左右半结肠癌多组学分子特征差异的研究进展

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摘要 结肠癌(colon cancer, CC)是一种常见的恶性肿瘤, 其发病率和死亡率均占癌症前列。根据解剖学位置, CC可分为左半结肠癌(left-sided colon cancer, LCC)和右半结肠癌(right-sided colon cancer, RCC), 两者在临床特征上表现出较大的差异。近些年来, 随着生物学技术和测序技术的发展, 从多组学角度分析LCC和RCC分子特征和微环境差异的研究也越来越多, 从而来揭示患者预后并指导其治疗。该文从基因突变、基因表达、miRNA表达、DNA甲基化、免疫微环境、共识分子亚型以及免疫治疗这几个方面来阐述LCC和RCC在分子特征和治疗差异上的研究进展。

关键词 左半结肠癌; 右半结肠癌; 多组学; 分子特征; 免疫微环境

Recent Progress of Differences between Left-Sided and Right-Sided Colon Cancer on Multi-Omics Molecular Characteristics

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Abstract CC (colon cancer) is a type of the common malignant tumor with high morbidity and mortality. Based on the anatomical location of primary cancer site, CC can be divided into LCC (left-sided colon cancer) and RCC (right-sided colon cancer). There is a large heterogenicity between the patients with LCC and RCC in clinical characteristics. Due to the development of biological technology and sequencing technology, more and more studies have been conducted to identify the differences of molecular characteristics and microenvironment between LCC and RCC through multi-omics approach during the last decade. And these findings are used to guide the treatment and prognosis of CC patients. This review summarizes the recent progress of distinct molecular characteristics and treatment between LCC and RCC patients from the views of gene mutation, gene expression, miRNA expression, DNA methylation, immune microenvironment, immunotherapy and consensus molecular subtypes.

Keywords left-sided colon cancer, right-sided colon cancer; multi-omics; molecular characteristics; immune microenvironment

收稿时间: 2020-08-30 接受日期: 2020-10-14

浙江省自然科学基金(批准号: LY21C060002)、国家自然科学基金(批准号: 31970630)、浙江省属高校基本科研业务费(批准号: SJLZ2021001)、宁波市自然科学基金(批准号: 2017A610154、2019A610253)、宁波大学研究生科研创新基金(批准号: IF2020163)和宁波大学王宽诚教育基金资助的课题

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Received: August 30, 2020 Accepted: October 14, 2020

This work was supported by the Natural Science Foundation of Zhejiang Province (Grant No.LY21C060002), the National Natural Science Foundation of China (Grant No.31970630), the Fundamental Research Funds for the Provincial Universities of Zhejiang (Grant No.SJLZ2021001), the Natural Science Foundation of Ningbo (Grant No.2017A610154, 2019A610253), the Graduate Research Innovation Fund of Ningbo University (Grant No.IF2020163) and the K.C. Wong Magna Fund in Ningbo University

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URL: http://www.cjcb.org/arts.asp?id=5445

结肠癌(colon cancer, CC)是一种多阶段、由遗传和表观遗传等多层面改变不断累积而引起的恶性肿瘤。据GLOBOCAN的最新报告, 2018年全球有CC病例180万, 死亡病例86万, 位居常见癌症第三位, 癌症死因第二位[1]。事实上, CC并不是一种单一的癌症, 尤其是发生在左右半的CC具有很大的异质性, 可以说它们是两种不同类型的癌症。根据解剖学位置, 发生在远端三分之一的横结肠、脾曲、降结肠和乙状结肠的肠癌被定义为左半结肠癌(left-sided colon cancer, LCC), 而发生在阑尾、盲肠、升结肠、肝曲和近端三分之二的横结肠的肠癌则被定义为右半结肠癌(right-sided colon cancer, RCC)[2]。

LCC和RCC患者的临床表型存在较大差异。如HELVACI等[3]研究显示, RCC患者明显多于LCC(83.2% *vs* 16.8%), 且RCC患者中女性略多于男性, 而在LCC中则相反[4], RCC患者的平均年龄也略高于LCC患者[(61.90±13.79)岁*vs* (60.39±13.23)岁, P=0.035)][5]。此外, RCC患者的中位生存期低于LCC患者(18个月*vs* 23个月, P=0.011), 预后较差。在组织学方面, LCC组织常常表现出具有息肉形态的管状、绒毛状或传统的锯齿状腺瘤(traditional serrated adenoma, TSA)[6], 并在早期阶段是可以通过结肠镜检查被发现的。而RCC组织则多为无蒂锯齿状腺瘤/息肉(sessile serrated adenoma/polyp, SSA/P)或黏液性腺癌[7], 这种肿瘤不易被检测到, 往往到晚期时才会被发现, 这也是RCC预后较差的原因之一。除了临床表型之外, 左右半结肠癌之间的肿瘤微环境也存在较大差异。已有的研究结果显示, LCC的肿瘤纯度高于RCC[8], 然而RCC的免疫浸润程度却高于LCC[9], 如$CD8^+$ T淋巴细胞等。左右半结肠癌之间微环境的异质性也会导致这两种癌症预后和治疗反应的差异[10]。

LCC和RCC不同临床表现的最根本原因是它们来自不同的解剖学位置和胚胎学起源。近年来, 随着生物学技术和测序技术的发展, 从多组学角度分析左右半结肠癌差异的研究也越来越多, 这更好地阐明了LCC和RCC发生发展的不同分子机制, 并为其辅助治疗和免疫疗法提供了有力的依据。本文主要从基因突变、基因表达、miRNA(microRNA)表达、DNA甲基化、免疫微环境、免疫治疗以及基于多组学特征构建的共识分子亚型这几个角度来阐明LCC和RCC分子差异及临床治疗的研究进展。

1 基因突变

致癌基因和抑癌基因的突变在肿瘤发展机制中扮演着重要的角色, 这两者在左右半结肠癌之间存在异质性, 并导致了肿瘤遗传不稳定性、生存预后、治疗和肿瘤微环境的差异。染色体不稳定性(chromosome instability, CIN)和微卫星不稳定性(microsatellite instability, MSI)是两种主要的遗传不稳定性亚型。

CIN是非整倍体肿瘤的主要特征, 由有丝分裂期间染色体分离错误导致[11]。大部分(75%) LCC患者具有CIN特征, 而RCC患者中CIN只占30%左右[12]。CIN亚型患者的第一个基因突变往往发生在腺瘤样结肠息肉基因(adenomatous polyposis coli, *APC*)上, APC是Wnt途径的重要负调节因子, 是β-catenin降解体复合物的组成部分, 可促进Wnt效应物β-catenin的蛋白酶体降解。APC一旦发生突变失活, 将导致β-catenin降解体复合物存在缺陷, 则过量的β-catenin会积聚在细胞质中并转移到细胞核中以激活转录因子TCF/LEF(T-cell factor/lymphoid enhancing factor), 从而引起*C-myc*等大量癌基因的激活, 致使结肠黏膜发展成异常隐窝灶, 这是CIN发生的早期事件。当CIN患者进展到较大的腺瘤和早期癌时需要激活*KRAS*、*TP53*的突变和18q染色体杂合性缺失(loss of heterozygosity, LOH)。而在小部分CIN患者中, III类磷酸肌醇-3-激酶复合物A(phosphoinositide-3-kinase class A, *PIK3CA*)的突变激活则发生在肿瘤晚期, 致使腺瘤逐渐转变成浸润癌[13]。通过对TCGA左右半CC突变谱的分析, 我们发现, 除了*APC*和*TP53*, RCC中CIN亚型关键基因的突变率均高于LCC(表1)。据研究报道, *APC*和肿瘤蛋白p53(tumor protein p53, *TP53*)的突变足以产生明显的CIN特征, 其他突变基因的存在仅对CIN产生微小的贡献[13], 这也许是LCC比RCC具有较多CIN亚型患者的主要原因。

基因突变引发的另一种CC亚型, MSI, 是由错配修复缺陷(defective mismatch repair, dMMR)引起的高突变表型, 即肿瘤突变负担(tumor mutation burden, TMB)>12个突变/Mb, 占所有CC病例的15%~20%, 常见于RCC[14], 因为RCC中的TMB比LCC高(28.7% *vs* 5.3%, 表1)。MSI型CC根据其是否具有遗传性可分为遗传性非息肉性结肠癌(hereditary non-polyposis colon cancer, HNPCC)和散发性MSI[15]。HNPCC, 即LS(lynch syndrome), 约70%的

表1 CIN、MSI和超突变亚型关键基因的突变率

Table 1 Mutation rate of key genes of CIN, MSI and ultra-mutation subtypes

亚型 Subtypes	基因 Genes	染色体位置 Chromosomal location	基因产物的功能 Function of gene product	类型 Type	LCC	RCC
CIN	/	/	/	/	75%	30%
	APC	5q21	Inhibition of Wnt signaling	Anti-oncogene	84.0%	70.1%
	TP53	17p13	Cell cycle arrest, apoptosis	Anti-oncogene	70.2%	43.1%
	SMAD2	18q21	Intracellular signal transmitter of TGF-β pathway	Anti-oncogene	3.1%	4.6%
	SMAD4	18q21	Intracellular signal transmitter of TGF-β pathway	Anti-oncogene	8.4%	14.4%
	DCC	18q21	Netrin 1 receptor	Anti-oncogene	6.1%	10.3%
	PIK3CA	3q26	Cell proliferation and survival	Oncogene	14.5%	35.9%
	KRAS	12p12	Cell proliferation and survival	Oncogene	32.1%	48.2%
	NRAS	1p13	Cell proliferation and survival	Oncogene	4.6%	5.1%
MSI	/	/	/	/	2.4%	28.5%
	TMB	/	Hyper- mutation (>12/Mb)	/	5.3%	28.7%
	MLH1	3p22	DNA mismatch repair (MMR)	Anti-oncogene	2.3%	4.6%
	MSH2	2p21-p16	DNA mismatch repair (MMR)	Anti-oncogene	1.5%	5.6%
	MSH6	2p16	DNA mismatch repair (MMR)	Anti-oncogene	1.5%	7.7%
	PMS2	7p22	DNA mismatch repair (MMR)	Anti-oncogene	0.8%	5.1%
	BRAF	7q34	Cell proliferation and survival	Oncogene	2.3%	23.1%
Ultra-mutation	*POLE*	12q24	DNA repair and chromosomal, DNA replication	Anti-oncogene	4.6%	8.7%
	POLD1	19q13	DNA repair and chromosomal, DNA replication	Anti-oncogene	0	10.8%

LCC: 左侧结肠癌; RCC: 右侧结肠癌; MSI: 微卫星不稳定; CIN: 染色体不稳定; TMB: 肿瘤突变负担。/: 非典型基因。TCGA中发生单核苷酸变异(SNV)的患者占总体样本量的比值。

LCC: left-sided colon cancer; RCC: right-sided colon cancer; MSI: microsatellite instability; CIN: chromosome instability; TMB: tumor mutation burden. /: atypical gene. The ratio of samples with SNV (single nucleotide variation) to the general population in the TCGA.

LS见于RCC[16], 由突变的错配修复(mismatch repair, MMR)基 因(*MLH1*、*MSH2*、*MSH6*、*PMS2*等)的显性遗传引起[17], 并且永远不会与*BRAF*突变共存[18]。散发性MSI肿瘤也常见于RCC, 由*MLH1*的两个等位基因启动子高甲基化导致, 且80%~90%的散发性高突变癌症具有*BRAF* V600E突变, 其与*MLH1*启动子甲基化也存在很强的相关。因此, *BRAF* V600E突变或*MLH1*甲基化的存在有助于将散发性MSI与LS患者区分开。在RCC中, MSI亚型关键基因的突变率和肿瘤突变负担均高于LCC, 尤其是*BRAF*基因的突变(LCC *vs* RCC: 2.3% *vs* 23.1%, 表1), 这可能是RCC更容易发生MSI亚型的主要原因。

*RAS/BRAF*基因是RAS/RAF/MEK/ERK信号通路的关键组成部分, 往往参与肿瘤的发生发展作用。*KRAS*、*NRAS*和*HRAS*分别是RAS基因家族的三个成员, 该家族成员的第12、13或61密码子突变可将这些基因转化为致癌基因, 其中以*KRAS*突变(*KRAS*^{MUT})最为常见[19]。与*KRAS*野生型(*KRAS*^{WT})相比, *KRAS*第12密码子突变患者的死亡率更高[20], 而13密码子突变则与III期CC患者的预后不良相关[21]。*BRAF*野生型(*BRAF*^{WT})患者的中位总生存期(overall survival, OS)也明显优于*BRAF*突变型(*BRAF*^{MUT}, 60个月*vs* 18个月)[22]。*RAS/BARF*^{MUT}的患者对抗表皮生长因子受体(epidermal growth factor receptor, EGFR)治疗具有耐受性[23]。其中, *KRAS*^{MUT}是食品药品监督管理局(food and drug administration, FDA)批准用于预测耐受抗EGFR治疗药物(cetuximab和panitumumab)的生物靶标, 而*KRAS*^{WT}患者是FDA批准的抗EGFR治疗的对象(表2)。针对*BRAF*^{MUT}, FDA批准了结肠癌三联疗法, 即encorafenib+cetuximab+binimetinib或dabrafenib+panitumumab+trametinib(表2)。在KOPETZ等[24]对*BRAF*^{MUT}患者的研究中, 三联疗法组的中位OS为9.0个月, 对照组(抗EGFR治疗)为5.4个月(P<0.001)。

PI3K/AKT/mTOR信号通路的激活通常由

表2 FDA批准的药物(1级或者2级)和生物学证据支持的药物(4级)

Table 2 FDA-approved drugs (level 1 or 2) and biological evidence supports drugs (level 4）

等级 Level	基因改变 Genes-alterations	药物 Drugs	肿瘤类型 Tumor type	LCC	RCC
1	*KRAS*-wildtype[1]	Cetuximab, panitumumab, regorafenib	Colon cancer	67.9%	51.8%
1	MMR genes-dMMR	Pembrolizumab, nivolumab, nivolumab+ipilimumab	Colon cancer	3.1%	36.9%
2	*BRAF*-mutation[2]	Encorafenib+cetuximab+binimetinib, dabrafenib+panitumumab+trametinib	Colon cancer	2.3%	23.1%
R1	*KRAS*-mutation[2]	Cetuximab, panitumumab	Colon cancer	32.1%	48.2%
R1	*NRAS*-mutation[2]	Cetuximab, panitumumab	Colon cancer	4.6%	5.1%
4	*MTOR*-mutation[2]	Everolimus, temsirolimus	All solid tumors	6.1%	10.8%
4	*FGFR2*-mutation[2]	Erdafitinib, debio1347, BGJ398, AZD4547	All solid tumors	1.5%	3.1%
4	*CDKN2A*-mutation[2]	Ribociclib, palbociclib, abemaciclib	All solid tumors	0	1.5%
4	*NF1*-mutation[2]	Cobimetinib, trametinib	All solid tumors	0.8%	8.7%
4	*PTEN*-mutation[2]	GSK2636771, AZD8186	All solid tumors	3.8%	7.7%
4	*ATM*-mutation[2]	Olaparib	All solid tumors	9.9%	16.9%
4	*KRAS*-mutation[2]	Binimetinib, cobimetinib, trametinib	All solid tumors	32.1%	48.2%
4	*FGFR1*-mutation[2]	Debio1347, erdafitinib, BGJ398, AZD4547	All solid tumors	2.3%	3.1%
4	*FGFR3*-mutation[2]	BGJ398, AZD4547, erdafitinib, debio1347	All solid tumors	1.5%	5.6%
4	*CDK12*-mutation[2]	Nivolumab, cemiplimab, pembrolizumab	All solid tumors	3.8%	10.3%

1: 1级, 在此适应症中, 可预测对FDA批准药物反应的FDA认可的生物标志物; 2: 2级, 在此适应症中, 可预测对FDA批准药物反应的NCCN或其他专家小组推荐的标准治疗生物标志物; R1: R1级, 在此适应症中, 可预测对FDA批准药物耐药的标准治疗生物标志物; 4: 4级, 可预测对药物反应有力的生物学证据支持的生物标志物, 但生物标志物和药物均不是标准治疗。LCC: 左侧结肠癌; RCC: 右侧结肠癌; MSI: 微卫星不稳定; dMMR: 错配修复缺陷。[1]TCGA中未发生单核苷酸变异(SNV)的患者占总体样本量的比值; [2]TCGA中发生单核苷酸变异(SNV)的患者占总体样本量的比值。

Level 1: FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication. Level 2: standard care biomarker recommended by the NCCN or other expert panels predictive of response to an FDA-approved drug in this indication. Level R1: standard of care biomarker predictive of resistance to an FDA-approved drug in this indication. Level 4: compelling biological evidence supports the biomarker as being predictive of response to a drug, but neither biomarker nor drug is standard care. LCC: left-sided colon cancer; RCC: right-sided colon cancer; MSI: microsatellite instability; dMMR: deficiency mismatch-repair. [1]The ratio of samples without SNV (single nucleotide variation) to the general population in the TCGA; [2]The ratio of samples with SNV (single nucleotide variation) to the general population in the TCGA.

*PIK3CA*等基因的扩增突变引起, 该通路可促进肿瘤细胞的增殖、存活和生长, 并且与耐药性有关[25]。*PIK3CA*基因负责编码p110α蛋白, 该蛋白为PI3K酶的亚基[26]。OGINO等[27]研究发现, 在*KRAS*^{WT}的患者中, *PIK3CA*^{MUT}可导致不良的生存预后, ROSTY等[28]又进一步发现, 在*BRAF*^{WT}的患者中, *PIK3CA*^{MUT}也与CC患者死亡率增加有关。同时, *PIK3CA*^{MUT}也会对抗EGFR治疗产生耐受性。在抗EGFR治疗中, 与*PIK3CA*^{WT}(应答率36.8%)相比, *PIK3CA*外显子20突变患者对抗EGFR治疗的应答率为0(P=0.029), 中位无进展生存期(progression free survival, PFS, P=0.013)和中位OS(P=0.0057)也较低[29]。对CC治疗的进一步研究发现, 阿司匹林与*PIK3CA*^{MUT}患者的总生存期较长有关, 而与*PIK3CA*^{WT}患者无相关性[30]。具体而言, 阿司匹林可抑制环氧合酶2(cytochrome c oxidase subunit II, COX2)表达, 从而下调PI3K的致癌作用[31]。

EGFR受体位于RAS/RAF/MEK/ERK和PI3K/AKT/mTOR信号通路的共同上游, 由配体诱导激活后可对CC产生增殖作用, 因此靶向EGFR的单克隆抗体可用于CC患者的治疗。然而, EGFR下游效应分子(如*RAS/BRAF*、*PIK3CA*等)的突变可独立激活RAS/RAF/MEK/ERK和PI3K-AKT-mTOR信号通路, 从而增加了肿瘤对抗EGFR治疗的耐受性[32]。可见, *RAS/BRAF*和*PIK3CA*突变对CC患者的预后和治疗起着重要的作用。通过对TCGA数据的总结发现, RCC患者中这三者的突变率均高于LCC(表1), 这可能是RCC生存率和抗EGFR治疗应答率较低的原因之一。在ARNOLD等[33]抗EGFR治疗的队列研究中, 与化疗患者相比, LCC患者获得了显著疗效, 其PFS的风险比(hazard ratio, HR)为0.78(95%

CI: 0.70~0.87), 而RCC的疗效却不明显, 其中位PFS的HR为1.12(95% CI: 0.87~1.44)。同时, 高*BRAF*和*PIK3CA*突变使得RCC患者可能更适合三联疗法和阿司匹林治疗, 具体的实验和临床验证有待进一步探索。

除了上述差异外, 基因突变与肿瘤微环境之间也存在紧密联系。高TMB会诱发免疫浸润[34]和高水平的程序性死亡受体–配体1(programmed death-ligand 1, PD-L1)表达[35], 这部分肿瘤往往发生在结肠右侧。TMB还与肿瘤纯度呈负相关, 这也解释了RCC患者肿瘤纯度更低的原因[36]。LCC患者中的Wnt/β-catenin途径激活与人类癌症的免疫排斥具有很强的相关性[37], 作为LCC的主要驱动力, *TP53*突变在免疫浸润中起负调节作用[38], 导致LCC免疫浸润程度低于RCC。

2 mRNA转录调节

关键基因表达的紊乱是癌症发生的主要原因, 包括原癌基因的激活和抑癌基因的失活, 使细胞增殖分裂功能失调, 从而导致癌症的发生[39]。基于转录组测序数据对左右半CC的系统分析显示, 相比于对应的正常癌旁组织, LCC和RCC相对于各自正常组织的差异表达基因存在大量重叠, 差异方向较一致, 仅少数几个基因在左右半CC的表达趋势完全相反[2], 如*SLC6A4*(solute carrier family 6 member 4)和*HOXB13*。Homeobox基因*HOXB13*在LCC中下调, 而在RCC中却上调。*SLC6A4*的表达趋势正好相反, 在LCC中表达上调, 而在RCC中却下调。*SLC6A4*编码神经递质5-羟色胺(5-hydroxy tryptamine, 5-HT), 该递质可抑制结肠的炎症反应[40], 这可能是LCC免疫浸润程度较低的原因之一。除此之外, LCC中出现的趋化因子(例如*MS4A1*和*BACH2*等)特异性下调也是导致其免疫浸润水平低于RCC的原因之一[2]。

尽管很多基因在LCC和RCC中相较于正常组织同时表现高表达或低表达水平, 但其肿瘤间的表达却存在差异, 从而导致左右半CC细胞的功能存在异质性。其中, Homeobox基因是常见的肿瘤间差异表达基因。例如, *HOXC6*、*HOXC4*、*HOXC9*、*PITX2*(paired like homeodomain 2)、*BARX2*、*DLX1*(distal-less homeobox 1)、*HOXB2*、*HOXB6*、*HOXB8*、*DMBX1*(diencephalon/mesencephalon homeobox 1)、*ONECUT2*(one cut homeobox 2)、*ONECUT3*、*PAX5*(paired box 5)、*PAX9*、*EMX1*(empty spiracles homeobox 1)和*ARX*(aristaless related homeobox)在RCC中的表达高于LCC, 而*HOXB13*、*PRAC1*和*PRAC2*在RCC中的表达则低于LCC[41-44]。有趣的是, 在LCC中下调的*HOXB13*, 其表达水平却高于RCC。据报道, *HOXB13*可能通过下调*TCF4*(transcription factor 4)和*C-myc*对细胞生长起抑制作用[45]; 又有文献报道, *HOXB13*具有促进肿瘤增殖、侵袭和转移的能力[46]。因此, *HOXB13*在左右半CC中的功能差异有待进一步研究。除了Homeobox基因, 影响细胞代谢的基因也存在肿瘤间差异, 如葡萄糖转运体*SLC2A1*在RCC中表达水平更高[47], 而参与脂肪酸降解和氧化磷酸化的几个线粒体代谢基因却下调, 包括*G6PC*(glucose-6-phosphatase catalytic)、*FABP1*(fatty acid binding protein 1)、*CPT1A*(carnitine palmitoyltransferase 1A)、*CPT2*、*ACAT1*(acetyl-CoA acetyltransferase 1)、*ACAA2*、*ACOX1*(acyl-CoA oxidase 1)、*EPHX2*(epoxide hydrolase 2)和*EHHADH*(enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase)等[2], 这可能与RCC中肿瘤细胞增殖速率更快、糖酵解和侵袭性更强有关。

还有部分差异表达基因已通过临床验证可影响左右半CC的预后和治疗。例如, FLOT1的高表达与RCC患者的预后较差、侵袭性和增殖性更强有关[48], 而MRE11的高表达则与LCC患者更好的生存率有关[49]。*AREG*和*EREG*的表达是抗EGFR治疗反应的预测因子, 这两个基因在左右半CC之间的表达不存在统计学差异, 然而在$KRAS^{WT}$的患者中, 这两个基因在LCC患者中的表达水平却高于RCC患者[50], 这与上一节得出LCC患者更适合抗EGFR治疗的结论相吻合。

3 DNA甲基化

在肿瘤患者中, DNA甲基化异常是一种常见的表观遗传现象, 启动子CpG岛的高甲基化导致抑癌基因失活, 而促癌基因则发生全局低甲基化而被激活[51-52]。总体而言, RCC患者更容易发生CpG岛甲基化表型(CpG island methylator phenotype, CIMP), 这部分患者往往与MSI亚型呈正相关[53]。RCC患者中高甲基化CpG位点比例较多, 约40%的下调基因与高甲基化相关, 20%的上调基因与低甲基化相关; 而LCC患者中则显示出较少的高甲基化位点及更多的

低甲基化位点, 约33%的下调基因与高甲基化相关, 27%的上调基因与低甲基化相关[2]。

研究表明, LCC和RCC患者的DNA甲基化差异位点也大多涉及Homeobox基因富集。Homeobox基因如*MEIS1*、*PRAC1*(PRAC1 small nuclear protein)、*PRAC2*和*HOXC*基因(*HOXC4*、*HOXC5*、*HOXC6*等)在RCC患者中发生高甲基化[54-55], 其中, *PRAC2*启动子中的甲基化与转录呈正相关[56]。*PRAC2*位于*HOXB13*和*PRAC*的基因组区域之间, 在前列腺、直肠、结肠和睾丸中高表达, 可能在细胞核中起作用[57]。在LCC中, Homeobox基因如*HOXB5*、*HOXB7*和*CDX2*都是常见的高甲基化基因[44]。*CDX2*是一种肠特异性转录因子, 与肿瘤分化、增殖、细胞黏附和迁移有关[58], 该基因通过抑制Wnt/β-catenin信号转导, 从而抑制结肠癌细胞的增殖和肿瘤形成[59]。

除了Homeobox基因, 左右半结肠癌其他的差异甲基化基因经常与DNA转录和细胞增殖等功能有关。如RCC中$p16^{INK4a}$、$p14^{ARF}$和*FHIT*等基因的甲基化程度均高于LCC[60]。抑癌基因$p14^{ARF}$和$p16^{INK4a}$的表达都能导致细胞周期阻滞[61-62], 这两者的高甲基化使RCC肿瘤细胞的增殖能力增强。此外, *FHIT*在肿瘤中发挥凋亡作用, 在RCC中高甲基化会促进肿瘤细胞的增殖[63], 这可能也是左右半CC存在预后差异的原因之一。

4 miRNA表达

miRNA是一类短的非编码RNA, 通过抑制RNA翻译或促进RNA降解调节基因的表达[64]。和对应正常癌旁组织相比, 大多数异常表达的miRNA在LCC和RCC中均发生一致的变化, 但是存在部分特异性差异表达的miRNA。

在RCC中特异性上调的miRNA大多与细胞代谢、细胞生长和细胞增殖有关, 例如miR-23a、miR-181d、miR-576和miR-31等, 这些miRNAs在LCC中不存在差异表达。miR-23a与包括G6PC和PPARGC1在内的几种线粒体蛋白相关, 抑制了RCC的氧化磷酸化。miR-181d和miR-576通过靶向细胞周期基因*BCL2*和*CCND1*, 从而抑制细胞凋亡[2]。此外, miR-31在RCC中与*BRAF*突变、高MSI表型有关[65], 并通过靶向*TNS1*促进结肠腺癌进展[66]。而miR-1288则是目前报道的LCC中唯一特异性上调的miRNA, 该基因的高表达与LCC良好的预后有关[67]。

除了特异性差异表达的miRNAs, RCC和LCC相比也存在一些差异表达的miRNAs, 这些miRNAs使LCC和RCC的侵袭性和免疫浸润程度产生差异。如在RCC中, miR-155的转录水平高于LCC[44], 该miRNA可直接调节β-catenin, 并增强RCC肿瘤细胞的侵袭力[68]。miR-147b和miR-224的表达则在LCC中更高, 且与免疫下调有关。miR-147家族受Toll样受体(Toll-like receptor, TLR)信号转导途径的刺激而表达, 但是其又能拮抗TLR诱导的炎症反应, 三者之间形成了一个负反馈循环[69]。此外, miR-224与*CXCR4*、*SMAD4*和*KRAS*的表达呈负相关, 这些基因通过产生IgA调节肠道免疫[41]。可见, miRNA调节在LCC免疫浸润的调节上起着重要的作用。

5 肿瘤免疫微环境

已有的研究显示, RCC患者免疫浸润程度比LCC患者高[9]。与LCC相比, RCC组织中高密度肿瘤浸润淋巴细胞(tumor infiltrating lymphocytes, TILs)更为常见, 且$CD8^+$ T淋巴细胞浸润水平更高, 细胞毒活性和干扰素-γ(interferon-γ, INF-γ)信号更强, 以及抗原呈递元件(antigen processing machinery, APM)更加丰富, 即从右侧到左侧的免疫活性呈负梯度变化[10]。

虽然RCC患者具有更强的$CD8^+$ T淋巴细胞浸润, 但是该细胞介导的抗肿瘤反应可能被高浓度VEGF所阻断, 使癌症组织发生免疫逃逸[70]。在炎症反应的“伤口愈合”阶段VEGF可促进新血管的生成, 这为肿瘤的生长提供了氧气和营养物质, 也促进了肿瘤的转移。此时, 具有抑癌作用的炎症反应和免疫细胞浸润反而促进了肿瘤的生长[71]。这种情况下, 免疫系统显然有助于结肠肿瘤的生长和增殖。在RCC中, 和血管生成相关的因子(例如eNOS和EPHB4)明显富集[72], 右侧肿瘤获得了更强的营养供给和生长能力。较低的免疫敏感性和较高的肿瘤生长能力之间形成恶性循环, 使其能够逃避免疫检测的肿瘤逐渐在RCC中成为优势群体。除此之外, RCC的炎症指数[NLR(neutrophil-to-lymphocyte ratio)、PLR(platelet to lymphocyte ratio)、SII(systemic immune-inflammation index)]低于LCC。高血管生成因子和低炎症指数都是抗VEGF(即bevacizumab)治疗获益的潜在因素[70]。据报道, 在接受辅助化疗伴抗VEGF治疗后, 尽管LCC患者的生存率依然高于RCC患者[73], 然而, 在RCC中, 辅助化疗伴抗VEGF治

疗与仅辅助化疗患者的生存率存在差异, 中位PFS分别为12.6和9.0个月(P=0.017), 而LCC中, 这两种疗法之间不存在生存差异(P=0.458)[72]。可见, RCC患者可能更适合抗VEGF治疗。

LCC中抗肿瘤CD56^{bright} NK细胞亚群的浸润程度高于RCC, CD56^{bright} NK细胞高浸润与患者预后更好有着紧密的关联[74]。研究表明, 抗EGFR治疗药物之一cetuximab的Fc片段可以结合NK细胞上的Fc受体FcγRIII(即CD16), 从而引发一系列细胞事件, 最终导致含有细胞毒性颗粒酶颗粒的释放和INF-γ的分泌, 随后杀死肿瘤细胞[75]。在*KRAS*^{WT}患者中, LCC患者对cetuximab的反应率高于RCC患者[76]。此外, 在肿瘤组织中, 程序性死亡受体-1(programmed cell death-1, PD-1)和PD-L1的表达会导致NK细胞应答降低, 使体内产生更具侵略性的肿瘤, 而PD-1和PD-L1阻滞则会引起强烈的NK细胞反应[77]。由此推测, PD-1抑制剂与cetuximab的联合疗法可能在*KRAS*^{WT}的患者中取得更好的疗效, 然而, 具体的实验和临床验证有待进一步探索。

6 免疫疗法

免疫检查点如PD-1、PD-L1和细胞毒性T淋巴细胞相关蛋白-4(cytotoxic T lymphocyte-associated antigen-4, CTLA-4)是重要的免疫系统抑制分子, 可以抑制T细胞活化[78]。免疫检查点抑制剂(immune checkpoint inhibitors, ICIs)治疗, 即通过抑制PD-1、PD-L1和CTLA-4受体活性而激活免疫反应[79], 其中, CD8$^+$ T细胞的浸润激活是ICIs发挥抑癌作用的必要条件[80]。最近的研究已经确定了ICIs的几种阳性预测标志物, 包括高微卫星不稳定性/错配修复缺陷(MSI high/dMMR, MSI-H/dMMR)[81]和较高的TMB[82], 而这两者又好发于RCC。FDA于2017年5月23日批准, pembrolizumab可用于治疗MSI-H/dMMR的实体瘤[83], 在KEYNOTE-164的队列研究中, 149例MSI-H/dMMR癌症患者在经过pembrolizumab治疗后的客观缓解率(objective response rate, ORR)为39.6%, 并产生了持久的临床效益[84]。在checkmate-142的单药队列研究中, 对于fluorouracil、oxaliplatin和irinotecan治疗无效的MSI-H/dMMR结肠癌患者, PD-1抑制剂nivolumab治疗后的ORR达31.1%, 持续12周以上的疾病控制率为68.9%[85]。随着进一步研究发现, CTLA-4单克隆抗体ipilimumab+nivolumab的联合疗法被认为比单独使用nivolumab更为有效, 联合疗法的ORR达55%[86]。超高突变负担的CC也经常位于右侧, 这部分CC患者常常由DNA聚合酶(POLE或者POLD1)的核酸外切酶结构域缺陷突变引起(表1)。有文献报道, 在多种癌症类型中, 存在高频率*POLE/POLD1*突变的患者在ICIs治疗中生存率更好[87]。因此, 免疫治疗(如pembrolizumab、nivolumab或nivolumab+ipilimumab)可能是MSI-H(高CD8$^+$ T淋巴细胞浸润)或者超高突变负担RCC患者的不错选择(表2)。

7 共识分子亚型

在结肠肿瘤中, 分子亚型识别对疾病的预后和治疗往往具有指导性的作用。根据不同的分类方案, 结肠癌分为数量不等的子类型, 且不同分类方案的子类型之间存在一定的差异[88-90], 这使得分子亚型的临床应用受限。为统一分子亚型分类, 国际结直肠癌分型联盟通过结合各组学参数, 包括基因突变、甲基化、miRNA、基因活性、免疫活性、细胞代谢和临床特征等数据, 进行共识分子亚型(consensus molecular subtypes, CMS)分类, 将结肠癌患者归为CMS1(MSI/immune)、CMS2(canonical), CMS3(metabolic)和CMS4(mesenchymal)[91]。通过对该项研究的系统性总结显示, CMS1和CMS3亚型以RCC患者为主, 而CMS2和CMS4集中了更多的LCC患者。CMS1具有高突变率、高*BRAF*突变、高CIMP、高MSI、高免疫浸润和低拷贝数变异(copy number variation, CNV)等特点, PD-1靶点的激活意味着CMS1可能是预测免疫治疗反应的靶点之一; CMS2患者的CNV最频繁, 呈现出上皮性分化及Wnt和C-myc信号通路的高度激活, 作为C-myc通路靶点的miR-17-92在CMS2中发生显著上调; CMS3患者激活了多种代谢途径, 并发生了更为频繁的*KRAS*突变; CMS4患者的整体生存率最差, 上皮细胞向间充质转化(epithelial-mesenchymal transition, EMT)通路和β转化生长因子信号激活, 与成纤维细胞相关的基质衍生基因也高表达。分子亚型共识的鉴定为不同CC患者的临床诊断提供了分子学依据, 同时也可以用于指导左右半结肠癌的特异性治疗。

8 总结和展望

由于解剖学、胚胎学起源和环境的不同, 左右半结肠癌的分子学特征存在差异。总体来说, RCC

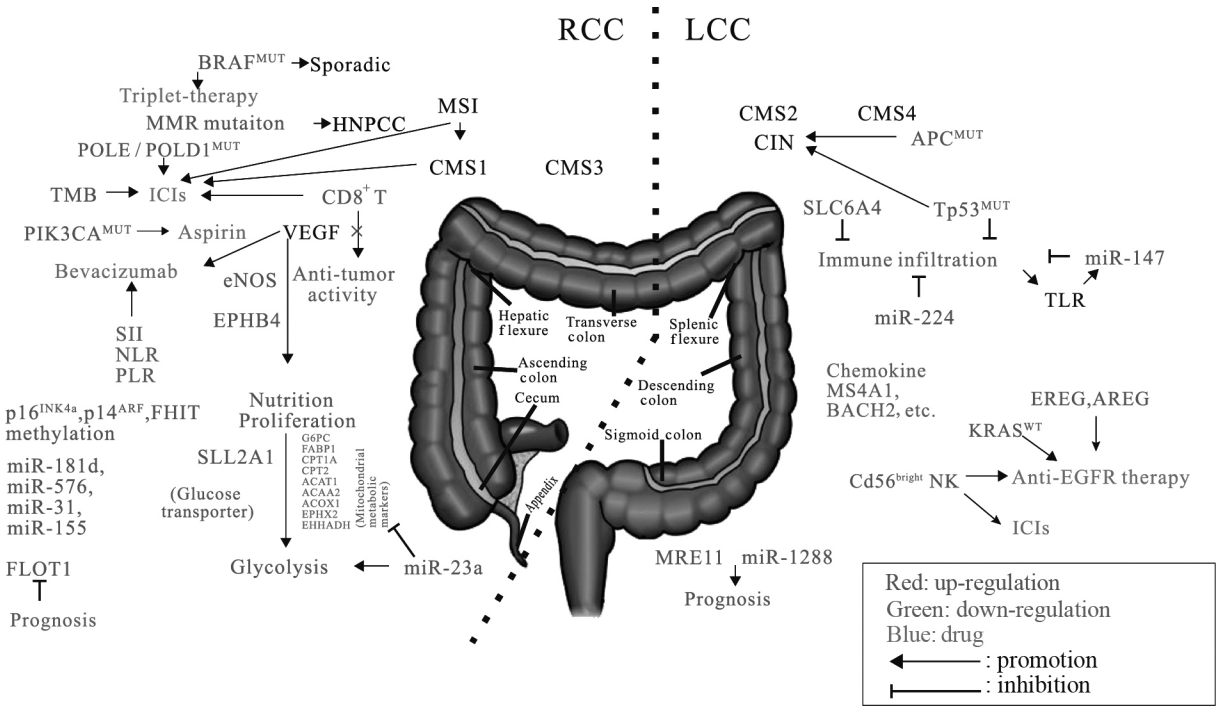

图1 左右半结肠癌主要的多组学分子特征差异

Fig.1 The main multi-omics molecular characteristics differences between left-sided and right-sided colon cancer

患者更倾向于高MSI、高CIMP表型, 而LCC患者则更倾向于CIN表型。这些表型之间没有固定的界限, 往往存在一定的重叠部分。例如, 高达25%的MSI可表现出染色体异常, 约12%的CIN表现出高MSI状态等[92]。此外, 虽然大部分CIMP表型具有MSI阳性/CIN阴性特点, 然而依然有多达33%的CIMP阳性肿瘤表现出高度的染色体畸变[93]。

Homeobox基因是左右半结肠癌中常见的差异基因, 比如*HOXB13*在LCC中表达下调, 在RCC中上调; *PRAC*、*PRAC2*和*HOXC*基因在RCC中高甲基化; *CDX2*和*HOXB*基因在LCC中高甲基化等。各种Homeobox基因在正常结肠组织中的不同解剖位置也存在梯度差异[94], 提示该类基因的异常可能对肿瘤间差异起主导作用。如图1所示, 与LCC相比, RCC中的炎性细胞浸润程度更高, 但是炎性细胞(如$CD8^+$ T)抗肿瘤活性被VEGF抑制, 反而产生了有助于肿瘤的形成、生长和破坏的能力。LCC的众多趋化因子被抑制, 并且SLC6A4、miR-147b和miR-224的高表达与*TP53*的高突变都对LCC的免疫浸润产生了负调节作用。葡萄糖转运体SLL2A1的增加和氧化磷酸化的抑制使得RCC的糖酵解增加, 这是肿瘤发生的一个重要特征。除此之外, 其他存在于左右半结肠癌组学之间的差异基因或者免疫细胞往往导致RCC更具侵袭力, 对三联疗法、阿司匹林、ICIs和bevacizumab产生较好的疗效, 而LCC则更适合抗EGFR疗法, 并且预后更佳。基于oncokb数据库(www.oncokb.org)的药物治疗靶点, 我们系统评估了各药物靶点基因在LCC和RCC患者中的突变率(表2)。该综述中左右半结肠癌之间的分子学特征和药物敏感性差异可为CC患者的个性化治疗提供一定的指导意义。

参考文献 (References)

[1] BRAY F, FERLAY J, SOERJOMATARAM I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. CA Cancer J Clin, 2018, 68(6): 394-424.

[2] MUKUND K, SYULYUKINA N, RAMAMOORTHY S, et al. Right and left-sided colon cancers-specificity of molecular mechanisms in tumorigenesis and progression [J]. BMC Cancer, 2020, 20(1): 317.

[3] HELVACI K, ERASLAN E, YILDIZ F, et al. Comparison of clinicopathological and survival features of right and left colon cancers [J]. J BUON, 2019, 24(5): 1845-51.

[4] GERVAZ P, USEL M, RAPITI E, et al. Right colon cancer: left behind [J]. Eur J Surg Oncol, 2016, 42(9): 1343-9.

[5] OZTURK E, KUZU M A, OZTUNA D, et al. Fall of another myth for colon cancer: duration of symptoms does not differ between right- or left-sided colon cancers [J]. Turk J Gastroenterol, 2019, 30(8): 686-94.

[6] MCCARTHY A J, SERRA S, CHETTY R. Traditional serrated adenoma: an overview of pathology and emphasis on molecular

pathogenesis [J]. BMJ Open Gastroenterol, 2019, 6(1): e000317.
[7] SEGEV L, KALADY M F, PLESEC T, et al. The location of premalignant colorectal polyps under age 50: a further rationale for screening sigmoidoscopy [J]. Int J Colorectal Dis, 2020, 35(3): 529-35.
[8] SHI C, DING K, LI K Z, et al. Comprehensive analysis of location-specific hub genes related to the pathogenesis of colon cancer [J]. Med Oncol, 2020, 37(9): 77.
[9] PATEL M, MCSORLEY S T, PARK J H, et al. The relationship between right-sided tumour location, tumour microenvironment, systemic inflammation, adjuvant therapy and survival in patients undergoing surgery for colon and rectal cancer [J]. Br J Cancer, 2018, 118(5): 705-12.
[10] ZHANG L, ZHAO Y, DAI Y, et al. Immune landscape of colorectal cancer tumor microenvironment from different primary tumor location [J]. Front Immunol, 2018, 9: 1578.
[11] BAKHOUM S F, NGO B, LAUGHNEY A M, et al. Chromosomal instability drives metastasis through a cytosolic DNA response [J]. Nature, 2018, 553(7689): 467-72.
[12] SHEN H, YANG J, HUANG Q, et al. Different treatment strategies and molecular features between right-sided and left-sided colon cancers [J]. World J Gastroenterol, 2015, 21(21): 6470-8.
[13] CARETHERS J M, JUNG B H. Genetics and genetic biomarkers in sporadic colorectal cancer [J]. Gastroenterology, 2015, 149(5): 1177-90,e3.
[14] SHIN U S, CHO S S, MOON S M, et al. Is microsatellite instability really a good prognostic factor of colorectal cancer [J]? Ann Coloproctol, 2014, 30(1): 28-34.
[15] DE ANGELIS G L, BOTTARELLI L, AZZONI C, et al. Microsatellite instability in colorectal cancer [J]. Acta Biomed, 2018, 89(9S): 97-101.
[16] LYNCH H T, LYNCH P M, LANSPA S J, et al. Review of the lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications [J]. Clin Genet, 2009, 76(1): 1-18.
[17] LATHAM A, SRINIVASAN P, KEMEL Y, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-Cancer [J]. J Clin Oncol, 2019, 37(4): 286-95.
[18] YAMAMOTO H, IMAI K. Microsatellite instability: an update [J]. Arch Toxicol, 2015, 89(6): 899-921.
[19] BOS J L. Ras oncogenes in human cancer: a review [J]. Cancer Res, 1989, 49(17): 4682-9.
[20] IMAMURA Y, MORIKAWA T, LIAO X, et al. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers [J]. Clin Cancer Res, 2012, 18(17): 4753-63.
[21] YOON H H, TOUGERON D, SHI Q, et al. KRAS codon 12 and 13 mutations in relation to disease-free survival in BRAF-wild-type stage III colon cancers from an adjuvant chemotherapy trial (N0147 alliance) [J]. Clin Cancer Res, 2014, 20(11): 3033-43.
[22] MARGONIS G A, BUETTNER S, ANDREATOS N, et al. Association of BRAF mutations with survival and recurrence in surgically treated patients with metastatic colorectal liver cancer [J]. JAMA Surg, 2018, 153(7): e180996.
[23] YANG Q, HUO S, SUI Y, et al. Mutation status and immunohistochemical correlation of KRAS, NRAS, and BRAF in 260 Chinese colorectal and gastric cancers [J]. Front Oncol, 2018, 8: 487.
[24] KOPETZ S, GROTHEY A, YAEGER R, et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer [J]. N Engl J Med, 2019, 381(17): 1632-43.
[25] MARQUARD F E, JUCKER M. PI3K/AKT/mTOR signaling as a molecular target in head and neck cancer [J]. Biochem Pharmacol, 2020, 172: 113729.
[26] EDIRIWEERA M K, TENNEKOON K H, SAMARAKOON S R. Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: biological and therapeutic significance [J]. Semin Cancer Biol, 2019, 59: 147-60.
[27] OGINO S, NOSHO K, KIRKNER G J, et al. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer [J]. J Clin Oncol, 2009, 27(9): 1477-84.
[28] ROSTY C, YOUNG J P, WALSH M D, et al. PIK3CA activating mutation in colorectal carcinoma: associations with molecular features and survival [J]. PLoS One, 2013, 8(6): e65479.
[29] DE ROOCK W, CLAES B, BERNASCONI D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis [J]. Lancet Oncol, 2010, 11(8): 753-62.
[30] LIAO X, LOCHHEAD P, NISHIHARA R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival [J]. N Engl J Med, 2012, 367(17): 1596-606.
[31] THERKILDSEN C, BERGMANN T K, HENRICHSEN-SCHNACK T, et al. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: a systematic review and meta-analysis [J]. Acta Oncol, 2014, 53(7): 852-64.
[32] HSU H C, THIAM T K, LU Y J, et al. Mutations of KRAS/NRAS/BRAF predict cetuximab resistance in metastatic colorectal cancer patients [J]. Oncotarget, 2016, 7(16): 22257-70.
[33] ARNOLD D, LUEZA B, DOUILLARD J Y, et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials [J]. Ann Oncol, 2017, 28(8): 1713-29.
[34] WANG X, LI M. Correlate tumor mutation burden with immune signatures in human cancers [J]. BMC Immunol, 2019, 20(1): 4.
[35] DUDNIK E, PELED N, NECHUSHTAN H, et al. BRAF mutant lung cancer: programmed death ligand 1 expression, tumor mutational burden, microsatellite instability status, and response to immune check-point inhibitors [J]. J Thorac Oncol, 2018, 13(8): 1128-37.
[36] MAO Y, FENG Q, ZHENG P, et al. Low tumor purity is associated with poor prognosis, heavy mutation burden, and intense immune phenotype in colon cancer [J]. Cancer Manag Res, 2018, 10: 3569-77.
[37] LUKE J J, BAO R, SWEIS R F, et al. WNT/beta-catenin pathway activation correlates with immune exclusion across human cancers [J]. Clin Cancer Res, 2019, 25(10): 3074-83.
[38] LI L, LI M, WANG X. Cancer type-dependent correlations between TP53 mutations and antitumor immunity [J]. DNA Repair, 2020, 88: 102785.
[39] LIMA L, DE MELO T C T, MARQUES D, et al. Modulation of

all-trans retinoic acid-induced MiRNA expression in neoplastic cell lines: a systematic review [J]. BMC Cancer, 2019, 19(1): 866.

[40] SAVAS S, HYDE A, STUCKLESS S N, et al. Serotonin transporter gene (SLC6A4) variations are associated with poor survival in colorectal cancer patients [J]. PLoS One, 2012, 7(7): e38953.

[41] YANG L, LI L, MA J, et al. miRNA and mRNA integration network construction reveals novel key regulators in left-sided and right-sided colon adenocarcinoma [J]. Biomed Res Int, 2019, 2019: 7149296.

[42] LIANG L, ZENG J H, QIN X G, et al. Distinguishable prognostic signatures of left- and right-sided colon cancer: a study based on sequencing data [J]. Cell Physiol Biochem, 2018, 48(2): 475-90.

[43] SU C, ZHAO J, HONG X, et al. Microarraybased analysis of COL11A1 and TWIST1 as important differentiallyexpressed pathogenic genes between left and rightsided colon cancer [J]. Mol Med Rep, 2019, 20(5): 4202-14.

[44] HU W, YANG Y, LI X, et al. Multi-omics approach reveals distinct differences in left- and right-sided colon cancer [J]. Mol Cancer Res, 2018, 16(3): 476-85.

[45] JUNG C, KIM R S, ZHANG H, et al. HOXB13 is downregulated in colorectal cancer to confer TCF4-mediated transactivation [J]. Br J Cancer, 2005, 92(12): 2233-9.

[46] WANG X, SUN Y, XU T, et al. HOXB13 promotes proliferation, migration, and invasion of glioblastoma through transcriptional upregulation of lncRNA HOXC-AS3 [J]. J Cell Biochem, 2019, 120(9): 15527-37.

[47] HAN J, ZHANG X, YANG Y, et al. Screening and identification of differentially expressed genes expressed among left and right colon adenocarcinoma [J]. Biomed Res Int, 2020, 2020: 8465068.

[48] BAIG N, LI Z, LU J, et al. Clinical significance and comparison of flotillin 1 expression in left and right colon cancer [J]. Oncol Lett, 2019, 18(2): 997-1004.

[49] FAN C W, KOPSIDA M, LIU Y B, et al. Prognostic heterogeneity of MRE11 based on the location of primary colorectal cancer is caused by activation of different immune signals [J]. Front Oncol, 2019, 9: 1465.

[50] KURAMOCHI H, NAKAJIMA G O, HAYASHI K, et al. Amphiregulin/epiregulin mRNA expression and primary tumor location in colorectal cancer [J]. Anticancer Res, 2019, 39(9): 4729-36.

[51] JONES P A, BAYLIN S B. The epigenomics of cancer [J]. Cell, 2007, 128(4): 683-92.

[52] BERDASCO M, ESTELLER M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry [J]. Dev Cell, 2010, 19(5): 698-711.

[53] GALLOIS C, PERNOT S, ZAANAN A, et al. Colorectal cancer: why does side matter [J]? Drugs, 2018, 78(8): 789-98.

[54] BARNICLE A, SEOIGHE C, GOLDEN A, et al. Differential DNA methylation patterns of homeobox genes in proximal and distal colon epithelial cells [J]. Physiol Genomics, 2016, 48(4): 257-73.

[55] DIHAL A A, BOOT A, VAN ROON E H, et al. The homeobox gene MEIS1 is methylated in BRAF (p.V600E) mutated colon tumors [J]. PLoS One, 2013, 8(11): e79898.

[56] DE ALMEIDA B P, APOLONIO J D, BINNIE A, et al. Roadmap of DNA methylation in breast cancer identifies novel prognostic biomarkers [J]. BMC Cancer, 2019, 19(1): 219.

[57] OLSSON P, MOTEGI A, BERA T K, et al. PRAC2: a new gene expressed in human prostate and prostate cancer [J]. Prostate, 2003, 56(2): 123-30.

[58] RYAN E J, CREAVIN B, KHAW Y L, et al. Effects of CDX2 on prognosis and chemotherapy responsiveness in mismatch repair-deficient colorectal cancer [J]. BJS Open, 2018, 2(6): 456-63.

[59] YU J, LIU D, SUN X, et al. CDX2 inhibits the proliferation and tumor formation of colon cancer cells by suppressing Wnt/beta-catenin signaling via transactivation of GSK-3beta and Axin2 expression [J]. Cell Death Dis, 2019, 10(1): 26.

[60] DONG S M, LEE E J, JEON E S, et al. Progressive methylation during the serrated neoplasia pathway of the colorectum [J]. Mod Pathol, 2005, 18(2): 170-8.

[61] KO A, HAN S Y, CHOI C H, et al. Oncogene-induced senescence mediated by c-Myc requires USP10 dependent deubiquitination and stabilization of p14ARF [J]. Cell Death Differ, 2018, 25(6): 1050-62.

[62] LIU J Y, SOUROULLAS G P, DIEKMAN B O, et al. Cells exhibiting strong p16 (INK4a) promoter activation *in vivo* display features of senescence [J]. Proc Natl Acad Sci USA, 2019, 116(7): 2603-11.

[63] SILVEIRA ZAVALHIA L, WEBER MEDEIROS A, OLIVEIRA SILVA A, et al. Do FHIT gene alterations play a role in human solid tumors [J]? Asia Pac J Clin Oncol, 2018, 14(5): e214-23.

[64] CORREIA DE SOUSA M, GJORGJIEVA M, DOLICKA D, et al. Deciphering miRNAs' action through miRNA editing [J]. Int J Mol Sci, 2019, 20(24): 6429.

[65] NOSHO K, IGARASHI H, NOJIMA M, et al. Association of microRNA-31 with BRAF mutation, colorectal cancer survival and serrated pathway [J]. Carcinogenesis, 2014, 35(4): 776-83.

[66] MI B, LI Q, LI T, et al. High miR-31-5p expression promotes colon adenocarcinoma progression by targeting TNS1 [J]. Aging, 2020, 12(8): 7480-90.

[67] GOPALAN V, PILLAI S, EBRAHIMI F, et al. Regulation of microRNA-1288 in colorectal cancer: altered expression and its clinicopathological significance [J]. Mol Carcinog, 2014, 53 (Suppl 1): E36-44.

[68] LIU N, JIANG F, HAN X Y, et al. MiRNA-155 promotes the invasion of colorectal cancer SW-480 cells through regulating the Wnt/beta-catenin [J]. Eur Rev Med Pharmacol Sci, 2018, 22(1): 101-9.

[69] LIU G, FRIGGERI A, YANG Y, et al. miR-147, a microRNA that is induced upon Toll-like receptor stimulation, regulates murine macrophage inflammatory responses [J]. Proc Natl Acad Sci USA, 2009, 106(37): 15819-24.

[70] CHEN D S, HURWITZ H. Combinations of nevacizumab with cancer immunotherapy [J]. Cancer J, 2018, 24(4): 193-204.

[71] YI M, JIAO D, QIN S, et al. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment [J]. Mol Cancer, 2019, 18(1): 60.

[72] ULIVI P, SCARPI E, CHIADINI E, et al. Right- *vs* left-sided metastatic colorectal cancer: differences in tumor biology and bevacizumab efficacy [J]. Int J Mol Sci, 2017, 18(6): 1240.

[73] YOU X H, JIANG Y H, FANG Z, et al. Chemotherapy plus

bevacizumab as an optimal first-line therapeutic treatment for patients with right-sided metastatic colon cancer: a meta-analysis of first-line clinical trials [J]. ESMO Open, 2020, 4(Suppl 2): e000605.

[74] MINOO P, ZLOBEC I, PETERSON M, et al. Characterization of rectal, proximal and distal colon cancers based on clinicopathological, molecular and protein profiles [J]. Int J Oncol, 2010, 37(3): 707-18.

[75] FUJII R, SCHLOM J, HODGE J W. A potential therapy for chordoma via antibody-dependent cell-mediated cytotoxicity employing NK or high-affinity NK cells in combination with cetuximab [J]. J Neurosurg, 2018, 128(5): 1419-27.

[76] BRULE S Y, JONKER D J, KARAPETIS C S, et al. Location of colon cancer (right-sided versus left-sided) as a prognostic factor and a predictor of benefit from cetuximab in NCIC CO.17 [J]. Eur J Cancer, 2015, 51(11): 1405-14.

[77] HSU J, HODGINS J J, MARATHE M, et al. Contribution of NK cells to immunotherapy mediated by PD-1/PD-L1 blockade [J]. J Clin Invest, 2018, 128(10): 4654-68.

[78] WEI S C, DUFFY C R, ALLISON J P. Fundamental mechanisms of immune checkpoint blockade therapy [J]. Cancer Discov, 2018, 8(9): 1069-86.

[79] JENKINS R W, BARBIE D A, FLAHERTY K T. Mechanisms of resistance to immune checkpoint inhibitors [J]. Br J Cancer, 2018, 118(1): 9-16.

[80] FARHOOD B, NAJAFI M, MORTEZAEE K. $CD8^+$ cytotoxic T lymphocytes in cancer immunotherapy: a review [J]. J Cell Physiol, 2019, 234(6): 8509-21.

[81] CHANG L, CHANG M, CHANG H M, et al. Microsatellite instability: a predictive biomarker for cancer immunotherapy [J]. Appl Immunohistochem Mol Morphol, 2018, 26(2): e15-21.

[82] CHAN T A, YARCHOAN M, JAFFEE E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic [J]. Ann Oncol, 2019, 30(1): 44-56.

[83] MARCUS L, LEMERY S J, KEEGAN P, et al. FDA approval summary: pembrolizumab for the treatment of microsatellite instability-high solid tumors [J]. Clin Cancer Res, 2019, 25(13): 3753-8.

[84] LE D T, KIM T W, VAN CUTSEM E, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164 [J]. J Clin Oncol, 2020, 38(1): 11-9.

[85] OVERMAN M J, MCDERMOTT R, LEACH J L, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study [J]. Lancet Oncol, 2017, 18(9): 1182-91.

[86] OVERMAN M J, LONARDI S, WONG K Y M, et al. Durable Clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer [J]. J Clin Oncol, 2018, 36(8): 773-9.

[87] WANG F, ZHAO Q, WANG Y N, et al. Evaluation of POLE and POLD1 mutations as biomarkers for immunotherapy outcomes across multiple cancer types [J]. JAMA Oncol, 2019, 5(10): 1504-6.

[88] WANG W H, XIE T Y, XIE G L, et al. An integrated approach for identifying molecular subtypes in human colon cancer using gene expression data [J]. Genes, 2018, 9(8): 397.

[89] DE PALMA F D E, D'ARGENIO V, POL J, et al. The molecular hallmarks of the serrated pathway in colorectal cancer [J]. Cancers, 2019, 11(7): 1017.

[90] WIELANDT A M, HURTADO C, MORENO C M, et al. Characterization of Chilean patients with sporadic colorectal cancer according to the three main carcinogenic pathways: microsatellite instability, CpG island methylator phenotype and Chromosomal instability [J]. Tumour Biol, 2020, 42(7): 1010428320938492.

[91] GUINNEY J, DIENSTMANN R, WANG X, et al. The consensus molecular subtypes of colorectal cancer [J]. Nat Med, 2015, 21(11): 1350-6.

[92] SHEN L, TOYOTA M, KONDO Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer [J]. Proc Natl Acad Sci USA, 2007, 104(47): 18654-9.

[93] CHENG Y W, PINCAS H, BACOLOD M D, et al. CpG island methylator phenotype associates with low-degree chromosomal abnormalities in colorectal cancer [J]. Clin Cancer Res, 2008, 14(19): 6005-13.

[94] MISSIAGLIA E, JACOBS B, D'ARIO G, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features [J]. Ann Oncol, 2014, 25(10): 1995-2001.