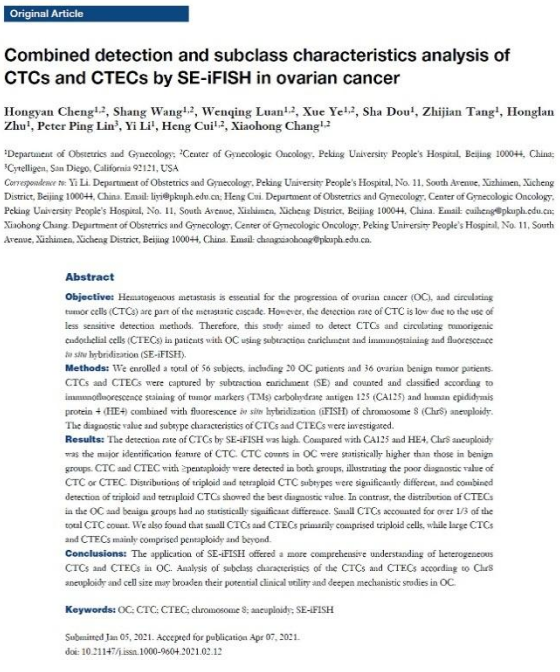
三倍体及四倍体 CD31 阴性 CTC 对卵巢癌具有特殊诊断价值

卵巢癌是我国女性发病率较高的肿瘤，病死率在妇科生殖系统肿瘤中高居首位。过去 10 年中，我国妇女的卵巢癌发病率增加了 30%，病死率增加了 18%。由于卵巢癌发病隐匿，且缺乏有效筛查手段，70%的患者就诊时已处于晚期。经过初次治疗后，70%的患者都会复发。为保障我国广大妇女的健康，利用完善的技术手段进行无创、有效地检测血液中的卵巢癌细胞，以达到早筛、早诊、早治疗及实时监测肿瘤复发的目的已刻不容缓！最近，著名的北京大学人民医院妇产科及妇科肿瘤中心崔恒、昌晓红主任团队与赛特生物密切合作，利用 SE-i•FISH 整合技术，对联合检测异倍体 $CD31^-$ 循环肿瘤细胞 (CTC) 及 $CD31^+$ 循环肿瘤血管内皮细胞 (CTEC)在卵巢癌诊断过程中的临床意义开展了深入研究。取得的重要成果刚刚在肿瘤专业期刊上得到发表 (Cheng et al., 2021 Chin J Cancer Res 33:256)。文章第一作者为北大人民医院妇科肿瘤中心程洪艳研究员。



Original Article

Combined detection and subclass characteristics analysis of CTCs and CTECs by SE-iFISH in ovarian cancer

Hongyan Cheng[1,2], Shang Wang[1,2], Wenqing Luan[1,2], Xue Ye[1,2], Sha Dou[1], Zhijian Tang[1], Honglan Zhu[1], Peter Ping Lin[3], Yi Li[1], Heng Cui[1,2], Xiaohong Chang[1,2]

[1]Department of Obstetrics and Gynecology; [2]Center of Gynecologic Oncology, Peking University People's Hospital, Beijing 100044, China; [3]Cytelligen, San Diego, California 92121, USA

Correspondence to: Yi Li. Department of Obstetrics and Gynecology, Peking University People's Hospital, No. 11, South Avenue, Xizhimen, Xicheng District, Beijing 100044, China. Email: liyi@pkuph.edu.cn; Heng Cui. Department of Obstetrics and Gynecology, Center of Gynecologic Oncology, Peking University People's Hospital, No. 11, South Avenue, Xizhimen, Xicheng District, Beijing 100044, China. Email: cuiheng@pkuph.edu.cn; Xiaohong Chang. Department of Obstetrics and Gynecology, Center of Gynecologic Oncology, Peking University People's Hospital, No. 11, South Avenue, Xizhimen, Xicheng District, Beijing 100044, China. Email: changxiaohong@pkuph.edu.cn.

Abstract

Objective: Hematogenous metastasis is essential for the progression of ovarian cancer (OC), and circulating tumor cells (CTCs) are part of the metastatic cascade. However, the detection rate of CTC is low due to the use of less sensitive detection methods. Therefore, this study aimed to detect CTCs and circulating tumorigenic endothelial cells (CTECs) in patients with OC using subtraction enrichment and immunostaining and fluorescence *in situ* hybridization (SE-iFISH).

Methods: We enrolled a total of 56 subjects, including 20 OC patients and 36 ovarian benign tumor patients. CTCs and CTECs were captured by subtraction enrichment (SE) and counted and classified according to immunofluorescence staining of tumor markers (TMs) carbohydrate antigen 125 (CA125) and human epididymis protein 4 (HE4) combined with fluorescence *in situ* hybridization (iFISH) of chromosome 8 (Chr8) aneuploidy. The diagnostic value and subtype characteristics of CTCs and CTECs were investigated.

Results: The detection rate of CTCs by SE-iFISH was high. Compared with CA125 and HE4, Chr8 aneuploidy was the major identification feature of CTC. CTC counts in OC were statistically higher than those in benign groups. CTC and CTEC with ≥pentaploidy were detected in both groups, illustrating the poor diagnostic value of CTC or CTEC. Distributions of triploid and tetraploid CTC subtypes were significantly different, and combined detection of triploid and tetraploid CTCs showed the best diagnostic value. In contrast, the distribution of CTECs in the OC and benign groups had no statistically significant difference. Small CTCs accounted for over 1/3 of the total CTC count. We also found that small CTCs and CTECs primarily comprised triploid cells, while large CTCs and CTECs mainly comprised pentaploidy and beyond.

Conclusions: The application of SE-iFISH offered a more comprehensive understanding of heterogeneous CTCs and CTECs in OC. Analysis of subclass characteristics of the CTCs and CTECs according to Chr8 aneuploidy and cell size may broaden their potential clinical utility and deepen mechanistic studies in OC.

Keywords: OC; CTC; CTEC; chromosome 8; aneuploidy; SE-iFISH

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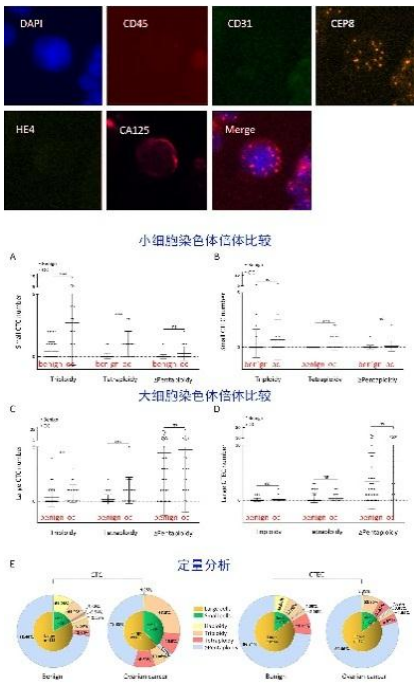
本文重点

- 卵巢癌患者的 $CD31^-$ CTC 及 $CD31^+$ CTEC 相对于卵巢良性瘤患者均有升高，其中 CTC 增高最明显
- 在有效区别 $CD31^+$ CTEC 的前提下，联合检测三倍体及四倍体 $CD31^-$ CTC（不包含 $CD31^+$ CTEC) 对卵巢癌极具诊断价值，而不加区分地检测异倍体细胞总数 (即 CTC + CTEC)，结果与卵巢癌诊断无显著相关性
- 卵巢癌小细胞 CTC 及 CTEC 分别在细胞总数中占比高达 1/3，且以 8 号染色体三倍体为主，而大细胞 CTC 及 CTEC 则以多倍体为主
- 20-30% 的患者可检测出表达了卵巢癌肿瘤标志物人附睾蛋白 4 (HE4) 、CA125 的 CTC 和 CTEC

结果分析

鉴于之前应用 SE-i•FISH 发现异倍体肿瘤血管内皮细胞与肿瘤的发生、进展、转移[1]紧密关联，肿瘤细胞内的染色体倍体数目与肿瘤细胞恶性度密切相关[2]，小细胞 CTC 与患者的不良预后高度关联[3, 4]，因此，我们使用赛特 6-通道 SE-i•FISH (HE4 + CA125) 方法对 56 例入组病人（20 例卵巢癌术前患者，36 例卵巢良性瘤）的 124 个临床标本进行了 CTC、CTEC 的富集，再从细胞三要素（核酸、蛋白、细胞形态）的角度出发，深入研究了这些 CTC、CTEC 的染色体异倍体特性、肿瘤标志物表达及各种大、小细胞和癌栓。结果显示，小细胞 CTC 在卵巢癌 (ovarian cancer, OC) 患者的细胞中占比 38.57%。小细胞三倍体、四倍体 CTC、小细胞四倍体 CTEC 及大细胞四倍体 CTC 在卵巢癌患者和良性瘤患者间存在显著性差异。



为了进一步确定 CTC 亚类细胞在卵巢癌诊断中的临床价值，我们应用 ROC 曲线分析了 CTC、CTEC 及各亚类细胞的敏感性与特异性。

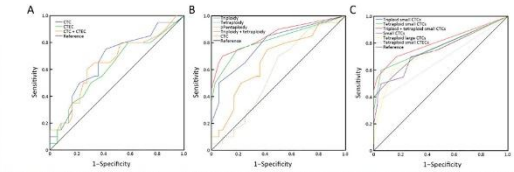


Figure 7 Potential clinical values of CTCs and CTECs. (A) ROC curves of CTC count, CTEC count, sum of CTC and CTEC; (B) ROC curves of CTCs classified by aneuploid of chromosome 8; (C) ROC curves of CTCs and CTECs classified by cell size and/or chromosome 8 aneuploidy. CTC, circulating tumor cell; CTEC, circulating tumorigenic endothelial cell; ROC, receiver operating characteristic.

Table 3 Optimum cut-off values and their respective sensitivity and specificity

Index	cut-off	AUC	Sensitivity (%)	Specificity (%)	P	95% CI
CTC	4.5	0.670	75.00	58.30	0.036	0.521–0.820
CTEC	1.5	0.616	80.00	41.57	0.150	0.459–0.773
CTC + CTEC	11.5	0.650	60.00	72.22	0.065	0.496–0.804
Triploid CTC	2.5	0.792	50.00	94.44	<0.001	0.663–0.920
Tetraploid CTC	0.5	0.821	75.00	80.51	<0.001	0.692–0.949
≥Pentaploid CTC	2.5	0.577	70.00	50.00	0.343	0.426–0.728
Triploid + tetraploid CTC	2.5	0.853	70.00	91.67	<0.001	0.738–0.969
Triploid small CTC	1.5	0.760	55.00	86.11	0.001	0.617–0.904
Tetraploid small CTC	0.5	0.781	60.00	94.44	0.001	0.638–0.923
Triploid + tetraploid small CTC	1.5	0.809	70.00	83.30	<0.001	0.674–0.944
Small CTC	3.5	0.745	50.00	94.40	0.003	0.598–0.892
Tetraploid large CTC	0.5	0.740	60.00	86.11	0.003	0.594–0.887
Tetraploid small CTC	0.5	0.675	35.00	100	0.031	0.515–0.835

CTC, circulating tumor cell; CTEC, circulating tumorigenic endothelial cell; AUC, area under the curve; 95% CI, 95% confidence interval.

结果显示，唯有同时检测三倍体、四倍体 CD31- CTC (cut-off: ≥ 2.5 cells) 或三倍体、四倍体 CD31- 小细胞 CTC (cut-off: ≥ 1.5 cells) 才具最高敏感性与特异性。只单纯计数 CD31- CTC 、 CD31+ CTEC 或 CD31- CTC 和 CD31+ CTEC 总数，对卵巢癌不具有诊断价值。

结论

本研究揭示了有效区分 CD31- CTC 及 CD31+ CTEC 是高特异性检测卵巢癌细胞的必要前提！联合检测三倍体及四倍体 CD31- CTC 或其小细胞 CTC 在卵巢癌诊断中极具临床价值。除此以外，有关 HE4+/CA125+ CTC、CTEC 及各个亚类细胞在后续手术及药物治疗卵巢癌过程中的特殊临床意义（包括术后及药物疗效评估、肿瘤耐药、转移的相关性及复发监测等）已在积极开展过程中。

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