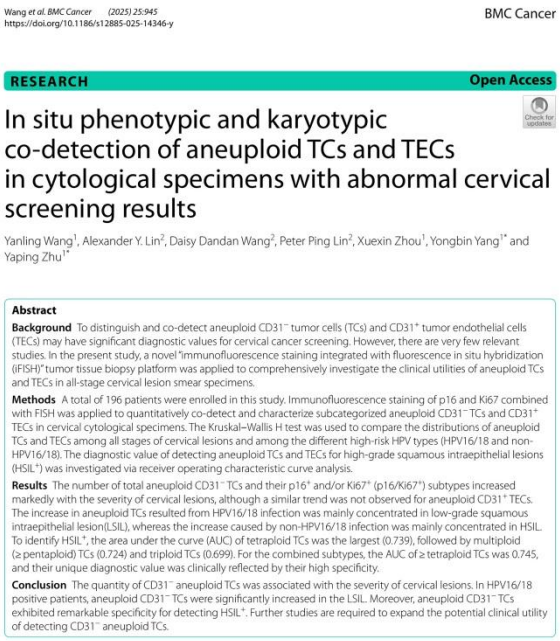
iFISH 高特异检测宫颈刮片肿瘤细胞的重要临床意义与应用

宫颈癌作为妇科肿瘤中的第四大癌种，全球每年新增发病例数 60 万，死亡 34 万，对广大妇女的生命健康构成了严重威胁。感染人乳头状病毒 (HPV) 是诱发宫颈癌的重要因素。从感染 HPV 发展到宫颈癌通常需要十多年，这为宫颈癌的早期防治提供了绝佳窗口期。此期间，基于细胞形态变化的宫颈刮片早期筛查肿瘤细胞是目前广泛采用的一线手段。然而，不同于病理组织切片样本中的肿瘤细胞具有易于识别的特定细胞形态，宫颈刮片中的细胞呈现各式各样的形态，而对此类形态万千的细胞筛查主要依赖病理科医生的水平与经验，缺乏统一的可视性客观标准及严格质控，判断极具主观性，由此造成的不可避免的假阴性、假阳性误诊已成为全球范围不可忽视的问题[1,2]。如何在这些形态差异极大的宫颈刮片细胞中高灵敏、**高特异**地检出肿瘤细胞是有效开展宫颈癌早期筛查的首要前提！

最近，上海交大附属上海第一人民医院妇产科祝亚平、杨永彬主任团队与赛特生物密切合作，应用“i•FISH 肿瘤组织活检”技术在未切片肿瘤组织中有效区分与联合检测完整异倍体 $CD31^-$ 肿瘤细胞 (tumor cell, TC)、$CD31^+$ 肿瘤血管内皮细胞 (tumor endothelial cell, TEC) 的基础上[3]，对 196 位伴有正常宫颈病变、HPV 阳性导致的不同程度宫颈病变（包括低级别上皮内瘤变 (LSIL)、高级别上皮内瘤变 (HSIL)及宫颈癌）的患者开展了宫颈刮片细胞染色体核型与蛋白表型的联合检测，在同一标本的细胞上原位、同步检测 8 号染色体异倍体及宫颈癌风险标志物 Ki67 和 *CDKN2A* 基因编码的 p16 蛋白表达。取得的重要成果刚刚得到发表 (Wang et al. 2025 BMC Cancer 25:945)。



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RESEARCH Open Access

In situ phenotypic and karyotypic co-detection of aneuploid TCs and TECs in cytological specimens with abnormal cervical screening results

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Abstract

Background To distinguish and co-detect aneuploid $CD31^-$ tumor cells (TCs) and $CD31^+$ tumor endothelial cells (TECs) may have significant diagnostic values for cervical cancer screening. However, there are very few relevant studies. In the present study, a novel "immunofluorescence staining integrated with fluorescence in situ hybridization (iFISH)" tumor tissue biopsy platform was applied to comprehensively investigate the clinical utilities of aneuploid TCs and TECs in all-stage cervical lesion smear specimens.

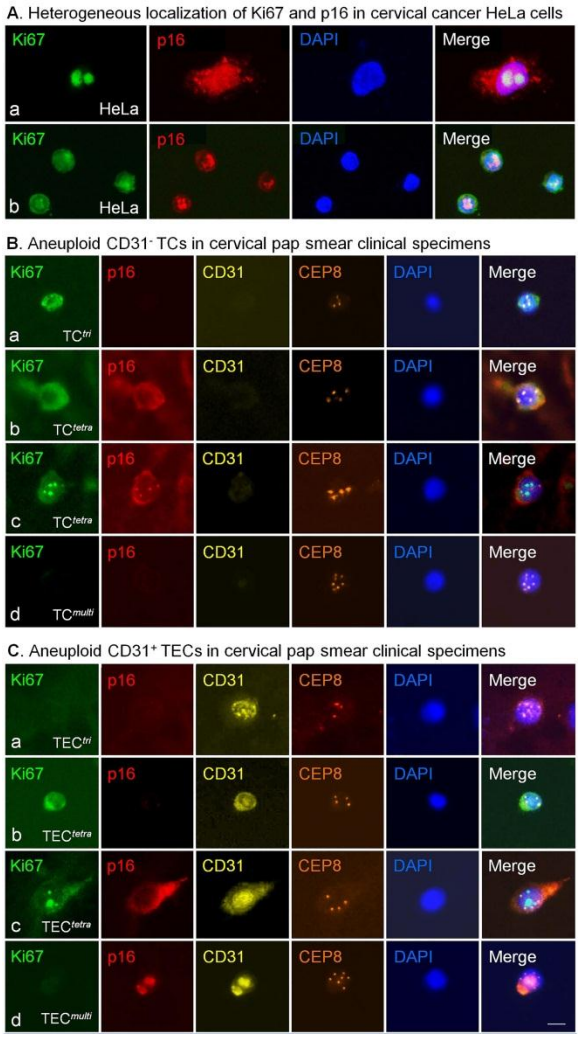
Methods A total of 196 patients were enrolled in this study. Immunofluorescence staining of p16 and Ki67 combined with FISH was applied to quantitatively co-detect and characterize subcategorized aneuploid $CD31^-$ TCs and $CD31^+$ TECs in cervical cytological specimens. The Kruskal–Wallis H test was used to compare the distributions of aneuploid TCs and TECs among all stages of cervical lesions and among the different high-risk HPV types (HPV16/18 and non-HPV16/18). The diagnostic value of detecting aneuploid TCs and TECs for high-grade squamous intraepithelial lesions ($HSIL^+$) was investigated via receiver operating characteristic curve analysis.

Results The number of total aneuploid $CD31^-$ TCs and their $p16^+$ and/or $Ki67^+$ ($p16/Ki67^+$) subtypes increased markedly with the severity of cervical lesions, although a similar trend was not observed for aneuploid $CD31^+$ TECs. The increase in aneuploid TCs resulted from HPV16/18 infection was mainly concentrated in low-grade squamous intraepithelial lesion(LSIL), whereas the increase caused by non-HPV16/18 infection was mainly concentrated in HSIL. To identify $HSIL^+$, the area under the curve (AUC) of tetraploid TCs was the largest (0.739), followed by multiploid (≥ pentaploid) TCs (0.724) and triploid TCs (0.699). For the combined subtypes, the AUC of ≥ tetraploid TCs was 0.745, and their unique diagnostic value was clinically reflected by their high specificity.

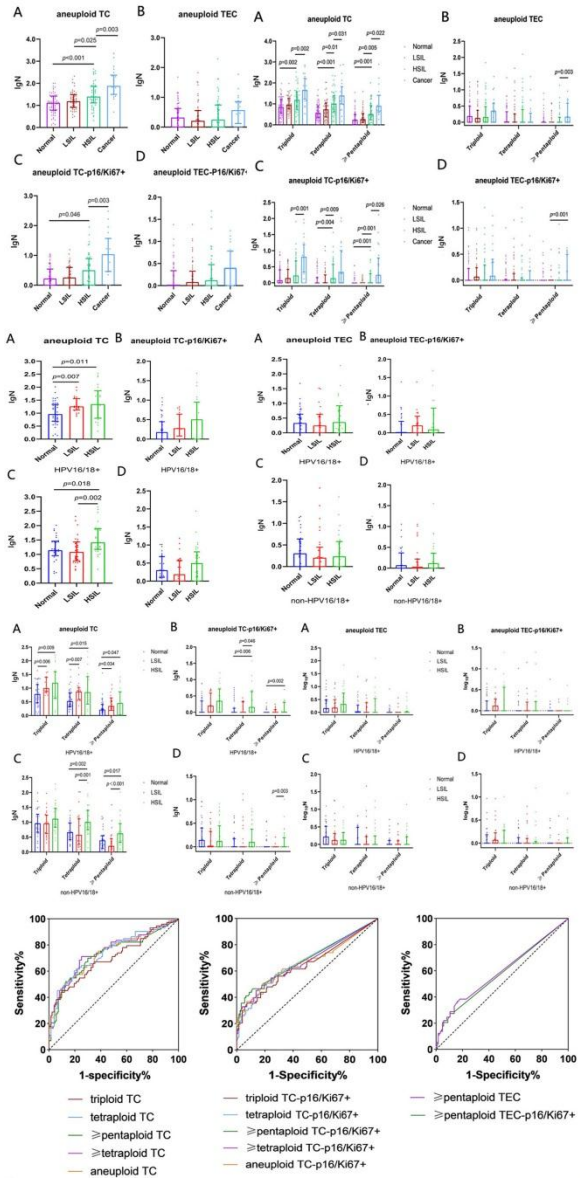
Conclusion The quantity of $CD31^-$ aneuploid TCs was associated with the severity of cervical lesions. In HPV16/18 positive patients, aneuploid $CD31^-$ TCs were significantly increased in the LSIL. Moreover, aneuploid $CD31^-$ TCs exhibited remarkable specificity for detecting $HSIL^+$. Further studies are required to expand the potential clinical utility of detecting $CD31^-$ aneuploid TCs.

鉴于细胞形态、Ki67 及 p16 染色是临床病理应用免疫组化 (IHC) 检查宫颈癌细胞的主要方法，本文作者首先采用免疫荧光染色技术检测了这两种蛋白在宫颈癌细胞 HeLa 内的分布，与以往已被报道的结果相一致，Ki67 分布于细胞浆、细胞核及核仁内[4,5]，

p16 则见于细胞浆和细胞核内[6,7]。同样地，Ki67 及 p16 在细胞内的高异质性分布也可见于 i•FISH 检测的宫颈刮片异倍体细胞 TC 或 TEC 中。



作者进一步在不同分期的宫颈病变及高风险 HPV (high risk HPC, hrHPV) (包括 HPV16/18 型与非 HPV16/18 型) 感染的正常宫颈刮片标本中，尤其针对伴有高级别上皮内瘤变 及宫颈癌患者应用 Kruskal–Wallis H test 重点研究了异倍体 $CD31^-$ 肿瘤细胞 (TC)、$CD31^+$ 肿瘤血管内皮细胞 (TEC) 的分布及相关染色体倍体、Ki67 及 p16 表达的变化。



结果要点

- 仅有部分而非全部检出的异倍体 TC、TEC 表达 Ki67 或 p16，揭示了 i•FISH 检测 TC、TEC 的灵敏性高于单纯 Ki67、p16 抗体染色

- 伴随着宫颈病变程度及宫颈癌从低级别向高级别的加深，宫颈刮片标本中异倍体 $CD31^-$ 肿瘤细胞 (TC) 的数量占比明显升高，其中 $Ki67^+$ 和 $p16^+$ TC (尤其是三倍体 TC) 的瘤标阳性检出率同样伴随升高
- HPV16/18 型感染所致的 TC 升高主要见于低级别上皮内瘤变 (LSIL) 患者，而由非 HPV16/18 型 (non-HPV16/18) 感染所致的 TC 升高主要见于高级别上皮内瘤变 (HSIL) 患者
- hrHPV 感染后“肉眼所见正常”的宫颈刮片标本中也可检测到异倍体 TC，其数量与 LSIL 标本中的 TC 近似，提示 i•FISH 检测的高灵敏性
- $CD31^-$ TC 与 HSIL 的高特异检出存在极为显著的相关性
- 有效区分与鉴别异倍体 $CD31^-$ TC 及 $CD31^+$ TEC 的重要意义：本实验结果只见于 $CD31^-$ TC，在 $CD31^+$ TEC 中没有观察到具有统计学意义的差异，若将这两类细胞不加区分地混检为异倍体细胞，上述实验结果不成立，此现象与在胶质瘤领域获得的研究成果相一致[8]

本研究为今后在更大样本上进一步应用 i•FISH 高灵敏、高特异地开展宫颈癌早筛提供了理论依据。

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