Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.04.Art094

Analysis of TGF-β Gene Expression in Carboplatin Treated Lung Cancer Cells

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Abstract

Cancer, characterized by uncontrolled cell growth, remains a formidable challenge in modern medicine. Among various treatment modalities, chemotherapy, a systemic approach using drugs to impede cancer cell proliferation, is a cornerstone of cancer therapy. This study aimed to analyze the trends in TGF-β gene expression in carboplatin-treated lung cancer cell line A549. The materials and methods included an MTT assay to assess cell survivability, RNA isolation using the TRIzol method, and further analysis by RT-PCR, with data statistically analyzed using SPSS software. Results showed that TGF-β gene expression was significantly lower in the A549 cell line treated with carboplatin compared to the untreated cell line. Specifically, the treated cells exhibited a 40% reduction in TGF-β expression, a statistically significant decrease ($p < 0.05$). Given that TGF-β is known to promote tumorigenesis, the observed reduction suggests that carboplatin may control tumor progression by downregulating TGF- β expression and the proliferation of cancer cells. In conclusion, our study demonstrates the effectiveness of carboplatin as a chemotherapy agent in inhibiting the proliferation of lung cancer cells (A549) in non-small cell lung cancer (NSCLC) by reducing TGF-β gene expression levels. These findings underscore the potential of carboplatin to modulate gene expression associated with tumor growth, offering a promising therapeutic strategy for NSCLC management. Future studies should explore the broader implications of TGF-β modulation in cancer treatment and investigate the potential of combining carboplatin with other therapeutic agents to enhance its efficacy.

Keywords: *A549 Cell Line, Carboplatin, NSCLC, Squamous Cell Carcinoma, TGF-β.*

Introduction

Lung cancer is the leading cause of cancer incidence and mortality worldwide, with approximately 2 million diagnoses and 1.8 million deaths [1]. Lung tumors are the most common cancer diagnosis among men and women (after prostate cancer and breast cancer, respectively). The main types are non-small cell carcinoma (NSCLC), which grows slowly, and the rapidly growing small cell carcinoma (SCLC). The main cause is smoking, which accounts for about 85% of all lung cancer cases. Lung cancer is often detected at later stages, limiting the treatment options [2]. As the availability and industrialization of tobacco increases in developing countries, the incidence of lung cancer exponentially increases worldwide. The average age of diagnosis is 70 years. Men are twice as likely to be diagnosed with lung cancer, largely reflecting the differences in tobacco use, although women may be more susceptible due to epidermal growth factor receptor (EGFR) mutations and greater estrogen exposure [3]. The greatest risk factor for development of lung cancer is

Received: 06.07.2024 ***Accepted: 19.08.2024*** ***Published on: 27.12.2024***
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tobacco use [4]. Despite advancements in treatment, lung cancer remains a significant challenge, necessitating improved therapeutic strategies. Secondhand smoking has also been shown to increase the risk of lung cancer by as much as 26% [5]. Effective prevention strategies include strict tobacco control, minimizing exposure to environmental risk factors, and early screening for those at high risk to help reduce lung cancer rates and improve survival outcomes [2]. Current treatment for lung cancer includes surgery, radiation therapy, and chemotherapy. Among chemotherapeutic drugs carboplatin, a platinum-based drug is commonly used due to its efficacy in inhibiting DNA replication and transcription in cancer cells [6].

Lung cancer is mainly divided into two types, i.e. Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). The A549 cell line is an established model system commonly used in cancer research, particularly in NSCLC. A549 cells were derived from human lung carcinoma and were extensively characterized by their morphological, biochemical and genetic characteristics. Many studies have used the A549 cell line to investigate various aspects of NSCLC biology, including tumor progression, metastasis, drug sensitivity, and molecular mechanisms underlying the disease. For instance, research has employed A549 cells to explore the role of long noncoding RNAs (lncRNAs) in NSCLC progression, revealing potential therapeutic targets for the disease [7]. In addition, A549 cells have been instrumental in elucidating the molecular mechanisms underlying drug resistance in NSCLC. Research has investigated the role of the Hippo pathway in mediating resistance to EGFR tyrosine kinase inhibitors, demonstrating that the upregulation of ANKHD1 inactivates Hippo signalling by increasing YAP protein levels and inhibiting YAP protein phosphorylation. This upregulation enhances cell proliferation and invasion, while depletion of YAP reverses these effects, highlighting the pathway's significance in drug resistance mechanisms [8]. Research is also being conducted using A549 cells to investigate the inhibition of cancer with natural products. For instance, studies have shown that the acetone extract of Rosa chinensis exhibits cytotoxic and apoptotic activity in the A549 cell line, indicating its potential as a natural therapeutic agent against NSCLC [9].

Transforming growth factor beta (TGF-β) signalling plays a dual role in cancer, acting as both a tumor suppressor and promoter depending on the tumor microenvironment and cellular context. TGF-β regulates a variety of cellular processes involved in cancer progression, including cell proliferation, apoptosis, differentiation, migration, invasion, and immune evasion [10]. Many studies have clarified the complex involvement of TGF-β in cancer. For example, an article provided a comprehensive review of TGF-β signaling in cancer, highlighting its context-dependent effects on tumor development and progression. The review discusses how TGF-β acts as a tumor suppressor early in carcinogenesis by inhibiting cell proliferation and inducing apoptosis but later promotes tumor progression by increasing epithelial-mesenchymal transition (EMT), which facilitates tumor invasion and metastasis [11]. In addition, several studies have investigated the role of TGF-β signalling in various types of cancer. Research has explored the mechanisms by which TGF-β promotes epithelial-mesenchymal transition (EMT) and metastasis in breast cancer, highlighting its contribution to tumor cell plasticity and proliferation. Other studies have shown that TGF-β signalling plays a crucial role in therapeutic resistance and immune evasion in cancer. For example, it has been demonstrated that TGF-β contributes to immune suppression and resistance to immunotherapy, suggesting that targeting TGF-β signalling could improve the efficacy of cancer immunotherapies [10, 12]. In addition, TGF-β signalling has been implicated in

therapeutic resistance and immune evasion in cancer. Studies have discussed the mechanisms by which TGF-β contributes to immune suppression and resistance to immunotherapy, suggesting strategies to target TGF-β signalling to enhance the efficacy of cancer immunotherapy [13]. In addition, another study explores TGF-β's role in OSCC tumor growth and metastasis, identifying it as an oncogene. It suggests that inhibiting TGF-β could treat OSCC. The research focuses on examining TGF-β expression levels in OSCC, given its dual role in buccal cavity carcinogenesis as both an enhancer and suppressor, providing insights into cancer progression[10]. Key molecular pathways such as PI3K/AKT/mTOR and MAPK/ERK are critical in lung cancer development, and targeting these pathways shows promise. Overall, these studies underscore the multifaceted role of TGF-β in tumor biology, influencing various aspects of cancer progression and treatment response. Further studies are needed to better understand the mechanisms underlying TGF-β-mediated tumorigenesis and to develop effective therapeutic strategies targeting TGF-β signalling in cancer.

Carboplatin is a chemotherapy drug that is used to treat various types of cancer, including ovarian, lung, and bladder cancer. It is a type of platinum-based chemotherapy agent that works by interfering with the DNA of cancer cells, ultimately preventing their ability to divide and grow. In addition to its role in lung cancer treatment, carboplatin also helps to fill a gap in cancer treatment more broadly. This second-generation drug plays a crucial role in various cancers, including ovarian and bladder. When coupled with other chemotherapeutic drugs such as gemcitabine, paclitaxel, or cisplatin, carboplatin-based regimens have demonstrated significant effectiveness in mitigating tumor load, boosting survival rates, and optimizing overall patient outcomes [14, 15]. Unlike its predecessor cisplatin, carboplatin is associated with fewer nephrotoxic and ototoxic side effects, making it a preferred option for patients with pre-existing kidney conditions or those who are more vulnerable to these toxicities [16]. Carboplatin exerts its cytotoxic effects primarily through DNA damage. Like cisplatin, carboplatin forms intrastrand and interstrand crosslinks with DNA, inhibiting DNA replication and transcription, ultimately causing cell death. Several studies explained the exact mechanisms of action of carboplatin. In the context of lung cancer, particularly NSCLC, carboplatin is often used in combination with other chemotherapeutic agents and its role beyond direct cytotoxicity. It has been found to modulate the tumor microenvironment, potentially reducing angiogenesis and altering immune cell infiltration, which can contribute to its overall anti-tumor effects [17]. A study by Kartalou and Essigmann investigated the formation and repair of carboplatin-induced DNA adducts, highlighting the role of nucleotide excision repair pathways in the removal of platinum-induced DNA damage [18]. Another study by Galluzzi et al. investigated the molecular mechanisms underlying carboplatin-induced apoptosis and showed the involvement of p53-dependent and -independent pathways in mediating cell death [19]. The efficacy and mechanism of action of carboplatin have been investigated in the context of NSCLC. Studies evaluating the combination of carboplatin and paclitaxel in patients with advanced NSCLC have shown improved overall survival and response rates compared to carboplatin alone [20]. Research is ongoing to better understand these mechanisms and to optimize carboplatin-based regimens, aiming to maximize therapeutic benefits while minimizing adverse effects.

The objective of this study is to evaluate the efficacy of carboplatin in inhibiting the growth and proliferation of lung cancer cells by analyzing the gene expression levels of TGF-β and providing insights into the potential therapeutic value of carboplatin for lung cancer

treatment. This study is novel in its specific focus on the TGF-β gene expression trends in carboplatin-treated lung cancer cell line A549. While carboplatin's efficacy is well-documented, examining its impact on TGF-β expression provides new insights into its mechanism of action and potential as a therapeutic target in NSCLC.

Materials and methods

Cell Culture and Treatment — A549 Cells Treated with Carboplatin

The A549 cell lines and normal lung cells were obtained from the National Center for Cell Science (NCCS), Pune, India. They were then cultured according to the provided cell culture instructions. It was filled with 15% baby cow blood at 37°C with low CO2 air in a humid box chamber called an incubator.

Cell Proliferation - MTT Assay

To see how carboplatin affected the NSCLC, an MTT assay was used to determine the survival of A549 cells. The cells were placed in a 96-circular well plate at the rate of 100,000 cells per well. After 12 hours, the cells were mixed with carboplatin for 48 hours at 37°C. This was done by increasing the dose slowly from 2.5 to high doses (from 0 to very high). The presence or absence of breast cancer cells was analyzed by using a normal medium as a control. All cells were cultured for an additional 48 hours. MTT reagent was then added to each small box and the cells were kept warm at 37°C for 4 hours. After the material was absorbed and the liquid was removed, 150 microliters of dimethyl sulphoxide (DMSO) were added to dissolve the dye. The amount of light that passed through in the wavelength range of 490 nm was checked using numerical readings with a small disk reader. The IC_{50} (level of drug required to cause 50% cell death compared to the normal control) in this test was 20 micrograms per millilitre. The test was performed three times, with six identical specimens being seen each time. Figure 1 illustrates the MTT assay plate.

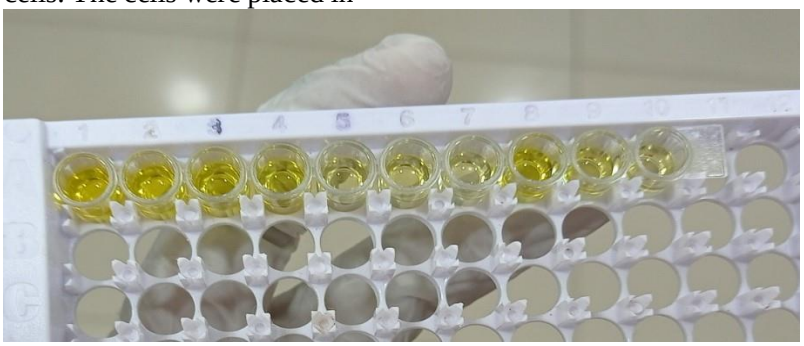

Figure 1. Illustrate MTT Assay Plate with Cells.

RNA Isolation – TRIzol Method

TRIzol reagent (Invitrogen, Carlsbad, USA) was used to obtain total RNA from both cancerous and normal tissues. This was done according to the manufacturer's instructions. The purity and amount of extracted RNA were checked using a Nanodrop 2000 Lite machine (Thermo Fisher Scientific, Waltham, MA). For further testing, the RNA was stored in a freeze chamber at -20°C[21].

Reverse Transcriptase Polymerase Chain Reaction (RT PCR)

A total of 10 μL was used within the Moloney murine leukaemia virus (M-MLV) reverse transcriptase. This process converts each RNA into complementary DNA or cDNA by heating and then immediate cooling. The mixture is then placed in a PCR (Thermo Fisher MiniAmp plus thermocycler) at 30°C for 10 minutes. It is placed at 42°C for 30 minutes and then it is allowed to fully warm up for another

five long periods, within which time it reaches the required temperature range and remains safe from start to finish. The amount of cDNA is measured using Nanodrop Lite and it is stored at -20°C until further investigation. The TGF gene was examined for how well it produces proteins from cDNA using a dye called SYBR Green (Takara, Japan). GAPDH was considered a normal control. TGF primer sequence (forward and reverse) and GAPDH primer sequence (forward and reverse) were used. The following thermocycling settings were used to duplicate all samples: melt for 30 seconds at 95°C, then repeat 40 times using the same 95°C temperature again to incorporate all the constituents, by starting with a faster stirring motion and ending with a slower mixing speed. Finally, TGF expression was measured using the 2-Cq method [10, 21].

GENE Expression Analysis

The TRIzol agent was used to obtain total RNA. A mixture of DNA was made from one strand. It contained 2 milligrams of RNA, a primer called oligo-dT, and a Superscript II reverse transcription tool. Real-time analysis (qRT-PCR) was performed on a machine called iCycler, separately for each, using tested primers and SYBR Premix Ex Taq II. Using a limit on the number of cycles, the expression of certain genes was measured. The GAPDH gene was used to ensure that component levels were balanced in the same mixtures [10, 21].

Statistical Analysis

The data was presented as mean standard deviation (SD). A t-test program was used to compare the amount of TGF-β in cancer tissue and normal nearby tissue. A p-value less than 0.05 was considered statistically significant [10, 21].

Results

Histomorphological Characterization of A549 Cells in Invitro Cultivation

The microscopic view of lung cancer exhibits squamous differentiation by formation of keratin and intercellular bridges as shown in Figure 2. When grown in vitro, these cells stick to the culture flask and develop into a monolayer. Cellular and Tissue Characterization of A549 Cells in Laboratory Cultivation. The tiny structures of the cells cultured in vitro allow us to see with great clarity the specific attributes of cellular maturation. The fact that this monolayer is tightly expressing itself to the culture flask demonstrates its ability to adapt properly to the natural growth patterns found in vivo.

Figure 2. Represents A549 Cell Line of Squamous Cell Type Non-Small Cell Lung Cancer.

Carboplatin Mediated Impact on A549 Cell Proliferation and Molecular Signaling in Lung Cancer

The proliferation rate has significantly reduced after treatment with carboplatin. Figure 3 depicts A549 cell proliferation before and after treatment with carboplatin. The cell proliferation levels of A549 were recorded to analyze the effect of carboplatin treatment on NSCLC cell viability. We see that the cell line viability is significantly reduced by the

exposure to carboplatin as seen by the decrease in the proliferation rate.

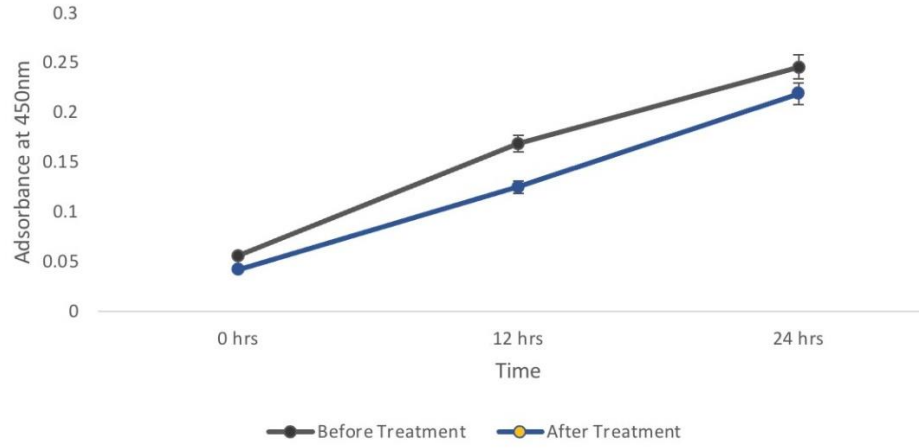

Figure 3. Represents the Cell Proliferation of A549 Cells Before and After Treatment with Carboplatin.

Expression Analysis of TGF- β Before and After Treatment with Carboplatin

The gene expression levels of TGF-β were recorded to analyze the molecular mechanism of action of carboplatin. We see that the gene expression is significantly reduced by the exposure to carboplatin as seen by the decrease in fold induction. Figure 4 represents the expression levels of TGF-β before and after treatment of A549 cells with carboplatin. The expression of TGF-β was significantly reduced after the treatment when compared to the normal cells. The X-axis represents treatment time both before and after therapy, and the Y axis represents fold change over control. Figure 5 represents the product images of qRT-PCR expression analysis.

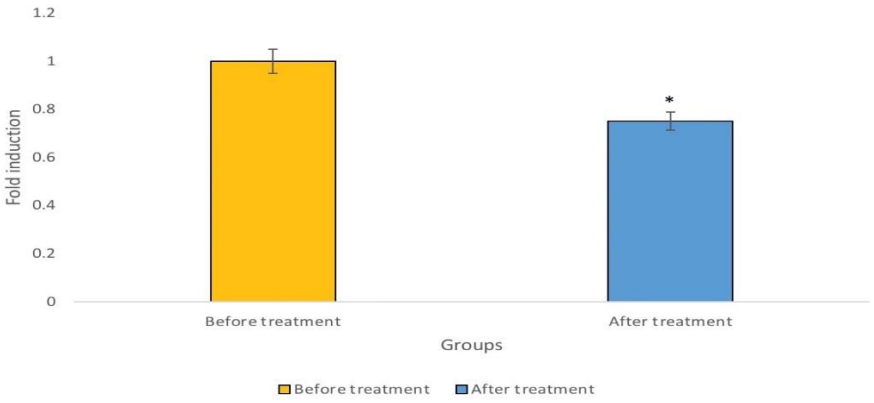

Figure 4. Represents the Expression Levels of TGF-β before and after Treatment with Carboplatin

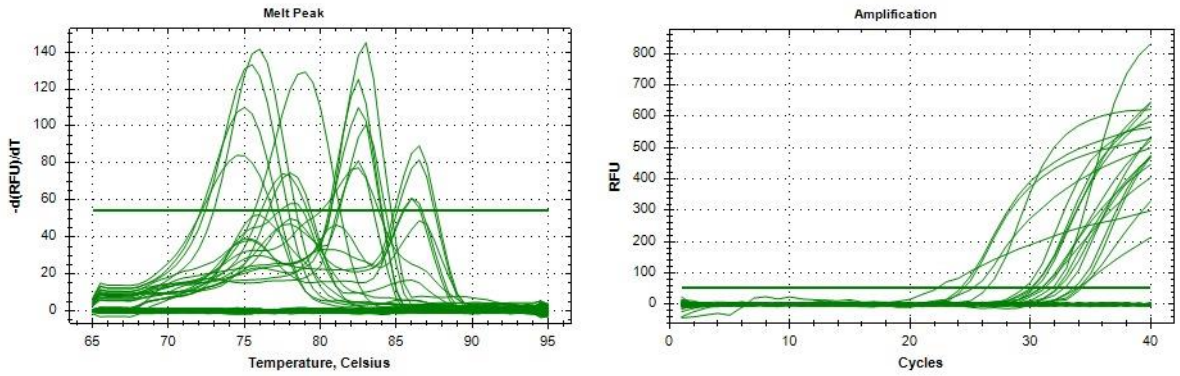

Figure 5. Represents the Product Images of RT-PCR Expression Studies.

Discussion

This present study was conducted to assess the molecular mechanism of action of carboplatin on the A549 cell line. Given the complexity of TGF-β signalling and its impact on NSCLC, further extensive in silico and genomic studies are necessary to fully elucidate the efficacy of carboplatin in the molecular suppression of cancer. Future research should also aim to identify and develop methods to suppress the mechanisms by which TGF-β signalling confers resistance to carboplatin therapy. Additionally, it is crucial to explore potential combination therapies involving carboplatin and TGF-β inhibitors. Such combinations may provide synergistic effects, improving the overall therapeutic efficacy and potentially reducing the required dosage of carboplatin, thereby minimizing its side effects.

This cell line is commonly used to study drug interactions and characteristics of NSCLC. The gene expression of TGF-β was targeted and analyzed in carboplatin-treated cell lines and untreated cell lines. From Fig 2, we see that the cell line viability of A549 is significantly reduced by the presence of carboplatin. This was inferred from the decrease in the cell proliferation rate over time. From Fig 3, we see that the gene expression of TGF-β is significantly reduced by exposure to carboplatin. This was inferred from the decrease in the fold induction.

Our findings are consistent with previous studies investigating the interaction between carboplatin and TGF-β signalling pathways in NSCLC. For instance, it has been shown that carboplatin treatment can activate TGF-β signalling in NSCLC cells, increasing cancer cell migration and invasion, which may contribute to tumor progression and metastasis, potentially leading to resistance to carboplatin therapy. Several studies have investigated the interaction between carboplatin and TGF-β signalling pathways in NSCLC. For example, Preca et al. showed that carboplatin treatment can activate TGF-β signalling in NSCLC cells, which increases cancer cell migration and invasion [22].

Conversely, studies have shown that TGF-β signalling can modulate the sensitivity of NSCLC cells to carboplatin. Research has found that inhibiting TGF-β increases the sensitivity of NSCLC cells to carboplatin-induced apoptosis, suggesting that targeting TGF-β signalling pathways may enhance the effectiveness of platinum-based chemotherapy in treating NSCLC tumors. This indicates a potential therapeutic strategy for overcoming resistance to carboplatin in NSCLC by combining it with TGF-β inhibitors [23]. This dual role of TGF-β in both promoting and inhibiting tumor progression underscores the complexity of the signaling pathways and their impact on chemotherapy outcomes.

The present study has analyzed the molecular mechanism of carboplatin in lung cancer solely on the basis of the gene expression rate of TGF-β. As this is a preliminary in vitro study, further extensive in

silico and genomic studies need to be conducted to analyze the efficacy of carboplatin in molecular suppression of cancer and to formulate methods to suppress the mechanism by which TGF-β signaling confers resistance to carboplatin therapy. While carboplatin is effective, there are some limitations for this study that can cause some side effects, necessitating further research into understanding and overcoming this resistance.

Conclusion

This present study has demonstrated the effectiveness of carboplatin as a chemotherapy agent. The gene expression of TGF-β is significantly reduced which inhibits tumor proliferation in the A549 cell line. Thus cisplatin, by the molecular mechanism of gene suppression, is an excellent drug for tumor suppression and cancer regression in NSCLC. Carboplatin has shown substantial efficacy in reducing tumor growth and proliferation, particularly in NSCLC. Studies have highlighted its role in DNA damage and apoptosis induction, contributing to improved patient outcomes.

Acknowledgement

Not applicable.

Conflict of Interest

The authors declare there is no conflict of interest.

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