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利用GFP示踪细胞内源性P53活性检测DNA损伤 GFP Reporting Endogenous P53 Transcriptional Activation as a Detector for DNA Damage

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摘要　DNA损伤的检测对预防癌症和遗传病等非常重要。采用分子克隆技术，将报告基因－绿色荧光蛋白（GFP）置于SV40基本启动子调控下，构建成对照载体pSV-GFP。在SV40基本启动子上游插入寡核苷酸P53RE，构建成示踪载体p53RE-GFP。转染NIH 3T3细胞，以GFP示踪细胞内源性P53的转录激活活性。紫外线照射或H2O2处理转化细胞使DNA损伤，诱导细胞内源性P53的表达。用激光扫描共聚焦成像系统（LSCIS）对细胞进行红、绿、蓝三色光融合成像，并测定GFP经488nm激发后发出的绿色荧光光密度，验证GFP示踪P53的特异性。p53RE-GFP转化细胞3T3-REG经紫外线照射或H2O2处理后，GFP的表达增高，处理后1hr光密度即达到最高水平，随后逐渐降低。血清“饥饿”－非DNA损伤处理的3T3-REG细胞，以及经紫外和H2O2处理的对照载体pSV-GFP转化细胞3T3-SVG，GFP的表达无明显增强。实验表明：GFP示踪内源性P53转录激活活性用于检测DNA损伤有很高的灵敏度和特异性，适宜推广应用。
Abstract　Indentifying and measuring DNA damage are important in hazard assessment. Reporter gene, green fluorescent protein(GFP), with P53 response element(P53RE) fusion gene was constructed and transfected NIH 3T3 cells. Transformed cells were treated with ultraviolet and H2O2 to make DNA damage and induce endogenous P53 expression. The GFP expression and the intensity of green fluorescence in cells were measured with Laser Scanning Confocal Imaging System(LSCIS). The fluorescence intensity increased rapidly aftern ultraviolet and H2O2 treatment and reached the maxism 1 hr later, then decreased slowly. The fluoorescent intensity of cells treated with fatal calf serum(FCS)drawing, a non DNA damage treatment, increased slightly 4hr later, as well as cells transformed with ?ontrol vector pSV-GFP after ultraviolet treatment. The results suggest that GFP as a roporter of P53 transcriptinal activation is a sensitive and specific detector for DNA damage.

关键词　绿色荧光蛋白　P53　DNA损伤　检测 Key words　GFP　P53　DNA damage　Detection
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