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Original Research Article

Expression of Metallothionein Isoforms in MDD_2 and MN_1 of Breast and Hepatic Cancer ($HEPG_2$) Cell Lines

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Abstract

The metallothionein (MT) expression is used as a prognastic factor for tumor progression and drug resistance in a variety of malihgnancies particularly breast, hepatic, prostatic, ovarial, head and neck, non - small cell lung carcinoma, and soft tissues sarcoma. The latest research outcomes indicate that MT levels in cancerous cells indicate the clinical stage of the disease or response to therapy. MT plays a key role in transport of essential heavy metals, detoxification of toxic metals and protection of cells against oxidation stress.

Aim: The goal of this study was to see the MT isoforms (MT1A, MT1E, MT1F, MT1H, MT1X, MT2A) expression in MN_1 (human breast cancer epithelial cell line containing wild type – p53) and MDD_2 (derived from MCF_7 cell line, containing mutated p53) and $HEPG_2$ cell lines.

Materials and Methods: After designing all the above set of primes and adjusting the gradient temperature for the Polymerase Chain Reaction (PCR). The RNA is extracted and made complementary DNA from it.

Results: After several numbers of polymerase reactions and number of repetitions our data suggest that MT isoforms are not expressed in MN and MDD_2 cell line, but they are expressed in $HEPG_2$ cell lines.

Keywords: Metallothionein Ioforms; p^{53}.

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Introduction

The metallothioneins are a family of low molecular weight proteins with a high cystine and metal ion contains like zinc [1]. The physiological function is not completely understood ,they are involved in diverse processes including metal homiostasis and detoxification, the oxidative stress response, inflammation and cell proliferation [2]. The aberrant expression of metallothineins has been described in a number of diseases, including crohn's disease cancers, alzheimeir's disease, amytrophic lateral sclerosis, menke's disease and wilson's disease.

The role of metal ion zinc in hepatic cells is regeneration of the hepatic cells after the injuries like stress and zinc abnormal homeostasis. In resting cells the total amount of detectable metallothinein were approximately 1.6 x106 and 4x105 molecules per cell. In exponentially growing cultures the cellular contains of all the isoforms were increased fourfold.

The tumor suppressor gene p53 which place a major role in genome integrity regulation of cell cycle and programmed cell death. The relationship between p53 and metallothinein show high expression of metallothineins in the cell with mutated p^{53} and in higher grade of tumor. In some studies it has been suggested that metallothinein regulates DNA binding activity of p53 to zinc transfer reactions. The maintenance of wild type p53 conformation is achieved by zinc ions.

Materials and Methods

The study was conducted at Molecular Pathology and Genetics Laboratory Medicine, Kamineni Institute of Medical Sciencs, Telangana.

Cell Culture

The cells of the human breast cancer of epithelial cell line origin MN-1,containing the wild type p^{53} and other MDD-2 cell line, containing the mutated p53 (PLMUDD-p53, mut-p53) both the cell lines are derived from MCF-7.

MN-1 and MDD-2 cell lines are cultured in the modified eagle's medium containing 10% fetal bovine serum, 2mM (1%), L-glutamine and insulin of 1-ml.

Primer Preparation

The metallothionein isoforms MT-1A, MT-1E, MT-1F, MT-1H, MT-1X, MT-2A for all the above primers are designed and set for detection of dimmers, hairpins and optimum temperature. The above primers were designed using ENSEMBLE and IDTNA.

Primers Table

The following primer sets are designed for the reverse transcription-polymerase chain reaction.

MT-1A

Upper 5′ CTCGAAATGGACCCCAACT3′

Lower 5′ ATATCTTCGAGCAGGGCTGTC3′

Product: 219 bp

MT-1E

Upper 5′ GCTTGTTCGTCTCACTGGTG3′

Lower 5′ CAGGTTGTGCAGGTTGTTCTA3′

Product: 284 bp

MT-1F

Upper 5′ AGTCTCTCCTCGGCTTGC3′

Lower 5′ ACATCTGGGAGAAAGGTTGTC3′

Product: 232 bp

MT-1H

Upper 5′ CCTCTTCTCTTCTCGCTTGG3′

Lower 5′ GCAAATGAGTCGGAGTTGTAG3′

Product: 315 bp

MT-1X

Upper 5′ TCTCCTTGCCTCGAAATGGAC3′

Lower 5′ GGGCACACTTGGCACAGC3′

Product: 151 bp

MT-2A

Upper 5′ CCGACTCTAGCCGCCTCTT3′

Lower 5′ GTGGAAGTCGCGTTCTTTACA3′

Product: 259 bp

All the above primer sets are optimized.

RNA-Isolation

The RNA-isolation from the MN-1 and MDD-2 cell lines was done by using TRI-REAGENT protocol. The RNA thus obtained is stored in DMDC-water in -800C.

Quantification of RNA

The quantification of RNA of MN-1 and MDD-2 is checked by using the nanovue (GE healthcare, Buckinghamshire). Concentration of MN-1 is 1749µg/ml, 260/A280 is 2.033µg/ml, 260/230 is 1.87 µg/ml. Concentration of MDD-2 is 1949 µg/ml, 260/280 is 2.0 µg/ml, 260/230 is 2.1 µg/ml.

Quality of RNA

The RNA was run in RNA gel the obtained picture is below:

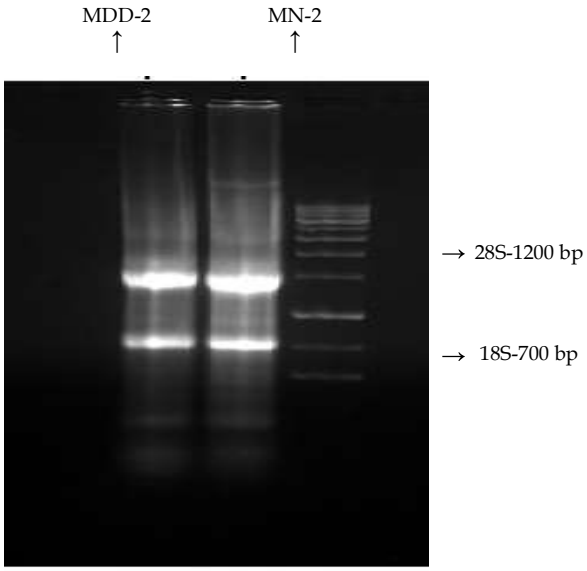


Fig. 1: RNA gel

CDNA Synthesis

DNA's treatment of the RNA was done using the promega kit and protocol.

Reverse Transcription Reaction (q Script cDNA Synthesis kit)

Reverse Transciptionisation of RNA is done using the RT-PCR protocol and conditions for PCR thermal cycle is:

1 Cycle: 220C, 5 mins

1 Cycle: 420C, 30 mins

1 Cycle: 850C, 5 mins

40C, hold

Gel Electrophoresis

All isoforms of metallothionein are run on the one percent agarose gel with 1X TAE buffer and Ethidium bromide is used in gel.

Gene ruler- DNA ladder, Low-Range, Ready -to-use.

Run at 100 volts for 45 to 75 mins and viewed under the UV lamp.

TAE buffer: Working Solution

1X

40mM Tris-acetate

1mM EDTA

Results

Metallothionein isoform expression in HepG2 cell lines. The expression of metallothionein isoform MT1-A, MT1-E, MT1-F, MT1-H, MT1-X, MT2-A is expressed in hepG2 cell lines not expressed in the MN-1, MDD-2 cell lines.

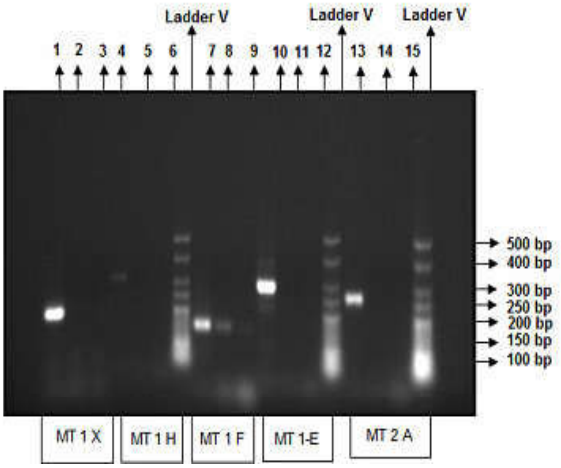


Fig. 2 MT isoform expression in $HEPG_2$

In the Figure 2 following are named as:

1. $HEPG_2$ with MT-1X primers
2. MN_1 with MT-1X primers
3. MDD_2 with MT-1X primers
4. $HEPG_2$ with MT-1H primers
5. MN_1 with MT-1H primers
6. MDD_2 with MT-1H primers
7. $HEPG_2$ with MT-1F primers
8. MN_1 with MT-1F primers
9. MDD_2 with MT-1F primers
10. $HEPG_2$ with MT-1E primers
11. MN_1 with MT-1E primers

12. MDD_2 with MT-1E primers
13. $HEPG_2$ with MT-2A primers
14. MN_1 with MT-2A primers
15. MDD_2 with MT-2A primers

Expected length of

- MT-1A primer is 219 bp.
- MT-1E primer is 284 bp.
- MT-1F primer is 232 bp.
- MT-1H primer is 315 bp.
- MT-1X primer is 151 bp.
- MT-2A primer is 259 bp.

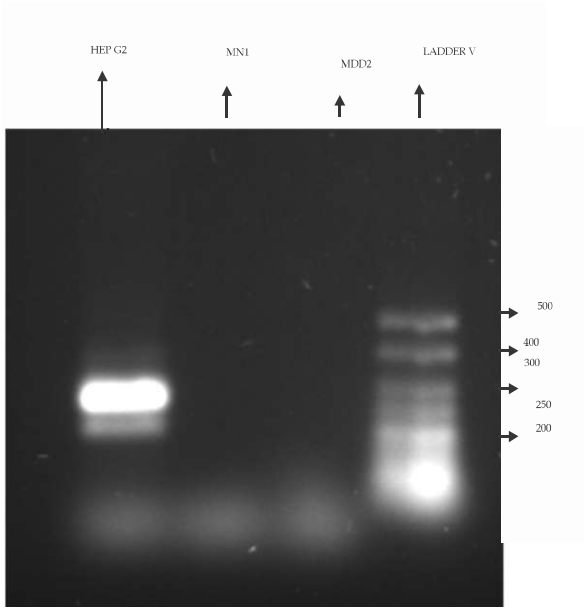
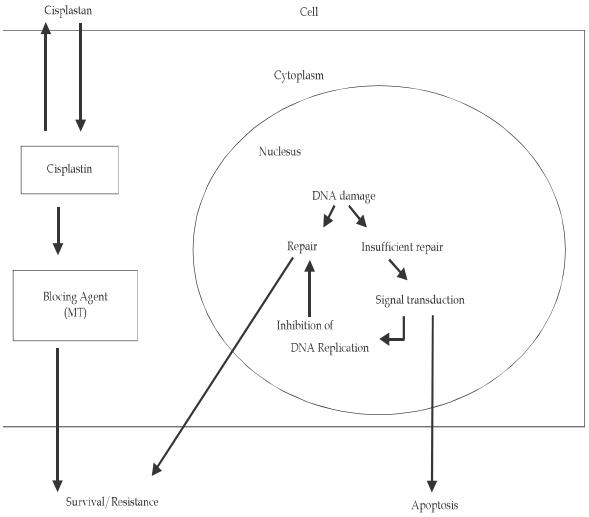


Fig. 3: MT-1A Primer (Expected lenth of MT-1A primer is 219 bp)

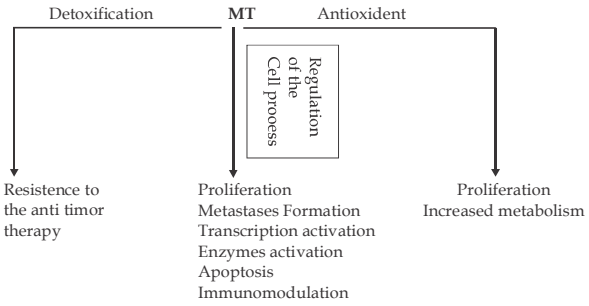
The metallothionein isoform expression in MN-1 and MDD-2 cell lines is not expressed.

Discussion

The mechanism of tumor cell protectio against cytotatic via synthesis of metallothioneins.



Main role of MT in healthy and tumor, cells and tissues



The aim of the study was to investigate the regulation and expression of MT expression. The results we achieved suggests that the hepatic tumor cells express the metallothoinein isoforms . Where as in thebreast cancer cell line the expression of the MT isoform is not possible only due to p53 active.

The tumor suppressor p53 protein which is regulator of the cell cycle progression apoptosis in responce to stimuli. The stability of p53 is attained by the phophorylation and acetylation.

The MT isoform in MN-1 and MDD-2 is not expressed may be due to active p53 active in both cell lines. The active p53 explemplifies the expression of the MT isoforms. The principal tumor supressor protein p53 mediated regulation of MT transcription in brest cancer cells, when the metals induce stress results in phosporylation of p53.

If the p53 is mutated then this leads to abnormality in normal cell homeostasis, chromosomal abnormalities and malignant transformation.

The MT isoforms are not expressed in cell lines with estrogen receptor positive cells and estrogen reseptor negative cells.

Recent studies suggest MTs expression is used as a prognostic factor for tumor progression and drug resistance in a variety of malignancies particulary breast, prostatic, ovarian, hepatic, head and neck, non – small cell lung carcinoma and soft tissue sarcoma. The role of MTs as a tumor disease marker or as a cause of resistance in cancer treatment. The latest research outcomes indicate that MT levels in peripheral blood and serum from cancer patients can provide much intesifying information about type or clinical stage of the disease, or responce to therapy. MTs play a key role in transport of essential heavy metals, detoxification of toxic metals and protection of cells gainst oxidation stress.

Serum MT levels of cancer patients are three times higher than control patients (6.5 µm). Thee elvated MT level in cancer cells are probably related to their increased proliferation and protection against apoptosis.

Prior publication: nil

Support: nil

Conflicts of interest: nil

Permissions: nil

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