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研究论文

未修饰的纳米金结合等位特异性扩增来检测**K-ras**基因点突变

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摘要 将等位基因特异性扩增的特异性与纳米金特殊的光学性质相结合, 发展了一种新的基因点突变检测方法. 以肿瘤中常见的K-ras癌基因第12位密码子作为点突变检测对象, 采用突变型引物对待测序列进行等位特异性扩增. 突变型样品扩增产物中大部分是双链DNA; 而野生型样品由于不能被顺利扩增, 产物中大部分是单链DNA. 以纳米金颗粒作为报告基团, 向两种不同基因型扩增产物中依次加入纳米金胶和盐溶液, 野生型基因扩增产物中的单链引物被吸附到纳米金颗粒表面, 使得纳米金在适宜浓度的盐溶液中不发生聚集; 突变型样品扩增产物中的双链DNA由于与纳米金颗粒间存在静电斥力而不能被吸附到纳米金颗粒表面, 纳米金在该浓度的盐溶液中发生聚集, 导致两种基因型的混合液在吸收光谱和颜色方面均存在显著差异, 从而实现了检测基因点突变的目的. 该检测方法直观、快速、简便, 实验成本低, 能够检测到pmol量级的样品, 为点突变检测提供了一种实用的新方法.

关键词 纳米金 表面等离子峰 等位特异性扩增 点突变

分类号

Detection of K-ras Point Mutation by Unmodified Gold Nanoparticles and Allele-specific Amplification

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Abstract A novel method for point mutation detection was developed. This approach combined the optical properties of gold nanoparticles with the specificity of allele-specific amplification (ASA). K-ras oncogene was studied for its possible point mutation at codon 12. In ASA, only the mutant K-ras gene can be amplified using the mutant-specific primers. Thus, the mutant amplification products were made of mostly double-stranded DNA, while the wild-type products were made of mostly unconsumed single-stranded DNA. Unmodified gold nanoparticles were used as reported groups. The amplification products were mixed with the gold colloid, followed by addition of salt solution. Because single- and double-stranded DNA have different electrostatic interactions with gold nanoparticles, only the gold nanoparticles in the wild-type mixtures did not aggregate due to the adsorption of ss-strand DNA. Therefore, the two kinds of genotype mixtures gave different absorption spectra and colors were observed easily by naked eyes. This new method is rapid, simple, low cost, and will offer a broad application for point mutation detection.

Key words gold nanoparticle surface plasmon peak allele-specific amplification point mutation

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