

早期肺癌术前三倍体小细胞 CTC 的重要临床意义

肺癌，作为肿瘤的头号杀手，对大众健康构成了严重威胁。随着肿瘤个体化治疗的不断开展，如何在治疗前有效判断肿瘤患者的疗效与预后，并在此基础上对患者实施个体化精准治疗，已成为广大医务工作者日益关注的重点。最近，北京大学肿瘤中心、北京肿瘤医院胸外科杨跃主任团队、胸内科方健主任团队和赛特生物密切合作，利用 SE-i-FISH 对早期肺癌患者术前循环异倍体小细胞 (small circulating aneuploid cell, sCAC) 的临床意义进行了开拓性研究，取得的重要成果刚刚在 *Frontiers in Oncology* 上发表 (Hong et al., 2021 *Front Oncol* 11:590952)。这是继北京胸科医院和赛特生物首次联合发现了晚期肺癌患者化疗前基线 Vimentin⁺ 间质型三倍体小细胞 sCAC^{tri} 与不良预后和肺癌定向肝转移紧密关联 [1]，以及汉中医院与上

海长海医院、上海张江转化医学中心应用 SE-i-FISH 揭示结直肠癌术前 CTC 的临床意义之后 [2]，再次证明早期肺癌患者体内手术前的三倍体小细胞 CTC 也与疗效、预后及复发密切相关。

本文重点:

- 针对 50 例早期肺癌患者 (I-III A) NSCLC 的研究发现，术前三倍体小细胞 sCAC^{tri} 与 TNM 分期密切相关，IIIA 患者 sCAC^{tri} 数目明显高于 I-II 期患者
- 术前 sCAC^{tri} 检测阳性，患者术后复发较快，无病生存期 DFS 显著较短
- 手术后患者体内 CAC 数目增加，其中 57% 的患者术后 sCAC^{tri} 数量增加



Small Cell Size Circulating Aneuploid Cells as a Biomarker of Prognosis in Resectable Non-Small Cell Lung Cancer

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Objective: The size distribution of circulating aneuploid cells (CACs) and its clinical significance were investigated in resectable non-small cell lung cancer (NSCLC).

Patients and Methods: A total of 50 patients with resectable NSCLC were enrolled in this study. Blood samples (50 pre-surgery and 35 post-surgery) were collected and used for the detection of CAC chromosome B heteroploidy through the subtraction enrichment and immunostaining fluorescence in situ hybridization (SE-i-FISH) method.

Results: Less than 20% small cell size and more than 80% large cell size CACs were detected. Karyotypes, including triploid, tetraploid, and multiploid, had varying distributions. The triploid subtype accounted for the majority of small cell size CACs, whereas the multiploid subtype accounted for the majority of large cell size CACs. We found that total small cell size and triploid small cell size CACs, but not large cell size CACs, derived from pre-surgery samples, were associated with shorter disease-free survival. Moreover, total small cell size and triploid small cell size CACs were associated with higher TNM stage and recurrence. Nevertheless, the variation between pre- and post-surgery CACs was not related to survival among patients with resectable NSCLC.

Conclusions: Pre-surgery small cell size CACs, especially the triploid subtype, could be regarded as a potential prognostic biomarker for patients with resectable NSCLC.

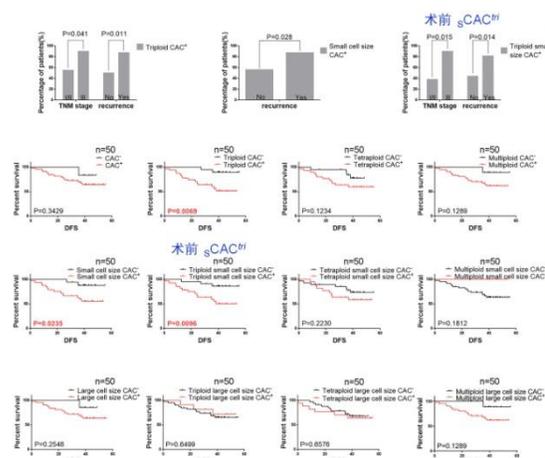
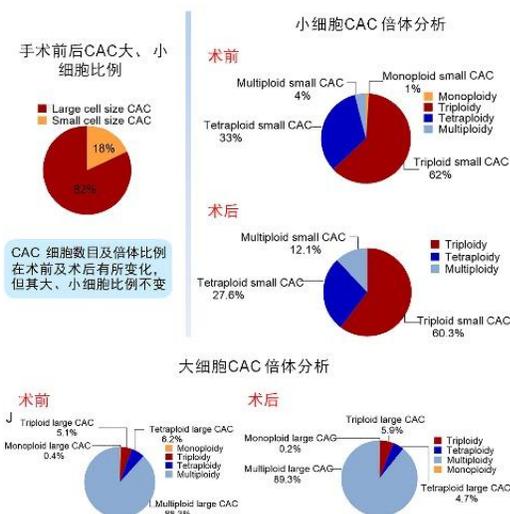
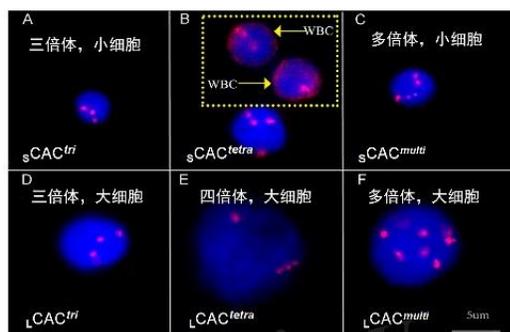
Keywords: non-small cell lung cancer, circulating aneuploid cells, prognosis, resection, biomarker

CAC 存在于手术前、后的早期肺癌患者体内

非血源性 (CD45⁻) 循环异倍体细胞 CAC 广泛存在于早期肺癌患者体内。本实验 50 位入组的早期肺癌患者手术前共检测出 554 个 CACs，其中的 35 位患者术后共检测出 628 个 CACs。这些 CAC 中，大细胞占比高达 82%，小细胞 CAC 只占比 18%，此比例在手术前、后维持不变。术后 CAC 总数增加的原因可能是手术过程导致部分肿瘤细胞直接入血，或手术操作激活了休眠 (dormant) 肿

瘤细胞^[3, 4], 被激活的肿瘤细胞随后进入血循环。

通过分析, 我们发现术前三倍体小细胞 $sCAC^{tri}$ 与早期肺癌的 TNM 分期和术后复发密切相关。38% 的 I/II 期患者含有 $sCAC^{tri}$, 而高达 90% 的 IIIA 肺癌患者可检测出 $sCAC^{tri}$; 81% 复发的患者术前呈 $sCAC^{tri}$ 阳性, 而没有术后复发的患者术前 $sCAC^{tri}$ 阳性率仅为 44%。



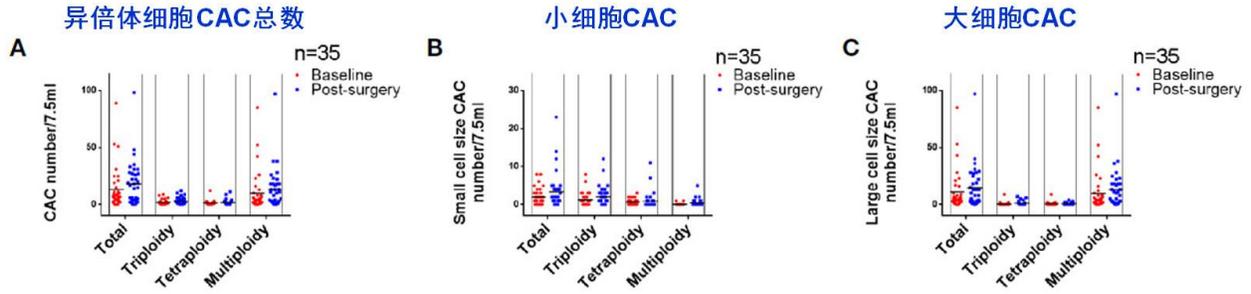
进一步的 8 号染色体倍体分析显示, 高于 60% 的小细胞 small CAC 在手术前、后均为三倍体 ($sCAC^{tri}$), 而大细胞 large CAC 中, 多倍体始终占主要部分 ($lCAC^{multi}$, >70%)。

术前可检测出 $sCAC^{tri}$ 的早期肺癌患者术后复发较快, 无疾病生存期 DFS 明显短于术前未检出 $sCAC^{tri}$ 的患者。在上述分析中, 术前多倍体大细胞 CAC 未显示特定的临床意义。

我们对患者术后的 CAC 也进行了定量分析。结果显示, 57% 的患者术后三倍体小细胞 $sCAC^{tri}$ 增加, 58% 的患者术后多倍体大细胞 $lCAC^{multi}$ 增加。这些术后增加的 CAC 的临床意义有待进一步研究。

术前三倍体小细胞 $sCAC^{tri}$ 的重要临床意义

术前与术后细胞数目比较



讨论

波形蛋白 Vimentin⁺ 的间质型肿瘤细胞与肿瘤的侵袭、转移及进展密切相关 [5]。北京胸科医院和赛特生物之前联合报道了晚期肺癌患者体内的 Vim⁺ 间质型 CTC 多为小细胞，这些化疗前存在的间质型三倍体小细胞 CAC (Vim⁺ sCAC^{tri}) 与肺癌患者的不良预后及肺癌肝转移高度相关 [1]。该报道与本研究结果相吻合，证实手术或化疗前的早期和晚期肺癌患者体内存在三倍体小细胞 CAC，尤其是间质型 Vim⁺ sCAC^{tri}，可作为肺癌患者不良预后及术后复发的 biomarker。针对治疗前存在这类细胞的患者，治疗手段可能应不同于不含此类细胞的患者。

除肺癌以外，北京清华长庚医院董家鸿院士团队和赛特生物也一同报道了肝癌患者术后出现的 EpCAM⁺ 多倍体小细胞 CAC 与复发密切相关 [6]，并且北大肿瘤中心沈琳主任团队和赛特生物多年前已发现胃癌三倍体 CAC 具有对顺铂内源性耐药的特性 [7]。虽然三倍体小细胞 sCAC^{tri} 在整体 CAC 中占比不高，但因其特殊意义，近年来已受到人们的密切关注！

循环异倍体细胞 CAC 主要由 CD31⁺ CTC 和 CD31⁺ CTEC (循环肿瘤血管内皮细胞) 组成，这两类细胞虽然功能各异，但相辅相成并可相互转化 [8-10]。不同于以往笼统的 CAC 检测，有效区分与同步检测 CTC、CTEC，不仅可避免两类细胞在分析实验结果时的相互干扰，亦可分别研究它们各自的临床意义。最近，CTC 与 CTEC 在肺癌 [11, 12]、乳腺癌 [13] 等肿瘤中的不同重要作用已被不断报道。如何在细胞三要素 (染色体倍体、肿瘤蛋白标志物表达、细胞形态) 的基础上，深入研究肺癌及其它瘤种术后出现的 CTC、CTEC 各亚类细胞的临床意义，以及如何优化手术操作来尽量减少手术过程造成的血中 CTC、CTEC 升高，将是今后相关研究的重点之一。

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