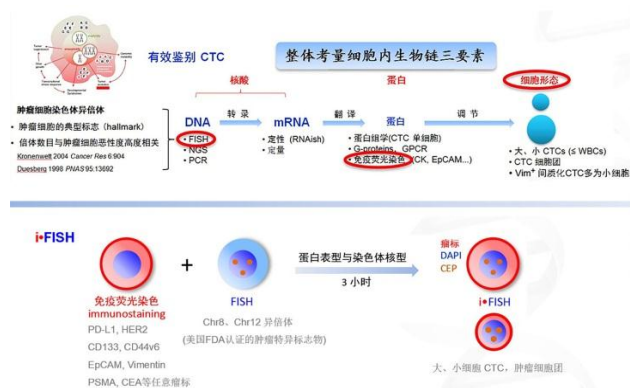


SE-iFISH 与细胞过滤法检测口腔癌 CTC-CTC 的临床对比

进入淋巴系统的肿瘤细胞 CD31⁻ CTC 及肿瘤血管内皮细胞 CD31⁺ CTEC 参与肿瘤淋巴通路的转移，它们最终汇入外周血，参与肿瘤的血路远端转移。口腔癌是发生于口腔部位的鳞状细胞癌，可通过淋巴系统或者血液转移到身体各个组织或器官，其中转移至头颈部位的可能性较大。因此，同步、原位联合检测 CTEC-CTC 是有效监测肿瘤转移及评估疗效的关键。

目前国内外 CTC 检测方法种类繁多，但大都只针对肿瘤细胞的单一生物学特性开展检测，从而严重制约了 CTC、CTEC 的准确检测与临床应用。相对于其它检测方法，SE-iFISH 在整体考量细胞三要素 (核酸、蛋白、细胞形态) 的基础上，针对 CTC、CTEC 的染色体异倍体、多种瘤标蛋白表达、及各种细胞形态 (大小、细胞团等) 进行全方位综合性检测，其显著特有技术优势已被广泛证实与报导。



为进一步验证赛特生物 SE-iFISH 与细胞过滤法在检测 CTEC-CTC 过程中的技术差异，最近，澳大利亚 Griffith 大学医学院、James Cook 大学医学院及香港大学对在香港玛丽医院 (Queen Mary Hospital) 接受手术治疗的 10 名口腔癌患者，独立比较了 SE-iFISH 与细胞过滤法的主要代表技术 ISET (Vona et al. 2000 Am J Pathol 156:57)在监测患者术前、术中及术后 MRD CTC、CTEC 动态变化过程中的应用，相关结果已得到发表 (Curtin et al. 2022 J Oral Pathol Med 51:249)。

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ORIGINAL ARTICLE

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A comparison of two methods for the detection of circulating tumour cells in patients with oral cavity cancer

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Abstract

Background: Circulating tumour cells (CTCs) detected in patient blood samples are relevant as diagnostic and prognostic markers offering insights into tumour behaviour and guiding treatment of cancer at an individualised level. The aim of this study was to ascertain the feasibility of detecting CTCs in oral squamous cell carcinoma (OSCC) using two different methods so as to determine the optimal method for the study of this cancer.

Methods: Comparison of the numbers of CTCs, circulating tumour micro-emboli (CTMEs) and circulating tumour endothelial cells (CTECs), was undertaken in forty clinical samples of oral squamous cell carcinoma (OSCC) determined by filtration (ISET[®]) and in situ fluorescent immunostaining (i-FISH, Cytelligen[®]) immunostaining and in situ hybridisation.

Results: i-FISH detected CTCs in 80% of samples compared with 40% of samples analysed by microfiltration. i-FISH detected CTCs in a further 40% of samples in which microfiltration did not detect CTCs. No CTC clusters were detected by microfiltration while i-FISH detected CTM in 12.5% of samples. i-FISH analysis detected CTCs in 20/40 samples.

Conclusion: These results highlight significant differences in detection of CTCs, CTM and CTCs between i-FISH and microfiltration when applied to OSCC samples, suggesting that technologies capable of detecting circulating aneuploid cells more accurately detect CTCs. i-FISH also detected CTM and CTEC not detected using ISET[®]. With proven prognostic relevance in adenocarcinomas, accurate enumeration of CTCs, CTMs and CTCs may be a clinically useful tool in the management of OSCC and may aid in the reduction of false-negative diagnoses.

KEYWORDS

circulating tumour cells, fluorescence in situ hybridisation, ISET, oral cancer

与细胞过滤法 (ISET) 结合单一检测核浆比的 H&E 染色鉴定 CTC 相比，赛特 SE-iFISH 检测 CD31⁻ CTC、CD31⁺ CTEC 及与肿瘤转移密切相关的肿瘤细胞团 CTM 的技术优势显而易见。在 40 例样本中，ISET 没有检测到 CTM 且无法检测到小细胞循环稀有细胞、CTEC 及肿瘤标志物的表达。SE-iFISH 可在 40% ISET 检测为阴性的样本中有效检出 CTC、CTEC。

检测方法	标本阳性率				
	CD31-CTC	CD31+CTEC	CTM	小细胞	瘤标表达
SE-iFISH	80%	40%	12.5%	+	+
小细胞过滤法(ISET)	40%	0	0	-	-

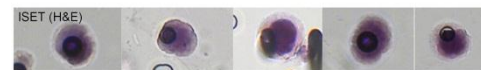


FIGURE 4 Images of CTCs isolated using ISET stained with H&E

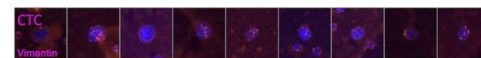


FIGURE 2 Images of CTCs isolated using iFISH stained with CEP-8 (red) and DAPI (blue)

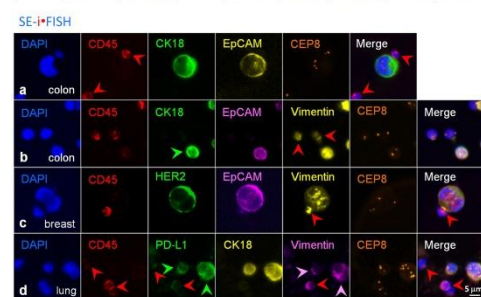
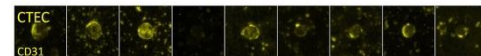


Figure 2. Multiple tumor biomarkers-iFISH. (a) (CK/EpCAM)-iFISH performed on enriched triploid colon cancer cell shows CD45⁺/CK18⁺/EpCAM⁺. WBCs (red arrows) are CD45⁺/CK18⁻/EpCAM⁻. (b) Tri-marker-iFISH indicates that the diploid tumor cell (green arrow) is CD45⁺/CK18⁺/EpCAM⁺/Vimentin⁺. Two of diploid WBCs (red arrows) are CD45⁺/HER2⁻/EpCAM⁻/Vimentin⁻. (c) (HER2/EpCAM/Vimentin)-iFISH reveals diploid breast cancer cell CD45⁺/HER2⁺/EpCAM⁺/Vimentin⁺. The WBC attached to the tumor cell is CD45⁺/Vimentin⁺ (red arrow). (d) Two of aneuploid lung cancer cells are CD45⁺/PD-L1⁺/CK18⁺/Vimentin⁺ (pink arrows). Depicted WBCs (red arrows) are CD45⁺/PD-L1⁻/CK18⁻, and one of WBCs is strong vimentin⁺ (red arrow).

Lin et al. 2017 Sci Rep 7:9789

作者对接受手术的患者在术前、术中、术后 1 天及 7 天进行了动态监测。SE-iFISH 结果显示：

- 术中：与术前相比，1 例患者 CTC 数目降低 (绿色)，2 例患者 CTC 及/或 CTEC 有所升高 (红色)
- 术后 1 天：除 1 例患者(P-OC) CTC-CTEC 降低外，大部分患者

的 MRD CTC-CTEC 数目有所增高

- 术后 7 天：10 例患者中，3 位患者 CTC 与术前比无明显变化，1 例显著下降归零，但另外 6 例患者 (包括术后 1 天降低的 P-OC 患者) MRD CTC 数目显著升高
- 细胞总数分析显示 CTC、CTEC 数目从术前至术后 7 天依次递增。

TABLE 2 Circulating tumour cell numbers compared

Patient code	术前 iFISH (baseline)			术中 iFISH (tumour-out)			术后1天 iFISH (post-op day 1)			术后7天 iFISH (post-op day 7)			ISET (baseline)			ISET (tumour-out)			ISET (post-op day 1)			ISET (post-op day 7)		
	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC	CTC	CTM	CTEC
P-SA	8	0	0	0	0	0	15	2	0	15	0	3	4	0	0	0	0	0	5	0	3	0	0	0
P-OB	7	0	0	10	0	0	11	0	1	0	0	0	4	0	0	2	0	0	4	0	0	0	0	0
P-OC	5	0	1	0	0	0	10	0	0	0	0	0	5	0	0	5	0	0	5	0	0	0	0	0
P-OD	12	3	5	10	1	0	10	0	0	0	0	0	3	5	4	13	0	0	0	0	0	0	0	0
P-DE	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P-DF	0	0	0	2	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P-DG	3	0	0	4	0	2	11	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P-DH	2	0	1	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P-DI	1	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
P-DJ	0	0	1	1	0	2	3	0	2	25	0	4	0	0	0	0	0	0	0	0	0	0	0	0

Abbreviations: CTC, Circulating tumour cells; CTEC, Circulating tumour-derived endothelial cells; CTM, Circulating tumour micro-emboli.

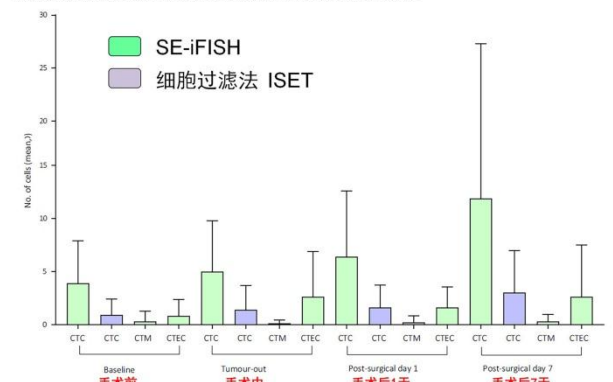


FIGURE 1 Comparison between CTC numbers enumerated using iFISH and ISET and comparison of cells found in blood samples at different timepoints

结论

系统性监测口腔癌患者从术到术后 MRD CTC、CTEC 的动态变化结果显示，术后 7 天检测患者 CTC-CTEC 的结果更为稳定、可靠。本实验再次证明了 SE-iFISH 全方位有效检测 CTC、CTEC 的独特技术优势，从而为进一步研究动态变化的 CTC、CTEC 在口腔癌及其它癌种中的预后价值提供了可靠技术保障。