocarcinoma of different organs, Drug Resist. Updates 71 (2023) 101002.
[2] S. Bekeschus, F. Saadati, S. Emmert, The potential of gas plasma technology for targeting breast cancer, Clin. Transl. Med. 12 (2022) e1022.
[3] Z. Zhu, H. Shen, J. Xu, Z. Fang, G. Wo, Y. Ma, K. Yang, Y. Wang, Q. Yu, J.H. Tang, GATA3 mediates doxorubicin resistance by inhibiting CYB5R2-catalyzed iron reduction in breast cancer cells, Drug Resist. Updates 69 (2023) 100974.
[4] X.Y. Liu, M.H. Zhu, X.Y. Wang, X. Dong, H.J. Liu, R.Y. Li, S.C. Jia, Q. Lu, M. Zhao, P. Sun, H.Z. Chen, C. Fang, A nano-innate immune system activator for cancer therapy in a 4T1 tumor-bearing mouse model, J. Nanobiotechnol. 20 (2022) 54.
[5] Y. Wang, H. Wang, Y. Song, M. Lv, Y. Mao, H. Song, Y. Wang, G. Nie, X. Liu, J. Cui, X. Zou, IR792-MCN@ZIF-8-PD-L1 siRNA drug delivery system enhances photothermal immunotherapy for triple-negative breast cancer under near-infrared laser irradiation, J. Nanobiotechnol. 20 (2022) 96.
[6] Y. Xue, X. Jiang, J. Wang, Y. Zong, Z. Yuan, S. Miao, X. Mao, Effect of regulatory cell death on the occurrence and development of head and neck squamous cell carcinoma, Biomark. Res. 11 (2023) 2.
[7] X. Zhang, B. Li, S. Huo, J. Du, J. Zhang, M. Song, B. Shao, Y. Li, Hexafluoropropylene oxide trimer acid exposure triggers necroptosis and inflammation through the Wnt/beta-catenin/NF-kappaB axis in the liver, Sci. Total Environ. 905 (2023) 167033.
[8] J. Yan, P. Wan, S. Choksi, Z.G. Liu, Necroptosis and tumor progression, Trends Cancer 8 (2022) 21–27.
[9] H.S. Chaouhan, C. Vinod, N. Mahapatra, S.H. Yu, I.K. Wang, K.B. Chen, T.M. Yu, C.Y. Li, Necroptosis: a pathogenic negotiator in human diseases, Int. J. Mol. Sci. 23 (2022).
[10] W. Gao, X. Wang, Y. Zhou, X. Wang, Y. Yu, Autophagy, ferroptosis, pyroptosis, and necroptosis in tumor immunotherapy, Signal Transduct. Targeted Ther. 7 (2022) 196.
[11] N. Cancer Genome Atlas Research, J.N. Weinstein, E.A. Collisson, G.B. Mills, K.R. Shaw, B.A. Ozenberger, K. Ellrott, I. Shmulevich, C. Sander, J.M. Stuart, The cancer genome atlas pan-cancer analysis project, Nat. Genet. 45 (2013) 1113–1120.
[12] C. Curtis, S.P. Shah, S.F. Chin, G. Turashvili, O.M. Rueda, M.J. Dunning, D. Speed, A.G. Lynch, S. Samarajiwa, Y. Yuan, S. Graf, G. Ha, G. Haffari, A. Bashashati, R. Russell, S. McKinney, M. Group, A. Langerod, A. Green, E. Provenzano, G. Wishart, S. Pinder, P. Watson, F. Markowetz, L. Murphy, I. Ellis, A. Purushotham, A. L. Borresen-Dale, J.D. Brenton, S. Tavare, C. Caldas, S. Aparicio, The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups, Nature 486 (2012) 346–352.
[13] P. Jezequel, O. Kerdraon, H. Hondermarck, C. Guerin-Charbonnel, H. Lasla, W. Gouraud, J.L. Canon, A. Gombos, F. Dalenc, S. Delaloge, J. Lemonnier, D. Loussouarn, V. Verriele, M. Campone, Identification of three subtypes of triple-negative breast cancer with potential therapeutic implications, Breast Cancer Res. 21 (2019) 65.
[14] C. Brueffer, J. Vallon-Christersson, D. Grabau, A. Ehinger, J. Hakkinen, C. Hegardt, J. Malina, Y. Chen, P.O. Bendahl, J. Manjer, M. Malmberg, C. Larsson, N. Loman, L. Ryden, A. Borg, L.H. Saal, Clinical value of rna sequencing-based classifiers for prediction of the five conventional breast cancer biomarkers: a report from the population-based multicenter Sweden cancerome analysis network-breast initiative, JCO Precis Oncol 2 (2018).
[15] L. Xu, X. Gao, J. Xing, Z. Guo, Identification of a necroptosis-related gene signature as a novel prognostic biomarker of cholangiocarcinoma, Front. Immunol. 14 (2023) 1118816.
[16] X.-N. Wu, D. Su, Y.-D. Mei, M.-Q. Xu, H. Zhang, Z.-Y. Wang, L.-L. Li, L. Peng, J.-Y. Jiang, J.-Y. Yang, D.-J. Li, H. Cao, Z.-W. Xia, W.-J. Zeng, Q. Cheng, N. Zhang, Identified lung adenocarcinoma metabolic phenotypes and their association with tumor immune microenvironment, Cancer Immunol. Immunother. 70 (2021) 2835–2850.
[17] F. Wu, J. Cai, C. Wen, H. Tan, Co-Sparse non-negative matrix factorization, Front. Neurosci. 15 (2021) 804554.
[18] Z. Gu, D. Hubschmann, Make interactive complex heatmaps in R, Bioinformatics 38 (2022) 1460–1462.
[19] Z. Wang, X. Wang, N. Zhang, H. Zhang, Z. Dai, M. Zhang, S. Feng, Q. Cheng, Pentraxin 3 promotes glioblastoma progression by negative regulating cells autophagy, Front. Cell Dev. Biol. 8 (2020) 795.
[20] A. Subramanian, P. Tamayo, V.K. Mootha, S. Mukherjee, B.L. Ebert, M.A. Gillette, A. Paulovich, S.L. Pomeroy, T.R. Golub, E.S. Lander, J.P. Mesirov, Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 15545–15550.

[21] N. Zhang, H. Zhang, Z. Liu, Z. Dai, W. Wu, R. Zhou, S. Li, Z. Wang, X. Liang, J. Wen, X. Zhang, B. Zhang, S. Ouyang, J. Zhang, P. Luo, X. Li, Q. Cheng, An artificial intelligence network-guided signature for predicting outcome and immunotherapy response in lung adenocarcinoma patients based on 26 machine learning algorithms, Cell Prolif. 56 (2023) e13409.
[22] A. Mayakonda, D.C. Lin, Y. Assenov, C. Plass, H.P. Koeffler, Maftools: efficient and comprehensive analysis of somatic variants in cancer, Genome Res. 28 (2018) 1747–1756.
[23] Z. Gu, Complex heatmap visualization, iMeta 1 (2022).
[24] S. Li, N. Zhang, S. Liu, H. Zhang, J. Liu, Y. Qi, Q. Zhang, X. Li, ITGA5 is a novel oncogenic biomarker and correlates with tumor immune microenvironment in gliomas, Front. Oncol. 12 (2022) 844144.
[25] X. Li, Z. Dai, X. Wu, N. Zhang, H. Zhang, Z. Wang, X. Zhang, X. Liang, P. Luo, J. Zhang, Z. Liu, Y. Zhou, Q. Cheng, R. Chang, The comprehensive analysis identified an autophagy signature for the prognosis and the immunotherapy efficiency prediction in lung adenocarcinoma, Front. Immunol. 13 (2022) 749241.
[26] T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, B. Li, X.S. Liu, TIMER2.0 for analysis of tumor-infiltrating immune cells, Nucleic Acids Res. 48 (2020) W509–W514.
[27] J.Y. Baik, Z. Liu, D. Jiao, H.J. Kwon, J. Yan, C. Kadigamuwa, M. Choe, R. Lake, M. Kruhlak, M. Tandon, Z. Cai, S. Choksi, Z.G. Liu, ZBP1 not RIPK1 mediates tumor necroptosis in breast cancer, Nat. Commun. 12 (2021) 2666.
[28] X. Ji, R. Wang, H. Tang, H. Chen, L. Bao, F. Feng, P. Jia, Necroptosis of osteoblasts was induced by breast cancer cells in vitro, Transl. Cancer Res. 9 (2020) 500–507.
[29] H. Yu, W. Lv, Y. Tan, X. He, Y. Wu, M. Wu, Q. Zhang, Immunotherapy landscape analyses of necroptosis characteristics for breast cancer patients, J. Transl. Med. 20 (2022) 328.
[30] S. Pu, Y. Zhou, P. Xie, X. Gao, Y. Liu, Y. Ren, J. He, N. Hao, Identification of necroptosis-related subtypes and prognosis model in triple negative breast cancer, Front. Immunol. 13 (2022) 964118.
[31] B. Thakur, Y. Kumar, A. Bhatia, Programmed necrosis and its role in management of breast cancer, Pathol. Res. Pract. 215 (2019) 152652.
[32] N.D. Mathewson, O. Ashenberg, I. Tirosh, S. Gritsch, E.M. Perez, S. Marx, L. Jerby-Arnon, R. Chanoch-Myers, T. Hara, A.R. Richman, Y. Ito, J. Pyrdol, M. Friedrich, K. Schumann, M.J. Poitras, P.C. Gokhale, L.N. Gonzalez Castro, M.E. Shore, C.M. Hebert, B. Shaw, H.L. Cahill, M. Drummond, W. Zhang, O. Olawoyin, H. Wakimoto, O. Rozenblatt-Rosen, P.K. Brastianos, X.S. Liu, P.S. Jones, D.P. Cahill, M.P. Frosch, D.N. Louis, G.J. Freeman, K.L. Ligon, A. Marson, E.A. Chiocca, D.A. Reardon, A. Regev, M.L. Suva, K.W. Wucherpfennig, Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis, Cell 184 (2021) 1281–1298 e26.
[33] C.L. Duurland, S.J. Santegoets, Z. Abdulrahman, N.M. Loof, G. Sturm, T.H. Wesselink, R. Arens, S. Boekestijn, I. Ehsan, M.I.E. van Poelgeest, F. Finotello, H. Hackl, Z. Trajanoski, P. Ten Dijke, V.M. Braud, M.J.P. Welters, S.H. van der Burg, CD161 expression and regulation defines rapidly responding effector CD4+ T cells associated with improved survival in HPV16-associated tumors, J Immunother Cancer 10 (2022).
[34] H. Li, K. Zhou, K. Wang, H. Cao, W. Wu, Z. Wang, Z. Dai, S. Chen, Y. Peng, G. Xiao, P. Luo, J. Zhang, Z. Liu, Q. Cheng, H. Zhang, A pan-cancer and single-cell sequencing analysis of CD161, a promising onco-immunological biomarker in tumor microenvironment and immunotherapy, Front. Immunol. 13 (2022) 1040289.
[35] W. Di, W. Fan, F. Wu, Z. Shi, Z. Wang, M. Yu, Y. Zhai, Y. Chang, C. Pan, G. Li, U.D. Kahlert, W. Zhang, Clinical characterization and immunosuppressive regulation of CD161 (KLRB1) in glioma through 916 samples, Cancer Sci. 113 (2022) 756–769.
[36] M. Domenichini, C. Ravelli, M. Corsini, S. Codenotti, E. Moreschi, A. Gogna, D. Capoferri, D. Zizioli, R. Bresciani, E. Grillo, S. Mitola, The D647N mutation of FGFR1 induces ligand-independent receptor activation, Biochim. Biophys. Acta Gen. Subj. 1867 (2023) 130470.
[37] N. Liu, H. Tian, Z. Yu, H. Zhao, W. Li, D. Sang, K. Lin, Y. Cui, M. Liao, Z. Xu, C. Chen, Y. Guo, Y. Wang, H.W. Huang, J. Wang, H. Zhang, W. Wu, H. Huang, S. Lv, Z. Guo, W. Wang, S. Zheng, F. Wang, Y. Zhang, T. Cai, E.E. Zhang, A highland-adaptation mutation of the Epas1 protein increases its stability and disrupts the circadian clock in the plateau pika, Cell Rep. 39 (2022) 110816.
[38] Y. Zhou, T.M. Bennett, P.A. Ruzycki, A. Shiels, Mutation of the EPHA2 tyrosine-kinase domain dysregulates cell pattern formation and cytoskeletal gene expression in the lens, Cells 10 (2021).
[39] D. Rodriguez-Contreras, S. Gong, J.J. Lebowitz, L.M. Fedorov, N. Asad, T.M. Dore, T.J. Phillips, C.P. Ford, J.T. Williams, K.A. Neve, Gait abnormalities and aberrant D2 receptor expression and signaling in mice carrying the human pathogenic mutation DRD2(I212F), Mol. Pharmacol. 103 (2023) 188–198.
[40] X. Niu, L. Chen, Y. Li, Z. Hu, F. He, Ferroptosis, necroptosis, and pyroptosis in the tumor microenvironment: perspectives for immunotherapy of SCLC, Semin. Cancer Biol. 86 (2022) 273–285.
[41] J. Sprooten, P. De Wijngaert, I. Vanmeerbeerk, S. Martin, P. Vangheluwe, S. Schlenner, D.V. Krysko, J.B. Parys, G. Bultynck, P. Vandenabeele, A.D. Garg, Necroptosis in immuno-oncology and cancer immunotherapy, Cells 9 (2020).
[42] R. Tang, J. Xu, B. Zhang, J. Liu, C. Liang, J. Hua, Q. Meng, X. Yu, S. Shi, Ferroptosis, necroptosis, and pyroptosis in anticancer immunity, J. Hematol. Oncol. 13 (2020) 110.
[43] W. Ye, C. Luo, C. Li, Z. Liu, F. Liu, CD161, a promising immune checkpoint, correlates with patient prognosis: a pan-cancer analysis, J. Cancer 12 (2021) 6588–6599.