Immune Score Indicator for the Survival of Melanoma Patients Based on Tumor Microenvironment

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Background: Tumor microenvironment (TME) refers to the cellular environment where tumors exist, including immune cells, fibroblasts, stromal cells, chemokines, etc. TME is closely related to the prognosis of various tumors; nevertheless, limited studies have established predictive prognosis models based on TME. This work aims to construct a survival prediction model for melanoma patients based on TME.

Methods: Data of 482 melanoma patients were extracted from The Cancer Genome Atlas (TCGA) database. Based on the infiltration of immune cells (Immune score), stromal cells (Stromal score), and tumor purity (Estimate score), the “Estimate” algorithm was used to construct 3 scores for each patient. To identify the differentially expressed genes (DEGs), Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using DAVID database and visualized using the R software. The STRING database was used to construct the protein-protein interaction (PPI) network and functional modules. FGD2 expression was confirmed via Western Blotting and quantitative reverse transcription PCR (RT-qPCR) analyses.

Results: Patients with higher immune scores estimate scores showed better OS than those with lower scores. All three scores were related to age and primary tumor stage. Further, DEGs between patients with high immune/stromal scores and low immune/stromal scores were screened. Eventually, 10 down-regulated DEGs and 201 up-regulated DEGs were identified as TME associated genes. Out of these, the FGD2 gene demonstrated close association with survival and was confirmed in the included melanoma patients.

Conclusion: In summary, TME is closely associated with the prognosis of melanoma patients. Besides, genes including FGD2 promote the TME-mediated regulation of melanoma.

Keywords: tumor microenvironment, the cancer genome atlas, melanoma, FGD2

Introduction

The tumor microenvironment (TME) is the initial internal environment where tumor cells proliferate. The main cell types in TME include stromal cells (fibroblasts, endothelial cells, and many more) and immune cells (T cells, B cells, etc.). Accumulating studies indicate that the tumor microenvironment regulates tumor immunosuppression, drug resistance, tumor invasion, metastasis, and growth.1,2

In the past decades, significant treatment efforts of cancers targeted tumor cells; nevertheless, with the growing research importance of TME, there has been a gradual shift in the concept of cancer treatment. Unlike the adaptive mutation and
acquired drug resistance produced by tumor cell accumulation, the immunotherapy approach targeting TME is stable.  As the most promising therapy in various cancers, immune checkpoint inhibitors (ICIs) are based on the immune escape in TME. Immune checkpoints are molecules producing costimulatory or inhibitory signals in the immune response, thus regulating the host immune response. Recent studies focused on the immune checkpoint PD-1 and its ligand PD-L1 signal axis. PD-L1, highly expressed in tumors, binds to PD-1 on the surface of T cells, inducing their depletion, thereby causing immune escape of tumor cells. Thus, the treatment of PD-1 or PD-L1 monoclonal antibodies to rescue the suppression of TME on T cells restores the normal activation of T cells. 3,4 Although the stromal cells in TME are not as important as immune cells in tumor immunotherapy, they regulate anti-tumor therapy. Commonly used methods minimize matrix hardness and fibrosis, thereby promoting immune cell infiltration and drug delivery. 5,6 Besides the therapeutic effect, TME mediates the prediction of cancer progression and response to immunotherapy. Reportedly, a Tumor Inflammation Signature (TIS) based on the expression of 18-gene signatures demonstrate satisfactory performance in predicting adaptive immune response. 7 In digestive system cancers, a prognostic immune score based on 22 types of immune cells shows satisfactory performance in predicting the survival of patients. 8

Melanoma is a tumor produced by melanocytes in the skin and other organs with high malignancy. Its early diagnosis and treatment are crucial for prevention. Melanoma incidence has increased at an annual rate of about 3% to 7%, hence one of the fastest-growing malignant tumors in recent years. The primary risk factors for melanoma include a history of long-term sun exposure, UV exposure history, local chronic injury, or irritation. Meanwhile, melanoma is cancer with highly activated TME. 9 As such, our research seeks to understand the prediction role of TME in melanoma and molecular mechanisms underlying TME regulation.

Materials and Methods

Data Acquisition and Score Construction

The data were obtained from the TCGA (The results here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga). database. Transcriptome data of 482 melanoma patients were identified and downloaded from the TCGA database using the R package “TCGA-Assembler”. Relevant clinical characteristics were also obtained and are shown in Table 1.

The TME score was analyzed using the R package “Estimate”; this algorithm was also used to obtain the three scores, including stromal score, immune score, and estimate score. A higher stromal score and immune score indicated higher infiltration of stromal and immune cells. The estimated score was the sum of the stromal and immune scores. A higher estimate score indicated lower purity of tumor cells.

Screening of Differentially Expressed Genes (DEGs)

The R software “Limma” package was used to normalize the expression of mRNAs based on transcript data derived from the TCGA database. Further, the “DEGseq” package was utilized to screen the DEGs between different groups. P<0.05 and Fold-change>1.5 or Fold-change<-1.5 were set as the screening filters of DEGs.

Gene Ontology, KEGG Pathway, and Gene Set Enrichment Analyses

For Gene Ontology (GO) and KEGG pathway analyses, all the screened DEGs were uploaded to the Database for Annotation Visualization and Integrated Discovery (DAVID, david.ncifcrf.gov/) online tool. Besides, concrete pathways and annotations were obtained using the above-mentioned tool and further visualized using the R software. GSEA database (http://software.broadinstitute.org/gsea/index.jsp) built-in standard datasets were used for gene set enrichment analysis (GSEA) analysis.

Protein Extraction and Western Blotting Analyses

Melanoma tissues samples were extracted from patients diagnosed with melanoma by (three independent) experienced physicians (based on Chinese guidelines for diagnosis and treatment of melanoma). The tissues of each group were digested and lysed using the 100ul RIPA lysate. After complete lysis, the lysate was centrifuged at 4 °C for 15 minutes. The supernatants were collected as the total protein extract. Then, the BCA assay was performed to quantify the proteins (Thermo Fisher Scientific, Waltham, MA, USA). Exactly 20μg proteins were then loaded and separated by 10% SDS-PAGE gels. The proteins were transferred to the PVDF membranes (0.45 mm,
Table 1 Relevant clinical characteristics of melanoma patients

| Id         | Futime | Fustat | Age | Gender | Stage     | T      | M   | N  |
|------------|--------|--------|-----|--------|-----------|--------|-----|----|
| TCGA-DA-A95Z | 396    | 0      | 87  | MALE   | Stage IV  | TX     | M1a | N0 |
| TCGA-FS-A1ZF | 470    | 1      | 78  | FEMALE | Stage IIC | T4b    | M0  | N0 |
| TCGA-DJ-A3J8 | 1992   | 1      | 48  | MALE   | Stage IB  | T2a    | M0  | N0 |
| TCGA-ER-A2NC | 1333   | 1      | 50  | MALE   | Stage IB  | T2a    | M0  | N0 |
| TCGA-RP-A693 | 10     | 0      | 77  | MALE   | Stage IV  | TX     | M1c | NX |
| TCGA-EB-A82C | 17     | 0      | 70  | FEMALE | Stage IIC | T4b    | M0  | N0 |
| TCGA-W3-A11R | 3379   | 1      | 71  | MALE   | Stage II  | T3     | M0  | N0 |
| TCGA-EF-A2EU | 2884   | 0      | 65  | FEMALE | Stage IA  | T1a    | M0  | N0 |
| TCGA-BF-A1PZ | 853    | 0      | 71  | FEMALE | Stage IIB | T4a    | M0  | N0 |
| TCGA-FS-A1ZQ | 4062   | 1      | 31  | MALE   | I/II NOS  | TX     | M0  | N0 |
| TCGA-EA-A20L | 412    | 1      | 79  | MALE   | Stage IV  | TX     | M1c | N0 |
| TCGA-FR-A44A | 5299   | 0      | 29  | FEMALE | Stage I   | T3a    | M0  | N0 |
| TCGA-DJ-A3J6 | 1766   | 1      | 54  | FEMALE | Stage IB  | T2a    | M0  | N0 |
| TCGA-EE-A3AE | 1658   | 0      | 52  | FEMALE | Stage IA  | T1a    | M0  | N0 |
| TCGA-GN-A262 | 4255   | 0      | 47  | FEMALE | unknown   | unknown| unknown | unknown |
| TCGA-ER-A2NE | 613    | 1      | 39  | MALE   | Stage 0   | T1s    | M0  | N0 |
| TCGA-DJ-A51R | 1941   | 0      | 60  | MALE   | Stage IIA | T3a    | M0  | N0 |
| TCGA-EB-A97M | 414    | 0      | 66  | MALE   | Stage IIC | T4b    | M0  | N0 |
| TCGA-WE-A82Q | 1923   | 0      | 48  | MALE   | Stage IIA | T3a    | M0  | N0 |
| TCGA-ER-A42K | 394    | 1      | 40  | FEMALE | Stage IIC | T4b    | M0  | N3 |
| TCGA-EE-A2MT | 2166   | 0      | 45  | MALE   | Stage IB  | T2a    | M0  | N0 |
| TCGA-DJ-A960 | 804    | 0      | 73  | MALE   | Stage IIB | T3b    | M0  | N0 |
| TCGA-XV-AAZY | 405    | 0      | 76  | FEMALE | Stage IIC | T4     | M0  | N3 |
| TCGA-EE-A2M6 | 3932   | 0      | 61  | MALE   | Stage I   | T1     | M0  | N0 |
| TCGA-GN-A264 | 3587   | 1      | 60  | MALE   | Stage IIC | T4b    | M0  | N1b|
| TCGA-ER-A19O | 938    | 1      | 56  | MALE   | Stage IIB | T3b    | M0  | N1b|
| TCGA-DJ-A5E9 | 301    | 0      | 75  | FEMALE | Stage IIIA| T3a    | M0  | N1 |
| TCGA-EE-A2MN | 1446   | 1      | 58  | MALE   | Stage I   | T2     | M0  | N0 |
| TCGA-DA-A1H4 | 1093   | 1      | 51  | MALE   | Stage IIC | T3b    | M0  | N2b|
| TCGA-EE-A3AB | 3733   | 0      | 30  | MALE   | Stage III | T0     | M0  | N2a|
| TCGA-DA-A3FB | 1319   | 0      | 39  | MALE   | Stage IIB | T2a    | M0  | N2b|
| TCGA-BF-AAP6 | 325    | 0      | 55  | MALE   | Stage III | T4b    | M0  | N2 |
| TCGA-FS-A1ZD | 1628   | 1      | 63  | MALE   | Stage IIA | T2b    | M0  | N0 |
| TCGA-DJ-A4Z3 | 505    | 0      | 73  | FEMALE | Stage IIC | T4b    | M0  | N1b|
| TCGA-DJ-A8GB | 938    | 1      | 48  | MALE   | Stage IIB | T3a    | M0  | N1b|
| TCGA-DA-A95V | 2193   | 0      | 83  | FEMALE | Stage IIC | T4b    | M0  | N0 |
| TCGA-EE-A25S | 1195   | 1      | 43  | MALE   | Stage IB  | T2a    | M0  | N0 |
| TCGA-DJ-A324 | 519    | 1      | 54  | MALE   | Stage IIC | T4b    | M0  | N3 |
| TCGA-FR-A8YE | 3176   | 0      | 41  | MALE   | Stage IA  | T1a    | M0  | N0 |
| TCGA-EE-A2GC | 2051   | 0      | 82  | MALE   | Stage IIB | T3b    | M0  | N0 |
| TCGA-EE-A29G | 2192   | 1      | 53  | MALE   | Stage IIIA| T4a    | M0  | N2a|
| TCGA-EE-A29S | 1864   | 1      | 79  | MALE   | Stage IIA | T3a    | M0  | N0 |
| TCGA-DJ-A3MO | 284    | 1      | 47  | MALE   | Stage III | TX     | M0  | N2c|
| TCGA-WE-A82Z | 2145   | 0      | 73  | FEMALE | Stage IIB | T3a    | M0  | N1b|
| TCGA-BF-A9VE | 440    | 0      | 77  | MALE   | Stage IIC | T4b    | M0  | N0 |
| TCGA-YD-A89C | 210    | 0      | 43  | FEMALE | Stage IA  | T1a    | M0  | NX |
| TCGA-EE-A2GT | 1365   | 0      | 77  | MALE   | Stage IIA | T3a    | M0  | N0 |
| TCGA-HR-A5NC | 0      | 0      | 90  | FEMALE | unknown   | unknown| unknown | unknown |
| TCGA-ER-A19G | 9188   | 0      | 48  | FEMALE | unknown   | unknown| unknown | unknown |
| TCGA-DJ-A3CB | 5065   | 0      | 39  | MALE   | I/II NOS  | T2     | M0  | N0 |
| TCGA-EB-A44P | 741    | 0      | 58  | FEMALE | Stage IIC | T4b    | M0  | N0 |

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| Id               | Futime | Fustat | Age | Gender | Stage       | T   | M   | N  |
|------------------|--------|--------|-----|--------|-------------|-----|-----|----|
| TCGA-EB-A6R0     | 608    | 1      | 58  | FEMALE | Stage IIIC  | T4b | M0  | N0 |
| TCGA-D3-A8GD     | 718    | 0      | 63  | FEMALE | Stage IIIC  | T4b | M0  | N3 |
| TCGA-ER-A197     | 424    | 1      | 83  | FEMALE | Stage IIIIB | T4b | M0  | N1a|
| TCGA-EE-A29X     | 545    | 1      | 58  | FEMALE | Stage IB    | T2a | M0  | N0 |
| TCGA-YD-A9TA     | 1496   | 0      | 75  | MALE   | unknown     | T2  | M0  | N0 |
| TCGA-EE-A2GE     | 5286   | 0      | 44  | MALE   | Stage I     | T3  | M0  | N0 |
| TCGA-EB-A57M     | 472    | 1      | 56  | MALE   | Stage IIIIB | T4b | M0  | N1 |
| TCGA-EB-A85J     | 360    | 0      | 66  | FEMALE | Stage IIIB  | T4a | M0  | N0 |
| TCGA-D3-A2JB     | 5110   | 1      | 70  | FEMALE | Stage I     | T3s | M0  | N0 |
| TCGA-D3-A1QB     | 2912   | 0      | 75  | FEMALE | Stage IIIC  | T0  | M0  | N2c|
| TCGA-D3-A2JE     | 841    | 1      | 75  | FEMALE | Stage IIIC  | TX  | M0  | N3 |
| TCGA-DA-A3F5     | 6873   | 1      | 45  | MALE   | Stage I     | T1a | M0  | N0 |
| TCGA-EE-A2M5     | 659    | 1      | 49  | MALE   | Stage I     | T2  | M0  | N0 |
| TCGA-D3-A2JA     | 3514   | 0      | 68  | MALE   | Stage IIIA  | T2a | M0  | N1a|
| TCGA-ER-A19H     | 4634   | 1      | 40  | MALE   | unknown     | unknown | M0  | N0 |
| TCGA-EE-A3JA     | 1618   | 1      | 44  | MALE   | Stage IB    | T2a | M0  | N0 |
| TCGA-FS-A4F8     | 5318   | 1      | 52  | MALE   | Stage I     | T1  | M0  | N0 |
| TCGA-WE-A82R     | 274    | 1      | 49  | MALE   | Stage IIIC  | T4b | M0  | N1b|
| TCGA-EE-A3JD     | 832    | 1      | 70  | MALE   | Stage III   | TX  | M0  | N2b|
| TCGA-Z2-A3S      | 2950   | 0      | 58  | MALE   | Stage I     | T1a | M0  | N0 |
| TCGA-ER-A198     | 1544   | 1      | 45  | MALE   | unknown     | unknown | M0  | NX |
| TCGA-ER-A42L     | 4533   | 0      | 49  | MALE   | Stage II    | T3  | M0  | N0 |
| TCGA-FR-A7U8     | 847    | 0      | 50  | MALE   | Stage IIIIC | TX  | M0  | N3 |
| TCGA-EB-A41B     | 291    | 0      | 76  | FEMALE | Stage IIIC  | T4b | M0  | N0 |
| TCGA-EB-A44O     | 81     | 0      | 69  | MALE   | Stage IIIIB | T4a | M0  | N0 |
| TCGA-GN-A267     | 1960   | 1      | 38  | MALE   | Stage IIIA  | T4a | M0  | N1a|
| TCGA-EE-A2MI     | 6225   | 1      | 43  | MALE   | Stage IIIB  | T4  | M0  | N0 |
| TCGA-ER-A19N     | 1341   | 1      | 47  | MALE   | unknown     | unknown | unknown | N0 |
| TCGA-EB-A3XC     | 650    | 0      | 74  | MALE   | Stage IIIIC | T4b | M0  | N0 |
| TCGA-EE-A2M7     | 877    | 1      | 66  | MALE   | Stage II    | T3a | M0  | N0 |
| TCGA-EB-A42Z     | 441    | 0      | 49  | MALE   | Stage IIIIC | T4b | M0  | N1b|
| TCGA-D3-A8G1     | 1780   | 1      | 68  | MALE   | Stage I     | T1a | M0  | N0 |
| TCGA-FR-A728     | 583    | 0      | 54  | MALE   | Stage IIIB  | T4b | M0  | N2a|
| TCGA-D3-A8GQ     | 884    | 1      | 66  | MALE   | Stage II    | T3  | M0  | N0 |
| TCGA-DA-A115     | 4107   | 0      | 27  | FEMALE | Stage IV    | T1a | M1c | N0 |
| TCGA-EE-A2GK     | 1665   | 0      | 46  | FEMALE | Stage I     | T1  | M0  | N0 |
| TCGA-BF-A5EO     | 703    | 0      | 65  | MALE   | Stage IIIC  | T4b | M0  | N0 |
| TCGA-ER-A3E5     | 1429   | 1      | 55  | MALE   | Stage III   | T4  | M0  | N0 |
| TCGA-EB-A40Y     | 977    | 0      | 65  | FEMALE | Stage IIIIB | T4b | M0  | N1a|
| TCGA-D3-A3CF     | 746    | 1      | 61  | FEMALE | Stage IIIIC | T4b | M0  | N3 |
| TCGA-XV-AB01     | 403    | 0      | 54  | FEMALE | Stage II    | T3  | M0  | NX |
| TCGA-EE-A29E     | 1940   | 0      | 54  | MALE   | Stage IIIIB | T3a | M0  | N1b|
| TCGA-DA-A1IC     | 2071   | 1      | 81  | MALE   | Stage IIIIB | T3a | M0  | N2c|
| TCGA-EE-A180     | 2889   | 1      | 69  | MALE   | Stage III   | T4a | M0  | N0 |
| TCGA-D3-A5GS     | 553    | 0      | 58  | MALE   | Stage IV    | T1b | M1c | N1b|
| TCGA-EE-A3AC     | 1948   | 0      | 47  | MALE   | Stage III   | T0  | M0  | N2b|
| TCGA-FS-A1ZZ     | 822    | 1      | 54  | FEMALE | Stage IIIB  | T3b | M0  | N0 |
| TCGA-WE-A8K1     | 1492   | 0      | 74  | MALE   | Stage IIIC  | T3b | M0  | N3 |
| TCGA-ER-A8YC     | 1059   | 1      | 78  | MALE   | Stage IIIB  | T3b | M0  | N0 |
| TCGA-FS-A1Z7     | 237    | 1      | 19  | MALE   | Stage IIIIC | T4b | M0  | N1b|

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**Table 1 (Continued).**

| Id             | Funtime | Fustat | Age | Gender | Stage       | T  | M | N |
|----------------|---------|--------|-----|--------|-------------|----|---|---|
| TCGA-FS-A1ZK   | 728     | 1      | 68  | MALE   | Stage II    | T4 | M0| N0|
| TCGA-DJ-A3CC   | 2644    | 0      | 69  | FEMALE | Stage IIIC  |    |   |   |
| TCGA-WE-A8JZ   | 731     | 0      | 70  | MALE   | Stage IIIB  | T4b| M0| N1a|
| TCGA-ER-A19M   | 1857    | 1      | 36  | MALE   | Stage IB    | T2a| M0| N0|
| TCGA-FS-A1ZN   | 730     | 1      | 43  | MALE   | Stage IIIA  | T4b| M0| N1a|
| TCGA-DJ-A8GL   | 2711    | 1      | 43  | MALE   | Stage IIIB  | T2a| M0| N1b|
| TCGA-EB-A5UN   | 1792    | 0      | 49  | MALE   | Stage IIIC  | T4b| M0| NX|
| TCGA-EJ-A17X   | 907     | 1      | 54  | MALE   | Stage IA    | T1a| M0| N0|
| TCGA-EJ-A2GD   | 10346   | 1      | 58  | FEMALE | Stage IIIB  | T4 | M0| N0|
| TCGA-EB-A3XE   | 180     | 0      | 77  | FEMALE | Stage IIA   | T3a| M0| N0|
| TCGA-FR-A726   | 0       | 0      | 90  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-D9-A4Z2   | 190     | 1      | 50  | MALE   | Stage III   | T4b| M0| N3|
| TCGA-FS-A4FC   | 1655    | 1      | 75  | FEMALE | Stage IIA   | T3a| M0| N0|
| TCGA-XV-AAZW   | 393     | 1      | 62  | FEMALE | Stage II    | T4 | M0| N0|
| TCGA-EB-A1NK   | 1039    | 0      | 48  | MALE   | Stage IIIC  | T4b| M0| N0|
| TCGA-EJ-A2GJ   | 2270    | 0      | 83  | MALE   | Stage IA    | T1a| M0| N0|
| TCGA-EJ-A20B   | 4070    | 0      | 66  | FEMALE | Stage II    | T3 | M0| N0|
| TCGA-EJ-A2MK   | 5487    | 0      | 18  | FEMALE | Stage IIA   | T4a| M0| N0|
| TCGA-GF-A3OT   | 301     | 0      | 58  | FEMALE | Stage IIC   | T3 | M0| N3|
| TCGA-FS-A1ZG   | 295     | 1      | 60  | FEMALE | Stage III   | T4b| M0| N2b|
| TCGA-EE-A182   | 447     | 1      | 84  | FEMALE | Stage III   | T4b| M0| N1b|
| TCGA-FS-A1ZJ   | 636     | 1      | 72  | FEMALE | Stage IV    | TX | M1| N0|
| TCGA-ER-A19W   | 4507    | 1      | 48  | FEMALE | unknown     | unknown| unknown| unknown|
| TCGA-ER-A19P   | 4930    | 1      | 47  | FEMALE | unknown     | unknown| M0| N0|
| TCGA-ER-A19C   | 1487    | 1      | 77  | MALE   | Stage I     | T2a| M0| NX|
| TCGA-EE-A5ES   | 490     | 0      | 76  | FEMALE | Stage IIC   | T4b| M0| N0|
| TCGA-D9-A1X3   | 551     | 0      | 63  | MALE   | unknown     | T4b| unknown| N2b|
| TCGA-DJ-A1Q7   | 4053    | 0      | 42  | FEMALE | Stage IB    | T1b| M0| N0|
| TCGA-EB-A4PO   | 326     | 1      | 82  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-DJ-A3MV   | 1378    | 0      | 38  | FEMALE | Stage IIIB  | T2b| M0| N2a|
| TCGA-EE-A2GM   | 2296    | 0      | 70  | FEMALE | Stage IIC   | T4b| M0| N0|
| TCGA-FR-A7U9   | 571     | 0      | 63  | FEMALE | Stage III   | T3b| M0| N3|
| TCGA-FS-A1ZB   | 1486    | 1      | 57  | MALE   | Stage II    | T3a| M0| N0|
| TCGA-QB-A6FS   | 220     | 0      | 49  | MALE   | Stage III   | T0 | M0| N3|
| TCGA-WE-A8ZY   | 1506    | 1      | 62  | MALE   | Stage IIA   | T3a| M0| NX|
| TCGA-EE-A2MF   | 8174    | 1      | 39  | FEMALE | Stage I     | T2 | M0| N0|
| TCGA-EB-A4XL   | 777     | 0      | 56  | FEMALE | Stage IIC   | T4b| M0| NX|
| TCGA-EE-A185   | 151     | 1      | 55  | FEMALE | Stage III   | T4b| M0| N3|
| TCGA-GN-A4U3   | 3708    | 0      | 30  | MALE   | Stage III   | T3a| M0| N1a|
| TCGA-EB-A3XB   | 796     | 0      | 63  | MALE   | Stage II    | T4 | M0| N0|
| TCGA-EE-A2A0   | 1424    | 1      | 77  | FEMALE | Stage IIA   | T3a| M0| N0|
| TCGA-DA-A3F3   | 319     | 1      | 52  | MALE   | Stage IIIB  | T0 | M0| N2b|
| TCGA-EE-A3AF   | 420     | 1      | 48  | FEMALE | Stage III   | T0 | M0| N3|
| TCGA-DJ-A3BZ   | 3976    | 0      | 63  | MALE   | Stage IIB   | T4a| M0| N0|
| TCGA-ER-A3ET   | 2829    | 1      | 64  | FEMALE | Stage IIIA  | T3a| M0| N1a|
| TCGA-RP-A694   | 21      | 0      | 71  | MALE   | Stage IV    | TX | M1c| NX|
| TCGA-EE-A29C   | 2402    | 1      | 20  | MALE   | Stage IB    | T2a| M0| N0|
| TCGA-EE-A2MH   | 516     | 1      | 66  | MALE   | Stage III   | T4a| M0| N0|
| TCGA-EB-A5UM   | 779     | 0      | 48  | FEMALE | Stage IIC   | T4b| M0| N0|
| TCGA-EE-A2MC   | 1871    | 1      | 73  | MALE   | Stage I     | T2 | M0| N0|

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Table 1 (Continued).

| Id          | Funtime | Fustat | Age | Gender   | Stage     | T | M | N  |
|-------------|---------|--------|-----|----------|-----------|---|---|----|
| TCGA-BF-A1PV | 14      | 0      | 74  | FEMALE   | Stage IIC | T4b | M0 | N0 |
| TCGA-GF-A6C8 | 62      | 0      | 62  | MALE     | Stage IIIB | T3b | M0 | N2b|
| TCGA-XV-A9VZ | 0       | 0      | 90  | FEMALE   | Stage II   | T4  | M0 | N0 |
| TCGA-GN-A4U7 | 317     | 1      | 56  | FEMALE   | Stage IIC  | T2b | M0 | N3 |
| TCGA-DA-A95W | 1136    | 0      | 52  | MALE     | Stage IIIB | TX  | M0 | N1b|
| TCGA-D3-A3C3 | 0       | 0      | unknown | FEMALE | I/II NOS  | TX  | M0 | N0 |
| TCGA-D3-A51K | 1002    | 0      | 51  | MALE     | Stage IIIB | Tis | M0 | N2b|
| TCGA-D3-A2JD | 361     | 1      | 58  | MALE     | Stage IIC  | T4b | M0 | N1b|
| TCGA-E6-A5U6 | 321     | 1      | 56  | MALE     | Stage IIIB | T4b | M0 | N1 |
| TCGA-WE-A82Z | 1794    | 0      | 57  | MALE     | Stage IIIB | T4a | M0 | N2c|
| TCGA-FS-A1ZH | 996     | 1      | 71  | FEMALE   | Stage IV   | T3b | M1c| N2c|
| TCGA-D3-A51F | 1695    | 0      | 51  | MALE     | Stage IIIIC| T4b | M0 | N1b|
| TCGA-BF-AAP2 | 405     | 0      | 62  | MALE     | Stage IIIB | T3b | M0 | N0 |
| TCGA-ER-A19L | 4000    | 1      | 35  | MALE     | unknown   | unknown | unknown | unknown |
| TCGA-W3-A825 | 1917    | 1      | 60  | MALE     | Stage II   | T3  | M0 | N0 |
| TCGA-GN-A26S | 2948    | 0      | 53  | MALE     | unknown   | unknown | unknown | unknown |
| TCGA-D3-A2JG | 3453    | 1      | 30  | MALE     | Stage IIIA | T3a | M0 | N1a|
| TCGA-EB-A55G | 2076    | 0      | 57  | FEMALE   | unknown   | unknown | unknown | unknown |
| TCGA-W3-A828 | 3683    | 1      | 66  | MALE     | Stage II   | T3  | M0 | N0 |
| TCGA-FS-A1ZA | 843     | 1      | 45  | FEMALE   | Stage IIIB | T4b | M0 | N2c|
| TCGA-OD-A75X | 9061    | 1      | 49  | MALE     | unknown   | TX  | M0 | N3 |
| TCGA-EB-A5SE | 401     | 1      | 73  | MALE     | Stage IIIB | T3b | M0 | N2c|
| TCGA-D3-A8GK | 5177    | 0      | 45  | MALE     | Stage II   | T3a | M0 | N0 |
| TCGA-BF-A4P4 | 335     | 0      | 61  | MALE     | Stage IIIC | T4b | M0 | N0 |
| TCGA-EE-A2MP | 7563    | 0      | 34  | FEMALE   | Stage I    | T2  | M0 | N0 |
| TCGA-FW-A50Y | 587     | 0      | 48  | MALE     | Stage III  | T3  | unknown | N1 |
| TCGA-EB-A5KH | 619     | 1      | 55  | MALE     | Stage III  | T0  | M0 | N1 |
| TCGA-EE-A2MM | 5107    | 1      | 63  | FEMALE   | Stage I    | T2  | M0 | N0 |
| TCGA-EB-A5FP | 454     | 1      | 65  | FEMALE   | Stage IV   | T4b | M1b| N1b|
| TCGA-FS-A1ZR | 347     | 1      | 36  | MALE     | Stage II   | T2  | M0 | N0 |
| TCGA-WE-A82T | 359     | 0      | 25  | MALE     | Stage IV   | T3b | M1b| N1b|
| TCGA-D3-A2J6 | 1321    | 1      | 65  | MALE     | Stage IIIB | T3b | M0 | N0 |
| TCGA-EE-A2Q9 | 2030    | 1      | 70  | MALE     | Stage IIIB | T3b | M0 | N0 |
| TCGA-EE-A2GS | 2470    | 1      | 28  | FEMALE   | Stage IB   | T2a | M0 | N0 |
| TCGA-D3-A1QA | 2765    | 0      | 55  | MALE     | Stage IB   | T2a | M0 | N0 |
| TCGA-D9-A1JW | 111     | 0      | 82  | MALE     | unknown   | T1a | M0 | N2a|
| TCGA-ER-A2ND | 710     | 1      | 57  | FEMALE   | Stage IIIC | T1b | M0 | N3 |
| TCGA-GN-A26C | 821     | 1      | 77  | MALE     | Stage IIIC | T4b | M0 | N2b|
| TCGA-EE-A2ME | 3141    | 1      | 51  | MALE     | Stage I    | T2  | M0 | N0 |
| TCGA-WE-A3A3 | 651     | 0      | 84  | FEMALE   | Stage IIIC | T4b | M0 | N2b|
| TCGA-BF-A3DM | 601     | 0      | 63  | MALE     | Stage IIIA | T2b | M0 | N0 |
| TCGA-D3-A3MU | 1209    | 0      | 53  | MALE     | Stage IIIA | T3a | M0 | N2a|
| TCGA-ER-A3YN | 2828    | 0      | 44  | MALE     | Stage IB   | T2a | M0 | N0 |
| TCGA-D9-A3Z3 | 678     | 0      | 39  | FEMALE   | Stage IIIB | T3a | M0 | N1b|
| TCGA-EE-A3J7 | 1949    | 0      | 43  | MALE     | Stage I    | T2  | M0 | N0 |
| TCGA-D3-A2JF | 1888    | 0      | 74  | MALE     | Stage IA   | T1a | M0 | N0 |
| TCGA-EE-A29L | 79      | 1      | 78  | MALE     | Stage IIIC | T4b | M0 | N3 |
| TCGA-FS-A4FB | 813     | 1      | 46  | FEMALE   | Stage III  | T2  | M0 | N1a|
| TCGA-D3-A5GN | 4129    | 0      | 15  | FEMALE   | Stage I    | T1  | M0 | N0 |
| TCGA-XV-AAZV | 412     | 0      | 56  | FEMALE   | Stage II   | T4  | M0 | N0 |

(Continued)
### Table 1 (Continued)

| Id                  | Futime | Fustat | Age | Gender | Stage          | T     | M | N       |
|---------------------|--------|--------|-----|--------|----------------|-------|---|---------|
| TCGA-D9-A149        | 1663   | 0      | 65  | FEMALE | Stage IIB      | TX    | M0| N1b     |
| TCGA-DA-A1H0        | 1096   | 1      | 37  | FEMALE | Stage IIB      | T1a   | M0| N1b     |
| TCGA-ER-A2NB        | 857    | 1      | 57  | MALE   | Stage IIC      | T4b   | M0| N2      |
| TCGA-D3-A1Q3        | 507    | 1      | 64  | MALE   | Stage IIC      | T4b   | M0| N0      |
| TCGA-D3-A2J7        | 3136   | 1      | 67  | MALE   | Stage III C    | T3b   | M0| N1b     |
| TCGA-ER-A2NG        | 1490   | 1      | 43  | FEMALE | Stage III C    | T3b   | M0| N3      |
| TCGA-FS-A1Z2        | 1413   | 1      | 40  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-DA-A95X        | 2249   | 0      | 62  | MALE   | Stage I B      | T2a   | M0| N0      |
| TCGA-FS-A4FD        | 2454   | 1      | 39  | MALE   | Stage III C    | T2    | M0| N3      |
| TCGA-GN-A26D        | 1460   | 1      | 72  | FEMALE | Stage II C     | T4b   | unknown| N0    |
| TCGA-3N-A9WC        | 2022   | 0      | 82  | MALE   | Stage I A      | T2b   | M0| NX      |
| TCGA-D3-A1Q6        | 2184   | 1      | 55  | MALE   | Stage III      | T4    | M0| N1b     |
| TCGA-ER-A2NF        | 877    | 1      | 53  | MALE   | Stage III C    | T3b   | M0| N3      |
| TCGA-FS-A1Z0        | 6164   | 1      | 32  | FEMALE | Stage I A      | T1a   | M0| N0      |
| TCGA-BF-A1Q0        | 831    | 0      | 80  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-EE-A2GR        | 1301   | 1      | 78  | MALE   | Stage II       | T4    | M0| N0      |
| TCGA-WE-AAA0        | 1229   | 0      | 47  | MALE   | Stage I A      | T1a   | M0| N0      |
| TCGA-EE-A29P        | 1716   | 0      | 73  | FEMALE | Stage II C     | T4b   | M0| N0      |
| TCGA-WE-A8K5        | 1860   | 1      | 65  | MALE   | Stage IV       | T2a   | M1c | N3      |
| TCGA-YG-AA3N        | 306    | 0      | 67  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-DA-A11B        | 1235   | 1      | 69  | FEMALE | Stage II C     | T2b   | M0| N2b     |
| TCGA-EB-A430        | 282    | 1      | 83  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-BF-A1PX        | 1525   | 1      | 46  | FEMALE | Stage II C     | T4b   | M0| N0      |
| TCGA-EE-A4F2        | 772    | 0      | 68  | MALE   | Stage II C     | T4b   | M0| NX      |
| TCGA-DA-A1Q0        | 378    | 0      | 63  | MALE   | Stage I A      | T2b   | M0| N0      |
| TCGA-BF-A4P2        | 688    | 0      | 56  | FEMALE | Stage IV       | T0    | M1c | N3      |
| TCGA-DA-A199        | 1620   | 0      | 71  | MALE   | Stage I A      | T1a   | M0| N0      |
| TCGA-BF-A4AP        | 297    | 1      | 86  | FEMALE | Stage III C    | T4b   | M0| N3      |
| TCGA-BF-A4P8        | 447    | 0      | 58  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-ER-A194        | 1354   | 1      | 77  | MALE   | unknown        | unknown| M0| N0      |
| TCGA-EB-A5UL        | 891    | 0      | 71  | MALE   | Stage III      | TX    | M0| N1      |
| TCGA-DA-A29H        | 1966   | 0      | 59  | FEMALE | Stage I A      | T1a   | M0| N0      |
| TCGA-D3-A51N        | 494    | 0      | 56  | FEMALE | Stage IV       | T0    | M1c | N3      |
| TCGA-EB-A55H        | 1740   | 0      | 60  | FEMALE | Stage III      | T4b   | M0| N0      |
| TCGA-EE-A2M2        | 292    | 1      | 60  | MALE   | Stage III      | T4b   | M0| N0      |
| TCGA-RP-A690        | 6      | 0      | 66  | FEMALE | unknown        | unknown| M0| N0      |
| TCGA-ER-A194        | 2588   | 1      | 67  | MALE   | Stage II B     | T3b   | M0| N0      |
| TCGA-ER-A194        | 549    | 1      | 73  | MALE   | Stage III C    | TX    | M0| N3      |
| TCGA-EB-A550        | 264    | 1      | 75  | FEMALE | Stage II C     | T4b   | M0| NX      |
| TCGA-DA-A199        | 1441   | 1      | 75  | FEMALE | Stage I        | T2    | M0| N0      |
| TCGA-EB-A194        | 39     | 0      | 37  | MALE   | Stage II C     | T4b   | M0| N0      |
| TCGA-3N-A9WB        | 518    | 1      | 71  | MALE   | Stage I A      | T1a   | M0| NX      |
| TCGA-W3-AA21        | 3195   | 1      | 26  | MALE   | Stage I        | T2    | M0| N0      |
| TCGA-D3-A8GC        | 2421   | 1      | 48  | MALE   | Stage III C    | TX    | M0| N3      |
| TCGA-FS-A1Z2        | 2421   | 1      | 63  | FEMALE | Stage II C     | T4b   | M0| N0      |
| TCGA-D3-A181        | 1026   | 1      | 82  | FEMALE | Stage II       | T3    | M0| N0      |
| TCGA-D3-A8GP        | 4638   | 0      | 77  | MALE   | Stage III      | T2    | M0| N2c     |
| TCGA-BF-AAP0        | 454    | 0      | 40  | FEMALE | Stage IV       | T4    | M1 | NX      |
| TCGA-DA-A181        | 1640   | 1      | 63  | FEMALE | Stage II C     | T4b   | M0| N0      |
| TCGA-D3-A5GO        | 4195   | 0      | 61  | MALE   | Stage II       | T4    | M0| N0      |
Table 1 (Continued).

| Id                  | Futime | Fustat | Age | Gender | Stage       | T  | M | N |
|---------------------|--------|--------|-----|--------|-------------|----|---|---|
| TCGA-D3-A51T        | 818    | 0      | 59  | FEMALE | Stage IIC   | T4b| M0| N1b|
| TCGA-ER-A19F        | 802    | 1      | 82  | MALE   | unknown     | unknown| M0| N0|
| TCGA-EB-A44R        | 315    | 1      | 52  | MALE   | Stage IIB   | TX | M0| N2b|
| TCGA-FS-A1Z4        | 854    | 1      | 62  | MALE   | Stage I     | T1 | M0| N0|
| TCGA-ER-A3YO        | 0      | unknown|     | FEMALE | I/I NOS     | T2 | M0| N0|
| TCGA-BF-AAPI        | 409    | 0      | 86  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-D9-A3Z1        | 468    | 1      | 66  | MALE   | Stage IIC   | T2a| M0| N3|
| TCGA-EB-A6L9        | 1109   | 0      | 55  | MALE   | Stage IIC   | TX | M0| N3|
| TCGA-ER-A42H        | 426    | 1      | 76  | MALE   | unknown     | unknown| unknown| unknown|
| TCGA-ER-A19S        | 1505   | 0      | 81  | FEMALE | unknown     | unknown| unknown| unknown|
| TCGA-ER-A1IA        | 3196   | 0      | 58  | MALE   | Stage IIC   | TX | M0| N3|
| TCGA-DA-A1II        | 6768   | 0      | 55  | MALE   | Stage III   | T0 | M0| N2a|
| TCGA-D3-A3CI        | 0      | unknown|     | MALE   | I/I NOS     | TX | M0| N0|
| TCGA-EB-A82B        | 390    | 0      | 58  | FEMALE | Stage III   | T4b| M0| N2|
| TCGA-EE-A29A        | 1927   | 1      | 68  | MALE   | Stage IIIA  | T3a| M0| N1a|
| TCGA-EB-A3I         | 568    | 0      | 34  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-FS-A4FS        | 874    | 1      | 77  | FEMALE | Stage IB    | T2a| M0| N0|
| TCGA-EB-A42Y        | 721    | 1      | 73  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-D3-A2JK        | 368    | 1      | 24  | MALE   | Stage IIC   | T4b| M0| N2b|
| TCGA-D3-A51J        | 4414   | 0      | 19  | MALE   | Stage III   | T0 | M0| N1b|
| TCGA-WE-A82X        | 1089   | 0      | 45  | MALE   | Stage IIIIB  | TX | M0| N1b|
| TCGA-EE-A29T        | 11252  | 0      | 51  | FEMALE | unknown     | TX | M0| NX|
| TCGA-ER-A19J        | 196    | 1      | 54  | MALE   | Stage IV    | TX | M1| N3|
| TCGA-W3-A11W        | 6666   | 0      | 64  | MALE   | Stage II    | T3 | M0| N0|
| TCGA-BF-A1PU        | 387    | 0      | 46  | FEMALE | Stage IIC   | T4b| M0| N0|
| TCGA-EB-A3XF        | 278    | 0      | 57  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-GN-A4U9        | 673    | 1      | 71  | MALE   | Stage IIC   | T2b| M0| N3|
| TCGA-EB-A4IS        | 774    | 0      | 77  | MALE   | Stage IIB   | T3b| M0| NX|
| TCGA-FS-A4FO        | 2367   | 0      | 67  | FEMALE | Stage IIB   | T4a| M0| N0|
| TCGA-BF-A5EP        | 335    | 0      | 75  | FEMALE | Stage IIC   | T4b| M0| N3|
| TCGA-EB-A41A        | 955    | 0      | 90  | MALE   | Stage IIC   | T4b| M0| N0|
| TCGA-ER-A193        | 2010   | 0      | 50  | FEMALE | Stage IIC   | T3b| M0| N0|
| TCGA-EB-A2J0        | 11217  | 0      | 24  | FEMALE | unknown     | unknown| unknown| unknown|
| TCGA-LH-A9QB        | 1832   | 1      | 74  | FEMALE | Stage III   | T0 | M0| N1b|
| TCGA-D3-A3CE        | 3826   | 0      | 74  | MALE   | Stage IB    | T2a| M0| N0|
| TCGA-D3-A5GL        | 1124   | 1      | 71  | MALE   | Stage III   | T4a| M0| N1|
| TCGA-EE-A3J5        | 425    | 1      | 87  | MALE   | Stage IIC   | T3b| M0| N1b|
| TCGA-EE-A29Q        | 2620   | 0      | 43  | MALE   | Stage IA    | T1a| M0| N0|
| TCGA-D3-A51E        | 5318   | 0      | 39  | FEMALE | I/I NOS     | T2 | M0| N0|
| TCGA-EE-A2GH        | 6699   | 0      | 34  | MALE   | Stage I     | T2 | M0| N0|
| TCGA-EE-A2A2        | 1814   | 0      | 71  | MALE   | Stage IIC   | T4b| M0| N1b|
| TCGA-GN-A9SD        | 1807   | 1      | 59  | FEMALE | Stage IA    | T1a| M0| NX|
| TCGA-EE-A183        | 818    | 1      | 48  | MALE   | Stage IIB   | T4a| M0| N0|
| TCGA-EE-A17Z        | 263    | 1      | 57  | MALE   | Stage IIB   | T4a| M0| N0|
| TCGA-GF-A6C9        | 480    | 0      | 78  | MALE   | Stage IIB   | unknown| unknown| unknown|
| TCGA-D9-A4Z5        | 218    | 0      | 68  | MALE   | Stage IIB   | T4a| M0| N0|
| TCGA-D3-A1Q1        | 504    | 1      | 79  | FEMALE | Stage IIC   | T1b| M0| N3|
| TCGA-EB-A3Y7        | 326    | 1      | 86  | FEMALE | Stage IIB   | T3a| M0| N2c|
| TCGA-ER-A3PL        | 1010   | 0      | 30  | MALE   | Stage IV    | T3b| M1a| N0|

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Table 1 (Continued).

| Id          | Futime | Fustat | Age | Gender | Stage   | T   | M | N  |
|-------------|--------|--------|-----|--------|---------|-----|---|----|
| TCGA-D3-A5GU| 3808   | 0      | 36  | MALE   | Stage IB| T1b | M0| N0 |
| TCGA-EF-A2GP| 423    | 1      | 80  | MALE   | Stage IIB| T4b | M0| N1a|
| TCGA-FS-A1YW| 6598   | 1      | 52  | MALE   | Stage IB| T1b | M0| N0 |
| TCGA-D3-A3JN| 2022   | 1      | 46  | FEMALE | Stage III| T0  | M0| N1b|
| TCGA-FS-A1ZC| 10870  | 1      | 51  | MALE   | I/II NOS| TX  | M0| N0 |
| TCGA-EF-A2MS| 4942   | 0      | 72  | MALE   | Stage II| T3a | M0| N0 |
| TCGA-WJ-A824| 6940   | 0      | 63  | MALE   | Stage I | T2  | M0| N0 |
| TCGA-FS-A1ZW| 1505   | 0      | 65  | MALE   | Stage III| T2b | M0| N1a|
| TCGA-D9-A1JX| 216    | 1      | 80  | FEMALE | unknown | TX  | M0| NX |
| TCGA-EF-A3JB| 6138   | 0      | 60  | MALE   | Stage III| T3a | M0| N1 |
| TCGA-EF-A2GI| 1482   | 0      | 39  | MALE   | Stage I| T1a | M0| N0 |
| TCGA-EF-A3JH| 4086   | 0      | 54  | MALE   | Stage I | T2  | M0| N0 |
| TCGA-D3-A2JP| 1812   | 0      | 37  | MALE   | Stage III| T0  | M0| N3 |
| TCGA-ER-A19Q| 1548   | 1      | 37  | FEMALE | unknown | unknown| M0| N0 |
| TCGA-FR-A8YD| 1103   | 1      | 56  | FEMALE | Stage II| T4b | M0| N0 |
| TCGA-BF-A3DJ| 464    | 0      | 36  | FEMALE | Stage III| T4b | M0| N1 |
| TCGA-EF-A20F| 2785   | 0      | 53  | MALE   | Stage I | T1  | M0| N0 |
| TCGA-EF-A3AG| 1265   | 1      | 25  | MALE   | Stage III| T0  | M0| N2c|
| TCGA-EF-A29V| 787    | 1      | 85  | MALE   | Stage III| T3b | M0| N1b|
| TCGA-EF-A2OH| 5118   | 1      | 56  | MALE   | Stage I | T2  | M0| N0 |
| TCGA-ER-A19E| 396    | 1      | 36  | FEMALE | Stage IB| T2a | M0| N0 |
| TCGA-GN-A4U5| 1156   | 0      | 61  | FEMALE | Stage IB| T2a | M0| NX |
| TCGA-EF-A3J3| 5237   | 1      | 42  | MALE   | Stage IB| T2  | M0| N0 |
| TCGA-FW-A3TU| 1691   | 1      | 72  | FEMALE | unknown | unknown| unknown| unknown|
| TCGA-EF-A2MD| 1438   | 1      | 52  | MALE   | Stage I| T3a | M0| N0 |
| TCGA-EF-A2GB| 1803   | 0      | 51  | MALE   | Stage III| T2b | M0| N1a|
| TCGA-XV-A9W5| 392    | 0      | 51  | MALE   | I/II NOS| T2  | M0| N0 |
| TCGA-GN-A8BL| 650    | 1      | 68  | FEMALE | Stage I| T4b | M0| NX |
| TCGA-BF-A5ER| 327    | 0      | 63  | MALE   | Stage I| T4b | M0| N0 |
| TCGA-BF-A4OX| 444    | 0      | 83  | MALE   | Stage I| T4b | M0| N0 |
| TCGA-EB-A44Q| 422    | 0      | 51  | FEMALE | Stage II| T4b | M0| N3 |
| TCGA-BF-AAP7| 318    | 0      | 76  | FEMALE | Stage I| T4b | M0| N0 |
| TCGA-ZZ-A8RT| 839    | 0      | 42  | FEMALE | Stage II| T3b | M0| N0 |
| TCGA-D3-A1Q8| 854    | 1      | 33  | MALE   | Stage IV| T0  | M1b| N3 |
| TCGA-EF-A2MB| 601    | 1      | 54  | FEMALE | Stage III| T3a | M0| N1 |
| TCGA-EB-A553| 226    | 0      | 62  | MALE   | Stage I| T4b | M0| N0 |
| TCGA-BF-A3DN| 717    | 0      | 81  | FEMALE | Stage III| T3b | M0| N3 |
| TCGA-ER-A3ES| 7514   | 1      | 25  | MALE   | unknown | unknown| unknown| unknown|
| TCGA-EB-A85I| 362    | 0      | 66  | MALE   | Stage II| T4b | M0| N0 |
| TCGA-FR-A69P| 478    | 0      | 34  | FEMALE | Stage III| TX  | unknown| N3 |
| TCGA-EF-A3AD| 875    | 1      | 50  | MALE   | Stage III| T0  | M0| N1b|
| TCGA-EB-A24D| 645    | 0      | 72  | MALE   | Stage III| T4a | M0| N2b|
| TCGA-D9-A4Z6| 561    | 1      | 54  | MALE   | Stage III| T3b | M0| N1b|
| TCGA-FR-A3R1| 685    | 0      | 69  | MALE   | Stage I| T4b | M0| N0 |
| TCGA-FS-A1ZY| 824    | 1      | 71  | MALE   | Stage I| T3b | M0| N0 |
| TCGA-FW-A3J3| 531    | 0      | 59  | FEMALE | Stage IV| unknown| M1| N0 |
| TCGA-EB-A4I2Q| 636   | 1      | 42  | FEMALE | Stage I| T4b | M0| N1 |
| TCGA-ER-A19K| 469    | 1      | 79  | FEMALE | Stage I| T4b | M0| N0 |
| TCGA-FW-A3TV| 411    | 0      | 57  | FEMALE | Stage II| T1  | M0| N2b|
| TCGA-EF-A2GN| 3106   | 1      | 67  | MALE   | Stage II| T2b | M0| N0 |
Table 1 (Continued).

| Id               | Futime | Fustat | Age | Gender | Stage      | T | M | N |
|------------------|--------|--------|-----|--------|------------|---|---|---|
| TCGA-FR-A7UA     | 1164   | 0      | 65  | FEMALE | Stage IB   | T2a| M0| N0|
| TCGA-DA-A3F2     | 1032   | 1      | 55  | MALE   | Stage IIIB | T4a| M0| N2b|
| TCGA-Z2-A33V     | 486    | 0      | 57  | FEMALE | Stage IA   | T1a| M0| N0|
| TCGA-FR-A2OS     | 368    | 1      | 49  | FEMALE | Stage IIC  | T4b| M0| N0|
| TCGA-EE-A2MQ     | 1315   | 1      | 70  | FEMALE | Stage IIIA | T3a| M0| N2a|
| TCGA-FR-A729     | 6716   | 0      | 38  | FEMALE | Stage I    | T1| M0| N0|
| TCGA-PS-A1YI     | 6953   | 1      | 55  | FEMALE | Stage II   | T3a| M0| N0|
| TCGA-BF-A3DL     | 769    | 0      | 84  | FEMALE | Stage IIIB | T3b| M0| N2|
| TCGA-YG-A3A3P    | 439    | 0      | 63  | FEMALE | Stage IIIB | T4a| M0| N0|
| TCGA-DA-A117     | 2703   | 0      | 62  | MALE   | Stage IIIB | T0| M0| N2b|
| TCGA-WE-A8K4     | 614    | 0      | 85  | MALE   | Stage IIB  | T4a| M0| NX|
| TCGA-EE-A2MR     | 4088   | 0      | 61  | FEMALE | Stage I    | T2| M0| N0|
| TCGA-EB-A3Y6     | 126    | 0      | 56  | FEMALE | Stage IIC  | T4b| M0| N0|
| TCGA-BF-AAOU     | 476    | 0      | 73  | FEMALE | Stage IIC  | T4b| M0| N0|
| TCGA-ER-A19D     | 383    | 1      | 46  | FEMALE | Stage IB   | T2a| M0| N0|
| TCGA-D3-A1Q9     | 961    | 1      | 72  | MALE   | Stage IIIB | T4b| M0| N2a|
| TCGA-D3-A2JC     | 2639   | 0      | 53  | FEMALE | Stage III  | T0| M0| N2b|
| TCGA-DA-A1HV     | 2329   | 0      | 75  | FEMALE | Stage IIIB | T0| M0| N2b|
| TCGA-EE-A2GL     | 2423   | 0      | 40  | FEMALE | Stage II   | T3a| M0| N0|
| TCGA-ER-A19T     | 270    | 1      | 51  | MALE   | Stage IV   | T4a| M1a| N3|
| TCGA-D3-A2JH     | 1280   | 0      | 68  | MALE   | Stage IB   | T1b| M0| N0|
| TCGA-GN-A268     | 1910   | 1      | 83  | FEMALE | Stage IIB  | T4a| M0| N0|
| TCGA-WE-A8K6     | 546    | 0      | 79  | MALE   | Stage IIIB | TX| M0| N1b|
| TCGA-GF-A2C7     | 21     | 0      | 48  | MALE   | Stage IIC  | T4b| M0| N0|
| TCGA-EE-A2ML     | 6590   | 1      | 35  | MALE   | Stage II   | T3a| M0| N0|
| TCGA-D3-A1Q4     | 3408   | 0      | 53  | FEMALE | Stage IIC  | T2b| M0| N1b|
| TCGA-D3-A5IG     | 0      | unknown| unknown| MALE | Stage 0    | Tis| M0| N0|
| TCGA-EE-A2A1     | 3527   | 0      | 46  | MALE   | Stage IB   | T2a| M0| N0|
| TCGA-GN-A269     | 170    | 1      | 70  | MALE   | Stage IIC  | T4b| M0| N3|
| TCGA-D3-A8GN     | 4897   | 0      | 27  | FEMALE | Stage II   | TX| M0| N0|
| TCGA-D3-A8GJ     | 7342   | 0      | 18  | MALE   | Stage II   | T3| M0| N0|
| TCGA-D3-A3ML     | 422    | 1      | 70  | MALE   | Stage IIIA | T3a| M0| N2a|
| TCGA-W3-AAIQ     | 2101   | 1      | 57  | MALE   | Stage III  | TX| M0| N1|
| TCGA-HR-A2OG     | 7      | 0      | 50  | FEMALE | unknown    | unknown| unknown| unknown|
| TCGA-EE-A3AA     | 3781   | 0      | 47  | MALE   | Stage III  | T0| M0| N2a|
| TCGA-FS-A4F4     | 2028   | 1      | 64  | MALE   | Stage II   | T3a| M0| N0|
| TCGA-EE-A29M     | 1729   | 0      | 33  | FEMALE | Stage IB   | T2a| M0| N0|
| TCGA-WE-A4A4     | 760    | 0      | 56  | FEMALE | Stage IIC  | TX| M0| N3|
| TCGA-DA-A112     | 5370   | 1      | 45  | MALE   | Stage III  | T4b| M0| N2b|
| TCGA-WE-A82M     | 3082   | 0      | 70  | MALE   | Stage IIIB | TX| M0| N1b|
| TCGA-FS-A1ZU     | 808    | 1      | 70  | FEMALE | Stage IIC  | T4b| M0| N0|
| TCGA-D3-A2JL     | 5219   | 0      | 43  | FEMALE | Stage II   | TX| M0| N0|
| TCGA-EB-A4OZ     | 620    | 0      | 41  | FEMALE | Stage IIC  | T4a| M0| N3|
| TCGA-ER-A196     | 1785   | 0      | 64  | FEMALE | Stage IIC  | T4b| M0| N0|
| TCGA-FW-A5DX     | 640    | 0      | 71  | MALE   | Stage IIC  | T4a| M0| N3|
| TCGA-EB-A6OZ     | 352    | 1      | 76  | MALE   | Stage II   | T3a| M0| N0|
| TCGA-D3-A8GS     | 3564   | 1      | 52  | MALE   | Stage I    | T1| M0| N0|
| TCGA-DA-A95Y     | 430    | 1      | 68  | MALE   | Stage IIC  | T4b| M0| N0|
| TCGA-EE-A2GO     | 3857   | 0      | 66  | FEMALE | Stage II   | T3b| M0| N0|
| TCGA-EE-A29W     | 5932   | 0      | 42  | MALE   | Stage 0    | Tis| M0| N0|

(Continued)
### Table 1 (Continued)

| Id          | Futime | Fustat | Age | Gender | Stage          | T | M | N |
|-------------|--------|--------|-----|--------|----------------|---|---|---|
| TCGA-EE-A29N| 566    | 1      | 78  | MALE   | I/II NOS       | TX | M0| N0|
| TCGA-EB-A55I| 590    | 0      | 78  | FEMALE | Stage IIIC     | T4b| M0| N2b|
| TCGA-DJ-A29| 723    | 1      | 75  | MALE   | Stage IIIC     | T4b| M0| N3 |
| TCGA-EA-A3J| 1562   | 0      | 75  | MALE   | Stage IIIB     | T3b| M0| N1a|
| TCGA-EE-A17Y| 828    | 1      | 69  | MALE   | Stage IIIB     | T3b| M0| N1a|
| TCGA-DJ-A3C8| 1409   | 0      | 58  | FEMALE | Stage IIIC     | TX | M0| N3 |
| TCGA-DJ-A3C7| 1429   | 0      | 57  | FEMALE | Stage III      | T0 | M0| N1b|
| TCGA-EE-A2MG| 3139   | 1      | 23  | MALE   | Stage I        | T2 | M0| N0 |
| TCGA-DJ-A1Q5| 3424   | 1      | 60  | MALE   | I/II NOS       | TX | M0| N0 |
| TCGA-EB-A24C| 632    | 0      | 56  | MALE   | unknown        | T4b| M0| NX |
| TCGA-XV-A9W2| 417    | 0      | 81  | MALE   | Stage I        | T1 | M0| N0 |
| TCGA-D9-A6EC| 2359   | 0      | 56  | MALE   | Stage IIIA     | T3a| M0| N1 |
| TCGA-BF-ASEQ| 323    | 0      | 63  | MALE   | Stage IIC      | T4b| M0| N0 |
| TCGA-W3-A11V| 1280   | 1      | 63  | MALE   | Stage II       | T3 | M0| N0 |
| TCGA-FS-A1ZP| 2273   | 1      | 52  | MALE   | Stage II       | T3 | M0| N0 |
| TCGA-GN-A4U4| 1197   | 0      | 73  | MALE   | Stage IIA      | T2b| M0| NX |
| TCGA-DJ-A8GE| 804    | 0      | 26  | MALE   | Stage IV       | TX | M1b| N0 |
| TCGA-EE-A3JB| 1044   | 1      | 59  | MALE   | Stage IIIA     | T4a| M0| N1a|
| TCGA-EB-A5SF| 369    | 1      | 78  | FEMALE | Stage IIC      | T4b| M0| NX |
| TCGA-GF-A769| 1070   | 1      | 39  | MALE   | Stage IIC      | T4b| M0| NX |
| TCGA-DJ-A8GM| 3259   | 1      | 73  | MALE   | Stage IIIB     | T3b| M0| N0 |
| TCGA-FS-A1ZM| 3080   | 0      | 74  | MALE   | Stage III      | T2 | M0| N2c|
| TCGA-YD-A9TB| 0      | unknown| unknown| FEMALE | unknown       | unknown| unknown| unknown|
| TCGA-EE-A3AH| 4222   | 1      | 30  | MALE   | Stage II       | T3b| M0| N0 |
| TCGA-GN-A266| 308    | 1      | 45  | MALE   | unknown        | unknown| unknown| unknown|
| TCGA-EB-A5V| 214    | 0      | 74  | FEMALE | Stage IIIB     | T3b| M0| N0 |
| TCGA-EB-A3XD| 1160   | 0      | 53  | FEMALE | Stage IIC      | T4b| M0| N0 |
| TCGA-EE-A29R| 440    | 0      | 48  | FEMALE | Stage IIC      | T3b| M0| N1b|
| TCGA-3N-A9WD| 395    | 1      | 82  | MALE   | Stage IIIA     | T2a| M0| N1a|
| TCGA-EE-A20C| 4601   | 1      | 59  | MALE   | Stage 0        | T1s| M0| N0 |
| TCGA-DJ-A8GV| 5101   | 1      | 25  | MALE   | I/II NOS       | TX | M0| N0 |
| TCGA-ER-A19A| 2365   | 0      | 79  | MALE   | Stage IV       | TX | M1| N0 |
| TCGA-ER-A2NH| 1264   | 0      | 49  | MALE   | Stage IIIC     | T3a| M0| N3 |
| TCGA-EE-A3J4| 3869   | 1      | 72  | MALE   | Stage II       | T3a| M0| N0 |
| TCGA-D9-A148| 4609   | 0      | 40  | MALE   | unknown        | TX | M1b| N3 |
| TCGA-FS-A1ZS| 4526   | 0      | 54  | MALE   | Stage I        | T2 | M0| N0 |
| TCGA-ER-A19B| 2993   | 1      | 42  | MALE   | unknown        | TX | M0| N0 |
| TCGA-GN-A8BLK| 1524   | 1      | 70  | MALE   | Stage IB       | T1b| unknown| NX |
| TCGA-W3-AAAIO| 122    | 1      | 85  | MALE   | Stage III      | TX | M0| N2 |
| TCGA-RP-A6K9| 0      | unknown| unknown| FEMALE | unknown       | unknown| unknown| unknown|
| TCGA-WE-A99Y| 370    | 0      | 37  | MALE   | Stage IIIIC    | T2a| M0| N3 |
| TCGA-EA-A3J1| 4648   | 1      | 48  | MALE   | Stage I        | T2 | M0| N0 |
| TCGA-EB-A6QY| 382    | 0      | 71  | MALE   | Stage IIC      | T4b| M0| N0 |
| TCGA-GF-A4EO| 591    | 0      | 74  | FEMALE | Stage IIIC     | T0 | M0| N3 |
| TCGA-DJ-A5GR| 5424   | 0      | 23  | FEMALE | Stage III      | T1b| M0| N1 |
| TCGA-DJ-A6EG| 698    | 1      | 56  | MALE   | Stage IIIA     | T4a| M0| N1 |
| TCGA-DA-A1I0| 620    | 1      | 63  | MALE   | Stage IV       | T4b| M1a| N3 |
| TCGA-FW-A3RS| 1124   | 0      | 68  | MALE   | Stage III      | TX | M0| N2 |
| TCGA-DJ-A5GT| 487    | 0      | 43  | MALE   | Stage IIIC     | T2b| M0| N3 |
| TCGA-EE-A184| 2073   | 1      | 72  | MALE   | Stage IB       | T2a| M0| N0 |
Merck Millipore, Billerica, MA, USA). The PVDF membranes were blocked with 5% bovine albumin (BSA) at room temperature for 1 h, then overnight incubated with FGD2 and GAPDH rabbit polyclonal antibodies (1:4000, Abcam, UK) at 4°C. The secondary antibodies were used at a dilution of 1:4000 and incubated at room temperature for 1 h. Eventually, the bands were visualized using the ECL reagents (Merck Millipore).

RNA Extraction, Reverse Transcription, and Quantitative PCR (RT-qPCR)
Melanoma tissues samples were extracted from patients diagnosed with melanoma by (three independent) experienced physicians (based on Chinese guidelines for diagnosis and treatment of melanoma). The total RNA was extracted using the Trizol Reagent (Invitrogen) from tissues based on the manufacturer’s instructions (Trizol, chloroform, and isopropanol were added in turn; the supernatant was centrifuged and quantified by absorbance value of 260nm and stored at -80°C). Subsequently, a reverse transcription kit (Takara Bio, Inc., Otsu, Japan) was used to reverse-transcribe RNA into cDNA in a 20ul system. Subsequently, the cDNA was used as a template, detected by the SYBR Green (Takara Bio) and ABI 7900HT Real-Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA). The primers used are shown in Table S1. The comparative cycle threshold values (2-ΔΔCt) were used to analyze the final results.

Statistical Analysis
The IBM SPSS 19.0 software was used for statistical analyses of all experimental data. Data were expressed as mean ± sd. Graphpad Prism version 7.0 software was used to visualize the statistical results. T-test was used to compare data between two groups, whereas One-way ANOVA was used to compare data between multiple groups; LSDt-test was used for pairwise comparison within the group. Overall Survival (OS) curves were drawn through the Kaplan–Meier analysis. The difference with P < 0.05 was considered statistically significant.

Results
Construction of Tumor Microenvironment Score
In total, transcript data of 482 melanoma patients were extracted from the TCGA-SKCM database; the R software “Limma” package was used for data standardization. The R software “Estimate” package was utilized to obtain three TME scores for each patient, respectively. Notably, higher stromal and immune scores indicated higher infiltration of stromal cells and immune cells. Estimate scores were the sum of the stromal score and immune score. A higher estimate score indicated lower purity of tumor cells. Patients with higher immune and

| Id              | Futime | Fustat | Age | Gender | Stage  | T  | M  | N  |
|-----------------|--------|--------|-----|--------|--------|----|----|----|
| TCGA-YG-AA3O    | 1154   | 1      | 62  | MALE   | unknown| unknown| unknown| unknown|
| TCGA-GN-A4U8    | 1487   | 0      | 51  | MALE   | unknown| unknown| unknown| unknown|
| TCGA-RP-A695    | 0      | unknown| unknown| MALE   | Stage IV| TX  | M1c | NX |
| TCGA-FS-A4F9    | 1035   | 0      | 80  | MALE   | Stage III| T4b | M0  | N3 |
| TCGA-EB-A44N    | 205    | 1      | 59  | MALE   | Stage II| T4b | M0  | N0 |
| TCGA-D9-A6EA    | 766    | 0      | 70  | MALE   | Stage II| T4a | M0  | N3 |
| TCGA-GN-A263    | 467    | 1      | 24  | MALE   | Stage IV| T4b | M1c | N3 |
| TCGA-EB-A51B    | 931    | 0      | 53  | MALE   | Stage II| T4b | M0  | NX |
| TCGA-D3-A3MR    | 3151   | 0      | 42  | MALE   | Stage III| T0  | M0  | N1b|
| TCGA-GN-A26A    | 988    | 1      | 63  | FEMALE | Stage IIIA| T3a | M0  | N1a|
| TCGA-DA-A1HY    | 4407   | 0      | 42  | MALE   | Stage III| T2b | M0  | N1 |
| TCGA-D3-A8GO    | 1478   | 1      | unknown| FEMALE | Stage I| T2  | M0  | N0 |
| TCGA-FS-A11X    | 2004   | 1      | 46  | FEMALE | Stage III| T3b | M0  | N2a|
| TCGA-EB-A2OH    | 1714   | 0      | 60  | MALE   | Stage III| T1b | M0  | N3 |
| TCGA-D3-A51H    | 1078   | 1      | 46  | MALE   | unknown| TX  | M0  | N0 |
| TCGA-ER-A195    | 524    | 0      | 61  | MALE   | Stage II| T4b | M0  | N0 |
| TCGA-D3-A8GR    | 3943   | 1      | 54  | FEMALE | Stage 0| Tis | M0  | N0 |
| TCGA-DA-A11A    | 2005   | 1      | 32  | FEMALE | Stage III| T2a | M0  | N1b|
estimate scores displayed better OS than those with lower scores (Figure 1).

**Tumor Microenvironment Score is Associated with Age and Tumor Size**
The relationship between TME scores (stromal score: Figure 2A, immune score: Figure 2B, estimate score: Figure 2C) and clinical features of patients (age, gender, pathological stages, etc.) was analyzed. Interestingly, higher TME scores were closely related to younger age (Figure 2 left panel) and earlier primary tumor stage (Figure 2 right panel).

**Screening for Tumor Microenvironment Associated Genes**
To evaluate the molecular mechanisms underlying the relationship between TME and survival, the patients were divided into two groups based on the median of the stromal and immune scores, respectively (Figure 3A, B, C and D). DEGs were screened between the two groups and further intersected based on stromal score and immune score. Consequently, 10 down-regulated DEGs and 201 up-regulated DEGs were identified. These DEGs were closely related to the TME, hence defined as TME associated genes (Figure 3E and F).

**Gene Ontology, KEGG Pathway, and Protein-Protein Interaction Analyses of Tumor Microenvironment Associated Genes**
Furthermore, GO and KEGG analyses were performed based on the TME associated genes. As a result, TME associated genes were closely related to T cell activation, cytokine-cytokine receptor interaction, etc. (Figure 4A and B). Moreover, a PPI network for TME associated genes was constructed, and Top20 hub-genes were calculated using the Cytoscape software (Figure 4C and D). The association of all DEGs and survival was analyzed through the Cox and Kaplan-Meier analyses. Consequently, 138 genes were confirmed to be associated with the survival of melanoma patients (Table S2). Further, 12 intersected genes were finally obtained between the Top 20 hub-genes and 138 survival-associated genes (Figure 4E). Among them, FGD2 showed the smallest q-value, hence was selected for subsequent analyses (Figure 4F).

**FGD2 is Associated with the Progression of Melanoma**
FGD2 was found to be associated with the progression of pan-cancer, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), and so on (Figure 5A). Further, were analyzed the association of FGD2 and clinical features. Consequently, higher expression of FGD2 indicated better survival (Figure 5B) and earlier primary tumor stage (Figure 5C). Nonetheless, FGD2 expression was not associated with lymph nodes metastasis, distant metastasis, pathological stage, and age (Figure 5D–G).

**Validation of FGD2 in Clinical Specimens**
To further verify the FGD2 expression, melanoma specimens and paired non-tumor skin tissues were used to perform Western blotting and RT-qPCR analyses (Figure 6A and B). As expected, FGD2 expression was significantly downregulated in melanoma...
Figure 2 Tumor microenvironment score associated with age and tumor size (A-C) The relationship between TME score and clinical features analyzed using One-way ANOVA.
Figure 3 Screening for tumor microenvironment associated genes. (A) Heatmap of DEGs between patients with high and low stromal scores; (B) Volcano map of DEGs between patients with high and low stromal scores; (C) Heatmap of DEGs between patients with high and low immune scores; (D) Volcano map of DEGs between patients with high and low immune scores; (E) Downregulated genes of the intersection of DEGs derived from immune and stromal scores; (F) Upregulated genes of the intersection of DEGs derived from immune and stromal scores.
compared to that in paired normal tissues (P<0.001). Also, FGD2 associated pathways were examined through GSEA analysis. As a result, FGD2 was associated with IL6-JAK, KRAS, TNF-α pathways, etc. All these pathways were closely related to the progression of melanoma and TME (Figure 6C). The relationship between FGD2 and various types of immune cells was assessed through ssGSEA analysis. As a consequence, FGD2 expression was closely related to the infiltration of T cells, B cells, and so on (Figure 6D and E). Generally, we confirmed the FGD2 downregulation in melanoma. Besides, downregulated FGD2 may modulate the TME by regulating the infiltration of immune cells. (WB original pictures are shown in the Supplementary Material)

Discussion

The tumor microenvironment is vital in the development of various tumors. Several studies have reported the role of part cells or factors in the TME of melanoma, including immune cells, immune checkpoints, etc.10,11 Nevertheless, limited information is available on the regulatory mechanisms of TME as a whole.

CIBERSORT is a gene expression-based deconvolution algorithm developed to examine the proportion of stromal and cells in tumor samples.12 Because of its excellent
performance, CIBERSORT has been utilized in TME research. Based on this algorithm, we calculated three TME scores for each patient, respectively. Stromal and immune scores indicated the infiltration of stromal and immune cells. The estimated score is the sum of stromal and immune scores indicating lower purity of tumor cells. In this scoring system, patients with higher immune and estimate scores demonstrated better survival. This also meant that patients with high infiltration of immune cells displayed better survival. Similar to other solid tumors, melanoma comprises a large number of immune cells, which potentially reflects tumor response. TME with high immune infiltration revealed strong antigenicity and can easily be detected by the immune system. Nonspecific innate immune mechanisms (including phagocytes, natural killer cells, etc.) and specific acquired immune
mechanisms (including CD4 + T cells, CD8 + T cells) are involved in the process of tumor cell clearance. Further, we analyzed the relationship between estimate score and clinical features. As a result, a higher estimate score was related to younger age and earlier primary tumor stage. That is, highly immune infiltration in the early stage inhibits tumor progression. With the secretion of cytokines in the TME, immune cells are inhibited, immune escape occurs, causing tumor progression.

We screened DEGs between patients with different TME scores to establish the related mechanisms underlying the regulation of TME. These DEGs were enriched in the T cell activation, cytokine-cytokine receptor interaction, and so on. T cells participate in killing tumors and the effective recognition of tumor cells is the premise of this role. In the TME, tumor cells exhibit selective inhibitory ligands and receptors, which regulate the function of T cells. In recent years, pharmacological modulators of these pathways (known as immune check-point therapy, specifically monoclonal antibody forms against PD-1 and CTLA-4) have been widely studied and utilized as novel immunotherapeutic agents against melanoma.

Considering the early success of immune checkpoint therapy, the development of immunotherapy targeting other costimulatory receptors activating the anti-tumor immune response is seemingly a convincing treatment approach.

In subsequent analyses, we verified that FGD2 may be the hub-gene of TME regulation in melanoma. Additionally, FGD2 was closely related to the progression of melanoma.

Figure 6 Validation of FGD2 in clinical specimens. (A) FGD2 expression in melanoma and paired non-tumor skin tissues validated by Western Blotting analysis (n=10). N: Normal tissues; T: Tumor tissues; (B) FGD2 expression of in melanoma and paired non-tumor skin tissues validated by RT-qPCR analysis (analyzed by Student’s t-test); (C) FGD2 associated pathways assessed by GSEA analysis; (D) The correlation of FGD2 and various types of immune cells assessed by ssGSEA analysis; (E) Top 6 FGD2 associated immune cells derived from the ssGSEA analysis. All experiments were conducted in triplicate.
Patients with high FGD2 expression demonstrated better survival. The protein encoded by this gene is a member of the guanine nucleotide exchange factors (GEFs) family which regulate cytoskeleton-dependent membrane rearrangements by activating the cell division cycle 42 (CDC42) protein. This gene is expressed in B lymphocytes, macrophages, and dendritic cells. In the B lymphocyte lineage, FGD2 levels change with the developmental stage. In both mature splenic and immature bone marrow B cells, FGD2 expression is suppressed upon activation through the B cell antigen receptor. Also, previous research approved FGD2 as a biomarker for head and neck squamous cell carcinoma. However, the roles of FGD2 in the response of tumors remain unclear. Through GSEA analysis, we established that FGD2 may regulate immune infiltration of various types of immune cells, including T cells, B cells, etc. Future studies should explore the role of FGD2 in the immune response of tumors.

In conclusion, we established a relationship between TME and the survival of melanoma patients. Consequently, we discovered a novel FGD2 gene that potentially regulates the TME in melanoma.

### Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Ethics Approval and Consent to Participate

All patients provided and signed informed consents. The study was approved by the Ethical Committee of the Affiliated Hospital of Qingdao University and experiments were performed as per the Ethical Committee’s guidelines and regulations. All procedures involving human participants were performed based on the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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### Author Contributions

All authors made a significant contribution to the work reported, including in the conception, study design, execution, data acquisition, analysis, and interpretation. Also, the authors participated in drafting, revising, or critically reviewing the article; provided final approval of the version to be published; agreed on the journal to which the article has been submitted, and remain accountable for all aspects of the work. Xuchao Ning and Renzhi Li equally contributed to this paper.

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### Disclosure

The authors declare no conflicts of interest concerning the publication of this article.

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