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肿瘤突变负荷与肿瘤浸润性免疫细胞在结直肠癌预后及进展中的作用

时姗姗，李霄，丁颖，宋国新，张智弘

(南京医科大学第一附属医院病理科，南京 210029)

[摘　要]　**目的：**本文旨在通过分析结直肠癌(colorectal cancer，CRC)中肿瘤突变负荷(tumor mutational burden，TMB)、肿瘤浸润性免疫细胞(tumor-infiltrating immune cells，TICs)及临床病理特征三者之间的相互关系，探讨TMB及TICs在CRC进展中的相互作用及临床意义。**方法：**下载癌症基因组(The Cancer Genome Atlas，TCGA)数据库中CRC患者的相关数据，包括肿瘤突变、基因表达及相关临床信息等。利用R语言分别计算每个肿瘤样本的TMB值及TICs百分比，并分析TMB值及TICs百分比与临床病理参数和5年总生存率的相关性。根据TMB中位值将肿瘤样本分成高TMB组(n=224)和低TMB组(n=246)，比较2组5年总生存率。筛选出差异表达基因和差异表达TICs，通过基因本体论(Gene Ontology，GO)、京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)及基因集富集分析(Gene Set Enrichment Analysis，GSEA)寻找其潜在相关的免疫机制和功能。**结果：**根据TCGA数据，>65岁CRC患者的TMB值更高，发生血管及淋巴结转移的肿瘤TMB值较低(P<0.001)。与低TMB组相比，高TMB组CRC患者的5年总生存率更低(P<0.05)。116个差异表达基因主要涉及药物代谢及白细胞跨内皮迁移信号通路；4种差异表达的TICs为$CD4^+$ T细胞、滤泡辅助性T细胞、M0及M1巨噬细胞。**结论：**TMB是一个重要的评价CRC预后和免疫治疗的指标，可以通过与TICs相互作用调节免疫微环境，从而影响CRC预后及免疫治疗的效果。

[关键词]　肿瘤突变负荷；肿瘤浸润性免疫细胞；肿瘤微环境；结直肠癌

Role of tumor mutational burden and tumor-infiltrating immune cells in the prognosis and progression of colorectal cancer

SHI Shanshan, LI Xiao, DING Ying, SONG Guoxin, ZHANG Zhihong

(Department of Pathology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China)

Abstract　**Objective:** This study aimed to investigate the association between tumor mutational burden (TMB), tumor-infiltrating immune cells (TICs) and clinicopathological features, and their clinical implications in the progression and prognosis of colorectal cancer (CRC). **Methods:** Data relating to CRC were downloaded from The Cancer Genome Atlas (TCGA) database, including genetic mutation files, RNA-seq data, and clinical information. TMB

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通信作者 (Corresponding author)：张智弘，Email: zhangzhih2001@aliyun.com

value and percentage of TICs of each tumor sample were calculated separately using R language; then correlation analysis was performed between these indices, clinicopathological parameters, and the 5-year overall survival rate. Based on the median TMB values, tumors samples were divided into a high TMB group (n=224) and a low TMB group (n=246). Five-year overall survival rates were compared between the 2 groups. Differentially expressed genes (DEGs) and TICs between high-TMB and low-TMB groups were screened and the potential immune mechanism and functions were identified by Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA). **Results:** Based on TCGA data, CRC patients >65 years had higher TMB values; but tumors with lymph node invasion and vascular invasion had a lower TMB values (P<0.001). Compared with the low-TMB group, CRC patients in the high-TMB group had shorter 5-year overall survival rate (P<0.05). A total of 116 DEGs involved in signaling pathway of drug metabolism and leukocyte transendothelial migration, and four special types of TICs, including $CD4^+$ T cells, follicular helper T cells, M0 and M1 macrophages, were identified. **Conclusion:** TMB is an important prognostic and immunotherapeutic index in CRC, which can interact with TICs to modulate the tumor microenvironment of CRC and therefore impact the prognosis of CRC and the efficacy of immunotherapy.

Keywords tumor mutational burden; tumor-infiltrating immune cells; tumor microenvironment; colorectal cancer

肿瘤突变负荷(tumor mutational burden，TMB)定义为肿瘤中每1 000 000个体细胞碱基突变的总数，一般认为TMB可刺激产生肿瘤特异性及高免疫原抗体[1]。研究[2]表明：在多种肿瘤中，高TMB提示患者的预后较差，但其在结直肠癌(colorectal cancer，CRC)中的预后作用目前仍不明确。研究[3-4]证实，在肿瘤中TMB值与细胞程序性死亡配体1(programmed death ligand 1，PD-L1)表达无相关性，却与检查点抑制剂免疫治疗的疗效显著相关。因此，2020年美国食品和药物管理局(Food and Drug Administration，FDA)批准pembrolizumab用于高TMB的实体瘤患者[5]。

CRC的肿瘤微环境(tumor microenvironment，TME)在近年来引起了广泛关注。TME包含多种不同类型的细胞，如肿瘤细胞、免疫细胞等，他们可以通过细胞间接触、细胞因子、趋化因子等相互影响，并受遗传、表观遗传及环境等多种因素的调控[6]。研究表明：肿瘤浸润性免疫细胞(tumor-infiltrating immune cells，TICs)与CRC的预后相关[7]；在原发性CRC中，调节性T细胞及自然杀伤(natural killer，NK)细胞增加提示肿瘤预后较好[8]；某些TICs还与CRC肿瘤免疫治疗的疗效相关[9]，如细胞毒性T细胞及辅助性T细胞1在免疫检查点抑制剂的治疗中起关键作用[10]，肠道的NK细胞减少可导致PD-L1表达上调[11]。但是，目前对TMB和TICs之间的关系及其作用机制仍不明确。

通过分析癌症基因组(The Cancer Genome Atlas，TCGA)数据库中的相关数据发现：年长的CRC患者的TMB值较高，发生血管及淋巴结转移的肿瘤TMB值较低。并且高TMB肿瘤的预后较差。通过筛选差异表达基因(differentially expressed genes，DEGs)及TICs发现这部分患者的特殊信号通路异常，提示TMB可能通过干扰浸润性免疫细胞的细胞间通讯，改变免疫耐受和免疫活性之间的平衡，影响CRC的TME，进而影响患者的生存及免疫治疗反应。

1 资料与方法

1.1 CRC 数据下载

所有CRC数据下载于TCGA数据库中的TCGA内部项目，包括基因突变、转录组数据及相关的临床资料(https://portal.gdc.cancer.gov/)。共获得551个肿瘤样本(重复样本81例)及48个正常对照样本的相关数据。

1.2 TMB 及相关 DEGs 的确定

TMB的定义为基因组中每1 000 000个体细胞碱基突变的总数，包括碱基替换、插入和缺失突变[12]。采用软件R (Vienna，New Zealand)中的“maftools”包分析肿瘤样本中的基因组突变，包含非同义和移码突变，检测下限为>5%。根据TMB中位值(重复样本求平均值)将肿瘤样本分成高TMB组(n=224)和低TMB组(n=246)。

采用软件R中的“limma”包处理RNA表达数据，通过“edgeR”包筛选出2个TMB组间的DEGs{log错误发现率(false discovery rate，FDR) <0.05及log|倍数变化(fold change，FC)| >1}。

1.3 TMB 的临床特征相关性分析

使用软件R中的Wilcoxon秩和检验分析不同TMB值与临床病理参数之间的相关性。Kaplan-Meier生存曲线及log-rank检验用于比较不同TMB组间的5年总生存率。

1.4 TMB 相关的 TICs 鉴别

采用软件R中的“CIBERSORT”包评估肿瘤样本的22种免疫细胞的比例，包括7种T细胞、3种B细胞、NK细胞及髓样细胞(https://cibersort.stanford.edu/)，保存结果并进行后续分析[13]。

采用软件R中的Wilcoxon秩和检验鉴别不同TMB组间的差异表达TICs (P<0.05)，并分析TICs百分比与TMB值之间的相关性。

1.5 GO、KEGG 及 GSEA

采用软件R的clusterProfiler、enrichplot及ggplot2包进行DEGs的基因本体论(Gene Ontology，GO)及京都基因与基因组百科全书(Kyoto Encyclopedia of Genes and Genomes，KEGG)富集分析。P值及q值定义为<0.05，FDR定义为<0.01。从Molecular Signature数据库下载C7基因集(version 6)，从Broad Institution (http://www.gsea-msigdb.org/)下载基因集富集分析(Gene Set Enrichment Analysis，GSEA)软件(4.1.0版本)。对所有肿瘤样本的转录组进行GSEA分析，P值及FDR均<0.05。

1.6 统计学处理

数据分析采用R软件4.0.3。均值比较采用t检验，其他比较采用Wilcoxon秩和检验。采用Kaplan-Meier生存曲线及log-rank检验进行生存分析。P<0.05为差异有统计学意义。

2 结果

2.1 CRC 中的基因突变

图1示CRC基因突变图谱。瀑布图为肿瘤样本中基因突变的情况(图1A)，突变率排名前10的基因分别为WNT信号通路的APC调节剂(APC regulator of WNT signaling pathway，*APC*) (77%)、p53肿瘤蛋白(tumor protein p53，*TP53*) (59%)、肌联蛋白(titin，*TTN*) (52%)、KRAS原癌基因(KRAS proto-oncogene，*KRAS*) (40%)、含有核膜蛋白1的血影蛋白重复序列(spectrin repeat containing nuclear envelope protein 1，*SYNE1*) (29%)、黏蛋白16(mucin 16，*MUC16*) (28%)、磷脂酰肌醇4,5二磷酸3激酶催化亚单位(phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha，*PIK3CA*) (25%)、脂肪非典型钙黏蛋白1(FAT atypical cadherin 4，*FAT4*) (24%)、兰尼碱受体2(ryanodine receptor 2，*RYR2*) (22%)及*OBSCN*(obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF) (20%)。最常见的突变类型是错译突变，最常见的单核苷酸变异方式为C>T(图1B)。突变率排名前20的基因之间的相关性见图1C。

2.2 TMB 与临床参数的相关性

图2示TMB值与临床参数之间的相关性。65岁以上CRC患者的TMB值更高，发生血管及淋巴结转移的肿瘤TMB值更低(P<0.001；图2A、2D、2E)。TMB值在不同CRC肿瘤分期中的差异也具有统计学意义(P<0.001，图2F)。但其在性别、肿瘤大小方面的差异无统计学意义(P>0.05，图2B、2C)。Kaplan-Meier生存曲线显示：高TMB的CRC患者5年总生存率更低(P<0.05，图2G)。

2.3 TMB DEGs 及其功能分析

不同TMB组之间筛选出116个DEGs。在高TMB组中，共有79个上调基因和37个下调基因。热图按差异程度列出40个DEGs(图3A)。GO分析表明：这些基因富集于上皮细胞内，涉及细胞分化、角质化及代谢过程的酶的调节；KEGG分析显示：这些基因富集在药物代谢及白细胞跨内皮迁移信号通路上(图3B)。GSEA分析显示：在高TMB组中，$CD4^+$和$CD8^+$ T细胞数目显著降低；而在低TMB组中，NK和$CD8^+$ T细胞数目显著升高(P<0.05，图3C)。

2.4 TICs 与临床参数的相关性

图4示CRC的TICs图谱。柱状图显示22种TICs在肿瘤样本中所占的百分比。根据不同的TICs中位值分组，22种TICs的高低两组的5年总生存率差异无统计学意义(P>0.05)，这与肿瘤免疫在线研究数据库肿瘤免疫评估资源(Tumor Immune Estimation Resource，TIMER)的分析结果相一致(https://cistrome.shinyapps.io/timer/)。

2.5 TMB 与 TICs 的关联性分析

图5示TMB与TICs的关联性分析。小提琴图显示2个TMB组之间22种TICs的百分比(图5A)。高TMB组中，活化的记忆性$CD4^+$ T细胞、滤泡辅助性T细胞和M1巨噬细胞所占百分比较高，而M0巨

噬细胞所占百分比较低(P<0.05)。散点图显示TMB值与TICs百分比的相关性(图5B)；TMB值与调节性T细胞、$CD8^+$ T细胞、活化的记忆性$CD4^+$ T细胞、滤泡辅助T细胞、M1巨噬细胞和活化NK细胞的百分比呈正相关；而与M0巨噬细胞的百分比呈负相关(P<0.05)。

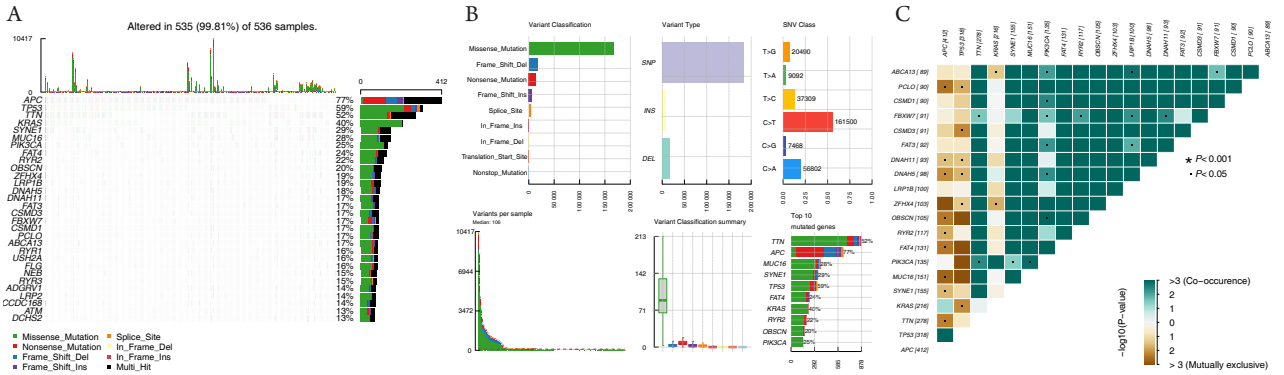

图1 CRC全基因组突变谱分析

Figure 1 Genome-wide mutation profiling in colorectal cancer

(A)CRC突变概况；(B)突变汇总、变异类型、变异方式、核苷酸转换类型、单样本突变总数、突变率前10基因；(C) 突变率前20基因之间的相关性。

(A) Landscape of mutation profiles in colorectal cancer samples; (B) Summary of variants, variant classification, type and single nucleotide variant class, total number of mutations in a single sample, top 10 mutation rate genes; (C) Association between top 20 mutation rate genes.

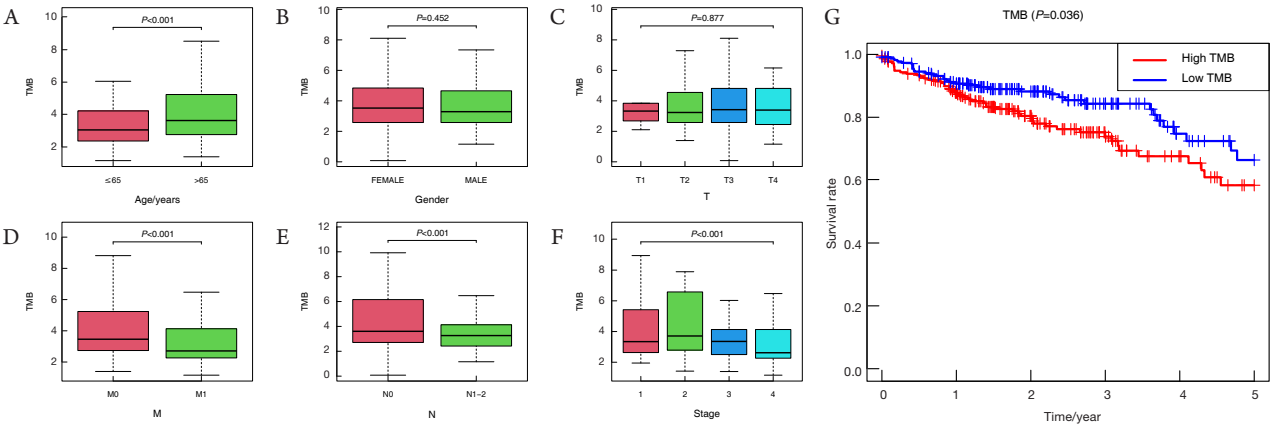

图2 TMB与临床参数之间的相关性

Figure 2 Correlation between TMB and clinical parameters

(A) 65岁以上CRC患者的TMB值更高(P<0.001)；(B、C)在性别、肿瘤大小方面TMB值的差异没有统计学意义(P>0.05)；(D、E)血管及淋巴结转移的肿瘤TMB值更低(P<0.001)；(F) TMB值在不同CRC肿瘤分期中的差异具有统计学意义(P<0.001)；(G) Kaplan-Meier生存曲线显示TMB高的CRC患者5年总生存率更低(P<0.05)。

(A) CRC patients >65 years old had higher TMB values (P<0.001); (B, C) There was no significant difference in TMB value in gender and tumor size (P>0.05); (D, E) Tumors with vascular and lymph node invasion had lower TMB values (P<0.001); (F) TMB values between different tumor stages have statistically significant difference (P<0.001); (G) Kaplan-Meier survival curve analysis showed that CRC patients with higher TMB values had a lower 5-year overall survival rate (P<0.05).

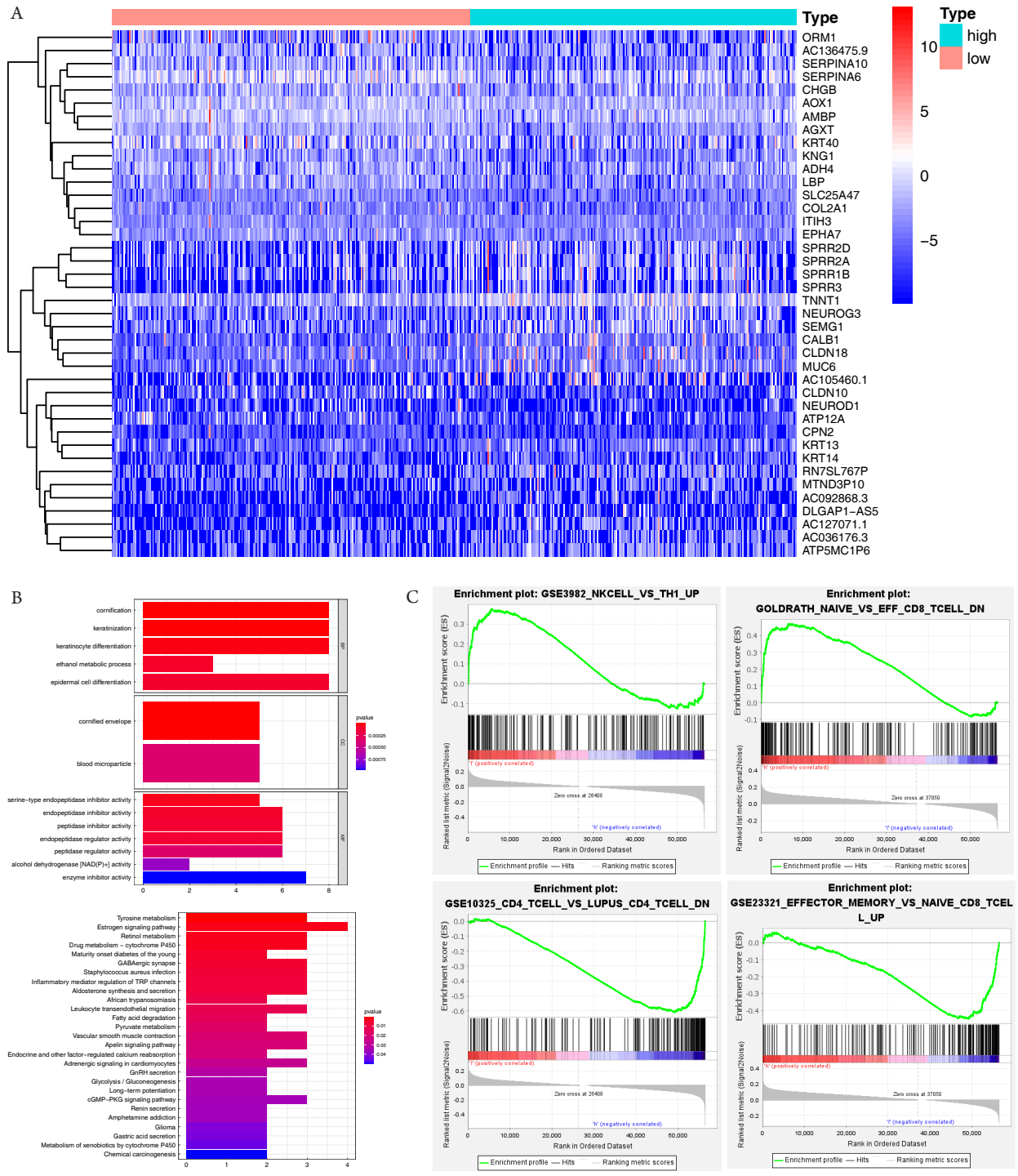

图3 TMB相关的DEGs及其功能性分析

Figure 3 TMB-associated DEGs and functional analyses

(A)热图显示排名前40的DEGs；(B) GO分析显示这些基因富集在上皮细胞，主要参与细胞分化、角质化以及对代谢过程的酶的抑制及活化调节，KEGG分析显示这些基因集中在药物代谢及白细胞内皮迁移信号通路；(C) GSEA分析显示高TMB组中$CD4^+$、$CD8^+$ T细胞数目显著降低，而在低TMB组中，NK、$CD8^+$ T细胞数目显著升高(P<0.05)。

(A) The top 40 DEGs in heat map; (B) GO analysis identified that these DEGs showed enrichment in epidermal cells and are mainly involved in cell differentiation, keratinization, and the inhibition and activation of enzymes in metabolism; KEGG analysis identified enrichment in pathways of drug metabolism and leukocyte trans-endothelial migration; (C) According to GSEA, the numbers of $CD4^+$ and $CD8^+$ were significantly decreased in the high-TMB group, while the numbers of NK and $CD8^+$ T cells were significantly increased in the low-TMB group (P<0.05).

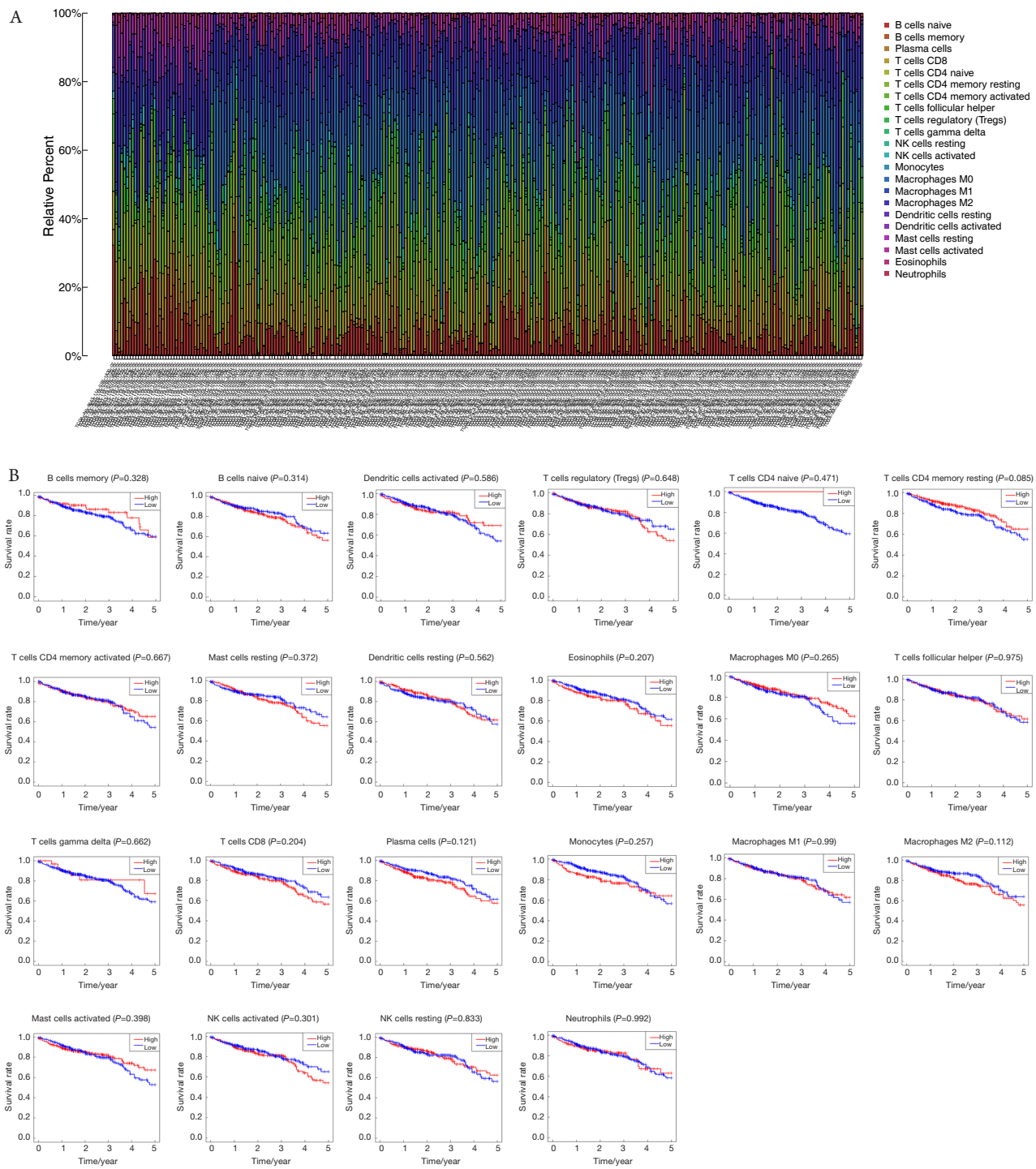

图4 CRC中的TICs概况

Figure 4 The profile of TICs in CRC

(A) 柱状图显示22种TICs在肿瘤样本中的百分比；(B)根据不同TICs的中位值分组，22种TICs的高低两组的5年总生存率差异无统计学意义(P>0.05)。

(A) Bar-plot shows the proportions of the 22 types of TICs; (B) There was no statistically significant difference in 5-year OS rates between high and low TIC median values in any of the 22 TICs (P>0.05).

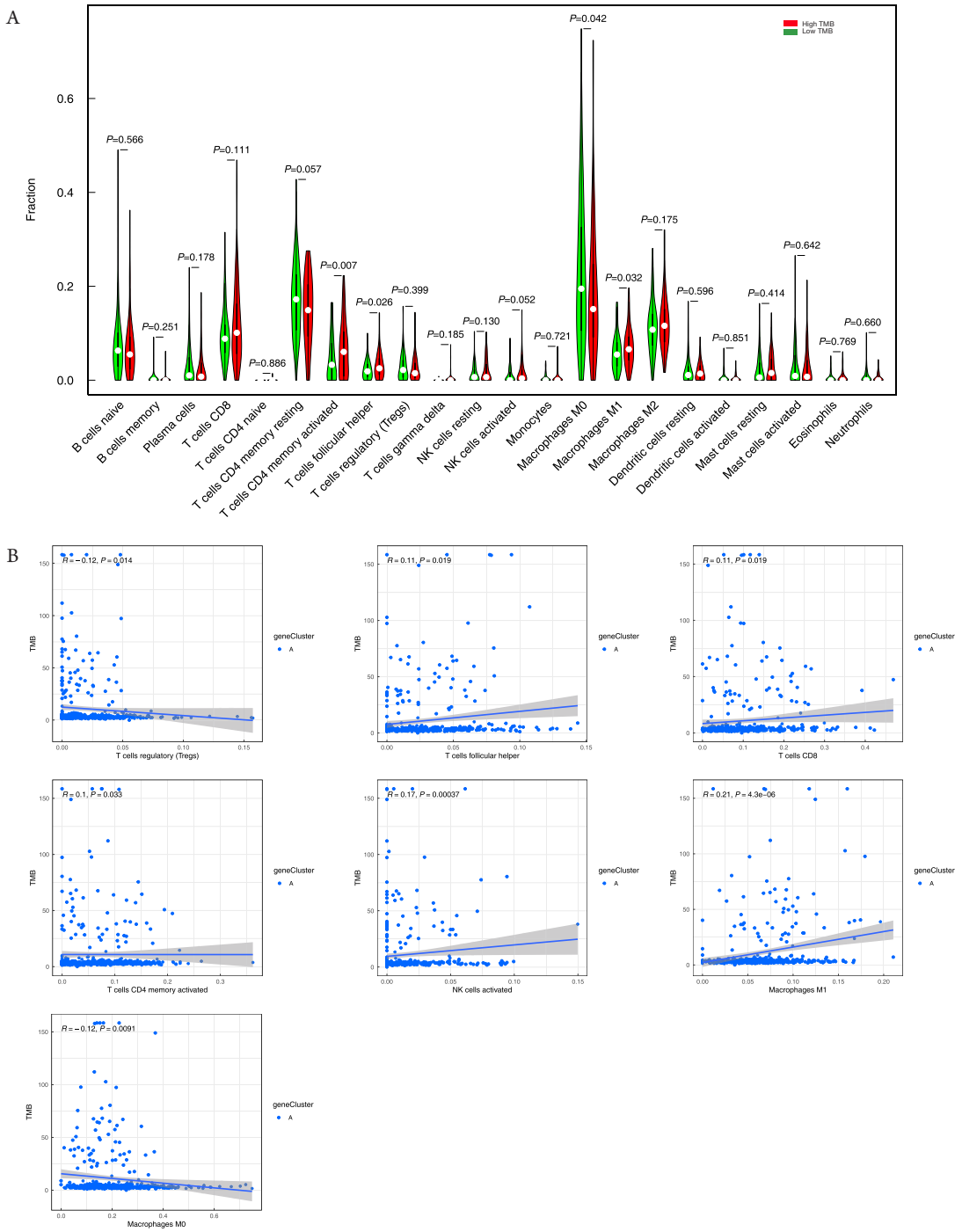

图5 TMB与TICs的关联性分析

Figure 5 Association analysis of TICs with TICs

(A)小提琴图显示2个TMB组之间TICs的百分比。高TMB组中，活化的记忆性$CD4^+$ T细胞、滤泡辅助性T细胞、M1巨噬细胞比例较高，而M0巨噬细胞比例较低(P<0.05)；(B)散点图显示TMB值与TICs百分比的相关性。TMB值与调节性T细胞、$CD8^+$T细胞、活化记忆$CD4^+$T细胞、滤泡辅助T细胞、M1巨噬细胞和活化NK细胞的百分比呈正相关，而与M0巨噬细胞百分比呈负相关(P<0.05)。

(A) Violin figure shows the distribution of TICs between the 2 TMB groups. Activated memory $CD4^+$ T cells, follicle-assisted T cells, and M1 macrophages showed higher levels of infiltration in the high-TMB group, while M0 macrophages showed lower levels (P<0.05); (B) Scatter diagrams further showed the association of TMB values and percentages of TICs. Regulatory T cells, $CD8^+$ T cells, activated memory $CD4^+$ T cells, follicle-assisted T cells, and M1 macrophages were positively correlated, while M0 macrophages were negatively correlated with TMB (P<0.05).

3 讨论

目前，免疫治疗已经广泛用于多种恶性肿瘤的治疗，但仅有部分患者产生应答并从中获益[5]。因此，寻找潜在标志物已成为免疫疗法的关键。有研究[4]发现：体细胞突变总指数TMB可作为CRC患者免疫检查点抑制剂治疗效果的评价指标。

本研究首先分析了CRC患者TMB值与临床参数之间的相关性，结果显示高TMB组CRC患者预后较差。近期针对微卫星稳定的CRC的研究[14]指出：高TMB患者具有更长的中位生存时间。目前，对TMB的定义缺乏相应的共识，比如检测方式、突变的范围、阈值的判定等[15]，这些都可能造成相冲突的实验结果。此外，TMB的预后价值还可能取决于肿瘤个体及TME之间的相互作用[16]。

在此基础上，本研究继续分析了CRC中TMB相关的基因表达谱，并筛选出DEGs。GO分析显示：DEGs富集于上皮细胞，涉及细胞分化、角质化及代谢过程的酶的调节。KEGG进一步分析显示：DEGs富集在药物代谢及白细胞跨内皮迁移信号通路。而GSEA富集分析发现：在高TMB组中，$CD4^+$、$CD8^+$ T细胞数量显著降低；在低TMB组中，NK、$CD8^+$ T细胞数量显著升高。$CD4^+$及$CD8^+$ T细胞是淋巴细胞中最具有杀伤活性的亚群。有研究[17]证实：预后较好的CRC患者肿瘤样本中常伴随$CD4^+$和$CD8^+$ T细胞浸润，这些细胞可以增强对肿瘤细胞的杀伤性。此外，$CD4^+$和$CD8^+$ T细胞还可以产生一种协同免疫检查点抑制剂作用的蛋白质干扰素γ(interferon γ，IFN-γ)[18]。而NK细胞也具有杀伤功能，在细胞程序性死亡蛋白1(programmed cell death protein 1，PD-1)表达上调的CRC肿瘤中易出现NK细胞衰竭[11]。因此推测高TMB的CRC肿瘤可以通过TICs诱导免疫反应，从而影响肿瘤的进展及治疗反应。

此外，本研究还分析了CRC中22种TICs。TICs作为TME的重要组成部分，参与调节肿瘤的发生及发展[19]。研究[9]表明TICs是CRC的潜在预后及免疫治疗的重要标志物。但本研究并未发现这22种TICs的百分比与CRC患者的预后具有相关性，该结果与肿瘤免疫在线研究数据库TIMER的分析结果相一致。但在不同的TMB组，某些TICs的百分比表现出差异表达，且还与TMB值具有线性关系。其中包括巨噬细胞M0和M1。在多种免疫相关的疾病中，巨噬细胞M0和M1的比例失衡[20]。随着肿瘤的进展，巨噬细胞浸润程度增加，其中涉及多种生长因子，如免疫抑制分子相关的蛋白水解酶及运动相关蛋白[21-22]。滤泡辅助性T细胞作为$CD4^+$ T细胞的特殊亚型，在促进B细胞分化及诱导免疫反应中发挥重要的作用[23]。这些差异表达的TICs的特殊性均提示高TMB的肿瘤可以通过直接或者间接的方式调节免疫细胞的浸润程度来影响TME的免疫特性[24-25]。

综上所述，TMB与CRC的临床指标息息相关，是一个重要的预后标志物。同时TMB和TICs之间相互作用导致TME改变，进而影响肿瘤进展，干预免疫治疗的效果。然而，目前临床中TMB的使用仍然存在一些限制，TMB的阈值及检测方法、检测范围[26]等均有待在今后的临床研究中一一解决。

参考文献

1. Steuer CE, Ramalingam SS. Tumor mutation burden: Leading immunotherapy to the era of precision medicine[J]? J Clin Oncol, 2018, 36(7): 631-632.
2. Cao D, Xu H, Xu X, et al. High tumor mutation burden predicts better efficacy of immunotherapy: A pooled analysis of 103078 cancer patients[J]. Oncoimmunology, 2019, 8(9): e1629258.
3. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy[J]. Science, 2018, 362(6411): eaar3593.
4. Schrock AB, Ouyang C, Sandhu J, et al. Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer[J]. Ann Oncol, 2019, 30(7): 1096-1103.
5. Subbiah V, Solit DB, Chan TA, et al. The FDA approval of pembrolizumab for adult and pediatric patients with tumor mutational burden (TMB) ≥10: A decision centered on empowering patients and their physicians[J]. Ann Oncol, 2020, 31(9): 1115-1118.
6. Li J, Byrne KT, Yan F, et al. Tumor cell-intrinsic factors underlie heterogeneity of immune cell infiltration and response to immunotherapy[J]. Immunity, 2018, 49(1): 178-193.e7.
7. Grossman JG, Nywening TM, Belt BA, et al. Recruitment of CCR2+ tumor associated macrophage to sites of liver metastasis confers a poor prognosis in human colorectal cancer[J]. Oncoimmunology, 2018, 7(9): e1470729.
8. Donadon M, Hudspeth K, Cimino M, et al. Increased infiltration of natural killer and t cells in colorectal liver metastases improves patient overall survival[J]. J Gastrointest Surg, 2017, 21(8): 1226-1236.
9. Guo L, Wang C, Qiu X, et al. Colorectal cancer immune infiltrates: Significance in patient prognosis and immunotherapeutic efficacy[J]. Front Immunol, 2020, 11: 1052.

10. Xu X, Xu J, Wu J, et al. Phosphorylation-mediated IFN-γR2 membrane translocation is required to activate macrophage innate response[J]. Cell, 2018, 175(5): 1336-1351.e17.
11. Seo H, Jeon I, Kim BS, et al. IL-21-mediated reversal of NK cell exhaustion facilitates anti-tumour immunity in MHC class I-deficient tumours[J]. Nat Commun, 2017, 8: 15776.
12. Jiang T, Shi J, Dong Z, et al. Genomic landscape and its correlations with tumor mutational burden, PD-L1 expression, and immune cells infiltration in Chinese lung squamous cell carcinoma[J]. J Hematol Oncol, 2019, 12(1): 75.
13. Chen B, Khodadoust MS, Liu CL, et al. Profiling tumor infiltrating immune cells with CIBERSORT[J]. Methods Mol Biol, 2018, 1711: 243-259.
14. Romero D. TMB is linked with prognosis[J]. Nat Rev Clin Oncol, 2019, 16(6): 336.
15. Samstein RM, Lee CH, Shoushtari AN, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types[J]. Nat Genet, 2019, 51(2): 202-206.
16. Wu HX, Wang ZX, Zhao Q, et al. Tumor mutational and indel burden: A systematic pan-cancer evaluation as prognostic biomarkers[J]. Ann Transl Med, 2019, 7(22): 640.
17. Narayanan S, Kawaguchi T, Yan L, et al. Cytolytic activity score to assess anticancer immunity in colorectal cancer[J]. Ann Surg Oncol, 2018, 25(8): 2323-2331.
18. Mucida D, Husain MM, Muroi S, et al. Transcriptional reprogramming of mature $CD4^+$ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes[J]. Nat Immunol, 2013, 14(3): 281-289.
19. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle[J]. Immunity, 2013, 39(1): 1-10.
20. Funes SC, Rios M, Escobar-Vera J, et al. Implications of macrophage polarization in autoimmunity[J]. Immunology, 2018, 154(2): 186-195.
21. Prenen H, Mazzone M. Tumor-associated macrophages: A short compendium[J]. Cell Mol Life Sci, 2019, 76(8): 1447-1458.
22. Zhu C, Kros JM, Cheng C, et al. The contribution of tumor-associated macrophages in glioma neo-angiogenesis and implications for anti-angiogenic strategies[J]. Neuro Oncol, 2017, 19(11): 1435-1446.
23. Qi H. T follicular helper cells in space-time[J]. Nat Rev Immunol, 2016, 16(10): 612-625.
24. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point[J]. Nature, 2017, 541(7637): 321-330.
25. Kim SI, Cassella CR, Byrne KT. Tumor burden and immunotherapy: Impact on immune infiltration and therapeutic outcomes[J]. Front Immunol, 2020, 11: 629722.
26. McNamara MG, Jacobs T, Lamarca A, et al. Impact of high tumor mutational burden in solid tumors and challenges for biomarker application[J]. Cancer Treat Rev, 2020, 89: 102084.

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