

基于乳酸代谢相关基因的头颈部鳞状细胞癌分子亚型和临床特征的生物信息学分析

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[摘要] **目的:** 筛选头颈部鳞状细胞癌(HNSCC)差异预后乳酸代谢相关基因(LRGs), 构建HNSCC的LRGs预后模型, 并阐明其潜在的作用机制。**方法:** 由癌症基因组图谱(TCGA)数据库和基因表达综合(GEO)数据库获取HNSCC基因表达及临床数据, 由GeneCards数据库中获得LRGs, 采用R软件筛选HNSCC的LRGs。采用单因素Cox回归分析得到预后相关基因, 基于预后相关LRGs鉴定出2种不同亚型, 采用Kaplan-Meier(K-M)曲线分析比较2组患者预后, 采用CIBERSORT算法进行2组患者间的免疫相关分析。采用多因素Cox回归分析和LASSO回归分析构建预后模型, 采用受试者工作特征曲线(ROC)和K-M生存曲线评估LRGs与HNSCC患者生存和预后的关系。采用GSE27020、GSE41613和GSE65858数据集验证预后模型。基于风险评分进行分组, 并进行免疫相关分析和肿瘤相关评分分析。**结果:** 通过TCGA数据库从HNSCC样本中差异分析筛选出1196个LRGs, 单因素Cox回归分析筛选出27个差异表达基因(DEGs)与HNSCC患者预后相关, 根据预后相关基因鉴定出2种不同的LRGs亚型(分组1和分组2), K-M生存曲线显示分组2患者总生存期(OS)明显高于分组1, 分组2患者免疫细胞浸润水平明显高于分组1。多因素Cox回归分析和LASSO回归分析筛选出9个LRGs, 包括次黄嘌呤磷酸核糖基转移酶1(HPR1)、淀粉样蛋白前体蛋白(APP)、糖原磷酸化酶(PYGL)、尿激酶型纤溶酶原激活物(PLAU)、大麻素受体2(CNR2)、斯钙素2(STC2)、核苷酸结合寡聚化结构域样受体1(NLRP1)、整合素连接激酶(ILK)和叉头框蛋白B1(FOXB1), 构建预后模型, K-M曲线和ROC曲线显示上述9个基因表达水平与HNSCC患者生存和预后有关联, 且均具有良好的1、2和3年生存预测作用, ROC曲线下面积(AUC)均大于0.650, 且预后模型的预后预测作用在GSE27020、GSE41613和GSE65858数据集中得到验证。根据风险评分分类的患者具有可区分的免疫状态。**结论:** 基于生物信息学方法筛选出的HNSCC差异表达LRGs与HNSCC患者生存和预后有关联, 由9个LRGs构建的预后模型可预测HNSCC患者的生存情况和治疗反应。

[关键词] 头颈部鳞状细胞癌; 乳酸代谢; 免疫浸润; 生物信息学; LASSO回归分析

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Bioinformatics analysis on molecular subtypes and clinical characteristics of head and neck squamous cell carcinoma based on genes associated with lactate metabolism

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ABSTRACT Objective: To select the differential prognostic lactic acid metabolism-related genes (LRGs) of the head and neck squamous cell carcinoma (HNSCC) to construct the LRGs prognostic model of HNSCC, and to clarify the potential mechanism. **Methods:** The HNSCC gene expression and clinical data were obtained from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) Databases, the LRGs were identified through GeneCards Database, and R software was used to screen out the LRGs of HNSCC; univariate Cox regression analysis was used to identify prognosis-related genes; two different subtypes were identified based on the prognosis-related LRGs; Kaplan-Meier (K-M) curve analysis was used to compare the prognosis of the patients between two groups; CIBERSORT algorithm was used to perform the immuno-correlation analysis between two groups; multivariate Cox regression analysis and LASSO regression analysis were used to construct the prognostic model; receiver operating characteristic curve (ROC) and K-M survival curve were used to assess the relationship between LRGs and survival and prognosis of the HNSCC patients. The prognostic model was validated by GSE27020, GSE41613, and GSE65858 datasets. The experiment were grouped based on risk score, and immune-related analysis and tumor score analysis were performed. **Results:** The TCGA Database differential analysis results showed that 1 196 LRGs were identified from HNSCC samples; univariate Cox regression analysis selected 27 differentially expressed genes (DEGs) associated with the prognosis of the HNSCC patients. Two different LRGs subtypes (Group 1 and Group 2) were identified according to the prognosis-related genes. The K-M survival curves results showed that the overall survival (OS) of the patients in Group 2 was significantly higher than that in Group 1, and the immune cell expression amount of the patients in Group 2 was also higher than that in group 1. The multivariate Cox regression and LASSO regression analysis results screened out 9 LRGs, including hypoxanthine phosphoribosyltransferase 1 (HPRT1), amyloid precursor protein (APP), glycogen phosphorylase L (PYGL), urokinase-type plasminogen activator (PLAU), cannabinoid receptor 2 (CNR2), stanniocalcin 2 (STC2), nucleotide binding oligomerization domain-like receptor protein 1 (NLRP1), integrin-linked kinase (ILK), and forkhead box B1 (FOXB1); the prognostic model was constructed. The K-M and ROC curve results indicated that the expression levels of above 9 genes were associated with the survival and prognosis of the HNSCC patients, providing good 1-year, 2-year, and 3-year survival prediction effect, and the area under ROC curve (AUC) values were all greater than 0.650. Furthermore, the predictive ability of the prognosis model was validated in GSE27020, GSE41613, and GSE65858 datasets. The patients classified based on the risk scores had distinguishable immune statuses. **Conclusion:** The differentially expressed LRGs of HNSCC screened by bioinformatics methods are related to the survival and prognosis of the HNSCC patients; the prognostic model constructed by 9 LRGs can predict the survival status and treatment response of the HNSCC patients.

KEYWORDS Head and neck squamous carcinoma; Lactic acid metabolism; Immune infiltration; Bioinformatics; LASSO regression analysis

头颈部鳞状细胞癌(head and neck squamous cell carcinoma, HNSCC)是全球第六大常见癌症^[1],且发病率逐年上升,预计至2030年将增加30%,即每年增加108万新发病例^[2-3],患者5年生存率仅为40%~50%^[4],晚期HNSCC患者生存率为34.9%^[5]。目前,HNSCC患者的主要治疗方法为手术治疗,放疗和化疗可作为晚期和肿瘤转移患者的辅助疗法。HNSCC患者的预后并不理想,因此研究HNSCC的分子机制,确定新的治疗靶点,构建有价值的预后模型,可为预测HNSCC患者预后和个体化用药提供参考,以改善其治疗效果。

肿瘤微环境(tumor microenvironment, TME)中乳酸的增加有利于癌细胞增殖和免疫逃逸。乳酸不仅是糖酵解的产物,也是正常组织和恶性肿瘤组织之间的关键调节剂。肿瘤转移、复发和预后较差均与较高的乳酸水平有关联^[6-7]。近年来,抑制乳酸代谢已被证实为针对恶性肿瘤的潜在治疗方法^[8]。研究^[9-12]表明:乳酸代谢相关基因(lactic acid metabolism-related genes, LRGs)在食管鳞状细胞癌、肺腺癌、乳腺癌和肾透明细胞癌等多种癌症的发生发展过程中发挥重要作用。但LRGs在HNSCC中的作用尚不明确。本研究对LRGs表达谱进行综合评估,基于2种LRGs亚型鉴定差异表达基因(differentially expressed genes, DEGs),构建HNSCC患者预后模型,以独立预测HNSCC患者的生存情况,同时探讨LRGs与HNSCC患者免疫细胞浸润和肿瘤相关评分的相关性,为HNSCC的临床诊断和治疗提供理论依据和思路。

1 资料与方法

1.1 数据收集和预处理 由癌症基因组图谱(The Cancer Genome Atlas, TCGA)数据库下载HNSCC患者基因表达数据和临床数据作为训练队列,其中HNSCC组织样本522份,正常组织样本44份,并下载肿瘤突变谱。由基因表达综合(Gene Expression Omnibus, GEO)数据库下载GSE27020、GSE41613和GSE65858数据集作为验证队列。由GeneCards^[12]数据库提取5 034个LRGs。

1.2 LRGs差异表达分析 采用limma数据包对TCGA数据库中HNSCC组织和癌正常组织中LRGs mRNA表达水平进行分析($|\log_2FC|>1$, $P<0.05$),通过火山图和热图显示差异表达的LRGs。

1.3 聚类分析和TME分析 采用单因素Cox回归分析筛选出与预后相关的差异表达LRGs(differentially expressed-LRGs, DE-LRGs)。对筛选出的DE-LRGs进行Pearson相关分析。基于DE-LRGs mRNA表达水平,采用ConsensusClusterPlus数据包对TCGA数据库中HNSCC患者采用K-means算法进行聚类分析。聚类分析是通过迭代寻找K个分组的划分方案,在不同分组中找到数据均值,通过最小化损失函数确定分组的个数及对应的成员。损失函数:
$$j(c,u)=\sum_{i=1}^n$$

$\|x_i-\mu_{c_i}\|^2$,其中 x_i 为第 i 个患者对应的基因表达水平, c_i 为 x_i 所属的组, μ_{c_i} 为分组对应的均值, n 为患者总数。采用Kaplan-Meier(K-M)生存曲线分析比较不同分组患者的预后。采用CIBERSORT算法进行TME分析,预测K个分组患者肿瘤样本中浸润免疫细胞的组成。

1.4 预后模型的构建和验证 采用多因素Cox回归分析和LASSO回归分析对HNSCC的独立预后基因进行分析,构建预后模型,根据风险评分的中位数,将HNSCC患者分为高风险组和低风险组。采用K-M生存曲线和受试者工作特征曲线(receiver operating characteristic curve, ROC)判断预后模型的预测价值,采用GSE27020、GSE41613和GSE65858数据集验证预后模型。

1.5 肿瘤免疫细胞浸润分析 采用单样本基因富集分析(single-sample gene set enrichment analysis, ssGSEA)算法分析各样本的免疫细胞活性和免疫功能。采用CIBERSORT算法预测低风险组和高风险组患者肿瘤样本中浸润免疫细胞的组成,比较不同分组患者主要组织相容性复合体(major histocompatibility complex, MHC)的表达情况。

1.6 肿瘤相关评分与风险评分相关性分析 计算低风险和高风险组HNSCC患者血管生成活性评分、EMT评分、致瘤细胞因子评分和干性评分,采用Pearson相关分析方法分析上述4种肿瘤相关评分与风险评分的相关性。

1.7 统计学分析 采用R统计软件(4.2.0版本)进行统计学分析。采用“Survival”软件包进行K-M生存分析、单因素Cox分析和多因素Cox分析,采用“glmnet”软件包进行LASSO回归分析,采用timeROC软件包绘制ROC曲线,根据ROC曲线评估患者预后模型的准确性,采用GSVA软件包进行ssGSEA分析。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 LRGs 差异表达分析结果 基于 GeneCards 数

据库获得 5 034 个 LRGs。分析 TCGA 数据库中 HNSCC 组织和癌旁正常组织样本的表达数据, 获得 1 196 个 DE-LRGs。见图 1。

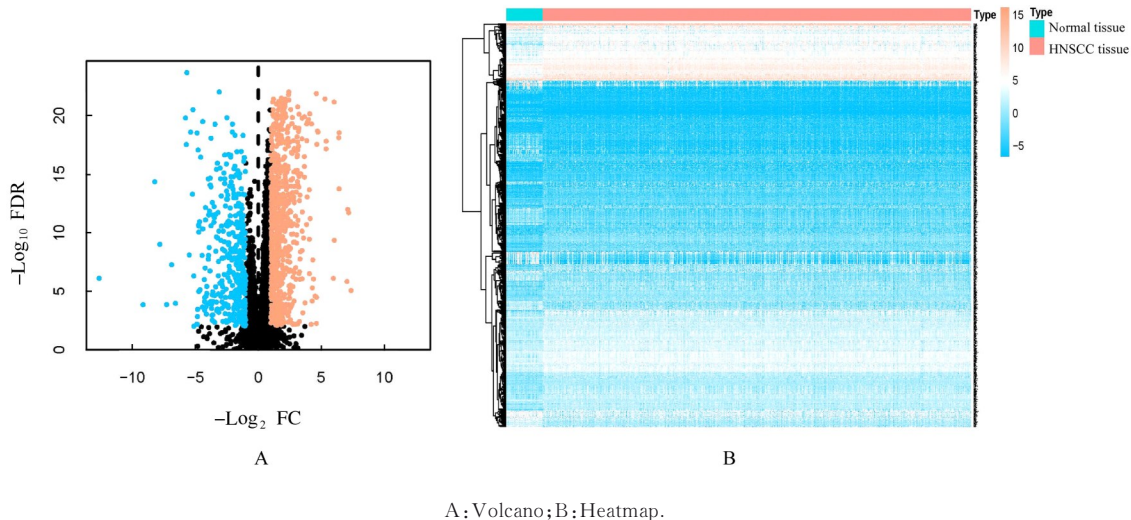


图 1 HNSCC 组织和癌旁正常组织中 LRGs mRNA 表达水平

Fig. 1 Expression levels of LRGs mRNA in HNSCC tissue and paracancerous normal tissues

2.2 聚类分型的鉴定及其与 TME 的相关性分析

单因素 Cox 回归分析得到 27 个 HNSCC 预后相关基因, 其中 16 个为保护因素, 11 个为危险因素 (图 2A)。相关分析结果显示: 大多数预后相关基因之间存在明显相关性 (图 2B)。进一步利用 27 个预后相关基因进行聚类分型, 当 HNSCC 患者分为 2 个亚组时, 聚类效果最好, 亚组内部一致性和稳定性较好 (图 3A~C)。K-M 生存曲线分析结果显示: 分组 2 患者的预后优于分组 1 (图 3D)。CIBERSORT 算法结果显示: 与分组 1 比较, 分组 2 患者免疫细胞浸润水平更高, CD4+T 淋巴细胞、CD8+T 淋巴细胞、中性粒细胞、巨噬细胞和树突状细胞浸润水平差异有统计学意义 ($P < 0.05$)。见图 4。

2.3 HNSCC 预后模型的构建 采用多因素 Cox 回归分析和 LASSO 回归分析对 HNSCC 患者的独立预后基因进行分析, 构建预后模型, 选择 9 个基因加入模型, 包括次黄嘌呤磷酸核糖基转移酶 1 (hypoxanthine phosphoribosyltransferase 1, HPRT1)、淀粉样蛋白前体蛋白 (amyloid precursor protein, APP)、糖原磷酸化酶 L (glycogen phosphorylase L, PYGL)、尿激酶型纤溶酶原激活物 (urokinase-type plasminogen activator, PLAU)、大麻素受体 2 (cannabinoid receptor 2, CNR2)、斯

钙素 2 (stanniocalcin 2, STC2)、核苷酸结合寡聚化结构域样受体蛋白 1 (nucleotide-binding oligomerization domain-like receptor protein 1, NLRP1)、整合素连接激酶 (integrin-linked kinase, ILK) 和叉头框蛋白 B1 (forkhead-box B1, FOXB1)。风险评分公式如下: 风险评分 = $0.015\ 195 \times \text{HPRT1 表达水平} + 0.002\ 176 \times \text{APP 表达水平} + 0.005\ 053 \times \text{PYGL 表达水平} + 0.002\ 458 \times \text{PLAU 表达水平} - 0.983\ 8 \times \text{CNR2 表达水平} + 0.023\ 846 \times \text{STC2 表达水平} - 0.090\ 1 \times \text{NLRP1 表达水平} - 3.414\ 72 \times \text{ILK 表达水平} + 0.641\ 958 \times \text{FOXB1 表达水平}$ 。根据风险评分中位数将 HNSCC 患者分为低风险组和高风险组。

TCGA 数据集中, 与低风险组比较, 高风险组 HNSCC 患者预后较差, 1、3 和 5 年的曲线下面积 (area under curve, AUC) 分别为 0.699、0.724 和 0.660 (图 5)。采用 GSE41613、GSE65858 和 GSE27020 数据集验证, 预后模型具有良好的效率 (图 6), 在 2 个临床亚组中, 与低风险组比较, 高风险组 HNSCC 患者预后较差。

2.4 HNSCC 患者肿瘤免疫细胞浸润分析

ssGSEA 结果显示: 低风险组 HNSCC 患者较高风险组患者有更高的免疫细胞浸润和更多的免疫相关通路 (图 7)。低风险组 HNSCC 患者浸润免疫

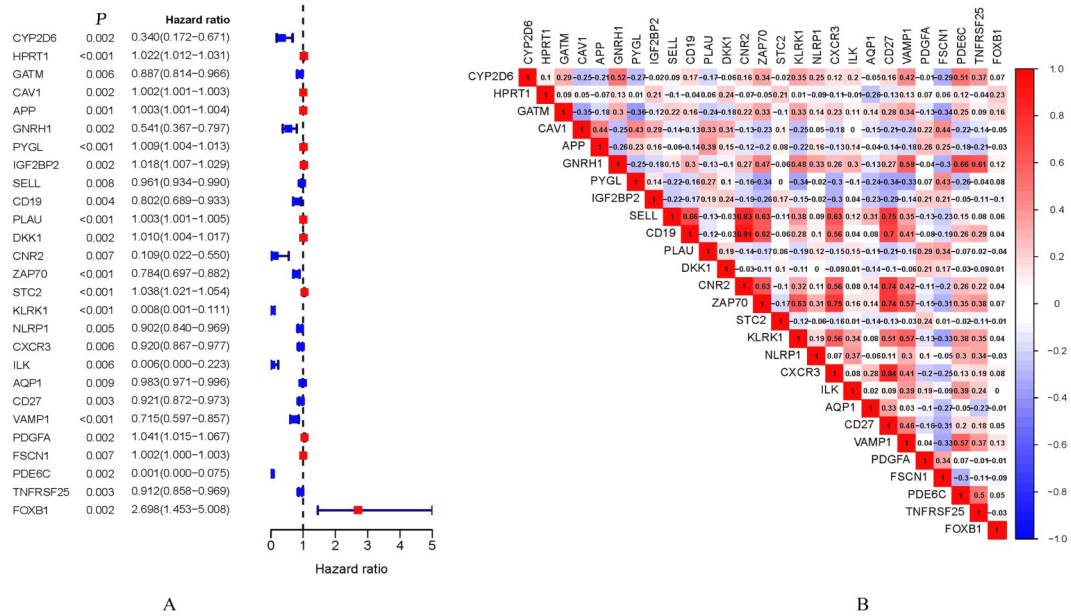
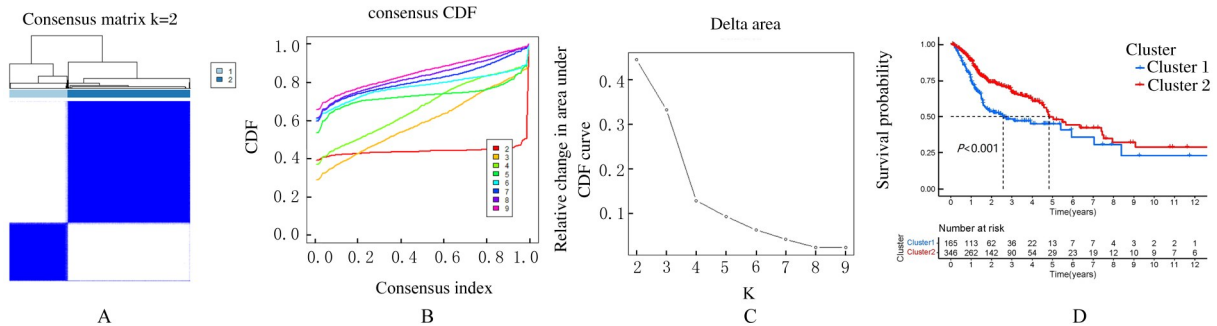


图2 HNSCC预后相关基因单因素Cox回归分析的森林图(A)和相关性分析(B)

Fig. 2 Univariate Cox regression analysis forest plot (A) and correlation analysis (B) on HNSCC prognosis-related genes



A: Consensus clustering matrix when k=2; B: Consensus clustering CDF when k valued 2 to 9; C: Relative change in area under CDF curve when k=2; D: K-M survival curve of survival difference between cluster 1 and cluster 2.

图3 聚类分析图(A~C)和聚类后HNSCC患者K-M生存曲线(D)

Fig. 3 Cluster analysis diagrams (A—C) and K-M survival curve (D) of HNSCC patients after cluster

细胞主要包括B淋巴细胞、浆细胞、CD8+T淋巴细胞、肥大细胞和树突状细胞(图8A),低风险组HNSCC患者MHC表达水平较高(图8B)。

2.5 HNSCC患者肿瘤相关评分与风险评分的相关性 高风险组HNSCC患者血管生成活性和致瘤细胞因子评分较高,低风险组HNSCC患者干性评分较高(图9),血管生成活性和致瘤细胞因子评分与风险评分呈正相关关系($r=0.85, P=8.8e-16; r=0.24, P=5.3e-08$),干性评分与风险评分呈负相关关系($r=-0.12, P=0.0061$)。

3 讨论

肿瘤组织中乳酸的积累是恶性肿瘤发展的关键和早期事件^[13]。近年来,乳酸代谢受到越来越多的关注,但HNSCC与乳酸代谢相关性分析的研究较少。本文作者分析了乳酸代谢与HNSCC发展的相关性,以确认其在癌症生物学中的作用。

本研究首先通过TCGA和GeneCards数据库获取1196个在HNSCC中差异表达的LRGs。进一步对HNSCC患者的总生存时间(overall survival, OS)进行单因素Cox回归分析,得到了27个预后相关基因,对预后基因进行进一步优化,纳入9个

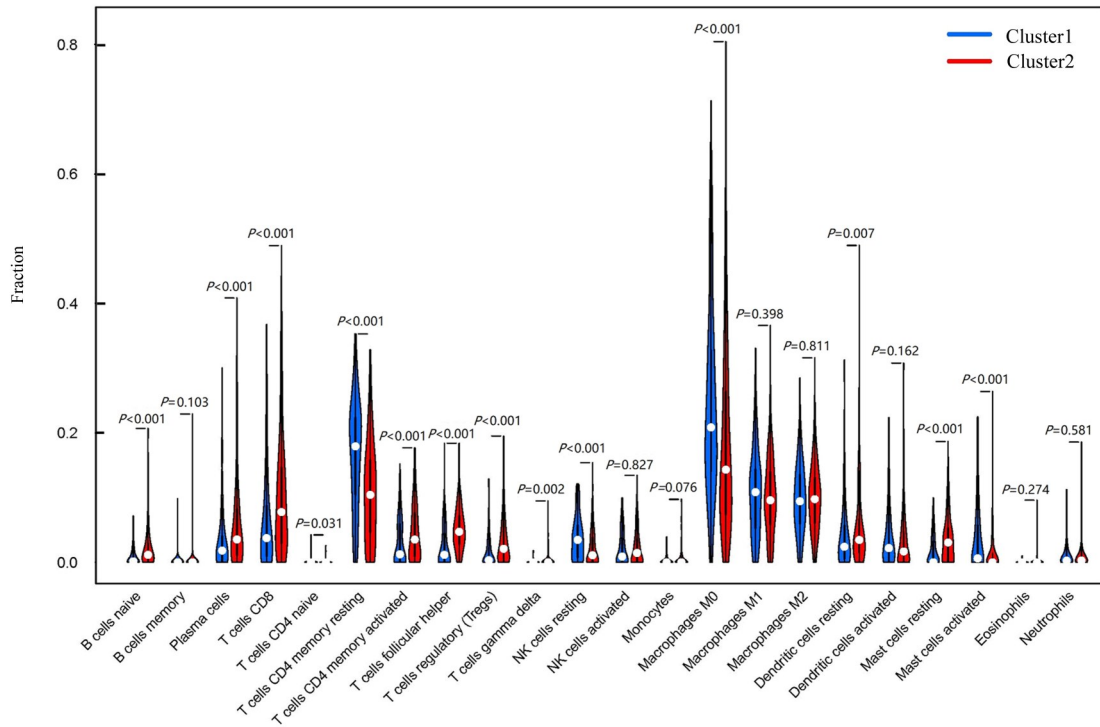
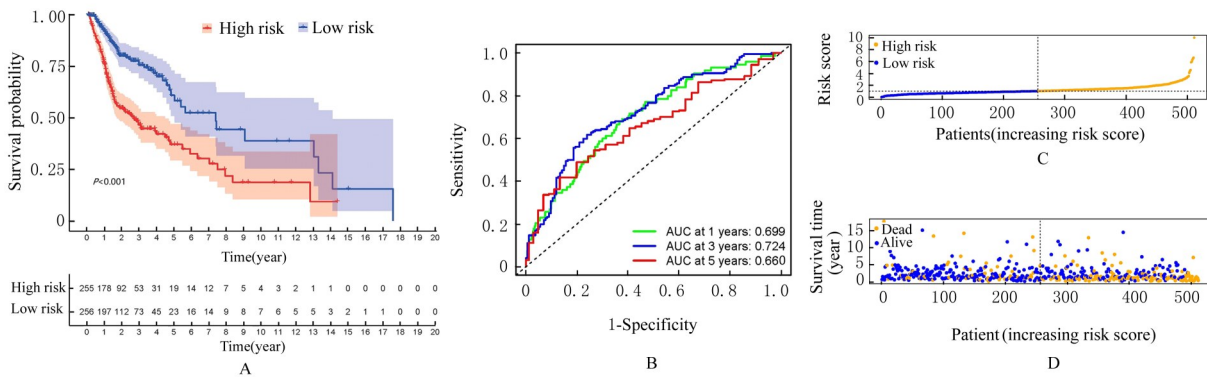


图 4 聚类分型后免疫相关分析

Fig. 4 Immuno-correlation analysis after cluster typing



A: Survival analysis; B: ROC analysis; C: Risk score; D: Survival status.

图 5 TCGA 队列的生存分析、风险评分、生存状态和 ROC 分析

Fig. 5 Survival analysis, risk scores, survival status, and ROC analysis in TCGA cohort

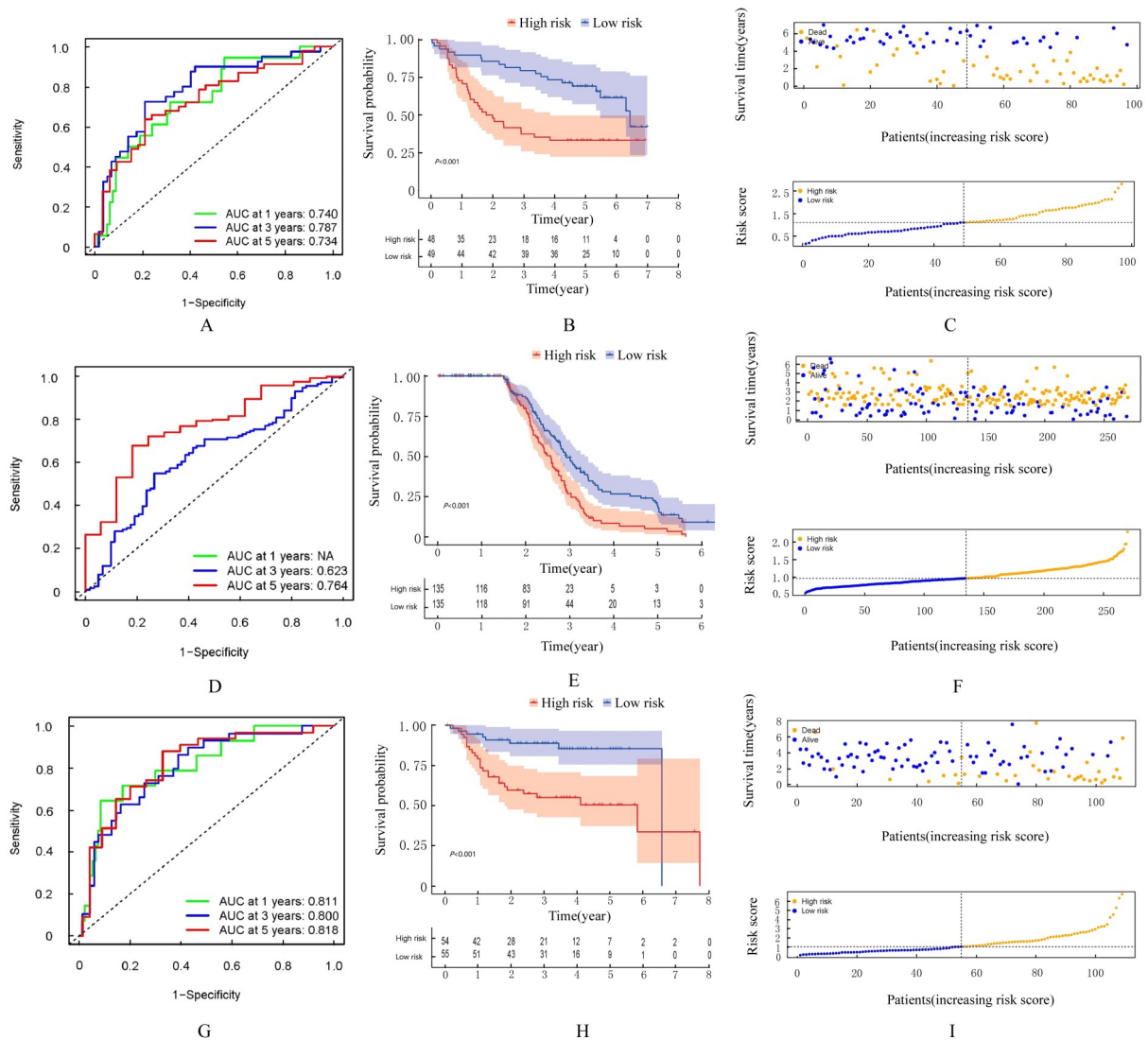
关键基因建立 HNSCC 预后模型, 包括 HPRT1、APP、PYGL、PLAU、CNR2、STC2、NLRP1、ILK 和 FOXB1, 并在 3 个外部队列中进行验证。此外, 通过聚类分析将 HNSCC 患者分为 2 组, 与分组 1 比较, 分组 2 患者的预后更好, 其免疫细胞浸润水平更高, 可能与分组 2 患者 OS 更长有关。

本研究中, 根据风险评分中位数将患者分为高风险组和低风险组, 2 组患者在预后和 TME 等方面均存在明显差异, 表明该预后模型可用于指导 HNSCC 患者的治疗, 具有预测 HNSCC 患者预后

的价值。

近年来, 免疫疗法在治疗皮肤癌、膀胱癌、肺癌、肾癌和错配修复缺陷肿瘤方面显示出明显效果^[14]。因此, 寻找可靠的生物标记物对 HNSCC 患者进行免疫治疗非常重要^[15]。

HPRT1 在多种组织中高表达, 与 HNSCC 患者预后不良有明显关联^[16], 可能是 HNSCC 潜在的治疗靶点。STC2 是一种分泌糖蛋白, 在调节钙稳态、葡萄糖稳态和磷转移中起重要作用, 是一种潜在的泛癌预后标志物和新的免疫治疗靶点^[17]。



A—C: GSE41613 cohort; D—F: GSE65858 cohort; G—I: GSE27020 cohort; A, D, G: ROC analysis; B, E, H: Survival analysis; C, F, I: Survival status (above) and risk score (below).

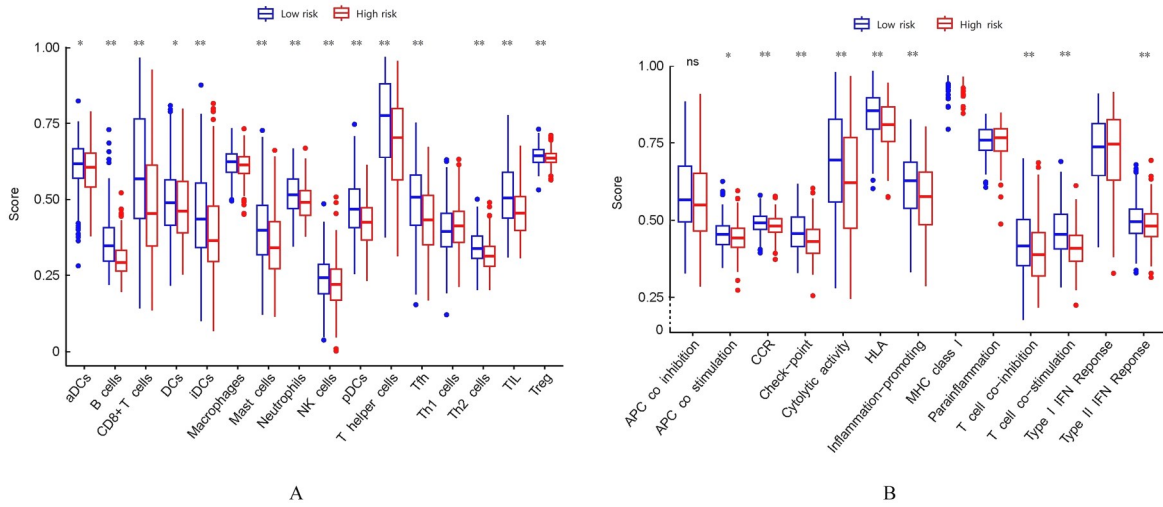
图6 GSE41613、GSE65858和GSE27020数据集中HNSCC患者的生存分析、风险评分、生存状态和ROC分析

Fig. 6 Survival analysis, risk scores, survival status, and ROC analysis of HNSCC patients in GSE41613, GSE65858, and GSE27020 datasets

NLRP1是一种先天性免疫传感器,与肺腺癌和胰腺癌^[18]等疾病发生有关。

APP对癌症免疫疗法具有耐药性,可能与使MHC-I APP失活的基因发生突变有关^[19]。PYGL是结肠癌发生的风险基因^[20],还与食管鳞癌^[21]、胰腺癌^[22]和胶质瘤^[23]的发生有关。PLAU可能是葛根苓连汤治疗溃疡性结肠炎的靶基因^[24],还与胰腺癌的预后^[25]及甲状腺癌^[26]、胃癌^[27]和乳腺癌^[28]的发生有关联。CNR2可通过内源性大麻素损害特异性T淋巴细胞的功能,导致肿瘤患者的OS缩短^[29]。ILK与整合素和生长因子受体信号通

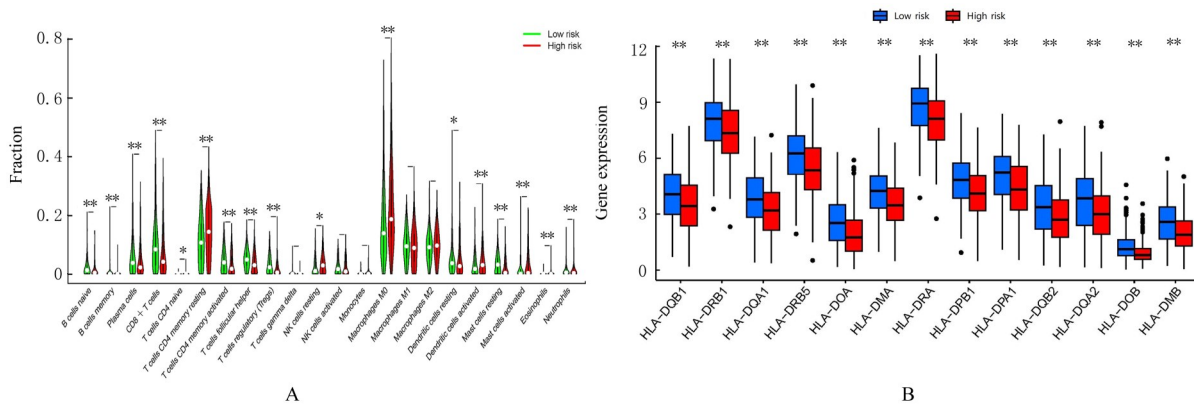
路有关,由于2种信号通路均有助于癌细胞的抵抗,ILK可能是潜在的新的肿瘤治疗靶点^[30]。FOXB1是叉头框(forkhead-box, FOX)基因家族的成员,FOX家族基因的失序会导致癌症的发生,全面研究FOX家族基因的表达谱、基因改变和表观遗传变化,有助于开发新的人类疾病治疗方法和预防手段^[31]。基于上述9个基因建立HNSCC预后模型,通过TCGA数据库和GSE65858、GSE27020及GSE41613数据集验证,高风险组HNSCC患者OS较短,从预后模型相关基因的回归分析来看,9个基因均具有较好的诊断性能。因



A: Immune cell infiltration; B: Immune-related functions or pathways. * $P < 0.05$, ** $P < 0.01$ vs high risk group.

图7 ssGSEA算法分析高风险组和低风险组HNSCC患者肿瘤免疫细胞浸润情况

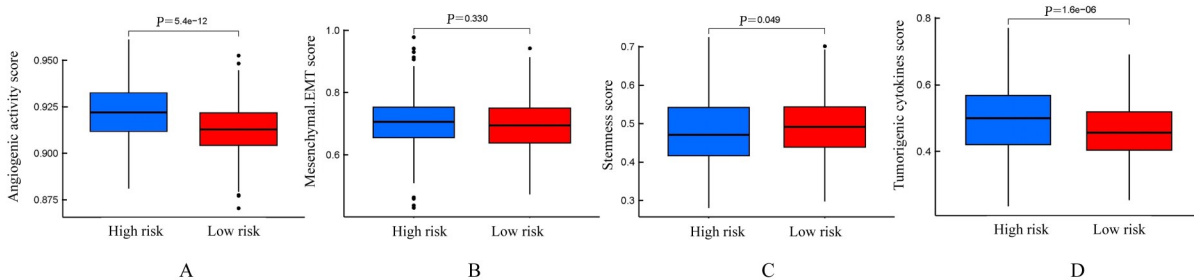
Fig. 7 Infiltration of tumor immune cells of HNSCC patients in high and low risk groups analyzed by ssGSEA algorithm



* $P < 0.05$, ** $P < 0.01$ vs high risk group.

图8 CIBERSORT算法分析高风险组和低风险组HNSCC患者肿瘤免疫细胞浸润情况(A)和MHC表达情况(B)

Fig. 8 Infiltration of tumor immune cells (A) and expressions of MHC(B) of HNSCC patients in high and low risk groups analyzed by CIBERSORT algorithm



A: Difference of angiogenic activity; B: Difference of mesenchymal-EMT; C: Difference of stemness score; D: Difference of tumorigenic cytokine.

图9 高风险组和低风险组HNSCC患者肿瘤相关评分

Fig. 9 Tumor-related scores of HNSCC patients in high and low risk groups

此该预后模型可以独立预测 HNSCC 患者的总体生存率。

在本研究中,低风险组 HNSCC 患者免疫细胞表达更丰富,提示其对免疫治疗更敏感。本研究结果表明:乳酸代谢对 TME 有一定的调节作用,可能有助于发现肿瘤免疫的调控机制,为 TME 的研究提供新的思路。

综上所述,本研究中由 9 个 LRGs (HPRT1、APP、PYGL、PLAU、CNR2、STC2、NLRP1、ILK 和 FOXB1) 构建的 HNSCC 的 LRGs 预后模型,可以预测 HNSCC 患者的生存情况和治疗反应。

利益冲突声明:

所有作者声明不存在利益冲突。

作者贡献声明:

杨紫煦参与研究设计、数据收集整理和论文撰写,苏畅参与研究设计,王波元参与数据收集和分析,刘冲参与论文校对和图片整合,李明贺参与论文审校。

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