Heliyon 10 (2024) e33595

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Multi-omics data analysis reveals the complex roles of age in differentiated thyroid cancer

Yu Zhang [a,b,1], Qi Chen [a,b,1], Lili Niu [c,d,1], Hu Huang [e], Zhou Yang [a,b], Tian Liao [a,b], Qing Guan [a,b,**], Jun Xiang [a,b,*]

[a] Department of Head and Neck Surgery, Fudan University Shanghai Cancer Center, Shanghai, 200032, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, 200032, China
[c] Central Laboratory, First Affiliated Hospital, Institute (College) of Integrative Medicine, Dalian Medical University, Dalian, 116021, China
[d] Pharmacy Department, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Shanghai, 201399, China
[e] Department of Thyroid and Breast Surgery, Jiangnan University Affiliated Hospital, Wuxi, 214000, China

ARTICLE INFO

*Keywords:*
Aging
Differentiated thyroid cancer
T cells
Immunotherapy
Somatic mutation

ABSTRACT

*Aims:* Age is a major risk factor for differentiated thyroid cancer (DTC); however, the mechanisms underlying aging-regulated progression of DTC remains unclear.
*Methods:* Based on multi-omics data (transcriptional files, somatic mutation files, methylation files) derived from the TCGA database, we comprehensively investigated the genomic and biological features associated with aging in patients with DTC.
*Results:* We confirmed that age was an independent risk factor for overall survival and progression-free survival of patients with DTC, and confirmed that 55 years of age (adopted in the 8th AJCC staging system) is an appropriate cutoff for patients with DTC rather than 45 years (adopted in the 7th AJCC staging system). Using 55 years as the cutoff, we demonstrated DNA methylation-driven transcriptional regulation during aging, and identified the landscape of somatic mutations in young and old patients with DTC along with two aging-related mutations: *TTN* and *EIF1AX*. Subsequently, we investigated the infiltration of immune cells in DTC, and found that old patients exhibited decreased $CD8^+$ T cells infiltration with lower cytotoxicity. Finally, we constructed a prognosis prediction model based on three age-related genes (*PTK2B, E2F1,* and *GHR*) that showed satisfactory performance in predicting patients prognosis.
*Conclusions:* We comprehensively investigated the complex interplay between age and biological features of DTC, which may provide new insights into the role of aging in DTC.

## 1. Introduction

Thyroid cancer (TC) is a common endocrine malignancy. There are five primary histological types of TCs: papillary, follicular (also known as differentiated), poorly differentiated, anaplastic (the most aggressive form), and medullary [1]. The clinical significance of

* Corresponding author. Department of Head and Neck Surgery, Fudan University Shanghai Cancer Center, No. 270, Dong'an Road, Shanghai, Shanghai 200032, China, Department of Oncology, Shanghai Medical College, Fudan University, No. 270, Dong'an Road, Shanghai 200032, China.
** Corresponding author. Department of Head and Neck Surgery, Fudan University Shanghai Cancer Center, No. 270, Dong'an Road, Shanghai, Shanghai 200032, China, Department of Oncology, Shanghai Medical College, Fudan University, No. 270, Dong'an Road, Shanghai 200032, China.
*E-mail address:* xiangjun@shca.org.cn (J. Xiang).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e33595
Received 15 April 2024; Received in revised form 21 June 2024; Accepted 24 June 2024
Available online 26 June 2024

thyroid nodules is based on the need to eliminate TC, which has a prevalence ranging from 7 % to 15 %, depending on factors such as age, sex, history of radiation exposure, family history, and other variables [2–4]. Differentiated TC (DTC), including papillary and follicular tumors, is the most common pathological type, accounting for >90 % of all TCs. Most patients show satisfactory survival and prognosis post-surgery; however, older patients are more likely to die of disease-specific causes [1].

Although the incidence rate of DTC in all age groups is increasing, it presents an inverted U-shaped curve according to age distribution. Peak incidence was observed between 50 and 54 years of age. With the gradual increase of age, the incidence rate was significantly reduced. Although TC incidence in the elderly population has gradually decreased, the mortality rate has increased significantly, especially for patients over 60-years-old, with a peak at 80–84 years. TC incidence in the elderly has gradually decreased, but the mortality rate has also gradually increased [2,3]. The association between DTC and age, is well-documented in several epidemiological studies. In a study of 31802 cases with papillary TC, a smooth linear relationship was observed between age and mortality rate [4].

Age at DTC diagnosis has been included in the American Joint Committee on Cancer (AJCC) staging system since the 2nd edition. However, as a continuous variable, age cutoff remains controversial [5]. Forty-five years of age was adopted as the cutoff age for the AJCC 7th edition and other major TC staging systems (GAMES [grade, age, metastases, extent, size], AGES [age, grade, extrathyroidal extension and tumor size], and National Thyroid Cancer Treatment Cooperative Study) [6]. Recently, several studies have questioned the performance of the 45 years age cutoff and suggested 55 years instead. Furthermore, previous studies have shown that an increase in the cutoff age from 45 to 55 years underscores 17 % of cases, whereas the survival curves of the lower stages were minimally affected [7,8]. Thus, in the most recent 8th edition of the AJCC, the age cutoff was set to 55 years [9].

Age plays an important role in DTC progression; however, the interplay between age-related changes and DTC initiation and progression remains unclear.

## 2. Material and methods

### 2.1. Data acquisition and processing

Multi-omics data of 512 patients with DTC were derived from the TCGA database, including transcriptional files, somatic mutation files, methylation files, and clinical information. The detailed clinical characteristics of patients were shown in Table 1. The mutation annotation files were visualized using the R software package "maftools." Transcriptional data were normalized to gene expression through the R software package "Limma." Moreover, "$P < 0.05$" and "Fold-change >1.5 or < -1.5" was set as the filter condition of differentially expressed genes (DEGs) between young (age <55-years-old) and old patients (age ≥55-years-old). DEGs were uploaded to the Database for Annotation Visualization and Integrated Discovery (david.ncifcrf.gov/) for Gene Ontology (GO) and KEGG pathway analyses. Similarly, we conducted a differential analysis of the methylation site, then converted it into the differentially Methylated genes (DMGs) using "$P < 0.05$" without considering fold-change.

**Table 1**
The association between age and clinical features in thyroid cancer patients.

| Factors | Age<55 | Age≥55 | Total | P-value | FDR |
|---|---|---|---|---|---|
| Sex | | | | 7.7e-3 | 0.03 |
| Female | 263(51.37 %) | 110(21.48 %) | 373(72.85 %) | | |
| Male | 80(15.63 %) | 59(11.52 %) | 139(27.15 %) | | |
| Race | | | | 0.09 | 0.18 |
| American | 1(0.24 %) | 0(0.0e+0 %) | 1(0.24 %) | | |
| Asian | 41(9.83 %) | 10(2.40 %) | 51(12.23 %) | | |
| Black | 15(3.60 %) | 12(2.88 %) | 27(6.47 %) | | |
| White | 222(53.24 %) | 116(27.82 %) | 338(81.06 %) | | |
| pT | | | | 6.6e-8 | 3.3e-7 |
| T1 | 101(19.80 %) | 42(8.24 %) | 143(28.04 %) | | |
| T2 | 126(24.71 %) | 43(8.43 %) | 169(33.14 %) | | |
| T3 | 112(21.96 %) | 63(12.35 %) | 175(34.31 %) | | |
| T4 | 3(0.59 %) | 20(3.92 %) | 23(4.51 %) | | |
| pN | | | | 0.01 | 0.04 |
| N0 | 139(30.09 %) | 90(19.48 %) | 229(49.57 %) | | |
| N1 | 168(36.36 %) | 65(14.07 %) | 233(50.43 %) | | |
| pM | | | | 0.30 | 0.30 |
| M0 | 191(64.75 %) | 95(32.20 %) | 286(96.95 %) | | |
| M1 | 4(1.36 %) | 5(1.69 %) | 9(3.05 %) | | |
| pStage | | | | 2.0e-28 | 1.2e-27 |
| I | 253(49.61 %) | 35(6.86 %) | 288(56.47 %) | | |
| II | 22(4.31 %) | 30(5.88 %) | 52(10.20 %) | | |
| III | 49(9.61 %) | 64(12.55 %) | 113(22.16 %) | | |
| IV | 18(3.53 %) | 39(7.65 %) | 57(11.18 %) | | |

Certain data are unavailable in the public database.

### 2.2. Immune infiltration assessment

To assess the infiltration of immune and stromal cells, we used R software package "ESTIMATE" to calculate immune score, stromal score, and ESTIMATE score. Higher immune, stromal, and ESTIMATE scores indicated greater infiltration of immune cells, stromal cells, and tumor purification, respectively [10].

To further calculate the infiltration of specific immune cells, we used the R software package "immuneeconv" to integrate the six latest algorithms, including xCell, CIBERSORT, TIMER, MCP-counter, EPIC, and quanTIseq. These algorithms have been benchmarked and each has a unique advantage [11].

### 2.3. Prediction of chemotherapeutic response

The chemotherapeutic response was predicted using Genomics of Drug Sensitivity in Cancer (GDSC; https://www.cancerrxgene.org/). The R package "pRRophetic" was used to predict the half-maximal inhibitory concentration of the samples. Default values were used for all parameters using the batch effect of combat and tissue types, and duplicate gene expressions were averaged.

### 2.4. Construction of prognosis prediction model based on aging related genes (ARG)

In total, 307 human ARGs were obtained from the Human Aging Genomic Resources (https://genomics.senescence.info/) (Table S1). First, we analyzed the association between these 307 ARGs and overall survival (OS) using Kaplan–Meier analysis and acquired 16 ARGs associated with OS. Subsequently, we performed the least absolute shrinkage and selection operator (LASSO) Cox regression analysis for further selection using R package "glmnet."

### 2.5. Self-collected DTC specimens

Overall, 145 patients with DTC were collected between July 2019 and July 2021. We excluded patients who had received adjuvant chemotherapy or radiotherapy pre-surgery and those with additional cancer diagnoses. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Fudan University Shanghai Cancer Center. Detailed clinical features of the DTC specimens are shown in Table 2.

### 2.6. Immunohistochemical (IHC) staining

Paraffin-embedded sections were deparaffinized in xylene, hydrated with ethanol (100, 90, 80, and 75 %), and heated in a microwave with sodium citrate for 3 min. After blocking in 5 % BSA, the sections were incubated with a CD8 rabbit polyclonal antibody (1:1000, Abcam, UK) overnight at 4 °C. The sections were then incubated with horseradish peroxidase conjugated rabbit secondary antibodies (1:200; ProteinTech Group, Inc., Wuhan, China) for 1 h at room temperature. Finally, sections were developed with 3,3′diaminobenzidine (DAB Substrate Chromogen System; Dako, Denmark) and stained with hematoxylin. Images of the sections were captured under a microscope (Olympus IX71, Japan).

### 2.7. RNA extraction and real-time quantitative PCR (RT–qPCR)

Twenty cases of anaplastic TC (ATC) and 20 cases of DTC were collected between June 2018 and June 2023 and deposited in liquid nitrogen. Detailed clinical features of these patients are provided in Tables S2 (DTC) and S3 (ATC).

Total RNA was extracted from the cells using the TRIzol Reagent (Invitrogen, CA, USA). Subsequently, cDNA synthesis was

**Table 2**
Clinical characteristics of 145 self-collected DTC patients.

| | | |
|---|---|---|
| Age | <55 | 76 |
| | ≥55 | 69 |
| Gender | Female | 101 |
| | Male | 44 |
| Stage | Stage I | 66 |
| | Stage II | 14 |
| | Stage III | 37 |
| | Stage IV | 28 |
| T stage | T1 | 41 |
| | T2 | 53 |
| | T3 | 37 |
| | T4 | 14 |
| M stage | M0 | 137 |
| | M1 | 8 |
| N stage | N0 | 82 |
| | N1 | 63 |

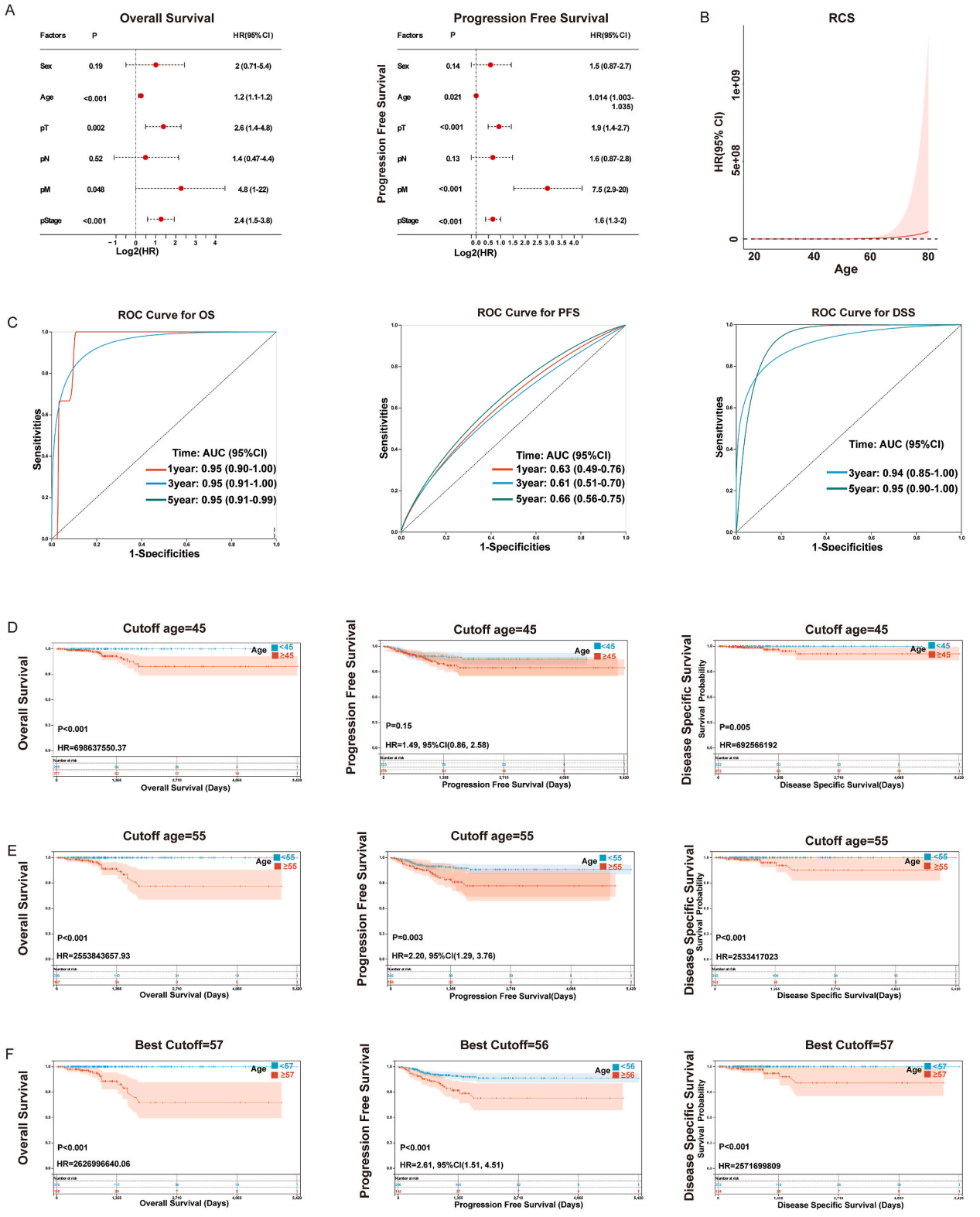


**Fig. 1. Age is an independent risk factor for the survival of patients with DTC.** A. Forest plot of univariate Cox analyses of OS and progression free survival. B. Restricted cubic spline (RCS) plot reveals association between age and OS. C. Age-dependent ROC curve showed good predictability AUC of 1-, 3-, 5-years survival. D–F. Kaplan–Meier survival analysis based on different age cutoffs (D. cutoff age = 45-years-old, E. cutoff age = 55-years-old, F. calculated best cutoff age.). HR: Hazard Ratio; ROC: Receiver Operating Curve; AUC: Area Under Curve.

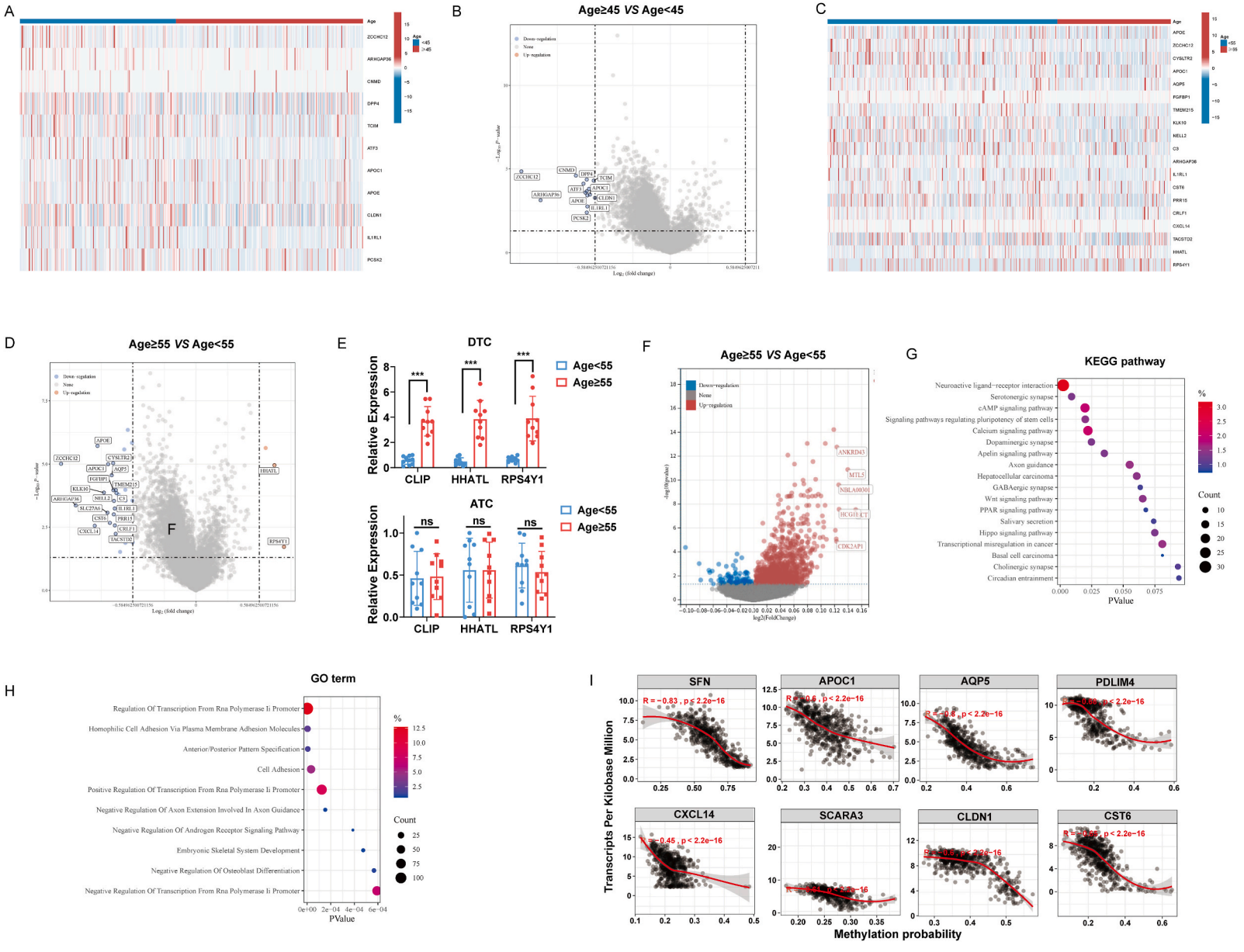


**Fig. 2. Effect of age on the transcriptional profiles of DTC.** A. B. Heatmap (A) and volcano plot (B) of DEGs analyzed using 45 years as the cutoff. DEGs screen threshold: Fold-change ≥1.5, *P*-value <0.05. C, D. Heatmap (C) and volcano plot (D) of DEGs analyzed using 55 years as the cutoff. E. mRNA expression of CLIP, HHATL and RPS4Y1 in patients with DTC (n = 20) and ATC (n = 20). F. Volcano map displayed differentially methylated genes (DMGs). DMGs Screen threshold: *P*-value <0.05. G, H. KEGG pathway (G) and Gene Ontology (GO) enrichment analysis (H) based on DMGs. I. The correlation between transcriptional expression and methylation probability performed by Spearman correlation analysis.

performed on total RNA using a PrimeScript™ RT Reagent Kit (Takara Bio, Otsu, Japan, RR037A). mRNA expression levels were quantified by RT-qPCR in triplicate using a SYBR Premix Ex Taq™ kit (Takara Bio, Kyoto, Japan, RR420A) and an ABI 7900HT Real Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA). The primers used are listed in Supplementary Table S4. The comparative cycle threshold (2 ΔΔCt) method was employed for data analysis.

### 2.8. Statistical analysis

SPSS 24.0 and R 4.2 were used for all analyses. One-way analysis of variance was used for comparison between multiple groups, and the least significant difference *t*-test was used for pairwise comparison within groups. The Wilcoxon rank-sum test was used for continuous variables that did not conform to a normal distribution (mainly applied in the analysis of the community screening population); $P < 0.05$ was considered statistically significant. The relationship between age, age-related genes, and OS was analyzed using the Kaplan–Meier method, which was evaluated using the log-rank test. Risk models were analyzed using time-dependent ROC curves. Restricted cubic spline (RCS) plots were used to explore the shape of the association between age and survival by fitting RCSs function with five knots.

## 3. Results

### 3.1. Age is an independent risk factor for the survival of patients with DTC

First, we analyzed the association between age and clinical features of patients with DTC (Table 1). Generally, age is closely associated with sex, tumor size (pT stage), lymph node metastasis (pN stage), and pathological stage.

Subsequently, we investigated the association between the major clinical features of patients with DTC (age, sex, and pathological stage) and survival by performing Cox analysis. Age was analyzed as a continuous variable and was significantly associated with OS (*P*-value <0.001) and progression-free survival (PFS; *P*-value = 0.021) (Fig. 1A). Through RCS, we found that the hazard ratio (HR) was stable before 60 years of age; after 60 years, it increased rapidly (Fig. 1B). Moreover, in the ROC curve analysis, age also showed a satisfactory area under the curve (AUC) for OS (1-, 3-, and 5-years AUC = 0.95), PFS (1-, 3,- 5-years AUC = 0.63, 0.61, 0.65), and disease-specific survival (DSS; 3 and 5 years AUC = 0.94, 0.95) (Fig. 1C). Subsequently, we performed Kaplan–Meier analysis using 45 and 55 years as cutoffs. Fifty-five years showed higher HR and lower *P*-value compared to 45 years old in OS ($P < 0.001$ *vs* $P < 0.001$; HR: 2553843658 *vs* 698637550), PFS ($P = 0.003$ *vs* $P = 0.15$; HR: 2.2 *vs* 1.49), and DSS analyses ($P = 0.005$ *vs* $P < 0.001$; HR: 692566192 *vs* 2533417023) (Fig. 1D and E). Moreover, we calculated the best age cutoffs for OS, PFS, and DSS as 57 ($P < 0.001$, HR = 2626996640), 56 ($P < 0.001$, HR = 2.61), and 57 years ($P < 0.001$, HR = 2571699809), respectively (Fig. 1F). Thus, 55 years of age was adopted in the 8th AJCC staging system as a better cutoff than 45 years of age.

### 3.2. Effect of age on transcriptional profiles of DTC

We further extracted the transcriptional profiles of patients with DTC from the TCGA database. Using 45 years as the cutoff, we screened 14 downregulated genes (Fig. 2A and B). Using 55 years as the cutoff, we screened 33 downregulated DEGs and three upregulated DEGs (Fig. 2C and D, Table 3). In addition, qRT-PCR was performed to validate that the three upregulated DEGs were upregulated in older patients with DTC (≥55-years-old) compared with younger patients (<55-years-old). We also detected their expression in patients with ATC, but found no significant difference between old and young patients, indicating that they are highly related to DTC over other types of TC (Fig. 2E). These screened DEGs further confirmed that 55 years was a better cutoff for distinguishing transcriptional association with age. The transcriptional differences between the two age groups were relatively small, and it is difficult to confirm whether age is the driver of these DEGs or it plays a role in transcriptional regulation.

DNA methylation is the main epigenetic modification contributing to transcriptional regulation. Herein, we further investigated DMGs between old (≥55-years-old) and young (<55-years-old) patients with DTC. Overall, 2742 hypermethylated genes and 472 hypomethylated genes were identified in older patients compared with younger patients (Fig. 2F). Further KEGG pathway and GO analyses indicated that these DMGs were enriched in cancer cell progression- and migration-associated pathways or terms, including cell adhesion, Wnt, Hippo, and PPAR signaling (Fig. 2G and H). Notably, 31/36 DEGs were also DMGs. Moreover, the transcriptional expression of DEGs showed a significant negative association with methylation (Fig. 2I). Thus, DNA methylation drives transcriptional regulation during aging.

**Table 3**
Differentially expressed genes between old and young DTC patients.

| Age≥55 *vs* Age<55 | |
|---|---|
| Downregulated | ANXA1, DUSP6, APOE, INHBB, CYSLTR2, ZCCHC12, APOC1, AQP5, DPP4, TMEM215, FGFBP1, KLK10, C3, CPAMD8, SCARA3, CLDN1, NELL2, ARHGAP36, ATF3, CLDN10, IL1RL1, SLC27A6, PRR15, IGSF1, PDLIM4, CST6, CRLF1, CXCL14, SFN, TACSTD2, PRSS2, KLK7, SFTPB |
| upregulated | CLIP, HHATL, RPS4Y1 |

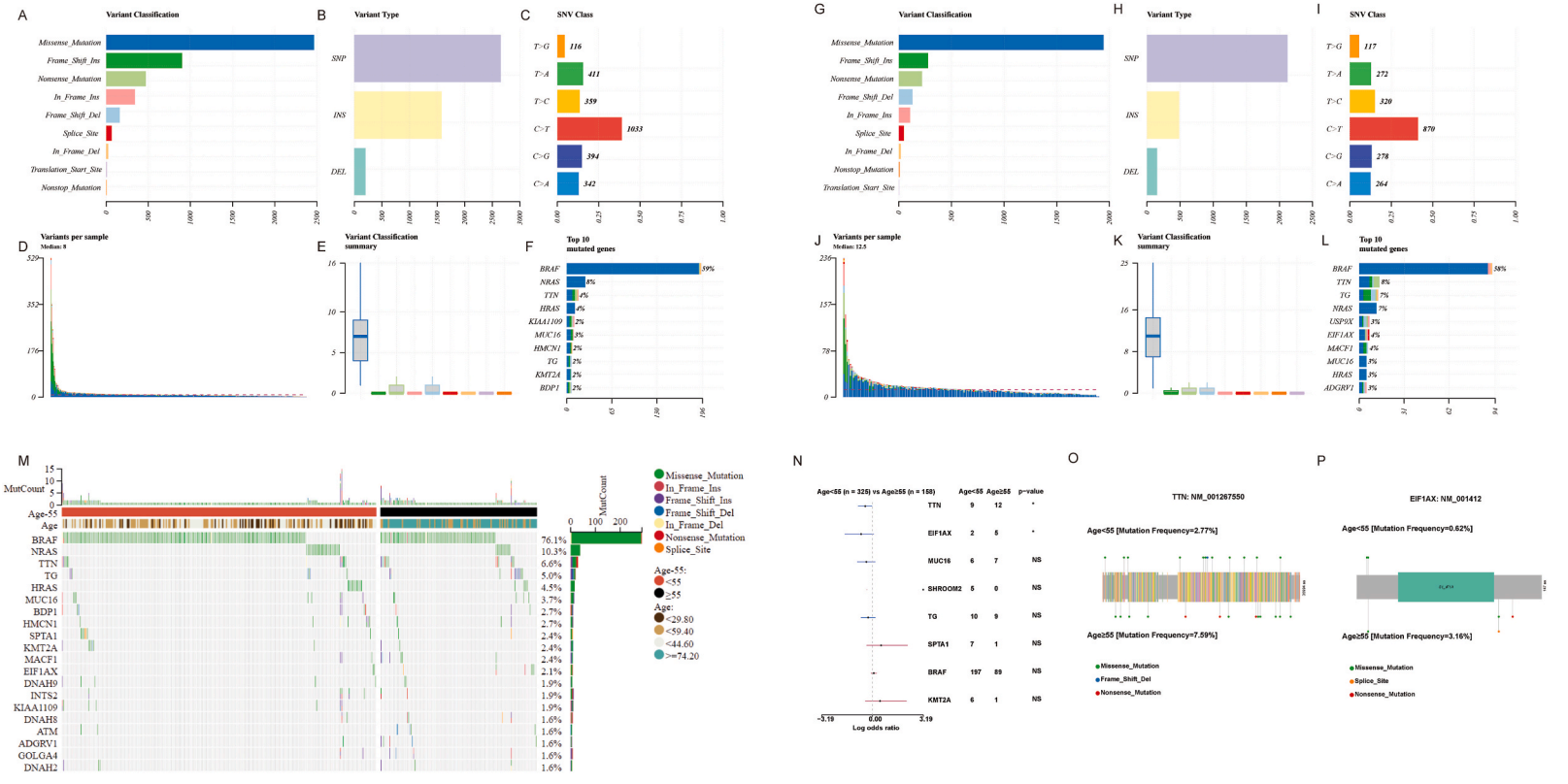


**Fig. 3. Effect of age on somatic mutations of DTC.** A–F. Landscape of somatic mutation in young patients (age <55-years-old). G–L. Landscape of somatic mutation in old patients (age ≥55-years-old). A, G. Number of variant classification. B. Number of variant type. SNP: single nucleotide polymorphism, DEL: deletion, insertion: INS. C, I. Number of SNP class. D, J. Variants per sample. E, K. Summary of variant classifications. F, L. Top 10 mutated genes. M. Top 20 mutated genes of all patients. N. Forest plot displayed different gene mutation frequency between young and old patients (*$P < 0.05$). O, P. "Lollipop" graph for specific mutation in TTN (O) and EIF1AX(P) protein domain. Mutation sites were shown on the x-axis, and the frequency of a particular mutation was represented by the height (y-axis).

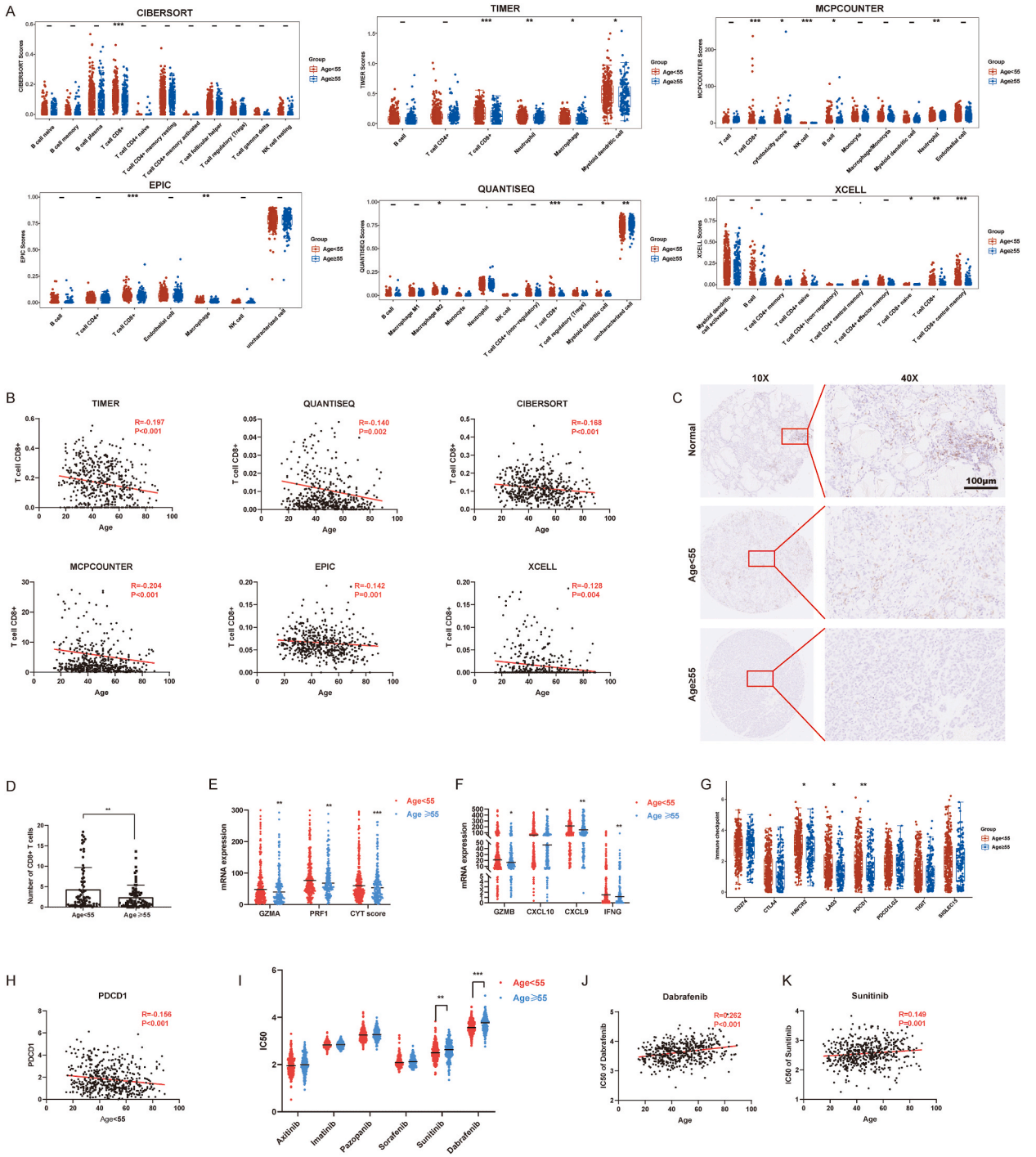


**Fig. 4. Age is associated with the infiltration and cytotoxicity of CD8$^+$ T cells.** A, B. The proportion of different immune cell types (A) and correlation (Spearman correlation analysis) between age and proportion of CD8$^+$ T cell (B) assessed using six different algorithms. C, D. IHC staining (C) and quantitation (D) of CD8 in self-collected DTC samples. (cases of age <55: 76; cases of age ≥55: 69). E. The mRNA expression of *GZMA*, *PRF1*, and calculated cytolytic activity (CYT) score (genomic mean of granzymes A [*GZMA*] and perforin [*PRF1*] mRNA expression). F. The mRNA expression of other CD8$^+$ T cell cytolytic indexes. G. The mRNA expression of immune checkpoints. H. The correlation between age and PDCD1 (PD-1) mRNA expression (Spearman correlation analysis). I. The prediction of 50 % of the inhibiting concentration ($IC_{50}$) score of different drugs from the Genomics of Drug Sensitivity in Cancer (GDSC) database. J, K. Correlation between age and $IC_{50}$ of dabrafenib (J) and sunitinib (K). (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

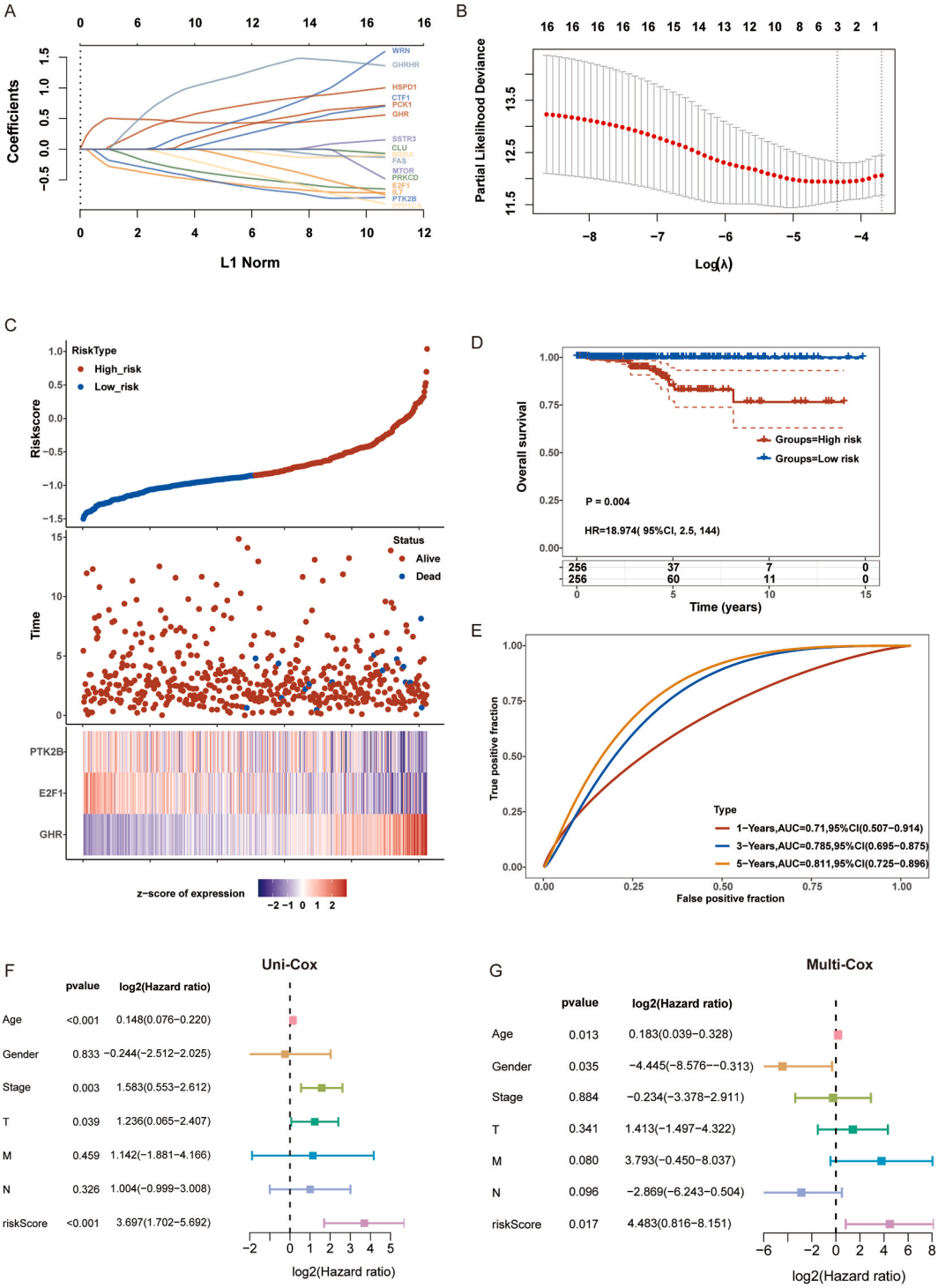


**Fig. 5. Prognosis prediction model constructed based on aging-related genes.** A. Selection of the optimal parameter (lambda) in the LASSO Cox model; dotted vertical lines were drawn at the optimal values using the minimum criteria. B. LASSO coefficient profiles of the 13 survival-associated ARGs with non-zero coefficients determined by the optimal lambda. C. Distribution of risk score, patient survival status, and expression of three ARGs adopted in risk model. D. The association between risk score and OS analyzed by Kaplan–Meier analysis. E. Time-dependent ROC curves for ARG-based OS prediction. F, G. Univariate (F) and multivariate (G) Cox regression analysis assessed the association between OS and risk score.

### 3.3. Effect of age on somatic mutations of DTC

We further investigated the somatic mutation of DTC cancer via R software ("maftools" Package) based on the TCGA database. In the comparison between variation classification, older patients (≥55-years-old) showed more missense mutation (70.4 *vs* 55.5 %%), but less frameshift insertion mutation (10.13 *vs* 20.3 %) and less in-frame insertion mutation (3.9 *vs* 7.7 %) compared to younger patients (<55-years-old) (Fig. 3A–G). In the comparison between variation types, older patients showed more single nucleotide polymorphisms (SNP) and deletions but fewer insertions than in younger patients (Fig. 3B–H). No obvious differences in the SNP types were observed between old and young patients (Fig. 3C–I). More variants were observed in older than in younger patients (median number of variants per young patient *vs* per old patient = 8 *vs* 12.5) (Fig. 3D–J). The order of the top 10 mutated genes in older patients was different from that in younger patients (Fig. 3E–M). Among these, *TTN* and *EIF1AX* were confirmed to be mutated more frequently in older patients than in younger patients (Fig. 3N). Moreover, we showed the specific mutation site, type, and frequency of *TTN* and *EIF1AX* in schematic representations of protein domain information. The mutation sites of *TTN* and *EIF1AX* in older patients were different from those in younger patients, with most additional mutations in older patients being nonsense mutations (Fig. 3O and P).

### 3.4. Age is associated with the infiltration and cytotoxicity of $CD8^+$ T cells

As a major fraction of the tumor tissue, infiltrating stroma and immune cells cause perturbing tumor signals in molecular studies and play an important role in cancer biology. Herein, the "ESTIMATE" algorithm was used to calculate the tumor purity (Estimate Score), immune score, and stromal score of patients with DTC. No significant association was found between age and the three scores (Fig. S1). To assess the reliability of the immune score evaluation results, we used TIMER, xCell, MCP-counter, CIBERSORT, EPIC, and quanTIseq to calculate the proportions of the main immune cell types in DTC. In all six algorithms, older patients showed a decreased proportion of $CD8^+$ T cells compared to younger patients (Fig. 4A). In addition, as a continuous variable, age showed a significant negative correlation with of $CD8^+$ T cells proportion in all six algorithms (Fig. 4B). Moreover, we assessed the infiltration of $CD8^+$ T cells in our self-collected 145 DTC samples using CD8 IHC staining (cases of age <55: 76; cases of age ≥55: 69). Similar to the mRNA data from the TCGA database, we confirmed decreased infiltration of $CD8^+$ T cells in older patients compared to younger patients (Fig. 4C and D). Subsequently, we assessed the cytolytic activity of the $CD8^+$ T cells using cytolytic activity (CYT) score, a new index of cancer immunity calculated from the genomic mean of granzyme A (*GZMA*) and perforin (*PRF1*) mRNA expression [12]. Older patients showed decreased CYT scores and mRNA expression of *GZMA* and *PRF1* (Fig. 4E). Additionally, we found that other $CD8^+$ T cell cytolytic indices, including GZMB, CXCL10, CXCL9, and IFNG, were decreased in older patients compared to younger patients (Fig. 4F). Older patients exhibited decreased infiltration of $CD8^+$ T cells with lower cytotoxicity.

As a part of cancer immunotherapy, immune checkpoints can stimulate or inhibit immune responses. We further investigated the expression of various checkpoints in DTC (Fig. 4G and H), and found that old patients showed decreased expression of HAVCR2/TIM3, LAG3, and PDCD1, suggesting that old patients may benefit less from immune checkpoints inhibitors (ICIs) therapy. Furthermore, we predicted the chemotherapeutic response for each sample based on the GDSC database. Older patients showed better responses to sunitinib and dabrafenib than younger patients (Fig. 4I–K). Therefore, sunitinib and dabrafenib are better choices than ICIs for elderly patients.

### 3.5. Prognosis prediction model constructed based on ARG

Multigene models have better predictive ability; therefore, we aimed to establish a prognosis prediction model based on ARGs. We obtained 307 ARGs from the Human Aging Genomic Resources database (Table S1). Using Kaplan–Meier analysis, we identified 16 ARGs associated with OS (Table S5, Fig. S2). Following the LASSO Cox regression model, three ARGs (*PTK2B, E2F1,* and *GHR*) were adopted to calculate the risk score (risk score = 0.4631* GHR-0.2124*E2F1-0.1125*PTK2B) for the survival prediction model (Fig. 5A–C). The three ARGs calculated risk scores showed a significant association with OS in Kaplan–Meier analysis ($P = 0.004$, HR = 18.974) (Fig. 5D). The risk score also showed a satisfactory AUC (1-, 3-, 5-years AUC = 0.71, 0.785, and 0.896, respectively) for predicting OS in the ROC curve (Fig. 5E). Finally, univariate and multivariate Cox regression analyses were performed to further analyze the prognostic characteristics of the ARG model. In univariate Cox regression analysis, age, stage, T stage, and risk score were significantly associated with OS in patients with DTC (Fig. 5F). In multivariate Cox regression analysis, the risk score was the primary independent predictor of OS in patients with DTC (Fig. 5G).

## 4. Discussion

Age is a well-established prognostic factor for the survival of patients with DTC and is included in the AJCC in Cancer TC staging system. One of the most important modifications in the 8th edition of the AJCC staging system is to increase the age cutoff for risk stratification in DTC from 45 to 55 years; however, whether this cutoff is suitable in clinical practice remains controversial. Herein, we assessed the performance of 45- and 55-years-old cutoff based on the clinical features of 512 patients with DTC. Fifty-five-years-old cutoff showed a higher HR and lower *P* value compared to 45 years, supporting the update of the 8th AJCC staging system.

Considering that the specific mechanisms whereby age-related genomic changes contribute to DTC progression remain unclear. First, we assessed the transcriptional files associated with age, and found that the transcriptional differences between the two age groups were relatively small. It was also difficult to determine whether these genes were associated with age or aggressiveness. Most DEGs were differentially methylated. DNA methylation changes gradually with age, and the methylation status tends to become

unstable. As DNA methylation inhibits gene expression, it is often described as a "silent" epigenetic marker. In animal genomes, more than half the genes contain short CpG dinucleotide-rich regions (approximately 1000 bp) called CpG islands, which are usually located at transcription start sites [13,14]. Thus, our findings reveal the epigenetic regulatory roles of aging in patients with DTC through the DNA methylation–mRNA axis.

Mutations such as gene rearrangements, deletions, insertions, substitutions, oncogenes, and tumor suppressors can be activated or inactivated [15]. With increasing age, cells divide more often, and mutations accumulate. The slow and gradual accumulation of mutations may also play an important role in aging. More variants occurred in older patients than in younger patients (median per patient: 12.5 *vs* 8); and most of the increased variants had missense mutations. Of which, the mutation frequencies of *TTN* and *EIF1AX* were significantly increased. *EIF1AX* encodes eukaryotic translation initiation factor 1A, facilitating 43S complex formation by stabilizing the binding of the 40S subunit to the eIF2-GTP-methionyl-initiator tRNA. As part of a sophisticated scanning system for mRNA in eukaryotes, it coordinates with other translation initiation factors to locate the correct start codon [16]. There is a 20 % risk of TC associated with *EIF1AX* mutations; this risk may be higher in nodules carrying an A113_splice mutation and *EIF1AX* mutations occurring in conjunction with RAS mutations. EIF1AX-A113 splice drives an ATF4-induced dephosphorylation of EIF2α, increasing protein synthesis to promote DTC tumorigenesis [17]. Titin is a large protein in the heart and skeletal muscle encoded by the *TTN* gene, which plays an important role in the structure and function of sarcomeres. Furthermore, previous studies have shown that *TTN* mutations are independent risk factors associated with tumor-associated macrophage infiltration in TC [18]. *TTN* mutations have also been reported to be associated with response to chemotherapy and immunotherapy in lung cancer [19,20].

In further immune infiltration assessments, we found that old patients showed decreased infiltration of $CD8^+$ T cells combined with lower cytotoxicity. $CD8^+$ T cells recognize and attack tumor cells that express tumor antigens, thereby improving the DFS of patients with DTC [21,22]. $CD8^+$ T-cells typically differentiate into cytotoxic T-cells (CTL) upon activation. CTL is a T cell that specifically kills target cells by secreting various cytolytic granules (including, granzyme, perforin, interferon). Decreased infiltration of $CD8^+$ T cells with low cytotoxicity in old patients indicates the potential of immunosuppression during aging. Combined with the lower expression of immune checkpoints, we suggest that older patients benefit less from immunotherapy than younger patients. However, the chemotherapy prediction assessment indicated that old patients responded better to dabrafenib and sunitinib. The BRAF inhibitor, and dabrafenib is effective against ATCs with the $BRAF^{V600E}$ mutations [23,24]. Sunitinib is a multitarget tyrosine kinase inhibitor (TKI), that inhibits PDGF, VEGFR, and c-KIT. Several clinical studies have reported the effectiveness of sunitinib as first- and second-line TKI therapy in patients with advanced dedifferentiated TC or medullary TC [25]. Age is associated with response to immunotherapy and chemotherapy and should be considered when formulating therapeutic strategies.

Finally, based on three ARGs (*PTK2B, E2F1,* and *GHR*), we established a prognostic prediction model with satisfactory performance. *PTK2B* encodes a cytoplasmic protein tyrosine kinase that regulates ion channels and maps kinase signaling in response to calcium. *E2F1* encodes E2F transcription factor 1 and functions as a tumor suppressor protein along with a target for transforming proteins from small DNA viruses. Previous studies have confirmed that these genes participate in the proliferation, migration, and progression of gastric, breast, and colon cancers [26–29]; however, few studies have demonstrated their role in DTC.

In conclusion, our research demonstrates that 55 years of age is a better cutoff for predicting the survival of patients with DTC. We demonstrated that aging is associated with DNA methylation-driven transcriptional regulation and accumulation of somatic mutations in *EIF1AX*. Aging contributes to the infiltration and cytotoxicity of $CD8^+$ T cells. Finally, we provided an ARG model for predicting TC prognosis and will continue to develop our model in the future by including more factors (mutations and immune cells), cases, and data.

## Funding

This study was funded by the Science and Technology Commission of Shanghai Municipality (20Y11914300).

## Data availability

Data from the TCGA database were downloaded from UCSC XENA (https://xenabrowser.net/). Any other data from this study can be obtained from the authors if requested.

## Ethics approval and consent to participate

The Ethics Committee of Fudan University Shanghai Cancer Center (050432-4-2307E) granted authorization for this study. Before this study, all individuals involved in the study provided informed consent by signing the necessary documents.

## CRediT authorship contribution statement

**Yu Zhang:** Resources. **Qi Chen:** Project administration, Data curation. **Lili Niu:** Formal analysis, Conceptualization. **Hu Huang:** Writing – review & editing, Project administration. **Zhou Yang:** Writing – original draft, Formal analysis. **Tian Liao:** Visualization, Investigation. **Qing Guan:** Funding acquisition, Conceptualization. **Jun Xiang:** Investigation, Funding acquisition.

## 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.

## Acknowledgements

Not applicable.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33595.

## References

[1] E. Chmielik, D. Rusinek, M. Oczko-Wojciechowska, M. Jarzab, J. Krajewska, A. Czarniecka, et al., Heterogeneity of thyroid cancer, Pathobiology 85 (1–2) (2018) 117–129.
[2] B.R. Haugen, E.K. Alexander, K.C. Bible, G.M. Doherty, S.J. Mandel, T.E. Nikiforov, et al., American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer, Thyroid 26 (1) (2015) 1–133, 2016.
[3] E.K. Alexander, G.M. Doherty, J.A. Barletta, Management of thyroid nodules, Lancet Diabetes Endocrinol. 10 (7) (2022) 540–548.
[4] R. Wong, S.G. Farrell, M. Grossmann, Thyroid nodules: diagnosis and management, Med. J. Aust. 209 (2) (2018) 92–98.
[5] B.E. Blackburn, P.A. Ganz, K. Rowe, J. Snyder, Y. Wan, V. Deshmukh, et al., Aging-related disease risks among young thyroid cancer survivors, Cancer Epidemiol. Biomark. Prev. : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 26 (12) (2017) 1695–1704.
[6] D.T. Hughes, M.R. Haymart, B.S. Miller, P.G. Gauger, G.M. Doherty, The most commonly occurring papillary thyroid cancer in the United States is now a microcarcinoma in a patient older than 45 years, Thyroid : official journal of the American Thyroid Association 21 (3) (2011) 231–236.
[7] M. Pizzato, M. Li, J. Vignat, M. Laversanne, D. Singh, C. La Vecchia, et al., The epidemiological landscape of thyroid cancer worldwide: GLOBOCAN estimates for incidence and mortality rates in 2020, Lancet Diabetes Endocrinol. 10 (4) (2022) 264–272.
[8] M.A. Adam, S. Thomas, T. Hyslop, R.P. Scheri, S.A. Roman, J.A. Sosa, Exploring the relationship between patient age and cancer-specific survival in papillary thyroid cancer: rethinking current staging systems, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 34 (36) (2016) 4415–4420.
[9] H.S. Kazaure, S.A. Roman, J.A. Sosa, The impact of age on thyroid cancer staging, Curr. Opin. Endocrinol. Diabetes Obes. 25 (5) (2018) 330–334.
[10] S.B. Edge, AJCC Cancer Staging Manual, vol. 7, Springer, 2010, pp. 97–100.
[11] I.J. Nixon, D. Kuk, V. Wreesmann, L. Morris, F.L. Palmer, I. Ganly, et al., Defining a valid age cutoff in staging of well-differentiated thyroid cancer, Ann. Surg Oncol. 23 (2) (2016) 410–415.
[12] I. Ganly, I.J. Nixon, L.Y. Wang, F.L. Palmer, J.C. Migliacci, A. Aniss, et al., Survival from differentiated thyroid cancer: what has age got to do with it? Thyroid, official journal of the American Thyroid Association 25 (10) (2015) 1106–1114.
[13] A.N. Berg, R.R. Seethala, The American Joint Committee on Cancer eighth edition: changes in thyroid carcinoma staging and an update on reporting, AJSP: Reviews & Reports 23 (3) (2018) 145–148.
[14] K. Yoshihara, M. Shahmoradgoli, E. Martínez, R. Vegesna, H. Kim, W. Torres-Garcia, et al., Inferring tumour purity and stromal and immune cell admixture from expression data, Nat. Commun. 4 (2013) 2612.
[15] B. Chen, M.S. Khodadoust, C.L. Liu, A.M. Newman, A.A. Alizadeh, Profiling tumor infiltrating immune cells with CIBERSORT, Methods Mol. Biol. 1711 (2018) 243–259.
[16] H. Wakiyama, T. Masuda, Y. Motomura, Q. Hu, T. Tobo, H. Eguchi, et al., Cytolytic activity (CYT) score is a prognostic biomarker reflecting host immune status in hepatocellular carcinoma (HCC), Anticancer Res. 38 (12) (2018) 6631–6638.
[17] M. Yu, W.D. Hazelton, G.E. Luebeck, W.M. Grady, Epigenetic aging: more than just a clock when it comes to cancer, Cancer Res. 80 (3) (2020) 367–374.
[18] S.C. Zheng, M. Widschwendter, A.E. Teschendorff, Epigenetic drift, epigenetic clocks and cancer risk, Epigenomics 8 (5) (2016) 705–719.
[19] S. Khambata-Ford, C.R. Garrett, N.J. Meropol, M. Basik, C.T. Harbison, S. Wu, et al., Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 25 (22) (2007) 3230–3237.
[20] A. Karunamurthy, F. Panebianco, J.H. S, J. Vorhauer, M.N. Nikiforova, S. Chiosea, et al., Prevalence and phenotypic correlations of EIF1AX mutations in thyroid nodules, Endocr. Relat. Cancer 23 (4) (2016) 295–301.
[21] G.P. Krishnamoorthy, N.R. Davidson, S.D. Leach, Z. Zhao, S.W. Lowe, G. Lee, et al., EIF1AX and RAS mutations cooperate to drive thyroid tumorigenesis through ATF4 and c-MYC, Cancer Discov. 9 (2) (2019) 264–281.
[22] X. Han, J. Chen, J. Wang, J. Xu, Y. Liu, TTN mutations predict a poor prognosis in patients with thyroid cancer, Biosci. Rep. 42 (7) (2022). BSR20221168.
[23] D. Xue, H. Lin, L. Lin, Q. Wei, S. Yang, X. Chen, TTN/TP53 mutation might act as the predictor for chemotherapy response in lung adenocarcinoma and lung squamous carcinoma patients, Transl. Cancer Res. 10 (3) (2021) 1284–1294.
[24] Z. Wang, C. Wang, S. Lin, X. Yu, Effect of TTN mutations on immune microenvironment and efficacy of immunotherapy in lung adenocarcinoma patients, Front. Oncol. 11 (2021) 725292.
[25] L.L. Cunha, E.C. Morari, A.C. Guihen, D. Razolli, R. Gerhard, S. Nonogaki, et al., Infiltration of a mixture of immune cells may be related to good prognosis in patients with differentiated thyroid carcinoma, Clin. Endocrinol. 77 (6) (2012) 918–925.
[26] Z. Yang, X. Wei, Y. Pan, J. Xu, Y. Si, Z. Min, et al., A new risk factor indicator for papillary thyroid cancer based on immune infiltration, Cell Death Dis. 12 (1) (2021) 51.
[27] V. Subbiah, R.J. Kreitman, Z.A. Wainberg, J.Y. Cho, J.H.M. Schellens, J.C. Soria, et al., Dabrafenib and trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 36 (1) (2018) 7–13.
[28] Y. Liao, Y. Gao, A. Chang, Z. Li, H. Wang, J. Cao, et al., Melatonin synergizes BRAF-targeting agent dabrafenib for the treatment of anaplastic thyroid cancer by inhibiting AKT/hTERT signalling, J. Cell Mol. Med. 24 (20) (2020) 12119–12130.
[29] S.M. Ferrari, M. Centanni, C. Virili, M. Miccoli, P. Ferrari, I. Ruffilli, et al., Sunitinib in the treatment of thyroid cancer, Curr. Med. Chem. 26 (6) (2019) 963–972.