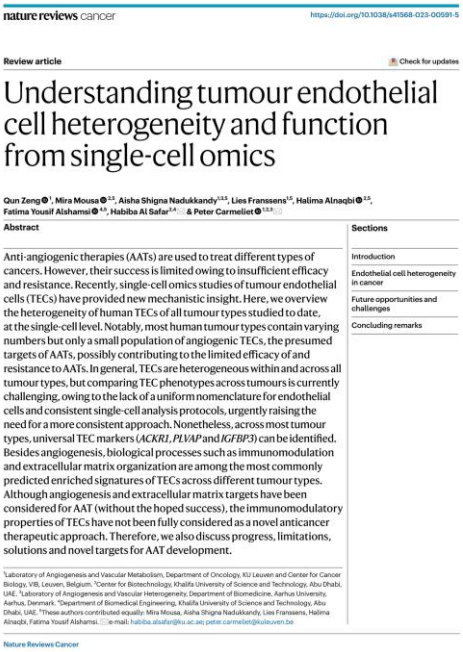
从《Nature》TEC 单细胞多组学分析看 CTEC 临床价值

不同于正常组织的血管内皮细胞 (EC)，肿瘤微环境 (包括肿瘤炎症微环境 inflammatory microenvironment in tumors) 的重要组成部分 –**“肿瘤血管内皮细胞”(TEC**, Tumor Endothelial Cell) 的重要意义近来已受到人们日益广泛的关注。临床一线用于抗肿瘤血管生成 (anti-angiogenic therapies, AATs) 的贝伐单抗，其疗效时常无法达到预期，目前认为主要原因是具有肿瘤血管生成功能的 TEC 在不同瘤种间以及同一肿瘤内具有高度异质性，导致贝伐单抗 AATs 疗效受到极大限制。TEC 已被证实：

- 参与肿瘤血管生成
- 促进肿瘤进展与转移[1]
- 抑制肺癌、肠癌等肿瘤癌患者体内的免疫机能[2, 3]
- 具有独特的转录组标记并对细胞凋亡具有更高的耐受性[4]
- 化疗后的肿瘤患者其 TEC 上的药物外排性转运子 (efflux transporter) 表达明显增高[5]

有鉴于此，人们已开始从多方面对 TEC 进行深入研究。最近，Carmeliet 等团队在联合发表的文章中 (Zeng et al. 2023 **Nat Rev Cancer** 23:544)，以 TEC 单细胞 RNA 测序 (scRNA-seq) 取得的一系列主要成果为主线，从 RNA 单细胞测序、多组学及功能分析、针对 TEC 特定靶标的多中心临床实验等方面对 TEC 进行了全面阐述，并对该领域今后的研究发展方向及临床意义做了深入探讨。



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Review article

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Understanding tumour endothelial cell heterogeneity and function from single-cell omics

Qun Zeng, Mira Mousa, Aisha Shigna Nadukkandy, Lies Franssens, Halima Alnaqbi, Fatima Yousif Alshamsi, Habiba Al Safar & Peter Carmeliet

Abstract

Anti-angiogenic therapies (AATs) are used to treat different types of cancers. However, their success is limited owing to insufficient efficacy and resistance. Recently, single-cell omics studies of tumour endothelial cells (TECs) have provided new mechanistic insight. Here, we overview the heterogeneity of human TECs of all tumour types studied to date, at the single-cell level. Notably, most human tumour types contain varying numbers but only a small population of angiogenic TECs, the presumed targets of AATs, possibly contributing to the limited efficacy of and resistance to AATs. In general, TECs are heterogeneous within and across all tumour types, but comparing TEC phenotypes across tumours is currently challenging, owing to the lack of a uniform nomenclature for endothelial cells and consistent single-cell analysis protocols, urgently raising the need for a more consistent approach. Nonetheless, across most tumour types, universal TEC markers (*ACKR1*, *PLVAP* and *IGFBP3*) can be identified. Besides angiogenesis, biological processes such as immunomodulation and extracellular matrix organization are among the most commonly predicted enriched signatures of TECs across different tumour types. Although angiogenesis and extracellular matrix targets have been considered for AAT (without the hoped success), the immunomodulatory properties of TECs have not been fully considered as a novel anticancer therapeutic approach. Therefore, we also discuss progress, limitations, solutions and novel targets for AAT development.

Sections

Introduction

Endothelial cell heterogeneity in cancer

Future opportunities and challenges

Concluding remarks

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不同瘤种的 TEC 功能各异

体内源自不同器官肿瘤的 TEC 量变趋势及临床意义因瘤种而异。作者首先将 TEC 按瘤种分类做了详尽介绍，并将其主要作用进行了汇总。由于 TEC 高异质性可导致不同瘤种 TEC 中的各种基因表达高低不等并具有不同功能，作者进一步以点-线图形式对 TEC 中的各种基因表达及多种功能做了详尽总结。scRNA-seq 显示，非典型趋化因子受体 1 (ACKR1)、胰岛素样生长因子结合蛋白 3 (IGFBP3) 及血浆外膜囊泡相关蛋白(PLVAP) 的基因在大部分瘤种的 TEC 中单一表达或全部表达。作者沿用了我们使用桑基图展现不同组分 (如细胞、蛋白) 与

各种生物或临床特征的关联性[6,7]，在本文中展示了上述 TEC 三种标志性基因表达如何与 TEC 的三种主要功能相对应 (包括肿瘤血管生成、细胞外基质重构 ECM remodeling、免疫调节)，进而又是如何通过“慢性炎症肿瘤微环境 (chronic inflammatory TME)”等因素促进肿瘤进展 (图 2b)。

不同癌种的 TEC 功能各异

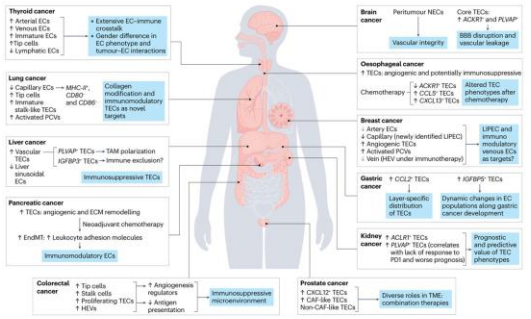

Fig. 1 | Body map of tumour endothelial cells characterized by single-cell RNA-sequencing in different cancer types. Overview of the key findings in different types of cancers, particularly highlighting the common increased tip cells and altered immunomodulatory function of tumour endothelial cells (TECs). This cross-organ comparison indicates the need for combination therapies targeting angiogenic, immunomodulatory and extracellular matrix (ECM) remodelling TECs. BBB, blood–brain barrier; CAF, cancer-associated fibroblast; EC, endothelial cell; EndMT, endothelial-to-mesenchymal transition; HEV, high endothelial venule; *IGFBP3*, insulin-like growth factor binding protein 3; LIPEC, lipid-processing endothelial cell; MHC-II, major histocompatibility complex class II; NEC, normal endothelial cell; PCV, post-capillary venule; PLVAP, plasmalemma vesicle-associated protein; TAM, tumour-associated macrophage; TME, tumour microenvironment. Grey arrow: trend without statistical significance.

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TEC三种主要标志物与相关临床意义的对应关系及如何促进肿瘤进展

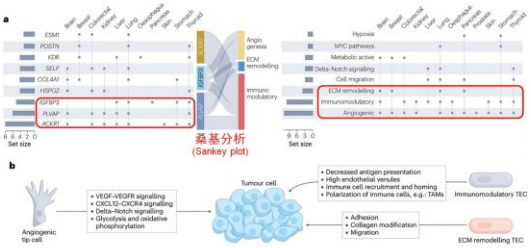

Fig. 2 | Common markers and functions of tumour-enriched endothelial cells. a, UpSet plot of top markers and functions of tumour-enriched endothelial cell subsets across different studies. The top 3 markers are mapped to the top 3 reported functions, as shown in the Sankey plot[52,56,57,59,60,62,63,64,65,66,67,68,69,70]. b, Summary of the ways in which tumour endothelial cells (TECs) can accomplish the top 3 functions to promote tumour progression. ECM, extracellular matrix; TAM, tumour-associated macrophage; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. The data presented in part a were extracted from the studies presented in Table 1 and are available in the Supplementary Information. The UpSet plot was generated with UpSetR software[100] and modified by Inkscape. The Sankey plot was plotted using *R* and the code is available in the Supplementary Information.

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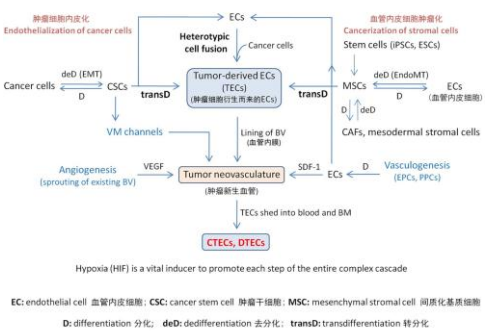
需要特别指出的是，上述三种标志物的基因高表达并不是在所有癌种的 TEC 中都能观察到，例如**乳腺癌 TEC 中检测不到这三种标识物的高转录 (transcription) mRNA**。此外，受细胞内蛋白合成的关键步骤 - “翻译(translation)”环节的调控，这三个高转录 mRNA 编码的蛋白在不同 TEC 中是如何表达的，其表达高低与否、甚至是否表达都是未知数。所有这些为使用上述三种标志物抗体检测不同瘤种的各种 TEC 带来了极大不确定性。

TEC 的重要共有特性：美国哈佛大学 Klagsbrun 团队 2004 年报道了不同于正常血管内皮细胞 ($CD31^+$ / 8 号染色体二倍体)，所有 TEC 均表现为 $CD31^+$ / 8 号染色体异倍体[8]，此特征被视为 TEC 通用标志，可用于有效检测不同瘤种中的各种高异质性 TEC。

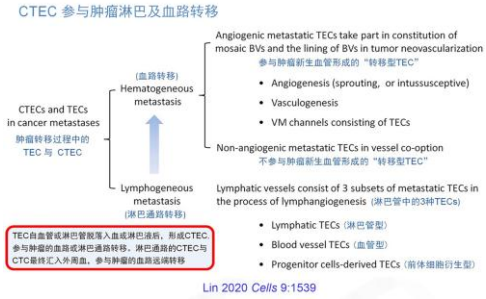
TEC 与 CTEC 的来龙去脉

“肿瘤细胞内皮化”以及**“血管内皮细胞肿瘤化”**是 TEC 的主要形成机制，具体过程包括“肿瘤细胞与正常血管内皮细胞融合”及可发生于体内或体外的“肿瘤细胞低氧转分化”(hypoxic transdifferentiation) 这两种形式[9]。TEC 来源于肿瘤细胞，是肿瘤细胞的另外一种特殊形式，与肿瘤细胞密不可分。

TEC 的形成：肿瘤细胞与正常血管内皮细胞融合或肿瘤细胞低氧转分化



CTEC 参与肿瘤淋巴及血路转移

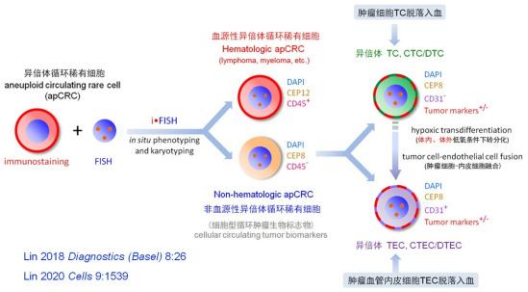


Lin 2020 *Cells* 9:1539

上海市第一人民医院肿瘤中心王红霞主任团队、德国慕尼黑大学 Gires 团队及赛特生物密切合作，应用赛特 SE-i•FISH 技术在国际上首先发现并报道了类似肿瘤细胞 TC 脱落入血形成“循环肿瘤细胞 CTC”，TEC 亦可脱落入血形成“**循环肿瘤血管内皮细胞 CTEC**”(circulating TEC，$CD31^+$异倍体)[10]。不同于正常的二倍体循环血管内皮细胞 CEC，广泛存在于各种肿瘤患者体内的异倍体 CTEC，可表达肿瘤细胞上的所有肿瘤标志物及干细胞标志物，包括 HER2、PD-L1、CA19-9、AFP、PSA、Ki67、SCCA1、CD44v6、EpCAM、Vimentin 等。脱落于淋巴管路的 CTEC 最终汇集到外周血参与肿瘤的血路远端转移。

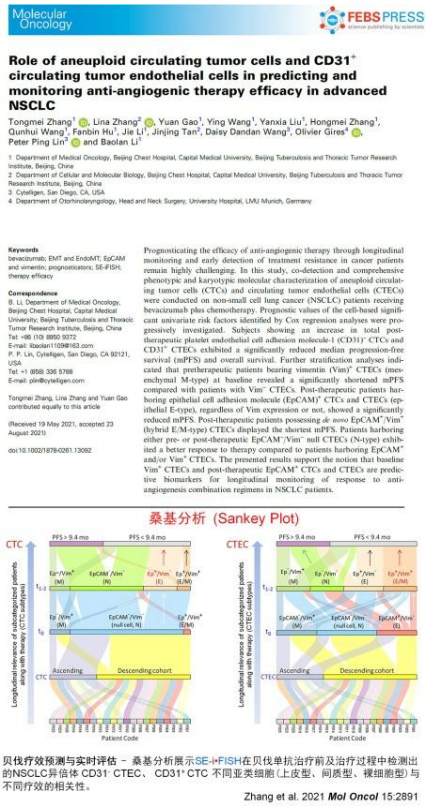
CTEC 的临床意义

多项已发表的研究证实，脱落入血的 CTC 经过失巢凋亡、血流剪应力及免疫系统攻击后，适者生存下来的 CTC 与原发灶上的肿瘤细胞相比，在基因及蛋白层面均发生了极大改变。CTEC 在血流中经历着相同筛选过程，存活下来的 CTEC 与原发灶 TEC 相比，各方面生物学性状已大不相同。**CTEC 在 TEC 的基础上又获得了移动能力，兼具着上述 TEC 功能、肿瘤细胞性状及血管生成的多重特性**。有别于小片段核酸 ctDNA，CTC 与 CTEC 作为血液中的一对相互协调、相互作用的“细胞型循环肿瘤生物标志物”(cellular circulating tumor biomarkers)，与肿瘤发生、进展、转移、耐药、MRD、复发等密切相关[9,11]。



- 异倍体 CD31⁻ **CTC** 与 CD31⁺ **CTEC** 构成了外周血中一对功能各异、但相辅相成的“细胞型循环肿瘤生物标志物”
- 依据染色体倍体数目、肿瘤标志物蛋白表达、细胞形态(大、小、细胞团)，可对 CTC 及 CTEC 进行**亚类分型**（裸细胞型为主，瘤标阳性有特殊临床意义）
- 不同亚类的 CTCs 与 CTECs 在肿瘤血管生成、及肿瘤的发生、进展、转移、耐药、MRD、复发等过程中相互协调、相互作用

北京胸科医院与赛特生物联合报道了 CTEC 在预测及实时评估贝伐单抗治疗非小细胞肺癌过程中的重要意义[7]，并在国际上首次揭示了不同于 $PD\text{-}L1^+$ CTC，$PD\text{-}L1^+$ CTEC 对免疫治疗药物 O 药 (Opdivo) 耐药并与患者较差预后密切相关[6]，从而为开展肿瘤患者精准个体化治疗提供了有效检测手段。



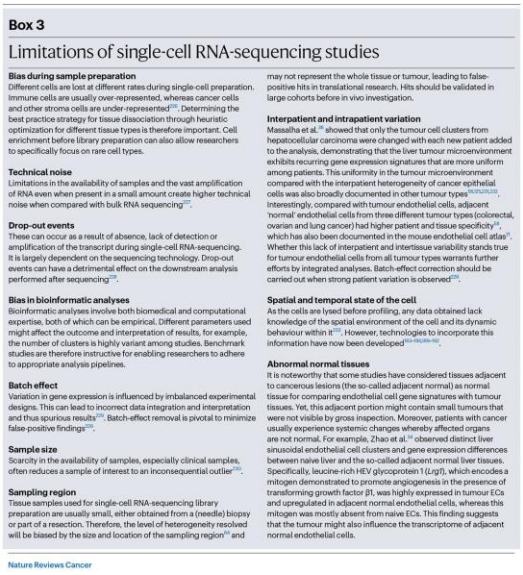
贝伐疗效预测与实时评估 – 桑基分析展示SE-i•FISH在贝伐单抗治疗前及治疗过程中检测出的NSCLC异倍体 $CD31^-$ CTEC、$CD31^+$CTC 不同亚类细胞（上皮型、间质型、裸细胞型）与不同疗效的相关性。

Zhang et al. 2021 ***Mol Oncol*** 15:2891

中国医学科学院肿瘤医院、国家癌症中心赫捷院士、院长团队证实了联合检测 CTEC-CTC 在区分良、恶性肺小结节及肺癌早筛中的重要作用[12]。此外，CTEC 在乳腺癌早诊[13]、新辅助化疗疗效评估[14]、监测 MRD[15]及胰腺癌术后复发[16]、监测胶质瘤血脑屏障破坏及评估疗效[17]等过程中的重要临床意义也已被相继报道。

肿瘤组织与 CTC-CTEC 单细胞测序

单细胞 RNA 测序 (scRNA-seq) 已被广泛应用于肿瘤细胞的研究，也是本文的主线。但文章作者同时也详尽列出了 scRNA-seq 的一系列局限性，其中最主要的一点就是如何在病理组织的各种混合细胞中精确获取具有不同功能及特性的肿瘤单细胞。此外，这些从**不可反复取材的、一次性病理活检组织**上分离的细胞既不能反映肿瘤不同时期的动态进展情况，也不能确定提取的细胞是否具有肿瘤转移能力。



Box 3

Limitations of single-cell RNA-sequencing studies

Bias during sample preparation
Different cells are lost at different rates during single-cell preparation. Immune cells are usually over-represented, whereas cancer cells and other stroma cells are under-represented[129]. Determining the best practice strategy for tissue dissociation through heuristic optimization for different tissue types is therefore important. Cell enrichment before library preparation can also allow researchers to specifically focus on rare cell types.

Technical noise
Limitations in the availability of samples and the vast amplification of RNA even when present in a small amount create higher technical noise when compared with bulk RNA sequencing[127].

Drop-out events
These can occur as a result of absence, lack of detection or amplification of the transcript during single-cell RNA-sequencing. It is largely dependent on the sequencing technology. Drop-out events can have a detrimental effect on the downstream analysis performed after sequencing[129].

Bias in bioinformatic analyses
Bioinformatic analyses involve both biomedical and computational expertise, both of which can be empirical. Different parameters used might affect the outcome and interpretation of results, for example, the number of clusters is highly variant among studies. Benchmark studies are therefore instructive for enabling researchers to adhere to appropriate analysis pipelines.

Batch effect
Variation in gene expression is influenced by imbalanced experimental designs. This can lead to incorrect data integration and interpretation and thus spurious results[129]. Batch-effect removal is pivotal to minimize false-positive findings[129].

Sample size
Scarcity in the availability of samples, especially clinical samples, often reduces a sample of interest to an inconsequential outlier[130].

Sampling region
Tissue samples used for single-cell RNA-sequencing library preparation are usually small, either obtained from a (needle) biopsy or part of a resection. Therefore, the level of heterogeneity resolved will be biased by the size and location of the sampling region[64] and may not represent the whole tissue or tumour, leading to false-positive hits in translational research. Hits should be validated in large cohorts before in vivo investigation.

Interpatient and intrapatient variation
Massalha et al.[28] showed that only the tumour cell clusters from hepatocellular carcinoma were changed with each new patient added to the analysis, demonstrating that the liver tumour microenvironment exhibits recurring gene expression signatures that are more uniform among patients. This uniformity in the tumour microenvironment compared with the interpatient heterogeneity of cancer epithelial cells was also broadly documented in other tumour types[18,20,21,131]. Interestingly, compared with tumour endothelial cells, adjacent 'normal' endothelial cells from three different tumour types (colorectal, ovarian and lung cancer) had higher patient and tissue specificity[28], which has also been documented in the mouse endothelial cell atlas[26]. Whether this lack of interpatient and intertissue variability stands true for tumour endothelial cells from all tumour types warrants further efforts by integrated analyses. Batch-effect correction should be carried out when strong patient variation is observed[129].

Spatial and temporal state of the cell
As the cells are lysed before profiling, any data obtained lack knowledge of the spatial environment of the cell and its dynamic behaviour within it[132]. However, technologies to incorporate this information have now been developed[10–18,28–32].

Abnormal normal tissues
It is noteworthy that some studies have considered tissues adjacent to cancerous lesions (the so-called adjacent normal) as normal tissue for comparing endothelial cell gene signatures with tumour tissues. Yet, this adjacent portion might contain small tumours that were not visible by gross inspection. Moreover, patients with cancer usually experience systemic changes whereby affected organs are not normal. For example, Zhao et al.[28] observed distinct liver sinusoidal endothelial cell clusters and gene expression differences between naive liver and the so-called adjacent normal liver tissues. Specifically, leucine-rich HEV glycoprotein 1 (*Lrg1*), which encodes a mitogen demonstrated to promote angiogenesis in the presence of transforming growth factor β1, was highly expressed in tumour ECs and upregulated in adjacent normal endothelial cells, whereas this mitogen was mostly absent from naive ECs. This finding suggests that the tumour might also influence the transcriptome of adjacent normal endothelial cells.

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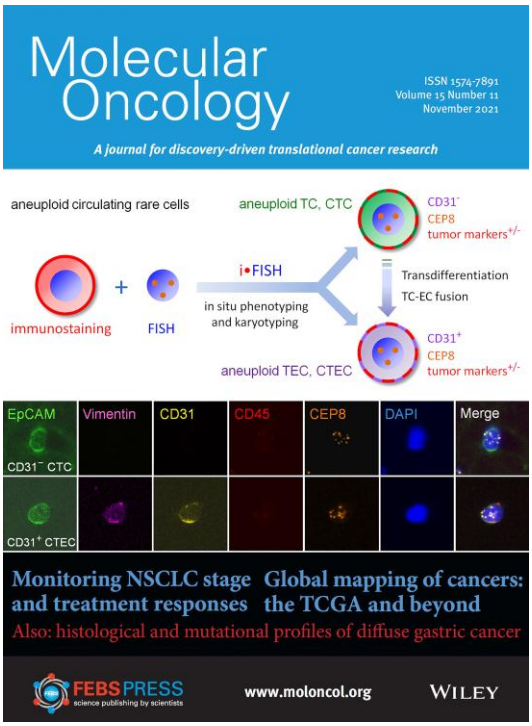
CTEC、CTC 是不断从进展变化的肿瘤组织上自然脱落入血的细胞，富含与肿瘤进展、转移、疗效相关的各种基因、蛋白的完整动态变化信息。赛特 SE-i•FISH 可持续、动态对这些细胞的染色体、瘤标、细胞形态 (细胞大小及细胞团等) 等进行多重原位检测 (欧洲生化学会 FEBS 官方肿瘤杂志“分子肿瘤学 **Mol Oncol**”封面专题报道[7]；生命科学新技术专栏特别报道 **Science** 2013 Vol. 341)，再结合 SE-i•FISH(NC)区分性检测活性及坏死 CTC、CTEC[18]，即可达到有效检测、锁定与肿瘤转移、药敏、耐药、MRD 等密切相关的各种 CTEC、CTC 亚类细胞的目的，从而为后续针对特定靶细胞开展精准单细胞测序提供了有力技术保障。此策略从实验理念到获取的数据信息与以往不加分类地对肿瘤细胞开展混合式单细胞测序相比，更具重要临床意义。



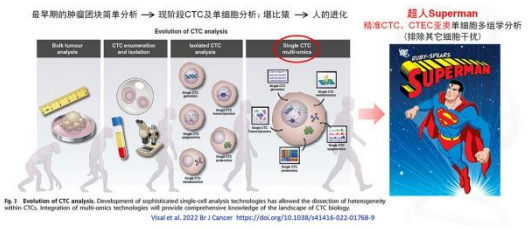
北大肿瘤中心沈琳主任团队与赛特生物及健为医学密切合作，对

SE-i•FISH 检测出的不同 CTC 亚类细胞开展单细胞测序研究，揭示了胃癌治疗过程中产生的大、小 CTC 细胞对化疗药物顺铂具有的不同耐药机制[19]。另一项 SE-i•FISH 单细胞测序研究揭示了乳腺癌 EpCAM$^+$ 异倍体小细胞 CTC 的多个特异性基因扩增，致使 CTC 粘附、增殖、上皮分化能力增加，该亚类 CTC 细胞在乳腺癌肺转移过程中起主要作用[20]。此外，不同于 CTC，研究发现众多 CTEC 存在着特征性 CDKN2A (P16) 基因点突变[11]。

展望 – 比对性精准靶向单细胞多组学分析

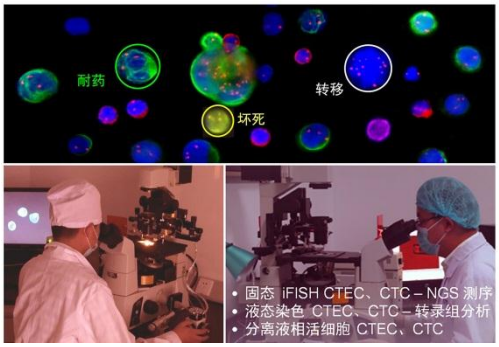
每个单细胞都具有多维度的丰富信息，单一 scRNA-seq 只能提供细胞的部分信息。如本文所言，**肿瘤单细胞多组学分析**，包括基因组学 (DNA 拷贝数、基因突变)、表观基因组学 (DNA 甲基化、染色质可及性 chromatin accessibility)、转录组学、蛋白组学、代谢组学、微扰 (perturbational)、空间 (spatial) 及时态 (temporal) 等，可为人们提供更加深入、全面的信息。最近，北京大学汤富酬教授团队和北大人民医院妇科肿瘤中心对卵巢癌组织样本中提取的肿瘤细胞开展了有意义的单细胞多组学分析研究，包括肿瘤单细胞甲基化[21]，为今后开展相关 CTC、CTEC 单细胞多组学分析奠定了基础。

肿瘤研究从早前的团块观察发展到如今的肿瘤单细胞多组学分析被形象地比喻为从猿向人的进化。随着实时、动态、全方位检测与挑取伴随肿瘤进展的 CTEC-CTC 技术日趋完善，在排除所有其它不相关细胞干扰的基础上，从同一张玻片上检出与分别挑取活性及坏死的、表达了不同肿瘤标志物及染色体倍体、具有不同临床意义的 CTEC、CTC 各种亚类单细胞，并对这些不同亚类细胞逐一进行精准单细胞分析及不同细胞间的深度比对性数据分析，此“比对性精准靶向单细胞多组学分析”将助力实现从人向超人的飞跃，是今后 CTEC、CTC、同时也是肿瘤液体活检的重要发展方向。



比对性精准靶向单细胞多组学分析

- SE-i•FISH锁定具有特殊临床意义的坏死及活性CTEC、CTC亚类细胞
- “固-液双相非激光单细胞分离系统（赛特）”逐一挑取锁定的待检单细胞
- 单细胞多组学分析



SE-i•FISH® 一网打尽 CTC、CTEC

一管血样，一次检测，两类细胞，多重指标

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