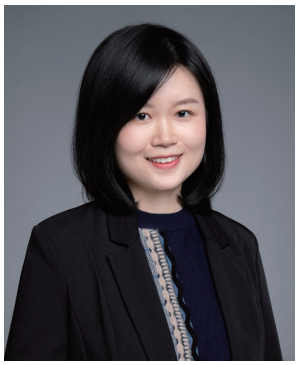

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三级淋巴样结构与抗肿瘤免疫

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摘要　三级淋巴样结构(tertiary lymphoid structure, TLS), 又称异位淋巴样器官(ectopic lymphoid organ, ELO), 是形成于非淋巴组织中的异位淋巴样器官, 通常发生在慢性炎症包括自身免疫病、感染性疾病和肿瘤等部位。TLS与淋巴结等淋巴器官有相似的结构, 包含T细胞、B细胞、滤泡树突状细胞等多种免疫细胞。在肿瘤中, TLS可以作为免疫细胞进入肿瘤组织的通道, 多与较好治疗反应和预后相关。诱导肿瘤组织中TLS形成是潜在的肿瘤治疗新策略。该文系统地综述了TLS的组成、结构、在肿瘤中的功能、与肿瘤预后的关联以及靶向TLS的相关治疗方案。

关键词　三级淋巴样结构; 肿瘤; 预后; 抗肿瘤免疫

Tertiary Lymphoid Structure and Antitumor Immunity

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Abstract　TLSs (tertiary lymphoid structures), also known as ELOs (ectopic lymphoid organs), are organized aggregates of immune cells that localize in non-lymphoid tissues at sites of chronic inflammation, such as autoimmune diseases, infectious diseases and tumors. The structure of TLS is similar with that of lymph node, which composes of T cells, B cells, follicular dendritic cells, etc. TLS serves as an entry for immune cells to infiltrate in tumor tissues. The presence of TLS commonly correlates with a better therapeutic response and a longer survival. Here, this article reviewed the composition, structure, function, the role of TLS in tumor prognosis and therapeutic strategies targeting TLS.

Keywords　tertiary lymphoid structure; tumor; prognosis; antitumor immunity

收稿日期: 2022-02-08　　接受日期: 2022-02-21

国家自然科学基金(批准号: 91942309、82125017、92057210、82002780)和广州市科技计划项目(批准号: 202103000070)资助的课题

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Received: February 8, 2022　　Accepted: February 21, 2022

This work was supported by the National Natural Science Foundation of China (Grant No.91942309, 82125017, 92057210, 82002780) and the Science and Technology Program of Guangzhou (Grant No.202103000070)

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1 TLS简介

三级淋巴样结构(tertiary lymphoid structure, TLS)是后天产生于非淋巴组织中的有组织的免疫细胞聚集结构, 在慢性炎症疾病如自身免疫病和感染性疾病组织中最早被发现, 浸润至慢性炎症部位的淋巴细胞自发形成含有生发中心(germinal center, GC)的B细胞滤泡区以及含有树突状细胞(dendritic cell, DC)和高内皮微静脉(high endothelial venule, HEV)的T细胞区。该过程被称为淋巴新生(lymphoid neogenesis)[1-3]。

肿瘤中的TLS最早在非小细胞肺癌[4]和黑色素瘤[5]中被发现, 随着研究的开展, 不同肿瘤中的TLS陆续被报道, TLS可见于肿瘤的间质、侵袭性边缘或中心部位, 更多出现在肿瘤的侵袭性边缘或间质[6]。TLS的结构类似于次级淋巴器官(secondary lymphoid organ, SLO), 根据细胞聚集区域, 可以分为T细胞区、B细胞区, T细胞区含有成熟DC和$CD3^+$ T细胞, 其附近的B细胞区具有GC特征, HEV和淋巴管(lymphatic vessel, LV)则位于TLS的边缘[7-8]。

2 TLS的主要组成及其功能

TLS主要由T细胞、B细胞、DC、滤泡树突状细胞(follicular dendritic cell, FDC)、滤泡网状细胞(follicular reticular cell, FRC)和HEV等组成, 各类细胞都具备重要的功能。

2.1 T细胞

TLS中的T细胞包括$CD8^+$ T细胞和$CD4^+$ T细胞。肿瘤免疫微环境中$CD8^+$细胞毒性T淋巴细胞(cytotoxic T lymphocyte, CTL)的功能通常会受抑制[9]。在非小细胞肺癌中, 研究者发现TLS中有一群高表达PD1的CTL, 其产生杀伤性细胞因子的能力降低, 却高表达趋化因子CXCL13。CXCL13的受体CXCR5主要表达在B细胞和滤泡辅助T细胞中, 说明TLS中一部分CTL具备募集B细胞和滤泡辅助T细胞的新功能[10-11]。

在TLS中, 辅助T细胞(helper T cell, Th)倾向于Th1分化[12]。滤泡辅助T细胞(follicular T helper cell, Tfh)主要定位于TLS-GC附近[13], 与亮区B细胞激活相关[14]。乳腺癌中存在一群$CXCR5^-CD4^+$ T细胞, 表达*BCL6*、*CD200*、*PD1*、*ICOS*等Tfh相关基因, 是TLS中分泌CXCL13的主要细胞, 研究者将其命名为TfhX13[15]。TfhX13与TLS的存在显著相关[13], 并且TfhX13能够招募B细胞并促进其向浆细胞(plasma cell, PC)和记忆B细胞分化[15], 说明TfhX13可能在TLS形成过程中具有重要作用。

在乳腺癌[16]、前列腺癌[17]中, 调节性T细胞(regulatory T cell, Treg)也存在于TLS中。Treg被选择性地招募到TLS中并可能通过抗原提呈被DC激活[16], 且Treg能抑制TLS中DC和T细胞功能[18]。值得注意的是, 在小鼠模型中Treg耗竭会引发瘤内HEV的新生[19], 提示Treg可能具有通过抑制HEV形成从而阻碍TLS生成的功能。

2.2 B细胞

在TLS中有大量B细胞聚集于TLS-GC中。TLS中B细胞的测序数据表明, 它们具有GC-B细胞基因特征, 即B细胞发生了抗原驱动的体细胞高频突变、抗体亲和力的成熟和类别转换[14,20–22]。此外, 我们前期的研究发现, 在新辅助化疗后的乳腺癌组织中有一群以$ICOSL^+$为特征的B细胞亚群, 它们多定位在TLS中, 并且与T细胞直接接触[23]。

TLS中的B细胞还可能具有抗原提呈功能。在卵巢癌远处转移灶中, 非典型记忆B细胞($CD27^-$)高表达MHCI、MHCII分子及CD40, 其与$CD8^+$ T细胞的共同出现与更好的预后相关[21]。在黑色素瘤中亦有相似发现, 高表达MHCI、MHCII分子的B细胞和T细胞的共同出现与更强的免疫检查点阻断治疗应答率、更长的总生存期相关, 而在缺乏TLS的肿瘤中的T细胞功能被抑制[24]。此外, 有研究者提出, TLS中存在一种抗原提呈B细胞(antigen-presenting B cell, BAPC; $CD86^{hi}CD21^{low}$), BAPC定位于TLS的淋巴滤泡中, 并在TLS数量较多的肿瘤中富集。从肿瘤引流淋巴结中分离的BAPC在体外能诱导自体T细胞反应, 局部激活幼稚T细胞并实现T细胞表型转换[25]。

TLS中的PC($CD138^+$)数量与B细胞的数量、TLS的数量均呈正相关[22,26]。有研究报道直接浸润肿瘤组织的PC与预后负相关[27]。然而, 在乳腺癌和高级别卵巢癌中TLS-PC与更长的总生存期相关[28-29]。

2.3 树突状细胞(DC)

成熟DC, 即表达溶酶体相关膜糖蛋白(lysosome-associated membrane protein, LAMP)的DC可以作为识别肿瘤组织中TLS的特征之一[4], TLS中的DC与肿瘤组织中的DC可能具有不同活化状态。在肾透明细胞癌中, $CD8^+$ T细胞的活化水平与TLS-DC的数量呈

正相关, 而与TLS之外的DC无关[30], 同时$CD8^+$ T细胞的功能衰竭与高密度的失能DC相关[31], 原因可能是TLS之外的DC抗原提呈功能受到抑制。

2.4 滤泡树突状细胞(FDC)

FDC促进抗原提呈至B细胞并促进B细胞表达高活性的抗体, 其出现是TLS-GC成熟的重要事件[32]。肿瘤中FDC的前体细胞仍未知, 但有研究表明, 在小鼠皮下注射淋巴结来源细胞诱导形成的TLS中, 激活的基质细胞与免疫细胞相互作用, 且其可能分化为FDC[33]。

2.5 滤泡网状细胞(FRC)

FRC形成紧密的网状支架, 是TLS中主要的基质支持细胞。

2.6 高内皮微静脉(HEV)

HEV是一种表达外周淋巴结血管地址素(peripheral lymphonode vascular addressin, PNAd)的特殊血管, 幼稚淋巴细胞和中央记忆淋巴细胞由此经循环作用进入淋巴器官[34]。HEV可以作为识别TLS的特征性标志[35], HEV的数目与肿瘤浸润淋巴细胞数呈正相关, 含有HEV的肿瘤高表达CCL19、CCL21、CCR6和L-选择素, 说明HEV是募集淋巴细胞的通道[36]。

3 TLS与肿瘤预后的关系

目前报道中, TLS与大部分肿瘤如非小细胞肺癌[4,37]、结直肠癌[32,38-39]、黑色素瘤[40-41]、乳腺癌[13]、膀胱癌[42-43]、头颈鳞状细胞癌[14]、胃食管腺癌[26]等的良好预后相关[44]。TLS见于各个分期的肿瘤, 是独立的预后预测因素(表1)。在非小细胞癌中, 对于早期和晚期的癌症患者, 高密度TLS患者组的总生存期(overall survival, OS)、无复发生存期(recurrence free survival, RFS)、无病生存期(disease free survival, DFS)均较低密度组更长[4,37,45]。此外, TLS还被报道与免疫治疗应答相关, 在接受免疫检查点阻断(immune checkpoint blockade, ICB)治疗的胃癌[46]、非小细胞肺癌[47]、晚期膀胱癌[48-49]、黑色素瘤[24,50]和软组织肉瘤[51]患者中, 高密度TLS与更好的治疗反应和预后相关。

但是, 在部分研究中, TLS的存在与更差的预后相关, 如肝癌中TLS与肿瘤进展相关[52], ER^+HER2^-乳腺癌中TLS与更高的肿瘤分期相关[53]。在肾透明细胞癌中, CXCL13和TLS均与无进展生存期(progression free survival, PFS)和OS呈负相关[54], 且SOBOTTKA等[55]在ICB无应答患者观察到了更高密度的TLS。TLS对预后的不同影响可能源于TLS分化程度、空间位置等方面的差异。

3.1 不同TLS分化程度与预后的关系

肿瘤中TLS存在不同的分化阶段, 它们可能在肿瘤免疫中发挥不同的作用。POSCH等[32]依据是否存在FDC和B细胞的分化情况, 将肿瘤中的TLS分为三个连续的发展阶段。早期TLS(early-TLS、E-TLS、$CD21^-CD23^-$): 密集的淋巴细胞簇, 不含有FDC; 初级滤泡样TLS(primary follicle-like TLS, PFL-TLS, $CD21^+CD23^-$): 存在FDC和B细胞作用网络, 但不含有GC; 次级滤泡样TLS(secondary follicle-like TLS, SFL-TLS, $CD21^+CD23^+$): 含有FDC和活跃的GC。结直肠癌和肝癌中, 相比于肿瘤中含有E-TLS的患者, 肿瘤中含有成熟TLS的患者呈现更低的复发率[32,56], 同时另一项研究观察到, 在早期肝癌或癌前病变中仅能观察到未成熟TLS(几乎为E-TLS), 且伴随免疫抑制分子(IL10RA、TGFB1、LILRB2)的过表达[57], 这些结果表明成熟TLS可能比早期TLS有更强的抗肿瘤功能。此外, TLS中Treg的比例也会影响其功能。肺腺癌小鼠中的TLS含有大量Treg[18]。在黑色素瘤中亦发现, TLS通过招募Treg细胞起到免疫抑制作用[58]。

3.2 不同空间位置的TLS与预后的关系

TLS可以分布在肿瘤组织中、肿瘤间质内或肿瘤旁。在肝癌中, 高密度的癌内TLS与低复发率相关, 而癌旁非肿瘤组织内TLS无此预后价值[56], 乳腺癌中高密度的癌周TLS则与最差的DFS和OS相关[59]。对962位肝内胆管细胞癌患者的回顾性分析也有相似发现, 高密度的癌内TLS指示良好预后, 而癌周TLS的存在与不良预后相关, 进一步探究, 在癌内TLS中, $CD4^+Bcl6^+$ Tfh与$CD4^+$ T细胞显著多于癌周TLS, 提示不同位置的TLS对预后的影响可能与其组成不同相关[60]。

肿瘤原发灶和转移灶中的TLS也可能具有不同功能。在结直肠癌转移性肺癌中, TLS的存在与更长的OS相关, 而肾细胞癌转移性肺癌却得出相反的结论, 进一步分析发现, 结直肠癌转移性肺癌TLS中有更多的成熟DC且其具有Th1极化基因特征, 而肾细胞癌转移性肺癌表达Th2极化、免疫抑制性的基因特征[61]。

表1 肿瘤TLS与预后的关系

Table 1 TLS in tumor prognosis

肿瘤类型 Tumor type	肿瘤分期 Tumor stage	患者数量 Number of patients investigated	TLS检测方法 Methods of TLS detection	检测标志物 TLS markers	阳性患者率% % of positive patients	TLS位置 TLS location	主要抗肿瘤细胞 Major antitumor cell	对预后影响 Impact on prognosis	对辅助治疗影响 Impact on adjuvant therapies	参考文献 Reference
Lung cancer	I-IV	150	mIF	CD3, CD20	NA	NA	NA	Prolonged RFS, HR=0.28 (0.13-0.58)	NA	[37]
	Early	74	IHC	CD3, CD20, DC-Lamp	NA	Tumor	NA	Prolonged OS, TLS^{lo} *vs* TLS^{hi} HR=1.88 (1.21-2.96); Prolonged DSS, TLS^{lo} *vs* TLS^{hi} HR=3.34 (1.64-8.68); Prolonged DFS, TLS^{lo} *vs* TLS^{hi} HR=2.11 (1.19-3.89)	NA	[4]
	I-II	74	IHC	CD3, CD20, DC-Lamp	NA	NA	B cell	No significant influence on DSS	NA	[22]
	IIA-IIIB	122	IHC	CD3, CD20, DC-Lamp	NA	NA	NA	NA	Newadjuvant chemotherapy: prolonged DSS, TLS^{lo} *vs* TLS^{hi} HR=4.2 (1.9-9.5)	[22]
	I-IV	362	IHC	CD3, CD8, CD20, DC-Lamp, PNAd	54	Tumor	Mature DC, $CD8^+$ T cell	Prolonged OS, HR=0.71 (0.62-0.81)	NA	[62]
	I-IIIA	20	HE	NA	78	NA	NA	NA	Anti-PD1: associated with better MPR	[47]
Breast cancer	I-IIIC	112	IHC	CD4, CD8, FoxP3	87.5	Peritumor	NA	Shortened OS, TLS^{lo} *vs* TLS^{hi} HR=0.277 7 Shortened DSS, TLS^{lo} *vs* TLS^{hi} HR=0.285 2	NA	[59]
	I-III	248	HE	NA	37.5	Peritumor	NA	Prolonged DFS in $HER2^+$ breast cancer, P=0.044	NA	[53]
	I-IV	996	IHC	CD3, CD4, CD8, CD20, CD23, CXCL13	NA	Tumor	NA	NA	Newadjuvant chemotherapy: associated with better pCR	[13]

续表1

肿瘤类型 Tumor type	肿瘤分期 Tumor stage	患者数量 Number of patients investigated	TLS检测方法 Methods of TLS detection	检测标志物 TLS markers	阳性患者率% % of positive patients	TLS位置 TLS location	主要抗肿瘤细胞 Major antitumor cell	对预后影响 Impact on prognosis	对辅助治疗影响 Impact on adjuvant therapies	参考文献 Reference
Hepatocellular carcinoma	NA	82	12-CGS	NA	18.0	Liver parenchyma	NA	Increased risk for late recurrence P=0.03, decreased OS after resection P=0.01	NA	[52]
	I-IV	273	HE	NA	47.0	Tumor	NA	Decreased risk for early recurrence, HR=0.46	NA	[56]
Intrahepatic cholangiocarcinoma	I-IV	39	mIHC	CD3, CD8, CD20	NA	Peritumor, intratumor	NA	Prolonged OS, P<0.01	NA	[60]
Melanoma	III-IV	32	mIF	CD4, CD8, CD20, CD21, FoxP3	NA	NA	TLS-B cell	NA	Anti-PD1: associated with higher response rate	[50]
	IV	160	IHC	CD3, CD8, CD20,	NA	NA	$CD8^+$T cell, $CD20^+$B cell	NA	Anti-PD1: prolonged OS, P=0.0012	[24]
Sarcoma	NA	93	IHC	CD3, CD20, DC-Lamp	11.8	NA	B cell	NA	Anti-PD1: associated with higher response rate and DFS	[51]
Head and neck squamous cell carcinoma	I-IV	50	IHC	CD4, CD20	NA	Peritumor, intratumor	GC-B cell	Prolonged PFS, HR=0.46	NA	[14]
Bladder cancer	II-III	542	IHC	CD3, CD8, CD79a, CD68	NA	Peritumor, intratumor	NA	Prolonged DSS, HR=0.32 (0.18-0.55)	NA	[42]
	Advanced	348	HE	NA	NA	NA	NA	Patients without ICB treatment: no significant influence on OS	Patients with ICB treatment: prolonged OS, HR=0.8 (0.68-0.94)	[48]
Urothelial cancer	III	24	IF	CD3, CD23	NA	Tumor	NA	NA	Anti-PD1+anti-CTLA4: associated with better pCR	[49]

续表1

肿瘤类型 Tumor type	肿瘤分期 Tumor stage	患者数量 Number of patients investigated	TLS检测方法 Methods of TLS detection	检测标志物 TLS markers	阳性患者率% % of positive patients	TLS位置 TLS location	主要抗肿瘤细胞 Major antitumor cell	对预后影响 Impact on prognosis	对辅助治疗影响 Impact on adjuvant therapies	参考文献 Reference
Colorectal cancer	II-III	109	IF	CD20, CD21, CD23, CXCL13	NA	NA	NA	Decreased risk for recurrence, SFL-TLS^{lo} *vs* SFL-TLS^{hi} SHR=3.73 (1.39-10.00)	NA	[32]
	II-III	351	IHC	CD3	78.6	Invasive margin	NA	Stage Ⅱ: prolonged DFS, HR=0.62 (040-0.97)	NA	[39]
	I-IV	28	IHC	CD3, CD20, CD21	NA	Peritumor, intratumor	NA	Associated with higher tumor stage, P<0.001	NA	[38]
Gastric cancer	I-IV	19	IHC	CD20	47.0	NA	NA	NA	Anti-PD-1: prolonged OS, P=0.045	[46]
Clear cell renal cell carcinoma	NA	100	HE	NA	40.0	NA	NA	Associated with higher PD-L1 expression	NA	[54]
	NA	500	12-CGS	NA	NA	NA	NA	Prolonged PFS P<0.001 Prolonged OS P<0.001	NA	[54]
	I-IV	8	HE	NA	NA	NA	NA	NA	Anti-PD1: associated with higher non-response rate	[55]
Metastatic melanoma	IIIB-IV	64	mIF	CD8, CD20, PNAd, Ki67	47.0	Tumor	NA	Prolonged OS, HR=0.51 (0.27-0.97)	NA	[41]
	IV	120	12-CGS	NA	NA	Peritumor, intratumor	NA	Prolonged OS, P=0.008	NA	[40]
Lung metastases of colorectal cancer	I-IV	140	IHC	CD3, CD8, CD20, DC-Lamp, PNAd	NA	Tumor	CD8$^+$ T cell, LAMP$^+$ DC	Prolonged OS, HR=0.54 (0.39-0.76)	NA	[61]
	I-IV	52	IHC	CD3, CD8, CD20, DC-Lamp, PNAd	NA	Tumor	CD8$^+$ T cell, LAMP$^+$ DC	Shortened OS, HR=2.68 (1.58-4.57)	NA	[61]

IF: 免疫荧光; IHC: 免疫组化; HE: 苏木精－伊红染色法; OS: 总生存期; RFS: 无复发生存期; DFS: 无病生存期; DSS: 疾病特异性生存期; DCR: 疾病控制率; pCR: 病理完全缓解; PR: 部分缓解; MPR: 病理学显著缓解; 12-CGS: 12趋化因子基因特征; HR: 风险比; SHR: 部分风险比; TLS: 三级淋巴样结构; NA: 未知。

IF: immunofluorescence; IHC: immunohistochemistry; HE: hematoxylin-eosin staining; OS: overall survival; RFS: recurrence-free survival; DFS: disease-free survival; DSS: disease-specific survival; DCR: disease control rate; pCR: pathologic complete response; PR: partial response; MPR: major pathologic response; 12-CGS: 12-chemokine gene signature; HR: hazard rate; SHR: substitution hazard rate; TLS: tertiary lymphoid structure; NA: not available.

4 TLS在肿瘤中的功能

TLS与肿瘤预后密切相关, 其在肿瘤微环境中发挥的功能逐渐被揭示。

4.1 TLS与体液免疫

从具有高密度TLS的肿瘤标本中分离的B细胞于体外培养, 在上清中可以检测更多的肿瘤抗原特异性的IgG、IgA抗体, 提示TLS中效应B细胞具备抗肿瘤作用[22]。在卵巢癌转移灶中的TLS-B细胞具有记忆表型, 产生抗体包括IgG1和IgG3[63]。此外, 肝细胞癌中, 位于肿瘤边缘与T细胞共定位(类似TLS结构)的$IgD^{-}IgG^{+}CD27^{-}CD38^{-}CD20^{+}$ B细胞, 能够通过分泌颗粒酶B和TRAIL直接杀伤肿瘤细胞[64]。

4.2 TLS与细胞免疫

HEV招募幼稚T淋巴细胞至瘤内, 在肺癌中, 幼稚T细胞与肿瘤的其他部位相比也在TLS中富集, 说明外周进入的幼稚T细胞可能优先定位在TLS[62,65]。与TLS^{low}肿瘤相比较, TLS^{hi}肿瘤亦有更多的效应记忆$CD8^{+}$ T细胞浸润, 伴随T细胞激活、Th1极化和T细胞毒性作用的相关基因的过表达[5,30,62], 说明TLS是效应T细胞浸润和活化的重要部位。TLS中CTL的活化受到了B细胞的推动, 研究发现TLS中B细胞与CTL表达穿孔素和颗粒酶B的水平呈正相关[21,24-25,66]。在一个脾切除的$LT\alpha^{-/-}$黑色素瘤小鼠模型中, SLO完全缺乏可诱导TLS生成, 同时在瘤内发生T细胞的招募和特异性T细胞反应[67], 提示TLS可以独立于SLO发生细胞免疫作用。

4.3 TLS与治疗诱导免疫

TLS与化疗和免疫治疗的疗效密切相关。对接受新辅助化疗前后的肿瘤组织进行对比, 我们发现接受新辅助化疗的乳腺癌中存在更多的TLS, 尤其是在对治疗有反应的肿瘤中, 定位于TLS中的$ICOSL^{+}$ B细胞能够激活T细胞, 产生抗肿瘤效应[23]。在临床研究中, 接受新辅助化疗联合免疫治疗的非小细胞癌患者中, 肿瘤局部TLS和淋巴细胞数目与患者病理完全缓解率呈正相关[68]。

免疫治疗方面, 在黑色素瘤[24,50]和软组织肉瘤[51]中, TLS-B细胞与患者对anti-PD1治疗的反应性及预后密切相关。非小细胞肺癌中, ICB治疗患者的不良预后与TLS中失调的Tfh-B-组织驻留记忆T细胞(tissue-resident memory $CD8^{+}$ T cell, Trm)相互作用有关[69]。

4.4 TLS的促瘤作用

据报道, TLS在少数肿瘤中具有促进肿瘤发生发展的作用。在肝细胞癌发生初期, 肝脏TLS中能够观察到增殖分化的肝癌祖细胞, 提示TLS可能为癌细胞提供了适宜的生长环境[52]。在前列腺癌中, TLS内含有免疫抑制表型的PC, 其通过分泌淋巴毒素(lymphotoxin, LT)激活前列腺癌干细胞, 从而促进肿瘤发展[70]。在$HER2^{-}$乳腺癌[53]和膀胱癌[71]中观察到肿瘤细胞浸润至部分TLS中, 而这一现象与淋巴结转移相关, 说明这些TLS可能为肿瘤的淋巴结转移提供了有利条件。

5 TLS的形成机制

并非在所有的肿瘤中都能观察到TLS, 这意味着肿瘤微环境需要具备一定的条件才能驱动TLS的形成, 然而, 目前大部分对于TLS形成的研究在自身免疫病模型或者慢性炎症疾病虽已被开展, 但在肿瘤中TLS的形成机制尚不清楚。

鉴于TLS与SLO结构相似, 有学者认为它们可能具有类似的形成过程。在SLO形成过程中, 淋巴组织诱导细胞(lymphoid tissue inducer cell, LTi)和淋巴组织组织细胞(lymphoid tissue organizer cell, LTo)的相互作用是形成过程中的关键事件, 二者通过LTα1β2-LTβR或TNF-TNFR1信号通路促进趋化因子(CCL19、CCL21、CXCL12、CXCL13)和黏附因子(VCAM1、ICAM1、MADCAM1)、血管内皮生长因子(VEGF-C)的释放, 招募淋巴细胞和刺激HEVs的生成[72], 趋化因子(CXCL13和CCL21)再进一步控制B细胞、T细胞的分离和区域化[73]。

在肿瘤TLS中, 多种免疫细胞和基质细胞被发现具有LTi或LTo细胞的表型特征, 这些细胞通过LTα1β2-LTβR或TNF-TNFR1信号通路诱导TLS形成。在小鼠黑色素瘤模型中, 肿瘤相关成纤维细胞(cancer-associated fibroblast, CAF)具备LTo细胞特征, 表达LTβR和TNFR1, 肿瘤内CD8 T细胞和$LT\alpha1\beta2^{+}$ B细胞则协同作为LTi细胞, $CD8^{+}$ T细胞促进淋巴细胞的初始聚集和网状结构的形成, B细胞被CAF表达的CXCL13招募, 随后通过LTβR信号通路驱动CAF增殖和TLS扩增[74]。在非小细胞肺癌中, 固有淋巴样细胞(innate lymphoid cell, ILC) NCR^{+} ILC3具有LTi细胞特征, 表达LTα、TNFα, 定位于TLS边缘且可以分泌细胞因子[75]。结直肠癌中的$NKp44^{+}$ ILC3高表达LTA、LTB, 通过LTα1β2与基质细胞作用诱导TLS形成[38]。在自身免疫病或者感染性疾病中, 成纤维细

胞[76-77]、B细胞[78]、Th 17细胞[79-80]、和M1巨噬细胞[81]起到类似LTi细胞的作用, 从而诱导TLS的发生。

淋巴细胞趋化因子与TLS的形成密切相关。在TLS^{hi}和TLS^{low}肿瘤中进行对比, TLS^{hi}肿瘤中有趋化因子呈高表达水平, 基于此, 研究人员开发出的12趋化因子(包含了CCL19、CCL21、CXCL13等趋化因子)表达水平特征也用于预测肿瘤中TLS的存在, 且已在多种肿瘤中得到证实[40,82-83]。趋化因子可由不同的基质细胞或免疫细胞分泌, 如乳腺癌中的CAF可分泌CXCL12、CXCL13[84], 卵巢癌中的$CD8^+$ T细胞可在TGFβ存在的情况下分泌CXCL13, 并募集B细胞[85], 而CCL19和CXCL12则与DC、B细胞和PC的定位相关[86]。

由此可见, TLS和SLO的形成过程可能有共同的下游信号分子, 而引发TLS出现的最初信号则与传统的淋巴器官形成过程有很大差异, 还有待进一步的研究。

6 靶向TLS治疗的新策略

鉴于TLS明确的抗肿瘤作用, 靶向TLS在肿瘤治疗中具有重要潜力, 如何诱导抗肿瘤TLS的形成也成为研究热点之一。有研究表明通过向小鼠直接输入LTo细胞可以促进TLS形成[87], 例如, 在C57BL/6小鼠中皮下注射淋巴结来源的成纤维基质细胞可以诱导TLS在原位形成, 而后注入MC38肿瘤溶解液处理过的DC后, 科研人员能观察到TLS中有IFNγ和细胞毒性效应分子的分泌; 而在TLS旁注射MC38肿瘤细胞, 成瘤明显受到抑制[87]。

B细胞是TLS的重要组成部分, 在胶质瘤小鼠模型中开展的两项研究中, 一项通过瘤内注射腺病毒表达的CD40L(CD40L表达于活化T细胞, 与CD40结合是B细胞活化的重要第二刺激信号)可以诱导TLS形成, 科研人员可观察到肿瘤的缩小甚至消失[88]; 另一项通过静脉注射激动性CD40抗体(αCD40), 科研人员在肿瘤组织附近的脑膜中可观察到TLS形成, 且TLS的形成依赖于B细胞, 同时治疗组的B细胞中的LTα表达水平升高, 提示αCD40可能刺激B细胞通过LTα信号促进TLS形成[89]。

趋化因子也被应用于TLS和抗肿瘤免疫的诱导。在胰腺导管腺癌(pancreatic ductal adenocarcinoma, PDAC)小鼠瘤内注射CXCL13、CCL21后, 能够观察到TLS的形成, 该治疗与吉西他滨联合应用相比单药有更显著的肿瘤抑制效应[90]。在小鼠实验中, 含有LTα1β2、CCL19、CCL21、CXCL12、CXCL13、RANKL缓释凝胶的海绵胶原支架可以募集淋巴细胞, 形成人工TLS, 其中富集的B、T细胞主要为记忆表型[91]。

肿瘤疫苗也被报道能够诱导TLS形成。高级别宫颈上皮内瘤变(CIN2/3)患者注射HPV疫苗后, 原病变的附近可以形成TLS, 而未注射疫苗的患者仅有弥漫性散在淋巴细胞浸润[92]。装有免疫调节因子的多孔3D打印支架和卵清蛋白(ovalbumin, OVA)能够在小鼠体内诱导人工TLS的形成, 抑制肿瘤生长, 并且可以与anti-PD1治疗协同作用, 显著延长小鼠生存时间[93]。

诱导TLS形成与免疫治疗结合使用时, 或许可以提高肿瘤对治疗的敏感性。近期围绕PDAC开展的几项研究表明, 在非免疫原性肿瘤中诱导TLS形成可能可以转换肿瘤的免疫表型, 将“非免疫原性”肿瘤变为“免疫原性”肿瘤, 增强免疫治疗应答反应。PDAC通常被认为是“非免疫原性”肿瘤, 缺乏肿瘤浸润效应淋巴细胞[94], 并且对免疫治疗的反应较差[95]。但在小鼠模型中, 静脉注射LIGHT-VTP(一种血管靶向肽)可以诱导TLS形成, LIGHT作为TNF超家族的成员之一, 其配体为LIGHTR和LTβR, 与anti-PD1、anti-CTLA4三联治疗能延长平均生存时间[96]; 由抗纤维化的α-mangostin和编码LIGHT的质粒组成的Nano-sapper能增加瘤内淋巴细胞浸润, 诱导TLS形成, 与对照组、化疗组(吉西他滨)、单独anti-PD1组相比较, Nano-sapper和anti-PD1联合治疗在小鼠中可以获得最大程度的肿瘤坏死以及最长的生存时间[97]。在PDAC患者皮内注射肿瘤疫苗GVAX, 并联合环磷酰胺治疗, 可以在瘤内形成TLS, 与更长的OS相关, 而在未接受疫苗治疗的患者中, 人们仅能观察到瘤内少量的淋巴细胞浸润[98]。尽管诱导或增强TLS功能可能改善抗肿瘤免疫, 但鉴于TLS在自身免疫疾病中作用, 这种干预可能同时促进自身反应性T和B细胞反应, 可能产生的副作用同样值得重视。

7 展望

TLS在抗肿瘤免疫中承担重要角色, 靶向TLS是潜在的肿瘤治疗方案。目前, 肿瘤TLS形成和作用机制仍不明确, 相关研究不仅有助于我们深入认识肿瘤微环境, 更对开发新的肿瘤治疗药物有重大意义。

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