INTRODUCTION

YKL 40, also known as Chitinase-3-likeprotein-1 (CHI3L1) is a secreted glycoprotein found in the human body. While YKL 40 is produced abundantly by many cells in the body, little is known about its function. The receptor to which it binds is unknown, but it has been shown to have a binding site for oligosaccharides and collagen. Its biological role also remains largely unclear, but recent studies have shown that YKL 40 may be linked to inflammation and tissue remodeling [1, 2].

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Certain cells in the body have been shown to uptake large amounts of YKL 40 under chronic, inflammatory conditions [1]. This is most evident in the macrophages of the lungs in diseases such as asthma, chronic obstructive lung disease, and pneumonia [3]. However, it can be seen in other diseases involving inflammation and tissue destruction, such as Sarcoidosis and Crohn’s Disease. It has also been noted in various forms of cancer, such as melanomas, glioblastomas, and various forms of leukemia [1,2]. By identifying how YKL 40 interacts with human cells, YKL 40 could be a potential biomarker to help diagnose and identify a variety of diseases, ultimately leading to more timely treatment.

YKL 40 also impacts the central nervous system. In vivo, astrocytes have been known to uptake YKL 40 in diseases involving inflammation of the brain [1]. This can be seen in a variety of conditions, such as Alzheimer’s disease, stroke, Pick’s disease, and HIV encephalitis. YKL 40 is thought to play a specific role in preventing neurodegeneration [2]. In vitro, these effects can be modeled with macrophages, astrocytes, and neuronal cell lines.

Another molecule that plays an important role in the immune response in the brain is Akt, or Protein Kinase B. Akt is a serine/threonine specific protein kinase that associated with cellular survival pathways [4]. It is the main signaling molecule located downstream of the PI3 kinase pathway, which is responsible for cell survival in immune responses [4]. It is normally found in the body in an inactive state, but can be activated by a variety of factors, including growth factors, extracellular stimuli, and oncogenic mutations. Activated Akt plays a major role in the growth of cells and production of neurotrophic factors that promote cell survival [4]. Because Akt can be activated by many factors, it is possible that YKL 40 can activate Akt in astrocytes and neurons, leading to increased cell survival and motility.

Hypothesis

It was hypothesized that U87 cells would have a higher initial concentration of intracellular YKL 40 than T98 cells based on previous research done with these cells. In addition, it was hypothesized that YKL 40 would activate Akt in astrocytes (U87 and T98 cells) as well as neuronal cell lines and do so in a concentration dependent manner.

MATERIALS AND METHODS

Cell Culture and Treatment

Both astrocytes (U87 and T98) and neurons (SH-SY5Y) were used in experimentation. Both cell types were cultured in minimal essential media with 10% fetal bovine serum and 1% Pen-Strep. Cells were cultured under standard conditions. For treatment, cells were cultured for 48 hours with varying levels of YKL 40 added to the media ranging from 0 to 300 ng/ml YKL 40. Cells were then lysed using a lysing buffer and intracellular protein was collected.

Western Blot

Intracellular protein content was analyzed using Western Blot. Protein sample and ladder were added loaded into an electrophoresis gel and run for approximately 40 minutes until ladder was legible. Protein on the gel was then transferred to a nitrocellulose membrane. After transfer, any protein binding sites on the gel were blocked using 5% milk for approximately 8 hours, diluted in 3% milk. After washing three times with PBS, a secondary antibody, also diluted in 3% milk, was applied for one hour. An primary anti- YKL 40 antibody was added for approximately 8 hours, diluted in 3% milk. After washing three times with PBS, a secondary antibody, also diluted in 3% milk, was applied for one hour. The gel was washed 3 times for PBS and a detection agent was then added to the gel. The membrane was then developed in a dark room, detecting any protein.

ELISA

The Sandwich ELISA method was used to detect intracellular YKL 40. The Microvue EIA kit was used, with a YKL 40 capture antibody. Blocking buffer was added to block any extra protein binding sites. Standard and protein samples were added to the wells on the plate, with any YKL 40 in the sample binding to the capture antibody. After incubating for one hour and washing four times with wash buffer, a primary detection antibody labeled with biotin was added to the wells. After incubating for one hour and washing four times with wash buffer, a secondary antibody was added to the wells, conjugated with horseradish peroxidase. After sitting for another hour and washing, a TMB substrate buffer was added
to the wells, inducing a color change. This reaction was allowed to proceed for one hour, stopping with stop solution. The plate was then put in a plate reader and then read at an optical density of 405 nm. The value given by the plate reader was then converted into a concentration after linearizing the standards.

RESULTS

Both Western Blot and ELISA were performed to characterize YKL 40 concentration in astrocytes. T98 were found to contain no YKL 40 at baseline and some YKL 40 after treatment with 300 ng/ml YKL 40. U87 were found to contain 600 ng/ml at baseline and about three times more after treatment. The results can be seen below in Figure 1.

![Figure 1. Astrocyte YKL 40 concentration results](image)

Western Blot was used to analyze Akt concentration in both neurons and astrocytes. Cell lysates from cells treated with a range of 0 to 200 ng/ml YKL 40 were tested for pAkt, pAkt at the threonine site, and pAkt and the serine site. A band of about 60 kDa was needed to confirm the presence of Akt. Western Blot results for astrocytes (U87 and T98) and neurons (SY5Y) can be seen in Figures 2 and 3.

![Figure 2. Western Blot results for neurons (SY5Y)](image)

![Figure 3. Western Blot results astrocytes (U87 T98)](image)

DISCUSSION

The results of the ELISA and Western Blot for YKL 40 concentration in astrocytes supported the hypothesis that U87 cells would have a higher initial concentration of YKL 40 than T98 cells. In addition, it was discovered that T98 do not produce YKL 40 before treatment while U87 produce about 600 ng/ml YKL 40. These results will be useful in future experiments when astrocytes are used. The results of the Western Blot for Akt do not support the original hypothesis that YKL 40 will activate Akt in a concentration dependent manner. The Western Blot showed that activated Akt was present in our samples, but wasn’t related to concentration. This means that YKL 40 may not be activating Akt, and it would not be a suitable biomarker for diseases that illicit an immune response from the Akt signaling pathway. The other possibility is that there is very subtle difference between the concentrations that cannot be detected by Western Blot. Performing an ELISA on the cell lysates would determine any smaller differences in concentration. One interesting finding from our Akt Western Blot was that there was a major difference between pAkt at the serine and threonine sites for between T98 and U87 cells. The T98 cells had a very weak signal in comparison to U87, which could either be due to human error or because T98 cells have a much lower initial concentration of YKL 40 in comparison to U87 cells. For future directions, all of the above experiments will be repeated. Also, an ELISA will be performed on the lysates from the Akt Western Blot to look for subtle differences.

CONCLUSION

While the concentration of YKL 40 in astrocytes was able to successfully be determined, a definitive link between YKL 40 and Akt could not be found. This could either be due to human error or due a lack of connection between the two molecules. If they are not connected, YKL 40 is not a suitable biomarker for certain inflammatory diseases. Further research will need to be done to determine the connection between YKL 40 and the immune response in the central nervous system.

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REFERENCES
