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A Study on the Expression Profile and Redox Potency of Carcinogenic Thioredoxin from Human Lung Adenocarcinoma Epithelial Cells.

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ABSTRACT

The aim of this study was to explore the expression pattern and redox potency of the mortality combating thioredoxin isolated from human adenocarcinoma cells of lung and this could not only serve as potential new diagnostic / predictive marker but also aid in targeted therapy. The expression profile of thioredoxin (Trx) in human lung adenocarcinoma epithelial cells (A549) and normal lung cancer cells wasstudied by RT PCR and recombinant cloning techniques. The expression profile was analysed by Agarose and SDS PAGE electrophoretic analysis. The gene sequence was confirmed by DNA sequencing and the protein by western blot using anti-thioredoxin antibody. The REDOX activity of the thioredoxins wastested and compared by insulin reduction assay. There was upregulated and widespread expression ofthioredoxinin adenocarcinomaof lung and this protein was more potent when compared with normal physiological thioredoxin protein. There was enhanced and exaggerated dithiol-disulphideoxido reductase catalytic activity by the malignant thioredoxin which was observed as increasing reduction of insulin disulphides by dithiothreitol that could be visualized spectrophotometrically at 650 nm (25°C) as turbidity formation resulting from the precipitation of the free insulin β chain.

Keywords: Recombinant DNA, thioredoxin, cloning, protein, E.coli

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INTRODUCTION

Lung cancer is still one of the leading causes of cancer death with adenocarcinoma being the predominate histological type. Apart from its etiologies of smoking, pollution, asbestos exposure etc. it has been shown to be influenced also by the redox state of the cells that determines p53 expression, cell proliferation, and apoptosis [1] .The redox state may also influence the resistance of the cancer cells to chemotherapy, thus affecting the prognosis of the patients [2].

The redox state of the cells is governed by the thioredoxin system of proteins. Trx is a small 11,500kDa disulfide-reducing enzyme with a redox-active Cys-Gly-Pro-Cys active site[3] . This ubiquitous protein has been shown to regulate p53 expression and facilitate p53-dependent induction of p21 [4]. It also regulates the expression of transcriptional factors such as TF111C, nuclear factor-κB, Jun/Fos, and AP-1 through redox control of these proteins, thus influencing their binding to DNA, [5]. Human Trx is identical to adult T-cell leukemia-derived factor secreted by human T-cell lymphotrophic virus-1-transformed lymphocytes and which has growth-promoting effects on transformed cells. Consequently, Trx also promotes cell proliferation, and in transfection experiments, it has been shown to increase the growth rate and colony formation of nonmalignant and malignant cells. In addition, Trx increases oxidant and drug resistance of various cells, and it may be up-regulated during drug exposure and be associated at least with cisplatin resistance [6].

The oxidative or hypoxic stress of the tumor environment resulting from abnormal angiogenesis causes unstable oxygen delivery which is known to up-regulate the expression of thioredoxin. [7]During carcinogenesis, tumor cells often become more resistant to hypoxia or oxidative stress-induced apoptosis and most studies on tumor oxygenation have focused on these two tumor environments. Thioredoxin is known to have important roles in both these cellular responses and several studies implicate thioredoxin as a contributor to cancer progression [8].

RESEARCH METHODOLOGY

The thioredoxin (Trx) from human lung adenocarcinoma epithelial cells (A549) and normal lung cancer cells was isolated by RT PCR and inserted into vector using recombinant cloning techniques. The expression profile was studied by Agarose and SDS PAGE electrophoretic analysis. The gene sequence was confirmed by DNA sequencing and the protein by western blot using anti-thioredoxin antibody. The REDOX activity of the thioredoxins was tested and compared by insulin reduction assay.

This study was approved by the Institutional ethical committee of the Sree Balaji Medical College and Hospital.

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Construction of the Clone

RT-PCR was carried out and the thioredoxin gene was isolated from normal and adenocarcinoma epithelial cell lines (A549) and amplified using the designed forward and reverse primers (TrxF/TrxR) with Nde1 and BamH1 restriction sites.(table 1)

Table 1: Primers designed for human Thioredoxin

Primer	Primer Sequence	Restriction enzyme
TRXF	5'GGGTTTCATATGGTGAAGCAGATCGAGAGCAAG3'	Nde I
TRXR	5'CGCGGATCCGACTAATTCATTAATGGTGGCTTC3'	Bam HI

The amplified gene was ligated and cloned into pRSETA vector as per manufacturer's protocol. Positive clones were selected by lysate PCR and sequenced in BIOSERV and confirmed by DNA sequencing.

Expression of Protein in *E.Coli*

The vector pRSET A based on T7 RNA polymerase was employed in the present study to express the recombinant construct in IPTG inducible BL21 (DE3) and salt inducible T7 expression host, GJ1158. The isolated thioredoxingenes (normal & malignant) were inserted into the multiple cloning sites and ampicillin resistant sequence was used for clonal selection. The protein expression was studied using SDS PAGE analysis &western blot and quantification by Bradford assay. The redox activity was assesed by Insulin reduction assay.

RESULTS

Thioredoxin which is encoded by 300 bp gene was isolated by RTPCR (fig1) and the expression analysis was studied using SDS PAGE and quantified by Bradford assay (fig 2). The protein expression from adenocarcinoma was compared with that of normal cells of same origin. There was upregulated and widespread expression of thioredoxin in adenocarcinoma of lung. Large quantities of protein generated from malignant cells were purified by column chromatography .The purified protein was confirmed by western blot(fig 3) using anti-thioredoxin antibodies.

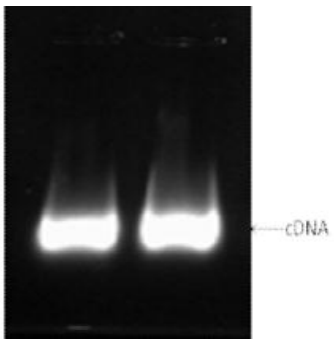

Figure 1: The malignant Thioredoxin gene

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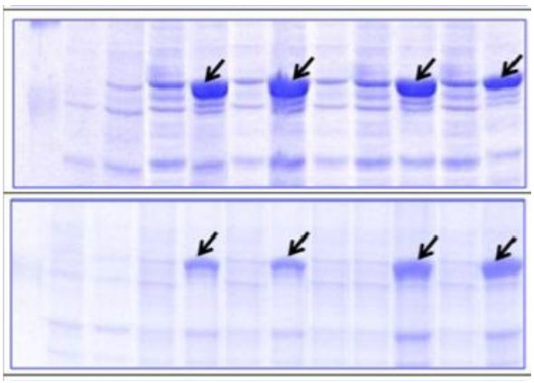

Figure 2: Expression analysis of Thioredoxin protein from malignant and non neoplastic cells-SDS PAGE

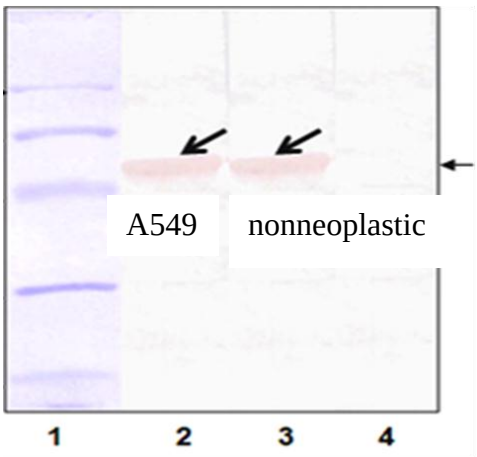

Figure 3: Western Blot of malignant &nonneoplasticthioredoxin

Thioredoxin was shown to catalyze the reduction of insulin disulfides by dithiothreitol[9]. An assay was developed which measures the rate of insulin reduction spectrophotometrically at 650 nm at 25°C as turbidity formation from the precipitation of the free insulin β chain. The implication of the dithiol-disulfide oxidoreductase activity of thioredoxin for the regulation of enzyme activities by thiol oxidation-reduction control is used in this assay [10].

The redox potency of the malignat protein when compared with normal physiological thioredoxin protein exhibited an exaggerated and enhanceddithiol-disulphideoxidoreductase catalytic activity visualized spectrophotometrically at 650 nm(25°C) as turbidity formation resulting from the precipitation of the free insulin β chain. Malignant thioredoxin resulted in a sustained and enhanced catalytic reaction visualized as thick white precipitate of free insulin β chain

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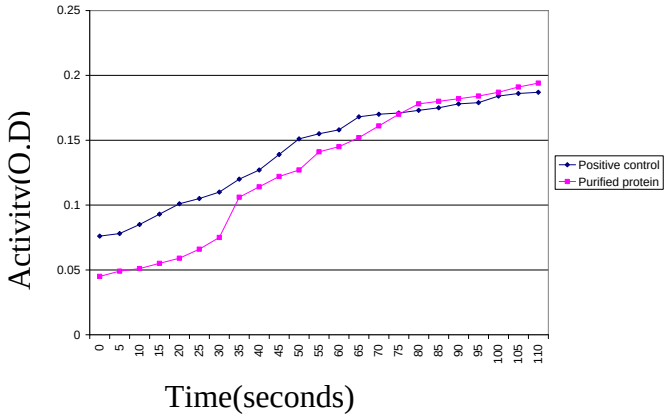

Figure 4: Insulin reduction assay by Thioredoxin

DISCUSSION

Thioredoxin has an active functional role in promoting cancer cell growth and that its increased expression is not just a consequence of cancer progression[11]. Thioredoxin is over expressed in many cancers and is considered as one of the enhancers of cancer cell growth, either through the direct stimulation of cancer cell growth or through the inhibition of cancer cell apoptosis. A recent in vivo study further highlights its importance in promoting cancer cell growth[12]. When two human lung carcinoma cell lines, expressing either high or low Thioredoxin levels, were injected subcutaneously into SCID mice the extent of tumor growth correlated with the levels of Thioredoxin expressed by the injected cells [13]. High levels of Thioredoxin expression have also been correlated with highly invasive and metastatic tumor activity both in vitro and in vivo [14]. Thioredoxin was shown to stimulate cell invasion in these cells and to promote overall matrix metalloproteinase (MMP) activity by preferentially inhibiting the MMP inhibitors. An in vivo study using mice injected with two human carcinoma cell lines expressing either high or low levels implicates Thioredoxin as an enhancer of tumors metastasis [15]. Tumor metastases were evident in the lung of mice injected with the higher Thioredoxin expressing cell line. Similarly, expression studies have also shown the highest levels of Thioredoxin expression in the most aggressive tumors isolated from patients diagnosed with breast, melanoma, thyroid, prostate or colorectal cancer [16].

Expression of Thioredoxin also results in patients developing resistance to chemotherapeutics [17] by scavenging intracellular toxic oxidants generated by various anticancer agent which suggests that Thioredoxin not only has an active role in cancer growth but also in cancer progression, through inhibition of apoptosis, stimulation of metastatic and invasive activity and through the involvement of chemotherapy resistance in cancer cells[18].

CONCLUSION

The malignant cells adopt several strategies to circumvent apoptosis and prolong survival; one such is the widespread expression of thioredoxin of enhanced potency. Thioredoxins are the major proteins taking part in the regulation of the redox state of cells.

There was a strong association between the overexpression of Thioredoxin in lung adenocarcinoma when compared to non-neoplastic tissues. The distribution of Thioredoxin expression in non-neoplastic lung suggests that this system possibly takes part in the defense of the lung to outside noxious stimuli. Thioredoxin system along with superoxide dismutases and enzymes associated with glutathione metabolism combat this free radical mediated cellular injury[19]

In conclusion, our results show enhanced wide spread expression of thioredoxin in lung adenocarcinoma suggesting that the involvement of the protein in regulating the redox balance in these tumors and, through this influence, the activity of transcriptional factors and tumor growth lung adenocarcinoma. They are also expressed in many cells of the non-neoplastic lung such as bronchial epithelial cells, alveolar macrophages, and regenerating type II pneumocytes, suggesting a part in cellular defense against outside noxious stimuli. Considering the varied of roles for Thioredoxin in malignancy, it is ideal to explore the detailed involvement of thioredoxin in various aspects of carcinogenesis that could possibly serve as a potential targets in handling one of the most leading cause of death, the cancer[20] .

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