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激光医学

激光共焦显微拉曼光谱技术在人舌鳞癌细胞检测中的应用

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【摘 要】**目的:**采用激光共焦显微拉曼光谱技术对人舌鳞癌细胞CAL-27进行检测,并将其与正常人体口腔黏膜上皮细胞(NHOK)进行对比和分析,从而从分子层面区分CAL-27和NHOK。**方法:**培养CAL-27和NHOK,并用激光共焦显微拉曼光谱对其进行检测,以及采用主成分分析法和线性判别技术对其进行分析。**结果:**对比CAL-27和NHOK光谱差谱,发现谱宽于735、959、1 334、1 575 cm^{-1}处,CAL-27的峰值比NHOK强,但在1 033 cm^{-1}处,则相对要弱。结合主成分分析和线性判别技术的灵敏度为96.8%,特异性为90.3%,诊断率为93.5%。受试者操作特性曲线下面积为0.971。**结论:**采用激光共焦显微拉曼技术可区分CAL-27和NHOK。

【关键词】人舌鳞癌细胞;正常人体口腔黏膜上皮细胞;激光共焦显微拉曼光谱;主成分分析法;线性判别技术
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Micro-Raman spectroscopy technique in the detection of human tongue squamous carcinoma cell

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Abstract: Objective To detect human tongue squamous carcinoma cell CAL-27 with micro-Raman spectroscopy technique, and distinguish CAL-27 from normal human oral keratinocytes (NHOK) in the molecular level by comparing CAL-27 with NHOK. **Methods** The cultivated CAL-27 and NHOK were detected with micro-Raman spectroscopy technique, and analyzed using principal component analysis combined with linear discriminate analysis (PCA-LDA). **Results** Comparing with NHOK, higher peak values were found at 735, 959, 1 334, 1 575 cm^{-1}, and lower peak value at 1 033 cm^{-1} in CAL-27. The PCA-LDA achieved a sensitivity of 96.8%, a specificity of 90.3%, and a diagnostic accuracy of 93.5%. The area under the receiver operating characteristic curve was 0.971. **Conclusion** Micro-Raman spectroscopy with PCA-LDA has the potential for distinguishing CAL-27 from NHOK.

Keywords: human tongue squamous carcinoma cell; normal human oral keratinocytes; micro-Raman spectroscopy; principal component analysis; linear discriminate analysis

前 言

舌癌是口腔癌中最常见的癌症,按国际抗癌联盟的分类,舌前2/3癌肿属口腔癌范畴;舌后1/3(舌根)属口咽癌范畴。舌癌的致癌因素与局部创伤和烟、酒嗜好有关。一般舌体癌98%以上为鳞癌[1-2]。

拉曼光谱分析技术结合一定的数据处理方法或者统计学分析方法,可以作为一种有效而可靠的肿瘤细胞检测技术。虽然已有多种肿瘤细胞(包括神经胶质瘤细胞[3]、乳腺癌细胞[4-5]、前列腺癌细胞[6]、口腔癌鳞癌细胞[7-8]等)的拉曼光谱的研究报道,但是对

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于人舌鳞癌细胞(CAL-27)的拉曼光谱研究几乎处于空白状态。如果要从分子水平上对CAL-27和正常人体口腔黏膜细胞(Normal Human Oral Keratinocytes,NHOK)的拉曼光谱进行深入探讨和分析,就有待于相关实验的开展和研究的深入。本研究正是从分子水平上分别对CAL-27和NHOK进行检测,为拉曼光谱技术用于鉴别和区分CAL-27和NHOK确立实验依据和奠定基础。

1 材料与方法

1.1 主要仪器和试剂

主要仪器为Renishaw inVia型激光共焦显微拉曼光谱系统(WiRE 3.2)。主要试剂为购自GIBOCO的DMEM细胞培养基粉末(Grand Island, NY, United States)。实验中的胚牛血清(FBS)和胰蛋白酶(含0.25% EDTA)购自天津TBD生物试剂公司。

1.2 细胞株及其培养

CAL-27购自上海瑞鹿生物科技有限公司,采用DMEM(高糖)培养液(含10% FBS)在CO_2恒温培养箱(37 ℃,含5%CO_2)进行贴壁培养,每天进行换液,当细胞生长到80%~90%致密时,用胰酶将细胞消化按1∶2的比例传代培养[9]。NHOK购自上海斯信生物科技有限公司,采用DMEM培养液,其余部分都与CAL-27的一样。

1.3 细胞的拉曼检测及数据处理

本研究使用雷尼绍公司的显微共聚焦拉曼光谱仪(Renishaw, inVia, UK)(图1),光谱测量范围为600~1 800 cm^{-1},光谱分辨率大约为1 cm^{-1},激发光源为785 nm半导体激光,最大激发功率大约为250 mW,激光光斑直径为5 μm。仪器配套的显微镜为莱卡DM2500,物镜放大倍数50×。取对数生长期的CAL-27和NHOK,再消化吹悬后,用移液枪吸取约50 μL的悬液滴到铝片上,然后将液滴用枪头拨开,使其尽量地展开,置于激光共焦显微拉曼光谱仪的显微镜载物台上,采用激发波长785 nm,采集次数4次,曝光时间2 s的参数进行采集,每个样品在不同位点测量3次,然后取平均谱。所有谱线都在相同条件下测量,光谱的获取与分析都使用雷尼绍公司的拉曼软件包WIRE 3.2[10]。

去除拉曼谱线背景噪声,5阶多项式拟合背景曲线后从原始谱线中扣除。为了便于比较不同样品光谱形状和谱峰的相对强度,对曲线下的光谱面积进行归一化处理。光谱的平滑和基线修正采用Vancouver拉曼算法,Vancouver是一个能进行批处理的扣除荧光背景噪声和曲线平滑的算法[11]。

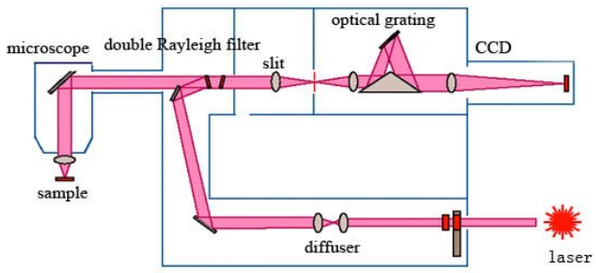
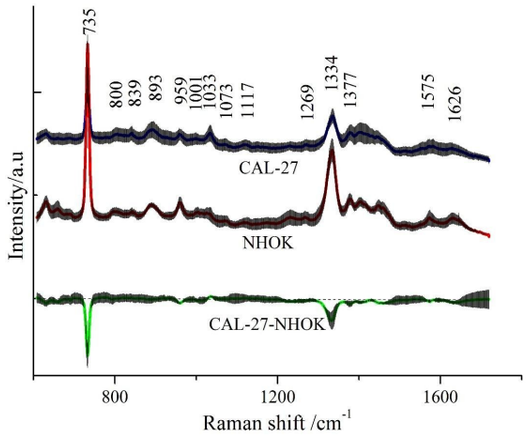


图1 Renishaw inVia型显微拉曼光谱仪的光路示意图

Fig.1 Schematic illustration of the optical path in a Renishaw inVia micro-Raman spectrometer

2 结 果

图2为CAL-27和NHOK的拉曼谱,可以发现CAL-27和NHOK的主要拉曼峰有735、839、959、1 001、1 033、1 073、1 117、1 269、1 334、1 575和1 626 cm^{-1},谱峰指认见表1。这些峰与以往报道的人口腔拉曼很相似[12-14]。另外,虽然两种细胞的拉曼峰大体是一致的,但也有差异,例如CAL-27在735、959、1 334和1 575 cm^{-1}处的峰值比NHOK的要强,但是在1 033 cm^{-1}相对弱。说明在鳞状癌细胞中DNA、蛋白及脂类合成增强,表现出比较明显的增殖活性[15]。



CAL-27: Human tongue squamous carcinoma cell; NHOK: Normal human oral keratinocytes; The solid lines indicated the average spectra and the shaded lines represented a standard deviation.

图2 CAL-27和NHOK的平均谱及差谱

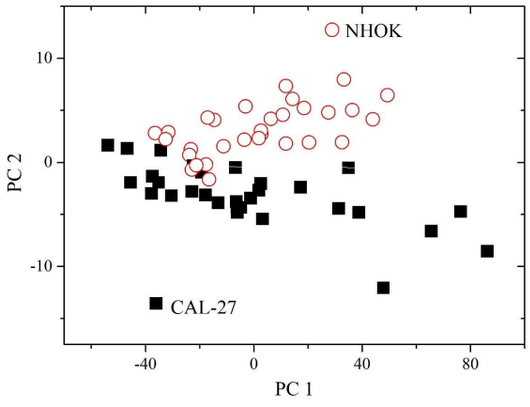
Fig.2 Average Raman spectra and differential spectra of CAL-27 and NHOK

为了进一步的区分CAL-27和NHOK的拉曼光谱,本研究采用主成分分析法进行分析,并利用前两个主要成份,即主成分1(PC1)和主成分2(PC2),作二维散点图,可以非常明显地区分肿瘤细胞和正常黏膜细胞(图3)。

表1 CAL-27与NHOK的拉曼光谱归属

Tab.1 Vibrational mode and major tentative assignments of identified CAL-27 and NHOK

Peak position/cm^{-1}	Vibrational mode and major assignments
735	A-nucleic acids[7]
839	Out of plane ring breathing tyrosine(O-P-O) stretch DNA[16]
959	C-C stretching mode of proteins[3], DNA [17]
1 001	C-C symmetric stretching mode of phenylalanine[14, 17]
1 033	C-H stretching mode of phenylalanine[17]
1 073	C-N stretching mode[5, 17]
1 117	C-N stretching mode of proteins, C-C stretching mode of carbohydrates[3]
1 269	Amide-Ⅲ[5]
1 334	Adenine, guanine[3, 17]
1 575	Adenine, guanine[3]
1 626	Amide of proteins[17]



The red circles were NHOK and the black squares were CAL-27.

图3 CAL-27和NHOK拉曼光谱经PCA主成分分析的散点图

Fig.3 Scatter plots of principal component analysis (PCA) for NHOK and CAL-27

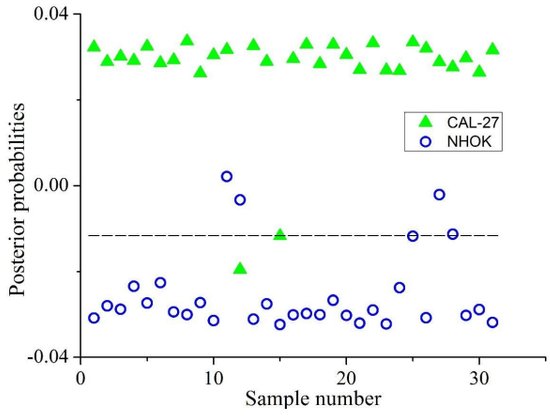


图4 CAL-27与NHOK的主成分分析线性判别散点图

Fig.4 Scatter plots of PCA-linear discriminate analysis (PCA-LDA) for CAL-27 and NHOK

采用主成分分析法和线性判别分析技术搜索CAL-27有关的特征拉曼光谱，并结合留一交叉检验法来绘制CAL-27与NHOK拉曼光谱的线性判别的散点图（图4）。该图分界线产生的诊断灵敏度为96.8%（30/31），特异性为90.3%（28/31），总诊断准确率为93.5%（58/62）。上述结果说明利用主成分分析法结合线性判别分析技术可为CAL-27的快速无创诊断发展了一条新的途径。

为了进一步验证构建的PCA-LDA算法的有效性，采用工作特征（Receiver Operator Characteristic, ROC）曲线方法来对该算法进行评估，该方法可直观地反映灵敏度和特异性关系。根据线性判别得分的结果，通过改变判别阈值来计算不同阈值下的灵敏度和特异性，再以真阳性率（灵敏度）为纵坐标，假阳性率（1-特异度）为横坐标，将得到的结果用曲线连接起来。本研究对PCA-LDA诊断模型所得到的判别函数得分进行ROC曲线（图5），该ROC线下的面积为0.971，该结果进一步验证了PCA-LDA法的诊断能力。

3 讨 论

本研究提出一种采用拉曼光谱对人体舌鳞癌细胞和NHOK进行高效、分子指纹的区分方法。该方法再结合主成分分析法和线性判别分析技术进行分析可获得灵敏度为96.8%，特异性为90.3%，诊断率为

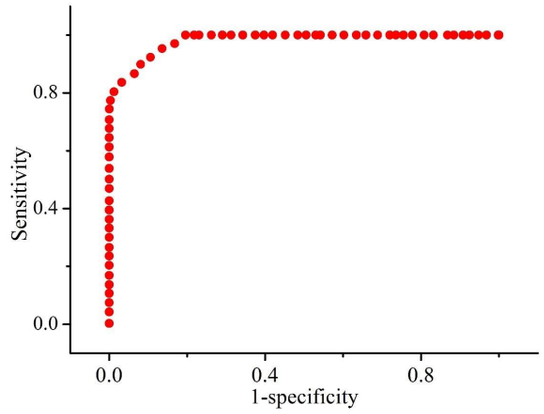


图5 PCA- LDA结合留一交叉检验法对CAI-27和NHOK的被试工作特征曲线

Fig.5 Receiver operating characteristic curve of PCA-LDA combined with leave-one-out cross-validation method for CAL-27 and NHOK

93.5%，该方法相对于荧光分光光度计具有无需添加标记剂等优点，有利于舌癌发生发展机制的研究。

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