



· 论 著 ·

结直肠腺癌差分化细胞群与临床病理学参数的相关性研究

彭 辉^{1,2}, 张志发¹, 朱贤强², 张潇涵², 秦海丽²

1. 广州中医药大学第二附属医院 / 广东省中医院病理科, 广东 广州 510120 ;
2. 广东省中医院珠海医院病理科, 广东 珠海 519000

[摘要] **背景与目的:** 基于分化程度的组织学分级在结直肠癌生物学行为的评估上存在局限性, 寻找更多的形态学指标将为结直肠癌患者的预后和分层管理提供更多依据。探讨结直肠腺癌差分化细胞群 (poorly differentiated clusters, PDC) 与临床病理学参数之间的关系及其意义。**方法:** 收集2019年1月—2020年10月在广东省中医院珠海医院行根治性手术切除的结直肠腺癌标本101例。其中男性54例, 女性47例, 年龄29~86岁, 中位年龄62岁, 肿瘤大小1.5~9.0 cm, 平均4.5 cm, 左半结肠癌42例, 右半结肠癌21例, 直肠癌38例。在光镜下观察H-E切片中结直肠腺癌PDC数量并进行分级, 分析PDC分级与结直肠腺癌其他临床病理学参数之间的关系。**结果:** 101例结直肠腺癌患者中, PDC分级G1级为42例 (41.6%), G2级为29例 (28.7%), G3级为30例 (29.7%)。结直肠腺癌中PDC与组织学分级、浸润深度、淋巴结转移、淋巴管血管侵犯、神经侵犯、远处转移分期及肿瘤芽 (tumor budding, TB) 呈正相关 ($P < 0.05$), 而与患者年龄、性别、肿瘤部位、肿瘤大小无关 ($P > 0.05$)。**结论:** PDC与结直肠腺癌侵袭性的生物学行为密切相关。PDC有可能是继TB之后的另一个比传统组织学分级更为有效的预后指标。识别和评估结直肠腺癌中PDC分级可以更好地预测结直肠腺癌的生物学行为, 从而更加准确地指导结直肠癌患者的治疗和预后评估。

[关键词] 结直肠腺癌; 差分化细胞群; 肿瘤芽

DOI: 10.19401/j.cnki.1007-3639.2021.09.007

中图分类号: R735.3 文献标志码: A 文章编号: 1007-3639(2021)09-0817-05

A study of correlation between poorly differentiated clusters and clinicopathological parameters in colorectal adenocarcinoma PENG Hui^{1,2}, ZHANG Zhifa¹, ZHU Xianqiang², ZHANG Xiaohan², QIN Haili² (1. Department of Pathology, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine/Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou 510120, Guangdong Province, China; 2. Department of Pathology, Zhuhai Hospital of Guangdong Provincial Hospital of Traditional Chinese Medicine, Zhuhai 519000, Guangdong Province, China)

Correspondence to: PENG Hui E-mail: 155343633@qq.com

[Abstract] **Background and purpose:** Histological grading based on the degree of differentiation has limitations in evaluating the biological behavior of colorectal cancer. Finding more morphological indicators will provide more evidence for the prognosis and stratified management of colorectal cancer. This study aimed to investigate the relationship between poorly differentiated clusters (PDC) and clinicopathological parameters in colorectal adenocarcinoma and its significance. **Methods:** Data of 101 patients with colorectal adenocarcinoma who underwent radical surgical resection in Zhuhai Hospital of Guangdong Provincial Hospital of Traditional Chinese Medicine from January 2019 to October 2020 were collected. Among them, there were 54 males and 47 females, ranging in age from 29 to 86 years with median age of 62 years. Tumor size ranged from 1.5 to 9.0 cm (mean 4.5 cm). There were 42 cases of left colon cancer, 21 cases of right colon cancer and 38 cases of rectal cancer. The number of PDC of colorectal adenocarcinoma in H-E section was observed under light microscope and graded, and the relationship between PDC grade and other clinicopathological parameters of colorectal adenocarcinoma was analyzed. **Results:** Among 101 cases of colorectal adenocarcinoma,

基金项目: 珠海市医学科研项目 (ZH2401330210005PWC)。

通信作者: 彭 辉 E-mail: 155343633@qq.com

42 cases (41.6%) were G1 grade, 29 cases (28.7%) were G2 grade, and 30 cases (29.7%) were G3 grade. In colorectal adenocarcinoma, PDC was positively correlated with histological grade, invasion depth, lymph node metastasis, lymphovascular invasion, nerve invasion, distant metastasis stage and tumor budding (TB) ($P < 0.05$), but not with patient age, gender, tumor site or tumor size ($P > 0.05$). **Conclusion:** PDC is closely related to the invasive biological behavior of colorectal adenocarcinoma. PDC is likely to be a more effective prognostic marker than traditional histological grading after TB. Identification and evaluation of PDC grading in colorectal adenocarcinoma can better predict the biological behavior of colorectal adenocarcinoma, and thus more accurately guide the treatment and prognosis evaluation of colorectal cancer.

[Key words] Colorectal adenocarcinoma; Poorly differentiated clusters; Tumor budding

2019版世界卫生组织(World Health Organization, WHO)消化系统肿瘤分册结肠癌章节描述了肿瘤芽(tumor budding, TB)和差分化细胞群(poorly differentiated clusters, PDC)的概念^[1],并将前者列入到结直肠癌推荐的基本报告内容中,但后者暂未作为要求项目。TB被认为是比传统组织学分级更为有效的预后指标^[2],而最近研究^[3]表明,PDC很有可能是继TB之后的另一个潜在预后指标,优于常规组织学分级和其他大多数组织学参数,且其分级有较好的观察者间一致性,国内相关报道少见。本研究旨在探讨PDC与各临床病理学参数,尤其是与TB之间的关系。

1 材料和方法

1.1 材料

收集2019年1月—2020年10月在广东省中医院珠海医院行根治性手术切除的结直肠腺癌标本109例,剔除术前化疗或放疗、非治疗性姑息切除、复发性或转移性、其他原因导致无法判读(如切片丢失或无法评估)的病例后,将剩余的101例患者作为研究对象。

1.2 方法

所有切片均经H-E染色,由2名病理科医师分别进行独立判读,肿瘤的TNM分期参照第8版美国癌症联合会(American Joint Committee on Cancer, AJCC)癌症手册分成T₁₋₄、N₀₋₂、M₀₋₁期,组织学分级根据2019版WHO标准分成高级别、低级别二类,PDC的定义为:出现在癌巢中的,由≥5个癌细胞组成的,但缺乏腺样结构的肿瘤细胞簇。按照Ueno标准^[4]进行量化计数,具体操作为:首先使用低倍显微镜扫描全部切

片,在肿瘤浸润前沿找出热点区域,然后使用20倍显微镜对热点区进行计数,根据PDC的数量分为G₁^{PDC}(<5群)、G₂^{PDC}(5~9群)和G₃^{PDC}(>10群)3个等级。其他用于评估的组织学参数包括淋巴管血管侵犯(lymphovascular invasion, LVI)、神经周围侵犯(perineural invasion, PNI)和TB。LVI和PNI均分为阴性和阳性,TB的定义为^[2]:在侵袭性前沿,一个单一的癌细胞或一簇<5个癌细胞的数量,根据在物镜下观察到的最高数量的芽数,放大倍数为×20,分为G₁^{TB}(<5个芽)、G₂^{TB}(5~9个芽)和G₃^{TB}(≥10个芽)3个等级。

1.3 统计学处理

基于PDC分级与其他临床病理学参数(年龄、性别、肿瘤部位、肿瘤大小、WHO组织学分级、TNM分期、LVI、PNI和TB)的关系,采用SPSS 25.0软件对数据进行分析,计数资料采用频数和百分比,计量资料采用均数、标准差、中位数、最小值和最大值进行描述性统计汇总,采用 χ^2 检验或Fisher精确概率法检验不同临床病理学特征人群的PDC分级情况的差异, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 基本参数统计

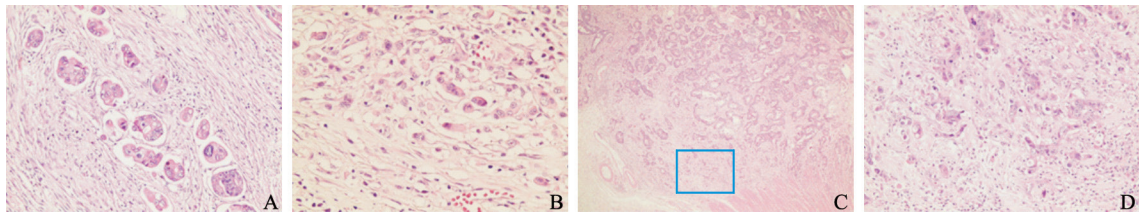
101例患者中,男性54例,女性47例,年龄29~86岁,平均年龄62岁,左半结肠癌42例,右半结肠癌21例,直肠癌38例,肿瘤平均最大径为4.5 cm,组织学分级低级别86例,高级别15例。G₁^{PDC}、G₂^{PDC}和G₃^{PDC}的病例数分别为42、29和30例,G₃^{PDC}形态见图1A;G₁^{TB}、G₂^{TB}和G₃^{TB}的病例

数分别为42、27和32例，G₃^{TB}形态见图1B。

2.2 PDC与其他病理学参数的相关性

PDC级别与组织学分级、T分期、N分期、M分期、脉管侵犯、外周神经侵犯及TB分级显著

相关 ($P < 0.05$)。而与性别、年龄、发病部位无关 ($P > 0.05$, 表1)。另外, 有近20%的低级别(高分化和中分化)结直肠癌, PDC分级为高级别(G₃), 如图1C、1D所示。



(H-E staining)

图1 PDC和TB的组织病理学形态

Fig. 1 Histopathological morphology of PDC and TB

PDC were defined as cancer cell clusters in the tumor stroma composed of ≥ 5 cancer cells and lacking a gland-like structure. The number of PDC in a single field of highest activity was then determined and graded as G₁ (<5 clusters), G₂ (5 to 9 clusters), or G₃ (≥ 10 clusters) under an objective lens with a magnification of $\times 20$. A: G₃^{PDC}. TB was defined as a single cancer cell or a cluster of <5 cancer cells at the invasive front and was graded as G₁ (<5 buds), G₂ (5 to 9 buds), or G₃ (≥ 10 buds) on the basis of the highest number of buds observed under an objective lens with a magnification of $\times 20$. B: G₃^{TB}. C, D: The same case. Figure D was a partial (blue box) enlargement of Figure C; 80% of this area could be seen with glandular tubular structures, which were classified as moderately differentiated according to traditional histological grading; but at the front of tumor infiltration, both PDC and TB were divided into G₃. The magnification of A and B was $\times 20$, the magnification of C was $\times 2$, and the magnification of D was $\times 10$.

表1 PDC与临床病理学特征的相关性

Table 1 Correlations between PDC and clinicopathological characteristics

Characteristic	Total n (%)	PDC n (%)			χ^2/z value	P value
		G ₁	G ₂	G ₃		
Gender					1.10	0.570
Male	54 (53.5)	20 (47.6)	16 (55.2)	18 (60.0)		
Female	47 (46.5)	22 (52.4)	13 (44.8)	12 (40.0)		
Median age/year	62	63	62	61	0.93	0.560
Site					0.25	0.881
Left colon	42 (41.6)	18 (42.9)	11 (37.9)	12 (40.0)		
Right colon	21 (20.8)	9 (21.4)	6 (20.7)	7 (23.3)		
Rectum	38 (37.6)	15 (35.7)	12 (41.4)	11 (36.7)		
Mean max diameter D/cm	4.5	4.3	4.4	4.6	0.21	0.781
WHO grade					15.52	<0.001
Low-grade	86 (85.1)	41 (97.6)	26 (89.7)	19 (63.3)		
High-grade	15 (14.9)	1 (2.4)	3 (10.3)	11 (36.7)		
T stage					21.04	0.001
T ₁	16 (15.8)	14 (33.3)	2 (6.9)	0 (0.0)		
T ₂	12 (11.9)	7 (16.7)	3 (10.3)	2 (6.7)		
T ₃	63 (62.4)	18 (42.9)	21 (72.4)	24 (80.0)		
T ₄	10 (9.9)	3 (7.1)	3 (10.3)	4 (13.3)		
N stage					20.82	<0.001
N ₀	50 (49.5)	31 (73.8)	10 (34.5)	9 (30.0)		
N ₁	27 (26.7)	8 (19.0)	11 (37.9)	8 (26.7)		
N ₂	24 (23.8)	3 (7.1)	8 (27.6)	13 (43.3)		
M stage					11.73	0.002
M ₀	85 (84.2)	41 (97.6)	23 (79.3)	21 (70.0)		
M ₁	16 (15.8)	1 (2.4)	6 (20.7)	9 (30.0)		

续表 1

Characteristic	Total <i>n</i> (%)	PDC <i>n</i> (%)			χ^2/z value	<i>P</i> value
		G ₁	G ₂	G ₃		
Lymphovascular invasion					27.71	<0.001
Negative	54 (53.5)	35 (83.3)	12 (41.4)	7 (23.3)		
Positive	47 (46.5)	7 (16.7)	17 (58.6)	23 (76.7)		
Perineural invasion					9.59	0.008
Negative	76 (75.2)	38 (90.5)	20 (69.0)	18 (60.0)		
Positive	25 (24.8)	4 (9.5)	9 (31.0)	12 (40.0)		
TB					35.02	<0.001
G ₁	42 (41.6)	30 (71.4)	10 (34.5)	2 (6.7)		
G ₂	27 (26.7)	9 (21.4)	8 (27.6)	10 (33.3)		
G ₃	32 (31.7)	3 (7.1)	11 (37.9)	18 (60.0)		
Total	101 (100.0)	42 (41.6)	29 (28.7)	30 (29.7)	-	-

3 讨 论

组织学分级等参数和TNM分期是WHO和AJCC认可的应用于判断结直肠癌患者预后和疗效的重要指标,但目前的评价体系存在一些问题,例如,肿瘤的分化程度是由分化最差的部分决定,还是占比最多的部分决定?WHO组织学分级是以后者(腺管状结构的比例)将结直肠癌划分为高分化、中分化和低分化三级或高级别、低级别二类(低级别相对应三分法的高分化和中分化,高级别相对应三分法的低分化),本研究对近年来广东省中医院珠海医院的结直肠癌分级进行了统计,按照此定义诊断结果为中分化的比例超过70%,从统计学分布上来看,构成比的偏差太大,代表性不强,可重复性差。多项研究^[5-7]发现,相对最近报道的肿瘤浸润前沿指标(如TB、PDC)而言,传统的组织学分级与预后虽然存在相关性,但相关系数为多个参数中最弱的一个。本研究结果也显示,有20%的G₃^{PDC}病例,按常规WHO组织学分级系统是判为低级别(高分化和中分化),说明包括PDC和TB在内的新型浸润前沿评价指标与常规WHO组织学分级不一致的情况并不少见。另外,部分TNM分期相同的结直肠癌患者,预后差异却很大。因此,迫切需要一些新的更加准确的形态学参数来对患者进行风险分层管理。PDC和TB等新型的肿瘤浸

润前沿预后评估指标就是在这种假设下产生的,TB先于PDC被文献报道,TB的研究更为深入和全面,是指肿瘤浸润的前沿(即最深处)间质内散在单个肿瘤细胞或小灶性肿瘤细胞群(<5个细胞),其被认为是比传统组织学分级和其他大多数组织学参数“更为有效的预后指标”^[8],并被2019版WHO消化系统肿瘤分册列入到结直肠癌推荐的基本报告内容中^[1],PDC暂未像TB一样列为常规报告项目,但WHO在TB章节中描述了PDC的概念。PDC虽起步较晚,但在显微镜判读时,比起TB的单个细胞或孤立细胞团,PDC是簇状细胞巢,体积更大,更容易被识别,可能更被阅片者所接受。

PDC是Ueno等^[4]提出的一种新的组织形态学特征。PDC与其他不良的组织病理学特征和较差的临床预后密切相关,PDC可以预测内镜切除的pT₁期结直肠癌的淋巴结转移情况,这有助于筛选出内镜切除恶性息肉后,需要追加外科根治切除的病例,对临床治疗有决定性的指导意义^[9]。PDC在Ⅱ期结直肠癌中具有特殊的应用前景,一项大型队列研究^[10]发现,PDC与Ⅱ期结直肠癌的预后显著相关。在患者选择辅助化疗时,使用PDC进行危险分层能起到重要作用^[5],PDC分级可以筛选出能在辅助化疗中获益的“高危”患者。另外,在进展期的病例中,PDC可以预测复发时间,这对调整术后监测具有指导意

义^[11]。此外,在关于PDC分级和传统组织学分级间可重复性的比较研究中,由5名观察者对100例结直肠癌切片进行评估,PDC分级的观察者间的总体加权Kappa值达到0.82(中度以上的一致性),而传统组织学分级的Kappa值为0.52^[4],相似的结论也出现在肠镜下活检标本中^[10],说明PDC分级在判读者间可重复性方面优于传统的组织学分级系统。

基于PDC数量的分级系统与常规组织学参数间有显著相关性,且优于常规组织学分级和大多数其他不良反应的组织学特征。一篇总结12项PDC与传统组织学分级研究的综述性文献^[12]指出,12项研究均显示PDC与不良临床后果相关,而仅有3项显示传统组织学分级与不良临床后果显著相关,且在这3项研究中有2项的PDC评分在预测不良结果方面优于常规组织学评分,预计将会有越来越多的研究支持传统的组织学参数与PDC的相关性。本研究验证了此推断,发现PDC分级与结直肠癌分化程度、TNM分期、淋巴结转移、淋巴管血管侵犯、TB等密切相关,而与患者年龄、性别和肿瘤大小无关,进一步证实PDC及其分级可作为一种预测结直肠癌侵袭性生物学行为的指标。本研究主要的局限性是缺乏生存随访数据,需要在后续研究中收集生存数据,尝试直接研究PDC与生存期数据的相关性。

总之,PDC等级与结直肠癌的侵袭行为相关,鉴于PDC等级在风险分层和可重复性方面的潜在优势,预计未来的分级分期系统很可能会考虑纳入PDC,当然,在将PDC分级纳入常规评估项目之前,其独立的预后意义应该在大样本的、多中心的研究中得到进一步验证,另外,最佳的截断值和标准化的评估方法还需要得到更广泛的认可。

[参 考 文 献]

[1] ARENDS M J, FUKAYAMA M, KLIMSTRA D S, et al. WHO classification of tumours of digestive system tumours [M]. 5th

Edition, Lyon: IARC Press, 2019: 140–155.

- [2] SLIK K, BLOM S, TURKKI R, et al. Combined epithelial marker analysis of tumour budding in stage II colorectal cancer [J]. *J Pathol Clin Res*, 2019, 5(1): 63–78.
- [3] BARRESI V, REGGIANI BONETTI L, BRANCA G, et al. Colorectal carcinoma grading by quantifying poorly differentiated cell clusters is more reproducible and provides more robust prognostic information than conventional grading [J]. *Virchows Arch*, 2012, 461(6): 621–628.
- [4] UENO H, KAJIWARA Y, SHIMAZAKI H, et al. New criteria for histologic grading of colorectal cancer [J]. *Am J Surg Pathol*, 2012, 36(2): 193–201.
- [5] NEARCHOU I P, KAJIWARA Y, MOCHIZUKI S, et al. Novel internationally verified method reports desmoplastic reaction as the most significant prognostic feature for disease-specific survival in stage II colorectal cancer [J]. *Am J Surg Pathol*, 2019, 43(9): 1239–1248.
- [6] BARRESI V, REGGIANI BONETTI L, IENI A, et al. Histologic prognostic markers in stage II A colorectal cancer: a comparative study [J]. *Scand J Gastroenterol*, 2016, 51(3): 314–320.
- [7] ROMITI A, ROBERTO M, MARCHETTI P, et al. Study of histopathologic parameters to define the prognosis of stage II colon cancer [J]. *Int J Colorectal Dis*, 2019, 34(5): 905–913.
- [8] RYAN É, KHAW Y L, CREAVIN B, et al. Tumor budding and PDC grade are stage independent predictors of clinical outcome in mismatch repair deficient colorectal cancer [J]. *Am J Surg Pathol*, 2018, 42(1): 60–68.
- [9] BARRESI V, BRANCA G, IENI A, et al. Poorly differentiated clusters (PDCs) as a novel histological predictor of nodal metastases in pT1 colorectal cancer [J]. *Virchows Arch*, 2014, 464(6): 655–662.
- [10] UENO H, HASE, HASHIGUCHI Y, et al. Site-specific tumor grading system in colorectal cancer: multicenter pathologic review of the value of quantifying poorly differentiated clusters [J]. *Am J Surg Pathol*, 2014, 38(2): 197–204.
- [11] KONISHI T, SHIMADA Y, LEE L H, et al. Poorly differentiated clusters predict colon cancer recurrence: an in-depth comparative analysis of invasive-front prognostic markers [J]. *Am J Surg Pathol*, 2018, 42(6): 705–714.
- [12] SHIVJI S, CONNER J R, BARRESI V, et al. Poorly differentiated clusters in colorectal cancer: a current review and implications for future practice [J]. *Histopathology*, 2020, 77(3): 351–368.

(收稿日期: 2021-04-27 修回日期: 2021-08-01)