

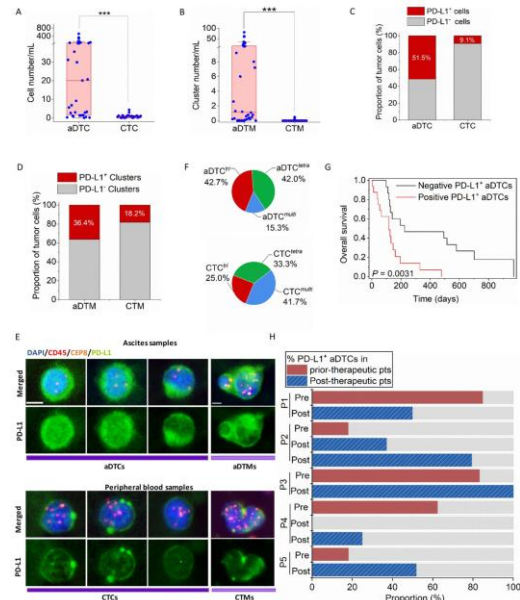
新进展 – 揭示 HER2+/PD-L1+ 恶性腹水异倍体肿瘤细胞 DTCs 的形成与免疫逃逸机制

常见于消化道 (gastrointestinal, GI) 肿瘤的腹膜转移 (peritoneal metastasis, PM) 具有极高的致死率。腹膜上的肿瘤细胞可脱落于腹腔, 形成恶性腹水中的播散型肿瘤细胞 (malignant ascites-disseminated tumor cells, maDTCs)。北京大学肿瘤中心暨北京肿瘤医院消化道肿瘤内科沈琳主任团队与赛特生物密切合作, 在 2020 年美国 AACR 肿瘤大会上已首次报道了应用 SE-i•FISH 研究 24 例 GI 肿瘤患者 (包括 15 例胃癌, 2 例胰腺癌, 1 例食管癌, 6 例结直肠癌) 恶性腹水中 HER2⁺ 及 PD-L1⁺ maDTCs 的成果。maDTCs 数量远远高于等体积外周血中的 CTCs 数量。15 例胃癌患者的检测结果显示, 11% 的异倍体 CTCs 及 50% 异倍体 maDTCs 为 PD-L1⁺。在所有 15 例胃癌病理组织活检均为 hHER2 阴性的患者体内, 血液中 HER2⁺/PD-L1⁺ 双阳的 CTCs 有 1.6% 检出率, 而恶性腹水中 HER2⁺/PD-L1⁺ maDTCs 的阳性检出率可高达 22%, 但 CTC 与 maDTC 两者之间无论细胞数量或瘤标表达互为独立, 无显著相关性 (Chen et al. 2020 Cancer Res 80:5381)。



流式细胞检测发现, 腹水中与免疫抑制功能密切相关的 Tregs 细胞浓度明显高于外周血, 提示 HER2⁺/PD-L1⁺ maDTCs 在 Tregs 参与构成的具有免疫抑制功能的肿瘤微环境中能够逃逸宿主免疫系统的攻击, 可作为临床治疗的潜在靶标, 用于治疗患有恶性胸水的 GI 肿瘤病人。

伴有腹膜转移的胃癌常因治疗无效，成为临床致死率最高的肿瘤。不同于组织中的肿瘤微环境 (tumor microenvironment, TME)，胃癌恶性腹水中具有免疫抑制功能的外源性肿瘤微环境 (extrinsic TME) 与疗效不佳密切相关，但其机理仍有待揭示。为此，沈琳主任团队在上述实验的基础上，应用赛特生物 SE-i•FISH 肿瘤液体活检及新格元 (Singleron) 单细胞 RNA 测序 (scRNA-seq) 技术，针对 51 例胃癌腹膜转移的患者进一步开展了腹水 TME 如何诱导、促进 PD-L1⁺ maDTC 的形成及免疫逃逸的深入研究，实验成果最近已得到发表 (Li et al. 2024 Gastric Cancer 27:519)。



SE-i•FISH 瘤标表达定量分析显示，异倍体 aDTCs、aDTMs 上的 PD-L1 荧光染色强度明显高于 CTCs 和 CTMs，提示 aDTCs、aDTMs 具有更高的 PD-L1 蛋白表达。

进一步的 scRNA-seq 研究证实，恶性腹水中存在两种不同的肿瘤相关巨噬细胞 (tumor-associated macrophages, TAMs)，分别为组织蛋白酶 (cathepsin, CTS) mRNA 高表达的 CTS^{high} TAMs、补体 complement 1Q mRNA 高表达的 C1Q^{high} TAMs。CTS^{high} TAMs 的主要作用是促进腹膜上的肿瘤细胞 (metastatic tumor cells) 脱落入腹腔，形成富含 aDTCs 的恶性腹水，C1Q^{high} TAMs 继而促进 aDTCs 的增生及介导 aDTCs 的免疫逃逸。腹水中 CTS^{high} TAMs 不断经历着向具有免疫抑制功能的 C1Q^{high} TAMs 的谱系转化 (lineage transition)，从而维持着恶性腹水中 aDTCs 的生成、增殖及免疫逃逸。

研究发现，与前述实验结果相同，aDTCs 及具有更高转移能力的 aDTC 细胞团 (aDTMs)、PD-L1⁺ aDTCs、PD-L1⁺ aDTMs 数目均高于等体积外周血中相应的各类 CTC 或 CTM。腹水中检出 PD-L1⁺ aDTCs 的患者，总生存期 (OS) 明显低于 PD-L1⁻ 患者 ($P=0.0031$)。

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ORIGINAL ARTICLE

Specific lineage transition of tumor-associated macrophages elicits immune evasion of ascitic tumor cells in gastric cancer with peritoneal metastasis

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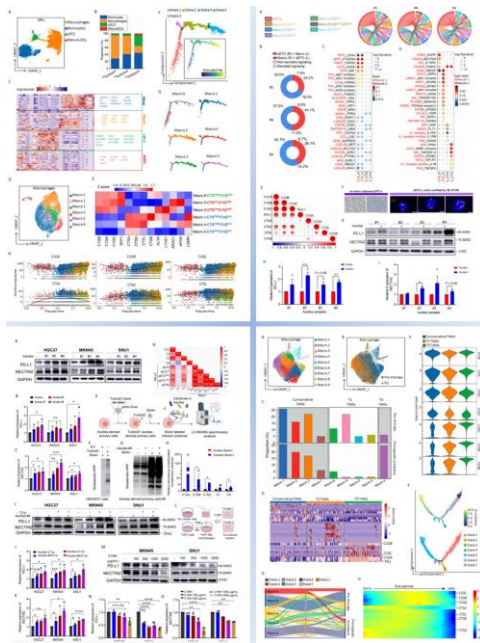
Abstract

Background Gastric cancer with peritoneal metastasis (PM-GC), recognized as one of the deadliest cancers. However, whether and how the tumor cell-extrinsic tumor microenvironment (TME) is involved in the therapeutic failure remains unknown. Thus, this study systematically assessed the immunosuppressive tumor microenvironment in ascites from patients with PM-GC, and its contribution to dissemination and immune evasion of ascites-disseminated tumor cells (aDTCs).

Methods Sixty-three ascites and 43 peripheral blood (PB) samples from 51 patients with PM-GC were included in this study. aDTCs in ascites and circulating tumor cells (CTCs) in paired PB were immunophenotypically profiled. Using single-cell RNA transcriptional sequencing (scRNA-seq), crosstalk between aDTCs and the TME features of ascites was inspected. Further studies on the mechanism underlying aDTCs-immune cells crosstalk were performed on *in vitro* cultured aDTCs.

Results Immune cells in ascites interact with aDTCs, prompting their immune evasion. Specifically, we found that the tumor-associated macrophages (TAMs) in ascites underwent a continuum lineage transition from cathepsin^{high} (CTS^{high}) to complement 1q^{high} (C1Q^{high}) TAM. CTS^{high} TAM initially attracted the metastatic tumor cells to ascites, thereafter, transitioning terminally to C1Q^{high} TAM to trigger overproliferation and immune escape of aDTCs. Mechanistically, we demonstrated that C1Q^{high} TAMs significantly enhanced the expression of PD-L1 and NECTIN2 on aDTCs, which was driven by the activation of the C1q-mediated complement pathway.

Conclusions For the first time, we identified an immunosuppressive macrophage transition from CTS^{high} to C1Q^{high} TAM in ascites from patients with PM-GC. This may contribute to developing potential TAM-targeted immunotherapies for PM-GC.



相关机理研究证实，C1q-补体激活路径促进了 C1Q^{high} TAMs 介导的 aDTCs 黏连蛋白-2 (NECTIN2) mRNA 高表达，以及 PD-L1 的 mRNA 及蛋白两个层面的高表达。

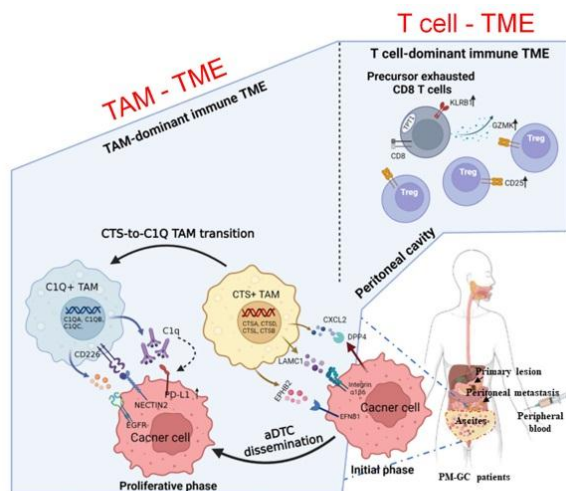
腹水中的不同 TME 及各类细胞的相互关联汇总如下：

恶性腹水中存在两类肿瘤免疫微环境 (immune TME)，TAM 为主的 TME (TAM - TME) 和 T 细胞为主的 TME (T cell - TME)。在 TAM 为主的 TME 中，TAM 不断从 CTS 谱系向 C1Q 谱系转化，从而确保了 aDTCs 的生成、增殖、免疫逃逸及治疗耐药过程的持续进行。而 T 细胞为主的 TME 中，含有大量具有免疫抑制功能的 Tregs，它们不构成免疫热肿瘤 (immune-hot) TME，其包含的 CD8⁺ T 细胞属于 CZMK⁺ 前体耗竭细胞 (precursor-exhausted cells)，不具备杀伤肿瘤细胞的功能。

随着人们对肿瘤腹膜转移、恶性腹水肿瘤细胞 DTC 的形成以及免疫抑制性肿瘤微环境认知的不断加深，将为临床有的放矢地开展针对伴有恶性腹水肿瘤的精准治疗提供更为客观的有效依据。

原文下载：

<http://www.cytointelligen.com/zlwz>，第 74R2 篇文章。



Graphical Abstract
Schematic of the immune TME of ascites and the crosstalk with aDTCs in patients with PM-GC. In ascites with TAM-dominant TME, the ascitic TAMs undergo CTS-to-C1Q transition to support multiple phases of aDTC dissemination, including aDTC homing, proliferation, immune escape, and therapeutic resistance. While in ascites with T-cell-dominant TME, infiltrated CD8⁺ T cells do not imply "immune-hot" TME. Infiltrated CD8⁺ T cells are GZMK⁺ precursor-exhausted cells that have lost their capacity to kill tumor cells. (Abbreviations: aDTC ascites-disseminated tumor cells, CTS cathepsin, TAM Tumor-associated macrophages, TME tumor microenvironment)