癌变.畸变.突变 ISSN: 1004-616x CN:44-1063/R 2004; 16(4) :196-198

论著

一种新的酶切保护PCR分析方法及其在二恶英类化合物检测中的应用

孙 唏 ，李 芳，陈启政，孙 纳，王小利，严 红，徐顺清

华中科技大学同济医学院环境医学研究所，湖北 武汉 430030

收稿日期 2004-2-21 修回日期 2004-3-26 网络版发布日期:

摘要 背景与目的:建立一种灵敏、快速、无放射性污染的生物方法来检测环境样本中的二恶英类化合物。材料与方法：二恶英类化合物可以活化胞浆内芳香烃受体(AhR)使之与一段包含特定序列的双链DNA结合，结合的DNA 因受蛋白质保护可抵抗核酸外切酶消化而保留下来，痕量的保留DNA通过PCR检测出来。根据此原理本研究将TCDD溶液加入到含AhR及相关蛋白的细胞溶质中，在体外与含二恶英反应元件的DNA作用形成二恶英-AhR-DNA复合物，用核酸外切酶ExoIII 和S1核酸酶进行消化后作PCR，通过琼脂糖电泳可以检测目的DNA是否存在；同时以有机溶剂DMSO设为对照。结果：在实验组用琼脂糖电泳可以检测到期望大小片段的DNA, 而对照组为阴性。结论：该方法可作为环境样本中二恶英污染的生物检测手段，具有灵敏、经济和快速的优点。

关键词 二恶英类化合物；芳香烃受体；二恶英反应元件；外切酶III；S1核酸酶

A Developmental Exonuclease Protection Mediated PCR Assay Applied to Detect the Dioxin-Like Chemicals

SUN Xi, LI Fang, CHEN Qi-zhen, et al

Institute of Environmental Medicine, Tongji Medical College,Huazhong University of Science and Technology,Wuhan 430030,China

Abstract BACKGROUND & AIM: For the non-radioactive, sensitive and rapid detection of the dioxin-like chemicals in the environmental samples, exonuclease protection mediated PCR assay was established. MATERIAL AND METHODS: 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) dissolved in DMSO, a typical and the most toxic ligand to transform AhR, was added into SD rat hepalic cytosol in vitro, which contained Ah receptors and relative proteins, and ligand-AhR-DRE complex are formed in addition of DNAs containing the sequence of DRE.With the digestion of Exonuclease III and S1 nuclease, free DNAs were digested to mononucleotide and bound DNA remained due to protein(AhR) protction and could be amplified by PCR.The effects of PCRs were shown by loading on 2 % agarose electrophoresis.DMSO was used as negative control was set up. RESULTS： Target DNA could be observed in the TCDD groups, but not in the control group. CONCLUSIONS： Exonuclease protection mediated PCR assay is a good non-radioactive tool to quantify the interaction of protein and DNA with high sensitivity and simple.

Keywords dioxin-like chemicals aromatic hydrocarbon receptor； dioxin responsive element； exonuclease III S1 nuclease

DOI

通讯作者 徐顺清 shunqing@public.wh.hb.cn