INDUCTION OF P53 IN MUSSEL *MYTILUS GALLOPROVINCIALIS* TISSUES

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Abstract

The tumor suppressor phosphoprotein p53 as a biomarker of genotoxic stress in marine mussel *Mytilus galloprovincialis* were investigated. The effect of direct and indirect acting genotoxic xenobiotic compounds on p53 protein family levels were determinated using the Western blot analysis and immunoassay detection. The well known DNA damaging agents: N-methil-*N*'-nitro-nitrosoguanidine (MNNG), 4-nitroquinoline-N-oxide (NQO), and benzo[a]pyrene (B[a]P) induced level of p53 in a time and dose dependent manner. The aim of the study was to establish the applicability of the p53 induction as a genotoxic stress estimation tool and promised use of p53 as a biomarker in marine ecotoxicological monitoring research.

Keywords : biomonitoring, ecotoxicology, genotoxic agents, musell Mytilus galloprovincialis, p53

Introduction

The investigation of tumor suppressor p53 gene and protein family is in the focus of much interest in last decade. Their sequence, biochemical properties and biological function were well described and characterized. Up to date the induction of the p53 protein as an indicator of genotoxic damage which leads to cell cycle arrest and DNA repair or apoptosis were investigated in several types of cell lines. The identification of genotoxic agents effects by p53 induction has arising interest in last several years (1).

A few scientific research teams in US showed attention on soft calm *Mya arenaria* p53 induction. The expression of *p*53 gene homologue in this organism (2) and structural and functional data for p53 and p73 gene products (3) were under investigation for a several years. The clam leukaemia were initially detected and characterized in hemolymph tumor cells (4). The differential expression of *p*53 family members between normal hemocytes and leukaemia cells were showed by approximately equal amounts of *p*53 but no *p*73 is detectable in normal hemocytes (5.)

Those investigations lead as to make an attempt to establish the p53 induction/level determination, as existing pathway in marine invertebrates upon genotoxic agents effects, in Mediterranean mussel *Mytilus galloprovincialis* tissues. In this study the preliminary results of p53 protein family members induction by chemical xenobiotics are presented.

Materials and methods

All chemicals were of the highest analytical or molecular biology grade. Six different Mouse monoclonal antibodies against human p53 and Goat Anti-Mouse IgG, Alkaline Phosphatase conjugate were purchased by Upstate Biotechnology, USA.

Mussels *Mytilus galloprovincialis* were collected in Rovinj area, Northern Adriatic, and transferred to the laboratory basin, treated with 0-10 g MMNG, NQO and B[a]P /g mussel and incubated in seawater with flow, at 16° C. After 2, 6, 12, 24, 48 and 168 hours the mussel gills and hemolymph were taken from each of the mussel. All the experiments were performed with 5 mussels in each sample group.

Discontinuous SDS electrophoresis and Western blot were performed using the BioRad Mini System. Immunochemical detection of p53 protein levels were performed using polyclonal rabbit anti-p53 proteins and goat anti rabbit-HRP conjugate.

Western blots were photographed and scanned. Although each protein lane contained the same amounts of proteins and the quantization of p53 specific bands was performed by densitometry of the whole lane areas.

Results and discussion

In present study the induction of the p53 protein family members in mussel tissues were examinated by using direct and indirect acting genotoxic xenobiotics.

The effect of 0-10 :g MMNG /g mussel shows a dose dependent response and highest level of p53 in the first few hours after treatment of mussels. Mussels injected with the same amount of indirect acting genotoxic agents: NQO and B[a]P also showed the dose-dependent p53 induction but the maximum level of protein (increased half-life of p53 protein) were determinated after several hours of incubation. This xenobiotic needs biological activation (*via* P450 pathway) which leads to high level of DNA damage and p53 induction.

This study shows that p53 induction in mussel *Mytilus galloprovincialis* tissues could be effective tool to identify environmental genotoxins. The p53 protein family induction/level as a biomarker in marine invertebrates has a promised role in the estimation of genotoxic potential in the marine environment. Their suitability for genotoxicity assessment of pollution impact on aquatic organisms and environmental monitoring, to predict the environmental changes upon induced genotoxic effects of mixed xenobiotics presented in marine environment, has to be under high interest and further investigation.

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