Original Article

Role of Transferrin Receptor Protein in Cancer Treatment

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ABSTRACT

Objective: This article aims to show the association of the transferrin receptors with cancer and take it as the target in cancer therapy.

Study Design: Prospective Experimental research study

Place and Duration of Study: This study was conducted at the Center for Applied Molecular Biology (CAMB) Punjab University Pakistan, from January 2021 till August 2021 for a period of 08 months.

Materials and Methods: Using the CellTiter 96 AQueous Assay (Promega), we determined the 50% growth inhibition values for the substances. Membrane proteins solubilized and separated on SDS PAGE, dyed, and digested in-gel using conventional methods. HPLC was used to identify the proteins. cDNA was generated using the Retroscript cDNA synthesis kit (Ambion). Time-delayed fluorescence was measured using a Wallac Victor plate reader (PerkinElmer) after each well was incubated with Europium-Streptavidin.

Results: Tumor cell apoptosis is triggered by GA binding to TfR. The results of these studies suggest that GA can be used for targeting the TfR in cancer therapy.

Conclusion: Targeting the TfR has been shown to be effective in the treatment of cancers but has shown some cytotoxic effects as well. The wide use of the TfR in the treatment of cancers has helped target specific receptors against cancer cells. In the future, it is expected that targeting TfR for cancer therapy can be improved to overcome the side effects of this therapy and highly target-specific drugs will be produced.

Key Words: Transferrin, Receptor 1, Cancer, Ferric, antibody, Expression

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INTRODUCTION

Ferric (iron) is an important biological element. It plays an important role in metabolism and other physiological processes. It has a function in every type of cell because it is related to heme formation and other proteins that have their role in the transportation of oxygen. With its role in oxygen transportation, it is found in all cells for energy production[(1)]. It acts as a cofactor in DNA replication. It is also the part of enzymes that play a role in repair and synthesis processes. While energy generation, iron produces oxygen free radicals in the cells[(2)]. These free radicals increase the oxidative stress in the cell and lead to the damage of DNA, lipids, and proteins.

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The high concentration of iron in the body can cause the formation of tumors and many other problems. Therefore, the proper maintenance of iron concentration in the body is necessary to avoid these kinds of complications. Likewise, the low concentration of iron can cause the death of the tumor cells[(3)].

Iron cannot be transported simultaneously in the cell; it needs another molecule for its transfer called 'transferrin'. It binds to the ferric iron and transports it to various parts of the body. It is a plasma glycoprotein present in the serum, milk, and melanin pigment[(4)]. Its function is to transfer iron within the body, mark inflammations, fight against foreign particles by helping the body's innate immune response and identify malignancies. The transferrin molecules also need a carrier protein called 'transferrin receptors[(5)]. These are the transmembrane glycoproteins. Various studies have clearly shown the role of the transferrin receptors in cancer therapy. A few methods are known to use transferrin in the treatment and diagnosis of cancer, one of them uses transferrin receptor 1 (TfR1). It targets intracellular iron reserves. It is based on the TfR1 mediated cytotoxic drug conjugates[(6)].

TfR1 is a type II transmembrane glycoprotein that binds to the transferrin molecule bound with ferric ions[(7)]. The binding of TfR1 with transferrin-iron (Tf-Fe) generates a clathrin-mediated endocytosis response. This process decreases the pH of the cell to 5.5 leading

to the release of the ferric ion[8]. This TfR1 is a recyclable molecule. The tumor cells have a high level of TfR1 and it helps in iron uptake. The clinical evidence has shown that there is a high level of TfR1 in cancer patients the antibody treatment against the TfR1 induced tumor is being studied as a cancer therapy because of the remarkable affinity of TfR1 towards the antibodies[9]. Therefore, TfR1 is considered the anticancer/antitumor agent for various types of cancers.

MATERIALS AND METHODS

Apoptosis Assays. Cellular viability assessment with propidium iodide, DAPI labeling, and cell cycle analysis. Caspase induction was performed. Using the CellTiter 96 AQueous Assay (Promega), we determined the 50% growth inhibition values for the substances.

Identification of GA Target. Membrane proteins solubilized and separated on SDS PAGE, dyed, and digested in-gel using conventional methods. HPLC was used to identify the proteins.

GA Target identification.

Conventional methods were used to solubilize, separate, dye, and digest membrane proteins. Identification of the proteins was conducted using HPLC.

cDNA synthesis, siRNA transfections, and real-time PCR: Ambion, Austin, Texas, chemically produced siRNA oligos for human transferrin receptors and caspase 8. Caspase-8 siRNA had a target sequence of 5 AAG GAA AGT TGG ACA TCC TGA 3 and TfR siRNA had a sequence of 5 AAC TTC AAG GTT TCT GCC AGC 3. Furthermore, Ambion provided human cyclophilin-control siRNA oligos and negative scrambled control siRNAs. In order to synthesize cDNA and run quantitative PCR assays, standard procedures were followed. cDNA was generated using the Retroscript cDNA synthesis kit (Ambion). In the Quantitect kit with normal settings and Sybrgreen inclusion, we performed quantitative PCR on the Light Cycler.

Assays for binding: The cells were then treated with 1 M tritium-GA with or without 20 M unlabeled GA at 37°C. A liquid scintillation counter was employed to measure bound tritium-GA at the pertinent time points. The TfR-coated wells were first treated with GAbiotin, followed by washing, and then incubated with nontagged analogs or binding washing buffer as a control. Time-delayed fluorescence was measured using a Wallac Victor plate reader (PerkinElmer) after each well was incubated with Europium-Streptavidin.

RESULTS

TfR Identified as the Molecular Target: A cell-surface target was activated by GA to induce apoptosis. Some GA derivatives were initially shown to be capable of withstanding bulky group changes while still inducing apoptosis. To better understand the target, we designed a biotin-conjugated GA that could be attached to fluorescein microspheres, streptavidin microspheres, or agarose conjugates. The binding of tritium-GA to Jurkat cells treated at 37 °C and 4 °C is saturable and temperature-dependent, which is inhibited by cold unlabeled GA. We used inactive-GA as a competition for determining the specificity of the bound protein. According to these results, GA mediates apoptosis through a cell surface receptor.

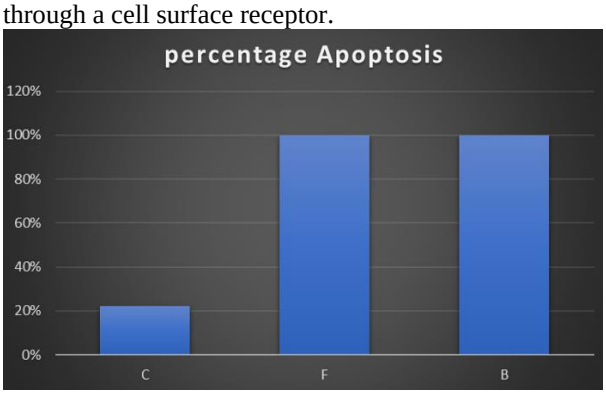

Figure No.1: The percentage of apoptosis when GA binds to receptor on the cell surface

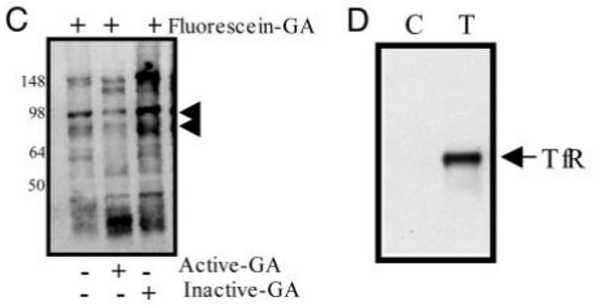

Figure No.2: Immunoblotting with anti-TfR antibody allowed detection of immunoprecipitated lysate

The downregulation of TfR affects cell apoptosis induced by GA: GA-induced apoptosis is more likely to occur in tumour cells that overexpress TfR (T47D and 293T). Proliferation rates did not vary with GA sensitivity, but TfRs expression was correlated with GA-induced apoptosis.GA-induced apoptosis is more likely to occur in tumour cells that overexpress TfR (T47D and 293T). A significant correlation was found between TfRs expression and GA-induced apoptosis, but no correlation was found between proliferation rates and GA sensitivity. Furthermore, we used paclitaxel, a recognized cytotoxic drug, to show that down-regulating TFR was not associated with reduced apoptosis. This indicates that cells lacking Tfr may not be resistant to every apoptotic trigger.

Mechanism of TfR-induced apoptosis: The apoptotic pathway is strongly activated by GA. The activation of caspase-8 by GA is similar to the activation of caspase-8 by anti-Fas. When certain regulatory molecules in this pathway are down-regulated, GA-induced cell death is delayed rather than inhibited. ADD loss has little to no

effect on GA-mediated apoptosis, based on these results.

Table No.1: Show the activity in apoptosis induction assay

Compound	Competition IC50 in TR binding assay, M	Fold caspase-3 activation	EC50
GA	5.1	19	535
Methyl-GA	4.6	23	325
Inactive-GA	>35	1.4	ND
unsaturated backbone	13.7	2	4160
saturated backbone	>33	1	ND

DISCUSSION

Brain Tumor: The regulation of the function of glioma cells (tumor of glial cells) and its progression in the brain cells is dependent on the TfR1 expression. It is observed that the level of TfR1 increases in brain cancer. Recent studies have shown that the TfR1 can be used in the prediction of glioblastoma prognosis and identification of targets to produce drugs[10].

Breast Cancer: Breast cancer is considered the most common and devastating cancer in females all over the world. The progression of this cancer also requires more uptake of iron. It can be identified by increased expression of TfR1. It helps in the identification of the biomarkers of cancer and its treatment at the early stages[11].

Colon Cancer: The level of transmembrane glycoprotein TfR1 is related to the rate of division of the tumor cells. Its expression is increased in the cancer cells; therefore, it is used as the target in cancer treatment [12].

Liver Cancer: Iron is considered a developmental factor for hepatocellular carcinoma (HCC) patients who also have hereditary hemochromatosis (HH). It is discovered that the wild type of HH is a complex of TfR with a mutation at two proteins, Cys 282 Tyr and His 63 Asp. It increases the binding strength of the TfR and Tf[13]. It eventually increases the uptake of iron and causes the rapid proliferation of HCC. The expression of the TfR gene increases with the increase in the stage of cancer. It was suggested that in HCC tissues, the level of miR-152 is decreased and TfR gene expression is increased. It is because of the downregulation of the miR-152 that mediates the post-transcriptional modification. Therefore, this process can be used in the treatment and diagnosis of HCC[14].

Ovarian Cancer: TfR level has a very vital role in ovarian cancer. It was proved that the metabolism of iron is interrupted during ovarian cancer by changing the targets. The level of TfR1 expression is raised in the tumor cells. This can be used in the diagnosis of cancer[15].

Prostate Cancer: The experimental studies have shown that the level of the serum transferrin receptors in males increases during this cancer. But the increasing stage of cancer does not affect the level of TfR1 expression. This raised expression of TfR1 is used as a biomarker for the diagnosis of this cancer[16].

Lung Cancer: It was suggested that the level of TfR1 increases in lung cancer along with the increase in the alpha-globin level. A receptor called the epidermal growth factor receptor controls the iron metabolism by binding to TfR1 and transporting it again to the cells of the lungs. This is a very important phenomenon in the proliferation of cancer and can be used in the therapy of lung cancer[17].

Leukemia (Blood Cancer): This is related to TfR1 in such a way that TfR1 is a carrier protein for iron transport and iron transport and metabolism is very high in the leukaemia's. The level of TfR1 is very high in blood cancer. Of the two types of leukaemia, T-cell leukaemia and B-cell leukaemia, the expression of the TfR1 is higher in the former[18]. The TfR1 causes an increase in the proliferation of the blood cells. The increase in the number of cells can increase the level of haemoglobin in the patients. Therefore, TfR1 can be used as a target for the treatment of leukaemia[19].

Cancer Therapy by TfR1: According to studies related to TfR1, curcumin can used be used as an effective drug in chemotherapy. It regulated the level of TfR1 and the iron regulatory proteins (IRP)[20]. It resuscitates the body's natural apoptosis phenomenon. The antibody therapy is used in it. The anti-transferrin receptor antibodies are designed against the transferrin receptors for the therapy of cancer. These are the monoclonal antibodies that inhibit the uncontrolled division of cells in T-cell leukaemia.

CONCLUSION

Cancer is a very deadly disease. It is caused by many reasons like mutations. The increase in TfR level is a very important cause of the progression of cancer. Targeting the increased level of TfR can be proved effective in cancer therapy. It is used in the diagnosis of cancer as well as in drug production and suggestion. Mainly, reviving apoptosis by inhibiting iron uptake is the key process to treat cancer. The drugs are being designed by taking all these points under consideration. The tumor-specific target therapy is considered the most promising way of treating cancer. Therefore, target-specific drugs should be produced. All these steps are taken to curb this fatal disease. To conclude, these therapies are being used worldwide and cancer is no more an untreatable malady.

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Author's Contribution:

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Final Approval of version:	Quratulain Maqsood

Conflict of Interest: The study has no conflict of interest to declare by any author.

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