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CELL CYCLE RESPONSE TO ISOCYANATES AND ONSET OF CYCLIN E EXPRESSION IN CULTURED MAMMALIAN CELLS

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Abstract: Cell cycle comprises the vital mechanism exposed to environmental toxic chemicals resulting in imbalance of cell cycle and finally ends with cancer. Cancer is a multistage process, which involves a series of genetic alterations that begins with genomic instability provoked by certain factors such as DNA damage, deregulation of CDKs, cyclins and end with the development of cancer. Key transitions in the cell cycle are regulated by the activities of various protein kinase complexes composed of cyclin and cyclin-dependent kinase (Cdk) molecules. An isocyanate is one of the highly reactive industrial intermediates, possesses the capability to modulate the bio-molecules by forming toxic metabolites and adducts which may cause adverse health effects. We find out the molecular mechanism of methyl isocyanate genotoxicity on cell cycle regulatory protein and also evaluate the expression of cyclin E protein in cultured mammalian cell lines (MM55.K & NIH/3T3) after exposure to isocyanate (N-sucinamidyl N-methylcarbamate). Extended exposure to isocyanates causes overexpression of Cyclin E and CDK2 and ultimately leads to genetic alteration in NIH/3T3 cells. This information could be useful to design of new approaches for risk assessment of potential industrial disasters.

Keywords: Cell cycle, Cyclin E, Cancer, Isocyanate, Genotoxic .

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INTRODUCTION

Signal transduction pathways represent the major communication network that transmits signals from cell surface to the nucleus in the form of metabolic messenger to activate specific genes. These pathways serve as an important function in integrating signals from individual pathways and to signal cell death, proliferation and differentiation [1, 2]. Progression through the cell cycle is regulated by inductive signals from outside the cell and intracellular signal pathways, while the cycle itself is regulated by cyclin-dependent kinases (CDKs). There CDKs & further phosphorylate by cyclins. This study provides the insights of the current state of knowledge for the biology of the cell cycle, with role of cyclin E, and a paradigm of cell cycle analysis. Cyclin E, Cyclin A and their partner cyclin-dependent kinases (Cdks) are key regulators of DNA synthesis and of mitosis. Cyclin A and Cyclin E are able to regulate both nuclear and cytoplasmic events because they both shuttle between the nucleus and the cytoplasm, mutations in the hydrophobic patch of cyclin A do affect Cdk binding and nuclear import. This has implications for the role of the hydrophobic patch as a substrate selection motif [3].

Cell cycle control is a highly regulated process that involves a complex cascade of events. Modulation of the expression and function of the cell cycle regulatory proteins including Cdks provides an important mechanism for inhibition of growth. Isothiocyanates have been shown capable of blocking cell cycle progression through the inhibition of multiple CDK activity [4, 5].

Isocyanates, the key components in the production of various industrial products, due to their electron loving chemistry, have been shown to form toxic metabolites and adduct in turn causing adverse health effects [6]. Yet, pathophysiological implications resulting from occupational and large scale accidental exposures still remain inexplicable. Therefore, there is an urgent need to delineate the molecular effects of isocyanates on cell cycle and cell cycle regulatory protein for; further understanding of cell survival mechanisms and cell death mechanisms, may have future practical application after exposure to occupational and environmental toxins.

Many investigations suggest that isocyanates and their family members are potential damaging agents at cellular levels; even they alter the cell cycle and its regulatory protein, so we are interested to assess the toxic insult at genomic level through MIC substitute exposure in cultured mammalian cells. For this purpose we have selected N- succinimidyl N- methyl carbamate which is a substitute of MIC that mimics its effect. In our study we have focus on the mechanisms underlying the genotoxic effect of MIC on cyclin E protein, a cell cycle regulatory protein in mammalian cultured cells.

MATERIALS AND METHODS

Experimental Material/ Agent/ Chemical:

N-Succinimidyl N-methylcarbamate, it is used as a substitute of methyl isocyanate (MIC), because it is only commercial available chemical which mimics the effect of MIC. When the cells were treated with this substitute, it produces DNA lesions in cells, because it has the capacity to react with the DNA molecules [7, 8]. N-succinimidyl N-methylcarbamate (Sigma Aldrich Laboratories, St. Louis, MO) dissolved in 2 mM DMSO at a final concentration of 0.005 mM (1 mg/ml) was used for investigations. Phytohemagglutinin and RPMI growth medium was procured from Gibco-BRL (Invitrogen Co, Carlsbad, USA).The culture petri-dishes were procured from Nalgene-Nunc Inc. (Roskilde, Denmark). Foetal bovine serum was obtained from HyClone Labs (Logan, Utah). For assessing the cell cycle regulatory proteins cyclin E antibodies procured from Abcam (Cambridge, UK) were used with appropriate dilutions.

Cell lines for Experiment

For implementing the investigation, the chosen mouse cell lines named, MM55.K (Mouse Kidney Epithelial Cells) and NIH/3T3 (Mouse Embryo Fibroblast Cells) were selected for our experiment.

Cell culture condition and medium

(I) Complete Growth Medium

The cells were grown in Dulbecco's modified Eagle's Medium with 4m M L – glutamine that is modified by ATCC to contain; 4.5 g/L Glucose, 1.5 g/L Sodium bicarbonate supplemented with 10% fetal bovine serum. This medium is formulated for use with a 5% CO_2 in air atmosphere. Complete culture medium described above supplemented with 5% (V/V) DMSO.

(II) Seeding of the cells for the Culture

Around 700,000 cells for Western blot and 200,000 cells for immuno fluorescence and FACS study were initially seeded into 100mm Petri dish and 60 mm Petri dish respectively; and were at 37^{o}C in presence of 5% CO_2 in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% Bovine calf serum and 1% antibiotic-antimycotic. Next day the grown cells, i.e.; 1400,000 cells for Western blot and 400,000 cells for immunofluorescence and FACS study were transfected with the experimental agent 5µl, N- Succinimidyl N-MethylCarbamate, and the experimental procedure was followed according to the 'experimental design'.

(III) ***Study design***

Different sampling intervals (n=5) ranging from 0-150 h were chosen. The cells were treated using a constant 0.005 mM concentration of N-succinimidyl N-methylcarbamate.

Quantitative Analysis of cyclin E protein Expression through Western Blot.

Briefly, cells were rinsed twice in PBS and lysed in the buffer (10% SDS, 1 M Tris pH- 7.6, 5 mM EDTA). The obtained cell lysates were centrifuged at 12 000 rpm for 10 min at 48C and the supernatant was collected. Protein concentrations were determined from the supernatant by Bradford assay. An amount of 100 mg protein was analysed through 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membrane in 25 mM Tris, 194 mM glycine and 20% methanol at 48C with semi-dryer transfer unit of Hoefer (Holliston, MA). Membranes were blocked with 5% non-fat milk in 0.1% PBST and incubated overnight at 48C with primary monoclonal antibody specific for cyclin E, CDK-2, (1:1000 dilutions). Membranes were washed two-to-three times for 20 min with 0.1% PBST and incubated for 2 h at room temperature with alkaline phosphatase conjugated secondary antibody (1:2500 dilutions). Membranes were washed and bound antibodies were analysed visually.

RESULTS

The results of western blotting demonstrate that the minute concentration of the MIC-substitute alters the cell cycle and by this process Cyclin E is expressed on nitrocellulose membrane.

A). Dried Gel: An intact protein profiling was observed after treatment of N-succinimidyl N-methyl carbamate in MM55.K & NIH/3T3 cell lines. First lane **M** indicate molecular weight marker (LMW-lower molecular weight marker), which is compared with our different passage (P1, P2, P3, P4, and P5) cells proteins (Image 2).

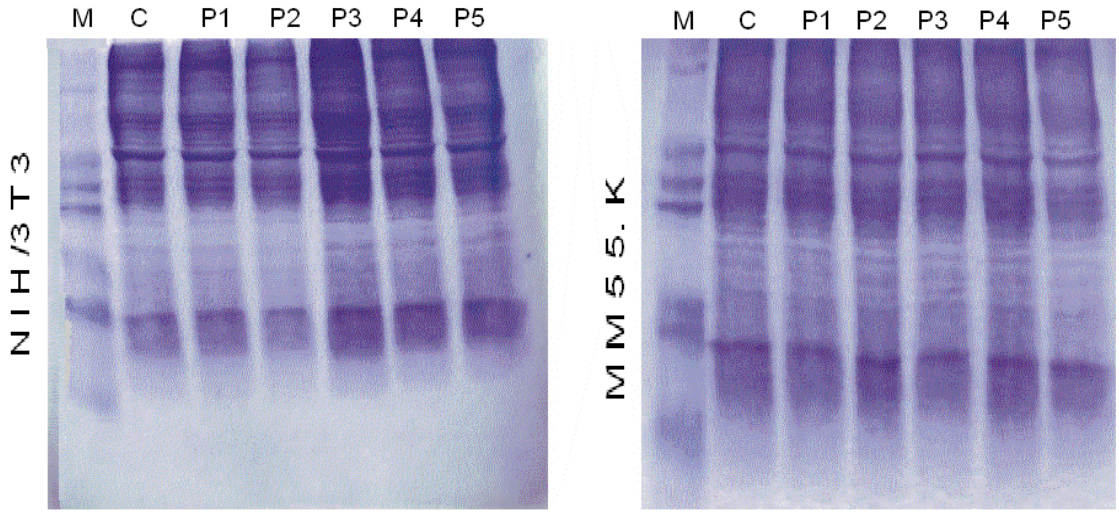

Image 1: A dried gel picture depicting the SDS-PAGE Protein profile.

B). <u>Nitrocellulose Membrane:</u> Western blot result shows the Cyclin E expression on nitrocellulose membrane in different passage but over expressed in passage second contrast to control. First lane C indicate Control, which is compared with our different passage (P1, P2, P3, P4, and P5) cells proteins (Image 2).

Image 2: Expression of Cyclin E protein observed through Western blotting.

DISCUSSION

Cyclin E is cell cycle regulatory protein. It mainly regulates the G_1/S phase transition of the cell cycle. It is involve in cell cycle regulation during DNA synthesis S phase. Cyclin E regulate the cell cycle with Cdk2, with its phosphorylations. Many organic and inorganic complexes affect the cyclin E activity few example of them.

Cyclin E expression negatively affects lung cancer prognosis. Its role in lung carcinogenesis was explored. Retroviral Cyclin E transduction promoted pulmonary epithelial cell growth, and small interfering RNA targeting of cyclin E repressed this growth [9]. Moreover, Galangin - a naturally occurring bioflavonoid, inhibited transition of cells from the G_0/G_1 to the S phases of cell

growth, likely through the nearly total elimination of cyclin D3. Expression of cyclin A and E was also suppressed [10].

In our Study two different mammalian cultured cells were implemented to study the toxic exposure of N-Succinimidyl N-Methylcarbamte, which is only available chemical that mimic the effects of Methyl isocyanates. Results from Western blot clearly suggest that the expression of Cyclin E gene, results indicating the over expression of cyclin E protein in second passage.

CONCLUSION

Cyclin E is a key regulator of cell cycle progression during G_1/S transition. The over expression of Cyclin E has been linked to the development and progression cancer. From our finding we are conclude that the Cyclin E over expression is due to deregulation of cell cycle after exposure of N–succinimedyl N-methyl carbamate in implemented cultured mammalian cells. Results suggest that this MIC substitute play a vital role in genetic alterations. Study predict that isocyanates toxicity potentially involve in DNA damage, so this investigation, besides decoding new scientific knowledge, would aid in preventive occupational management of future industrial disasters.

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REFERENCES

1. Chow S, Patel H, Hedley DW. Measurement of MAP kinase activation by flow cytometry using phospho-specific antibodies to MEK and ERK: Potential for Pharmacodynamic monitoring of Signal Transduction inhibitors. Cytometry. 2001; 46:72-78.

2. Jacobberger JW, Sramkoski RM, Frisa PS, Ye PP, Gottlieb MA, Hedley DW, Shankey TV, Smith BL, Paniagua M, Goolsby CL. Immunoreactivity of STAT5 phosphorylated on tyrosine 694 as a cell-based measure of Bcr/Abl kinase activity. Cytometry. 2003; 54:75-88.

3. Jackman M, Kubota Y, Elzen N, Hagting A and Pines J. Cyclin A- and Cyclin E-Cdk Complexes Shuttle between the Nucleus and the Cytoplasm. Mol Cell Biol. 2002; 13:1030-1045.

4. Suh SJ, Moon SK, Kim CH. Raphanus sativus and its isothiocyanates inhibit vascular smooth muscle cells proliferation and induce G (1) cell cycle arrest. Int Immunopharmacol. 2006; 6: 854-861.

5. Lee JY, Moon SK, Hwang CW, Nam KS, Kim YK, Yoon HD, Kim MG, Kim CH. A novel function of benzyl isothiocyanate in vascular smooth muscle cells: the role of ERK1/2, cell cycle regulation, and matrix metalloproteinase-9. J Cell Physiol. 2005; 203: 493-500.

6. Tamura N, Aoki K, Lee MS. 1992. Selective reactivities of isocyanates towards DNA bases and genotoxicity of methylcarbamylation of the DNA. Mutat Res 283:97-106.

7. Martinez J, Oiry J, Imbach JL and Winternitz F. Activated Nnitrosocarbamates for regioselective synthesis of N-nitrosoureas. J Med Chem. 1982; 25:178-82.

8. Takeda K. N-Succinimidyl N-MethylCarbamate. Tet Let. 1983; 24: 4569

9. Yan M, Steven F, Candice B, Xi L and Ziqiang Y, Vincent AM, David JR, Heather AB, Gregory JT, Eugene D, Sarah JF, and Ethan D. Transgenic cyclin E triggers dysplasia and multiple pulmonary adenocarcinomas. PNAS. 2006; 104:4089-4094.

10. Murray TJ, Yang X, and Sherr DH. Growth of a human mammary tumor cell line is blocked by galangin, a naturally occurring bioflavonoid, and is accompanied by down-regulation of cyclins D3, E, and A. Breast cancer Res. 2006; 8(2):R17.