中文摘要

戴奧辛（dioxin）是一群含多氯聯苯化合物之總稱，由於具有高度脂溶性，多經由食物鏈積存於生物體內，影響賀爾蒙之調控，甚至造成細胞癌化，其中2,3,7,8-TCDD(2,3,7,8-tetrachlorodibenzo-p-dioxin)是目前所知毒性最強之污染物。目前我國檢測戴奧辛大多使用氣相層析-高解析度質譜儀分析(GC/HRMS)等化學方法，然而隨著戴奧辛在生物體內毒理機制了解與日俱增，先後有 ELISA、CALUX 等生物檢測戴奧辛方法，由於具備快速、便宜、操作簡單等優勢，目前應用生物檢測方法來篩檢戴奧辛樣品逐漸成為主流。因此，本研究根據細胞辨識戴奧辛等環境污染物反應，分別以小鼠戴奧辛受器 mAHR (mouse aryl hydrocarbon receptor)-小鼠熱休克蛋白 90 mHSP90 (mouse heat shock protein 90)組合與人類戴奧辛受器 hAHR (human aryl hydrocarbon receptor)-人類戴奧辛受器核轉置蛋白 hARNT(human AHR nuclear translocator)組合，藉由螢光共振能量轉移技術(Fluorescence Resonance Energy Transfer，FRET)訊號偵測戴奧辛污染程度，在時間及成本上占有相對優勢，且較能真實反應環境汙染因子對生物體的毒害程度。實驗設計上，分別建構 mAHR-CFP 與 mHSP90-YFP 以及 hAHR-CFP 與 hARNT-YFP 的表達質體，送入大鼠肝癌 H4IIEC3 細胞中，結果發現螢光共振能量轉移訊號與戴奧辛污染程度有依賴關係，在 mAHR-CFP 與 mHSP90-YFP 組合部分，隨著不同劑量之戴奧辛而訊號下降；在 hAHR-CFP 與 hARNT-YFP 組合部分，則隨著不同劑量而訊號上昇。根據研究結果，未來具有作為輔助戴奧辛等環境污染物大量篩選檢測工具之可行性。

[關鍵字]戴奧辛、螢光共振能量轉移、戴奧辛生物檢測方法

Abstract

Dioxins comprise a group of compounds which contain a double aromatic ring-like structure. They are among the most prevalent and toxic environmental pollutants. Accumulation of dioxins in human tissues poses a potential threat to human health. Currently, analytical chemical procedures dominate dioxin-detection protocols. In this study, we established 2 set of fluorescence resonance energy transfer (FRET)-based dioxin-detection bioassays. We generated plasmids encoding fusion proteins of mAHR (Mouse aryl hydrocarbon receptor) and mHSP90 (Mouse heat shock protein 90) with CFP and YFP , and the other set by the same method, We generated plasmids encoding fusion proteins of hAHR (Human aryl hydrocarbon receptor) and hARNT (Human AHR nuclear translocator) with CFP and YFP, respectively. Constructs were transiently co-transfected into rat hepatoma cell line, H4IIEC3 cells. Our results showed that FRET signals were detected in mAHR-CFP and mHSP90-YFP co-transfected H4IIEC3 cells. Dioxin treatments down-regulated FRET signals in these transfected cells in a dose-dependent manner. On the other hand, no FRET signals were detected in hAHR-CFP and hARNT-YFP-transfected H4IIEC3 cells. In contrast, dioxin treatments up-regulated FRET signals in these transfected cells in a dose-dependent manner. This work highlighted the potential of using FRET technique in the detection of dioxin-like compounds.

Key words: Dioxin, Fluorescence resonance energy transfer, Dioxin bioassay