Combination of intensity modulated radiotherapy followed treatment with p38 MAPK activation inhibitor inhibits the proliferation of MCF-7 breast cancer cells

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\textbf{A B S T R A C T}

The present study was aimed to investigate the effect of intensity modulated radiotherapy (IMRT) followed by treatment with inhibitor for p38 MAPK, SB203580 on the rate of proliferation in drug resistant MCF-7 breast cancer cells. Interestingly, the results from immuno histochemistry and western blot assays revealed higher level of distribution of activated p38 MAPK in the drug resistant breast cancer tissues compared to the primary tissues. Treatment of the drug resistant MCF-7 cells with SB203580 led to a significant decrease in the phosphorylation of p38 MAPK. Exposure to IMRT caused a significant decrease in the rate of proliferation in drug resistant MCF-7 breast cancer cells (P < 0.05). MCF-7 cells were subjected to IMRT for 45 min followed by treatment with SB203580 for 12 h. The results from MTT assay revealed inhibition in the rate of proliferation of MCF-7 cells more efficiently compared to the IMRT or SB203580 when used separately (P < 0.02). The effect of IMRT and SB203580 on inhibition of MCF-7 cell proliferation showed synergistic relation. Since MAPK signaling pathway plays an important role in the development of drug resistance, therefore, inhibition of p38 MAPK activation by the combination of IMRT followed by treatment with inhibitor for p38 MAPK can be a promising strategy for breast cancer treatment. Thus combination of IMRT exposure and treatment with SB203580 can be used for the inhibition of drug resistant breast cancer.

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1. Introduction

Breast cancer at present is one of the most commonly detected cancer in females and leading cause of deaths due to cancer throughout the globe. It has been proposed that breast cancer is initiated and developed by the alterations in multiple genes. The breast cancer is currently treated using various chemotherapeutic agents which exhibit their effect through several mechanisms including, inhibition of microtubules (Li et al., 2003). These microtubule inhibiting chemotherapeutic agents suppress cancer growth by the arrest of cell cycle and induction of apoptosis (Blajeski et al., 2002; Shin et al., 2003). However, the use of chemotherapeutic agents over a long period has a serious disadvantage of the development of drug resistance (Morrison et al., 2012). There are reports that in around 20% of the patients suffering from breast cancer the early stage develops into the metastatic phase. Among these patients in more than 9% cases metastasis to the distant organs takes place. It has been estimated that the average survival time for the patients with metastasis to distant organs is less than 3 years despite treatment with the currently available chemotherapeutic agents (Stevanovic et al., 2006). Compared to the females the percentage of males suffering from breast cancer is very less and is around 1% in the US (Anderson et al., 2010). However, the average survival time of the males with metastatic stage of breast cancer is lower than those of females (Nahleh et al., 2007).

Intensity modulated radiotherapy (IMRT) is presently being used for the treatment of various types of cancers and it uses multiple beams on the cancerous tissues. The use of IMRT leads to the decrease in volume of the carcinoma tissue because of high beam whereas the normal tissue is exposed to the low beam radiation (Purdy, 2008). Studies have revealed that use of IMRT decrease the chances of development of the secondary carcinoma during one decade to less than 2% (Hall, 2006). It has been demonstrated...
that IMRT has more advantages for the treatment of various cancers compared to the chemotherapeutic agents (Ost et al., 2011; Murray et al., 2013). In the present study effect of IMRT followed by treatment with inhibitor for p38 MAPK, SB203580 on the drug resistant cancer tissues and the MCF-7 cells was investigated (Serasanambati and Chilakapati, 2016). It was observed that SB203580 and IMRT of MCF-7 cells led to a significant decrease in the phosphorylation of p38 MAPK compared to the control cells. IMRT caused a significant decrease in the rate of proliferation in drug resistant MCF-7 breast cancer cells. The effect of IMRT and SB203580 on inhibition of MCF-7 cells proliferation showed synergistic relation.

2. Materials and methods

2.1. Materials

Five breast cancer tissue samples were obtained from Southern Medical University, the Nanfang Hospital, Guangzhou, Guangdong. All the tissue samples were from the patients having drug resistant cancer which relapsed after treatment. From the same hospital five primary breast carcinoma tissues were also collected. All the tissues samples were stored under atmosphere of liquid nitrogen before the study was performed.

2.2 Ethical statement

Permission for the use of tissue samples in the present study was taken from the patients and a written consent was obtained confirming that they are aware of the study being performed. The approval for performing the study was taken from the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong.

2.3. Cell lines and reagents

The drug resistant breast cancer cell line, MCF-7 was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 U/ml) in an incubator with humidified atmosphere of 5% CO2 at 37°C.

2.4. Cell proliferation assay

The rate of proliferation in MCF-7 breast cancer cells was determined using MTT assay. The cells were seeded at a density of 2.5 × 10^4 cells per ml and cultured in a 96-well plate (Gibco Life Technologies; Carlsbad, CA, USA). Following serum starvation, the cells were exposed to IMRT for 45 min or incubated for 12 h with 10 μM concentration of SB203580. After treatment, the cells were washed twice with PBS and then lysed using lysis/extraction reagent including BCA protein assay kit. The protein samples were separated by electrophoresis using 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to the nitrocellulose membrane which was blocked for 2 h using 5% skimmed milk in Tris-buffered saline Tween-20 (TBST) buffer. Incubation of the membranes was performed overnight at 4°C with primary antibodies (polyclonal rabbit antibodies; 1:1,000 dilution) using 5% skimmed milk. After incubation with primary antibodies, the membranes were washed again with PBS and then incubated for 1 h with secondary anti-rabbit antibodies conjugated with horseradish peroxidase (1:20,000 dilution). Detection of the blots was performed using an enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotechnology, Piscataway, NJ, USA). The blots were analyzed using Quantity One 1D Analysis software (version 4.4; BioRad).

2.5. Western blot analysis

MCF-7 breast cancer cells at density of 2 × 10^6 cells per dish were put onto 6-cm dishes and incubated for 24 h. Following incubation, the cells were exposed to IMRT for 45 min or incubated for 12 h with 10 μM concentration of SB203580. The cells in some dishes were subjected to IMRT for 45 min and then incubated for 12 h with 10 μM concentration of SB203580. After treatment, the cells were washed twice with PBS and then lysed using lysis/extraction reagent including BCA protein assay kit. The protein samples were separated by electrophoresis using 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to the nitrocellulose membrane which was blocked for 2 h using 5% skimmed milk in Tris-buffered saline Tween-20 (TBST) buffer. Incubation of the membranes was performed overnight at 4°C with primary antibodies (polyclonal rabbit antibodies; 1:1,000 dilution) using 5% skimmed milk. After incubation with primary antibodies, the membranes were washed again with PBS and then incubated for 1 h with secondary anti-rabbit antibodies conjugated with horseradish peroxidase (1:20,000 dilution). Detection of the blots was performed using an enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotechnology, Piscataway, NJ, USA). The blots were analyzed using Quantity One 1D Analysis software (version 4.4; BioRad).

2.6. Immunohistochemical staining

The drug resistant and the primary breast carcinoma tissue samples were stored under liquid nitrogen atmosphere prior to use in the experiment. The samples were embedded in the paraffin wax, cut into thin 2 μm sections and then fixed using 10% paraformaldehyde. The standard avidin-biotin-peroxidase complex method was used for tissue section staining. Expression of the activated p38 MAPK in the tissue sections examined and measured as the mean of the gray value.

2.7. Statistical analysis

The data presented are the mean ± standard error and were analyzed for statistical significance using SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). For the determination of statistics an ANOVA was used and P < 0.05 was taken to indicate a statistically significant difference.

3. Results

3.1. P-p38 MAPK expression in the breast carcinoma tissues resistant to drug and primary tissues

Analysis of the presence of P-p38 MAPK protein using immunohistochemistry in the breast carcinoma tissues revealed higher levels both in the nuclei as well as cell cytosol (Fig. 1). However, the level was found to be comparatively higher in the breast carcinoma tissues resistant to drugs compared to the primary carcinoma tissues (P < 0.02). A significant (P < 0.02) difference was observed in the gray values for the levels of P-p38 MAPK protein among the tissues resistant to drug and the primary carcinoma tissues. The above findings were further confirmed by the analysis of P-p38 MAPK protein expression using western blotting assay. These results also showed a significantly (P < 0.02) higher level of P-p38 MAPK in the breast carcinoma tissues resistant to drugs compared to the primary carcinoma tissues (Fig. 2).
3.2. Treatment with p38 MAPK inhibitor inhibited the activation of p38 MAPK in drug resistant MCF-7 breast carcinoma cells

The MCF-7 cells were treated with inhibitor for p38 MAPK, SB203580 to examine its effect on activation of p38 MAPK. It was observed that the treatment of MCF-7 cells with SB203580 led to a significant decrease in the phosphorylation of p38 MAPK (Fig. 3). However, the phosphorylation of p38 MAPK was significantly higher in control cells.

3.3. IMRT inhibited the rate of proliferation in drug resistant breast carcinoma cells

The MCF-7 cells were subjected to IMRT and then analyzed for cell proliferation. It was observed that IMRT caused a significant decrease in the rate of proliferation in drug resistant MCF-7 breast cancer cells (P < 0.05) (Fig. 4).

3.4. Combination of exposure to IMRT followed by treatment with SB203580 inhibited proliferation of MCF-7 more effectively

MCF-7 cells were subjected to IMRT followed by treatment with SB203580. The results revealed inhibition in the rate of proliferation of MCF-7 cells more efficiently compared to the IMRT or SB203580 when used separately (P < 0.02). The effect of IMRT and SB203580 on inhibition of MCF-7 cells proliferation showed synergistic relation (Fig. 5).

4. Discussion

Intensity modulated radiotherapy (IMRT) is presently being used for the treatment of various types of cancers and it uses multiple beam radiations on the cancerous tissues [10]. The present study demonstrates the effect of a treatment strategy consisting of exposure to IMRT followed by treatment with inhibitor for p38 MAPK, SB203580 on activation of p38 MAPK and rate of proliferation in drug resistant MCF-7 breast cancer cells. The strategy efficiently inhibited the activation of p38 MAPK and proliferation of drug resistant MCF-7 cells.

The mitogen-activated protein kinase (MAPK) plays an important role in the induction of damage to the cell and development of resistance to drugs [Gotlieb et al., 2008]. MAPK regulates several vital pathways in the cells which include, p38 MAPK and ERK1/2. Activation of MAPK leads to the phosphorylation of B cell lymphoma-2 and its associated proteins which are involved in the development of drug resistance [Xing and Orsulic, 2005; Ohta et al., 2006]. MAPK induced signaling pathways are responsible for the increased rate of cell proliferation and drug resistance [Kuo et al., 2002; Lee et al., 2005]. In the present study immunohistochemistry and western blot assays revealed higher level of activated p38 MAPK protein in the breast carcinoma tissues both in the nuclei as well as cell cytosol. However, the level was found to be comparatively higher in the breast carcinoma tissues resistant to drugs compared to the primary carcinoma tissues. Studies have demonstrated that inhibition of the activation of MAPK pathways by chemotherapeutic agents enhances their anticancer potential.
Use of the strategy consisting of IMRT followed by treatment with inhibitor for p38 MAPK significantly inhibited the activation of p38 MAPK in the MCF-7 drug resistant breast cancer cells. The present study revealed that exposure of MCF-7 cells to IMR caused a significant decrease in the rate of proliferation in drug resistant MCF-7 breast cancer cells. On the other hand, when MCF-7 cells were subjected to radiotherapy followed by treatment with SB203580 the results revealed inhibition in the rate of proliferation of MCF-7 cells in synergistic manner.

5. Conclusion

Since MAPK signaling pathway plays an important role in the development of drug resistance, therefore, inhibition of p38 MAPK activation by the combination of IMRT followed by treatment with inhibitor for p38 MAPK can be a promising strategy for breast cancer treatment. Thus combination of IMRT and inhibitor for p38 MAPK can be used for the treatment of drug resistant breast cancer.
Conflict of interest

The authors declare that they have no conflicts of interest.

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