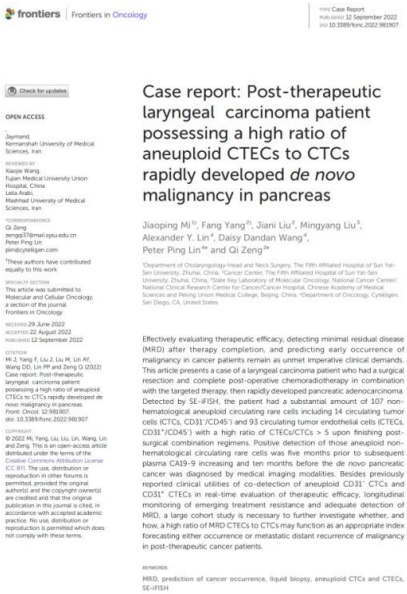
MRD 高比值 CTEC/CTC 与治疗后肿瘤发生密切相关

肿瘤微小残留病灶 (minimal residual disease, MRD) 的重要意义近来已受到国内外广大临床医生的密切关注。对全程治疗结束后肿瘤患者体内的 MRD CTC、CTEC 进行检测，可以帮助临床医生客观评估疗效、实时监测耐药、预测复发风险及治疗后出现的新发肿瘤，这对肿瘤个体化精准治疗具有十分重要的现实意义。最近，广州中山大学五院、中国医学科学院暨北京协和医学院肿瘤医院、国家癌症中心、国家肿瘤临床医学研究中心、分子肿瘤学国家重点实验室及赛特生物应用 SE-i•FISH 技术，针对肿瘤患者 MRD $CD31^-$ CTC 及循环肿瘤血管内皮细胞 $CD31^+$ CTEC 开展了深入研究，其中一例接受全程治疗后的喉癌患者 MRD 高比值 CTEC/CTC 与随后新发瘤高度相关，该案例与之前北京协和医院报道的 CTEC 与胰腺癌术后快速远端复发密切相关[1]的结果相似，引起了人们的格外关注，相关研究刚刚得到发表。中大五院肿瘤中心曾奇主任及赛特生物林平博士为本文通讯作者。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Case report: Post-therapeutic laryngeal carcinoma patient possessing a high ratio of aneuploid CTECs to CTCs rapidly developed *de novo* malignancy in pancreas

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Effectively evaluating therapeutic efficacy, detecting minimal residual disease (MRD) after therapy completion, and predicting early occurrence of malignancy in cancer patients remain as unmet imperative clinical demands. This article presents a case of a laryngeal carcinoma patient who had a surgical resection and complete post-operative chemoradiotherapy in combination with the targeted therapy, then rapidly developed pancreatic adenocarcinoma. Detected by SE-iFISH, the patient had a substantial amount of 107 non-hematological aneuploid circulating rare cells including 14 circulating tumor cells (CTCs, $CD31^-/CD45^-$) and 93 circulating tumor endothelial cells (CTECs, $CD31^+/CD45^-$) with a high ratio of CTECs/CTCs > 5 upon finishing post-surgical combination regimens. Positive detection of those aneuploid non-hematological circulating rare cells was five months prior to subsequent plasma CA19-9 increasing and ten months before the *de novo* pancreatic cancer was diagnosed by medical imaging modalities. Besides previously reported clinical utilities of co-detection of aneuploid $CD31^-$ CTCs and $CD31^+$ CTECs in real-time evaluation of therapeutic efficacy, longitudinal monitoring of emerging treatment resistance and adequate detection of MRD, a large cohort study is necessary to further investigate whether, and how, a high ratio of MRD CTECs to CTCs may function as an appropriate index forecasting either occurrence or metastatic distant recurrence of malignancy in post-therapeutic cancer patients.

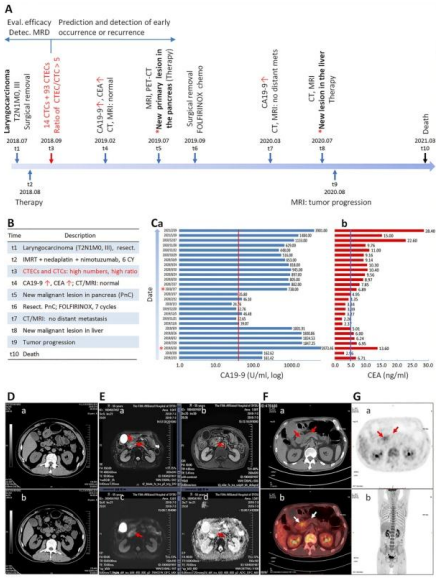
MRD, prediction of cancer occurrence, liquid biopsy, aneuploid CTCs and CTECs, SE-iFISH

案例要点

- 喉癌患者手术后接受放化疗、靶向全程联合治疗后，SE-i•FISH 检测出 107 个 MRD $CD31^+$ CTEC 及 $CD31^-$CTC，CTEC/CTC 比值> 5
- MRD CTC 及 CTEC 的检出较后期出现的 CA19-9 升高早 5 个月（此时影像学复查无异常），较影像学确诊新发胰腺癌早 10 个月
- 结果提示：全程治疗结束后检测 MRD CTC、CTEC，对客观评估疗效具有积极意义，高比值 CTEC/CTC 与肿瘤发生密切相关

案例描述

如图所示，原位喉癌（T2N1M0, III 期）手术切除后 (t1)，患者立即接受两个月的放疗、化疗（3 程奈达铂）和靶向（6 程尼妥珠单抗）联合治疗 (t2)。全程治疗结束后，采用 SE-i•FISH 技术检测 MRD (t3)。结果显示，患者体内仍存有大量 MRD $CD31^-$CTC 及 $CD31^+$ CTEC，且 CTEC 数目比 CTC 高出 5 倍以上。

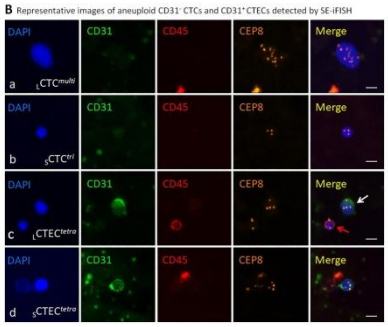


SE-i•FISH 检测 5 个月后，患者 CA19-9（161.42 U/ml, >cut-off 37 U/ml，蓝色动态检测）及 CEA（6.71 ng/ml, >cut-off 5 ng/ml，红色动态检测）持续升高 (t4)，但 CT、MRI 等一系列影像学检查结果正常。10 个月后(t5)，MRI 平扫+增强、MRCP、DWI、PET-CT 等影像学检查确诊新发胰头癌。患者于 2019.09 (t6) 手术切除胰腺病灶后，2020.07 (t8) 被确诊肝癌。经反复治疗无效后，患者于 2021.03 (t10) 病故。患者全程治疗结束后检测出 14 个 $CD31^{-}$CTC、93 个 $CD31^{+}$ CTEC，小细胞 CTC 在 CTC 总数中占比 28.6%，8 号染色体多倍体 (≥5 倍体) CTC 占比 57.1%，而在占据绝大多数比例 (95.7%) 的大细胞 CTEC 中，多倍体占比高达 86%。

A Comprehensive quantification and molecular characterization of aneuploid CTCs and CTECs

Categorization		haploid	diploid	triploid	tetraploid	multiploid	Sum1	% (Sum1/Total)	Total
$CD31^{-}$ CTC	Large	0	n/c	2	0	8	10	71.4%	14
	Small	0	n/c	3	1	0	4	28.6%	
	Sum2	0	n/c	5	1	8			
	% (Sum2/Total)	0	n/c	35.7%	7.1%	57.1%			
$CD31^{+}$ CTEC	Large	0	n/c	6	3	80	89	95.7%	93
	Small	0	n/c	3	1	0	4	4.3%	
	Sum2	0	n/c	9	4	80			
	% (Sum2/Total)	0	n/c	9.7%	4.3%	86.0%			

B Representative images of aneuploid $CD31^{-}$CTCs and $CD31^{+}$CTECs detected by SE-iFISH

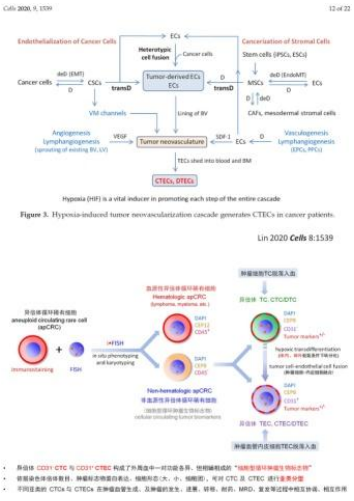


结论

- 本案例显示 MRD 高比值 CTEC/CTC 与治疗后 5 个月后出现的 CA19-9 增高及 10 个月后新发胰腺癌具有相关性
- 肿瘤患者接受全程治疗后（如手术、放化疗、靶向、介入等），检测 MRD $CD31^{-}$ CTC 及 $CD31^{+}$CTEC，尤其是使用瘤标-i•FISH（如 HER2、PD-L1、CA19-9、PSA、AFP、CEA 等）动态监测瘤标阳性 CTC、CTEC 或使用 i•FISH (NC) 区分性检测坏死或活性 CTC、CTEC，将有助于临床客观评估疗效、监测肿瘤早期发生及复发
- 需要密切关注与肿瘤早期发生及复发相关的不同亚类的 CTC、CTEC 及高比值 CTEC/CTC

讨论

人们对循环肿瘤血管内皮细胞 (CTEC) 的来龙去脉已经有了比较清晰的了解。异倍体 CTEC 可通过肿瘤细胞与正常血管内皮细胞相融合或肿瘤细胞在低氧环境下转分化而来，即肿瘤细胞内皮化，这些表达了 CD31 (PECAM-1) 的 CTEC，被喻为“披着羊皮的狼”。CTEC 兼具恶性肿瘤细胞与造血管功能内皮细胞的双重特性，与抗肿瘤血管生成药物 - 贝伐单抗疗效[2]、肿瘤转移及复发密切相关[3]。CTC 与 CTEC 构成了血液中的一对“细胞型循环肿瘤标志物”，在肿瘤发生、进展、转移、耐药等过程中相辅相成。

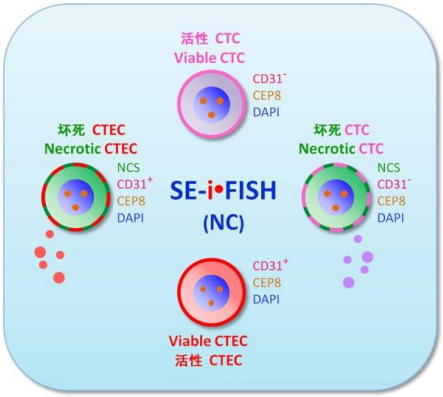


现已证实 CTC、CTEC 经外周血循环不断适应性筛选，与原发灶肿瘤细胞差异极大，包括对治疗药物不同的敏感性与耐药性等[4]，并可形成治疗后的 MRD[5]。

小细胞 (≤ 5 μm) CTC、CTEC 在不同瘤种中的重要临床意义近年来相继被报道，相关研究发现，小细胞 CTC 与肝癌术后快速复发[6]、肺癌肝转移[7]及不良预后[8]密切相关，且大细胞 (> 5 μm)、小细胞 CTC 具有完全不同的耐

药机理[9]。大量实验证实，肿瘤细胞染色体的倍体数目与肿瘤恶性度呈正比，异倍体数目越高，肿瘤恶性度越高[10, 11]。本案例检出的 MRD CTC 中，多倍体及小细胞 CTC 均占重要比例；在检出的 CTEC 中，多倍体占比高达 86%，这些多倍体 CTC、CTEC 与后期的肿瘤快速发生有密切相关性。

本案例中喉癌（鳞癌）与新发胰腺癌属不同病理类型，治疗后检测出的 MRD CTCs 及 CTECs 可能由不同来源的肿瘤细胞组成。它们当中一部分可能来自于耐药的原发灶肿瘤细胞，提示前期治疗未达预期效果[12]，另一部分可能是被多种外界压力（如手术等）诱发激活的蛰伏于机体不同部位的**休眠肿瘤细胞** (dormant cancer cells)[13]，后者可能是新发胰腺癌的主因。需要特别指出的是，外周血中的异倍体 $CD31^+$ CTEC 在肿瘤形成、转移、复发过程中的重要意义早已被报道，北京协和医院外科利用 SE-i•FISH 开展的临床研究显示，胰腺癌患者术后一个月 MRD 干性 $CD44v6^+$ CTEC/$CD44v6^+$ CTC 数目 ≥4 个，患者半年内复发[1]。与其类似，本案例患者治疗后检出的 MRD CTEC 数量是 CTC 的 5 倍以上 (CTEC/CTC >5)，随后间隔仅 5 个月相继出现了血中瘤标 CA19-9 增高及新发 (de novo) 胰腺癌，提示高比值 CTEC/CTC 在监测肿瘤早期发生及复发过程中具有重要临床意义，甚至比检测肿瘤标志物用于监测肿瘤发生更加灵敏！



Lin et al. 2021 ***Cancers*** 13:5108

随着可进一步区分、检测坏死及活性 CTC、CTEC 的赛特 SE-i•FISH(NC)® 技术被成功开发[14]，肿瘤治疗过程中可实现多点动态监测这类细胞，为降低肿瘤复发风险提供可靠依据[15]。临床上有针对性地消除活性 CTC、CTEC，将有助于控制、减少肿瘤转移。

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