Validation of antibody specificity using reverse short interfering ribonucleic acid (siRNA) transfection technique

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SUMMARY
The Human protein atlas project is a project, which is aimed to produce and validate antibodies towards all the human proteins, and to use these antibodies for large scale proteomics. Some of them were identified as the potential biomarkers. In this thesis, I optimized specific conditions for transfection of cells. The protein down-regulation was brought by transfection of sequence specific siRNA into the cells. Also, I worked on GAPDH+, and ACAT1 predesigned siRNA transfecting them on CACO-2 cells, STMN1 siRNA on T24 cells. The transfected cells were harvested on slide and stained with immunohistochemistry. Here we validated antibody specificity of ACAT1 through immunohistochemistry and conformed its result by western blot. T24 cells were transfected and protein down regulation was analyzed with the help of western blot. Thus, the antibody specificity of particular antibody of interest was verified.
**ABBREVIATIONS**

- siRNA: short interfering Ribonucleic acid
- GAPDH: Glyceraldehyde 3-phosphate dehydrogenase
- ACAT1: Acetyl-CoA acetyltransferase 1
- STMN1: Stathmin 1
- CACO-2 cells: Human colonic carcinoma cell line
- T24 cells: Human urinary bladder carcinoma cell line
- PrESTs: protein epitope signature tags
- IHC: immunohistochemistry
- WB: Western blot
- Opti-MEM: Optimal-Minimum Essential Medium
1. INTRODUCTION

Human protein atlas

Human protein atlas is a project which started with the goal of producing and validating antibodies towards all the human proteins [1]. Specific antibodies to human proteins were produced by conventional immunizations and stringent affinity purification using recombinant protein epitope signature tags (PrESTs) as immunogens and affinity ligands [2]. These help in producing recombinant protein fragments suitable for antibody generation [3]. The antigens were produced in large amount to ensure the quality of the antibodies [3].

Figure (1) Antibodies produced can be used for analysis of corresponding proteins in a wide range of assay platforms, including (i) tissue profiling, (ii) protein assays, and (iii) as capture (“pull-down”) reagents for purification of specific proteins and their associated complexes for structural and biochemical analyses [3].

These antibodies were later used for the study of the expression profiles in cells and tissues in tissue profiling [3], protein assays as shown in figure (1). The antibodies produced through PrESTs were validated through the four methods (i) Immunohistochemistry (IHC) (ii) Immuno-florescence (iii) Western blot (WB) (iv) Protein array.

Figure (2) The antibodies were validated through (i) Immunofluorescence, (ii) Western blot, (iii) Bioinformatics and literature search, (iv) Immunohistochemistry, (v) Protein Array [4].
All antibodies which were validated through these steps were given score according to their expression shown in WB and IHC, as shown in the figure (3.1). This reliability score is given as shown in figure (3.2) [5] from IHC and WB.

![Supportive, Uncertain, Not supportive](image)

**Figure (3.1)** According to the expression of antibodies shown in western blot and immunohistochemistry the quality of the antibodies are given as validity scores as shown in the Figure 3.1 [5].

![High, Medium, Low](image)

**Figure (3.2)** From the analysis antibodies through western blot and immunohistochemistry reliability scores are given as shown in Figure 3.2 [5].

The validated antibodies towards human proteins were published on the [www.proteinatlas.org](http://www.proteinatlas.org) website with IHC images and validation scores [1]. The study of the complete human protein gives us understanding on the gene expression.

The 11,260 human proteins which were 56 percent of the human protein coding genes were mapped against 14,500 different antibodies. Each antibody has been used for immunohistochemical staining of 48 normal human tissue samples in triplicates and 432 human cancer samples covering the 20 most common cancer types [5]. In addition to complete localization of human proteins, some of the antibodies were identified as potential biomarkers. Identifying potential biomarkers leads to a new level in dealing with the diseases and can be great importance in clinical field.

In this thesis, we used a different technique, short interfering Ribonucleic acid (siRNA) transfection assay, to analyse the antibody specificity. This assay is commonly used to study and down-regulate the expression of gene of interest [6]. Professor Andrew Z. Fire and Professor Craig C. Mello have introduced the mechanism of the RNA interference to the world and were awarded Nobel prize in the year 2006. In the present research world, the
double stranded RNA is used as a powerful tool to study the functions of gene of interest [7]. After the discovery of RNA interference (RNAi) it has been a very advanced tool for the research in biology [8]. When the double stranded RNAi (dsRNA) were introduced into the mammalian cells or human cancer cells, they are processed into the small interfering RNAs. They are processed into 22 nucleotides in length by an RNase enzyme Dicer. The siRNA are then processed into the RNA-Induced silencing complex (RISC) called silencing complex. Then these siRNA are transported to the messenger RNA by RISC complex. This RISC complex recognizes the messenger RNA (mRNA), silences the complementary mRNA, and degrades it [8]. With its promising approach RNAi has become an experimental model approach for silencing the expression of specific genes in mammalian cells [8]. The RNAi is a process where the sequence-specific, post-transcriptional gene silencing occurs in the cells IN Vivo. The mediator for this sequence specific mRNA degradation is 22 nucleotides siRNA which are generated by the ribonuclease III cleavage from longer dsRNAs [9]. Later the siRNA are produced commercially which has emerged as a new tool for the study in medical research and for therapeutic application [10]. The commercially manufactured siRNA are brought into cell cytoplasm by the transfection with the help of the transfecting reagents [10]. The sequence of the targeted gene has to be identified and the homologous double stranded siRNA were designed prepared commercially which are available from Ambion applied Biosystems Company Stockholm, Sweden. The siRNA transfection is transient, effective in gene silencing, simple, and highly reproducible and effective [11].

IHC is commonly used tool developed for understanding the histology of protein-protein interactions, different kinds of antibodies, over or under expression of genes. The protein of interest can be translocated within the cell or a tissue sample [12]. This technique mainly works on the antibody specificity. The technique involves in (I) preparation of slides which comprises specimen fixation, nonspecific site block, endogenous peroxide block. (II)Then the primary and secondary antibodies are bound to the tissue or a cell specimen on the slide. (III)Then the detection systems are employed to the slides containing the sample. Then the slides are counter stained with Hematoxylin and Eosin. (IV) After the staining and mounting procedures the interpretation and quantification of the obtained protein expression is done [13]. IHC is used in validation of disease targets as the expression of target in the affected tissue during the disease can be visualized [14].

WB is a technique which is used for analysis of the proteins fractioned on the basis of the molecular weight in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS) [12]. In addition, the proteins are immobilised on to a nitrocellulose membrane by the electrophoretic transfer [11]. Then the membrane is coupled with the HRP-conjugated antibody method with correct primary antibody for the antigens. The super signal west Dura-kit is used for producing the signals. The detection of the chemiluminesence is done with CCD camera by a chemidoc instrument. WB assay is most commonly used as confirmation tool of human immunodeficiency virus (HIV) [15].

We use this siRNA to transflect target sequence into cell cytoplasm and validate the antibody specificity for particular antibodies of interest. This transfection of siRNA involves
tranfecting reagent – Lipofectamine, siRNA ACAT1 for CACO-2 cells, STMN1 for T24 cells and a scrambled siRNA of human, rat, mouse was used as a control parallel to these sequence specific siRNA. Validation of the antibody specificity after the transfection involves (I) (IHC), (II) (WB).

Aim of the project is to validate antibody specificity of a couple of antibodies which were highly interested by using reverse siRNA transfection technique. Aim of the project also was to, set up the method in the HPA group, in order to make it available as one of the validation mechanisms for several antibodies of interest.

2. MATERIALS AND METHODS

2.1 Cell and cell culturing

2.1.1. Human colon carcinoma cell line (CACO-2)
CACO-2 cell line human colon adenocarcinoma cells [16]. The cells were commercially available. These cell lines were cultured in the Eagle’s Minimum Essential Medium (MEM) which is available commercially from Sigma Aldrich Company, Stockholm. The MEM is supplemented with 20% Fetal Bovine Serum (FBS), 5% L-Glutamine and 1% penicillin-streptomycin antibiotic solution which were preheated at 37ºc. This medium was used to culture the CACO-2 cells in a petridish and maintained at 37ºc under 5% CO₂ in the incubator. The subcultures from the second passage were used for the assay. For the reverse transfection, non-antibiotic medium was prepared. The medium was always pre-warmed before the usage.

2.1.2. Human bladder carcinoma cell line (T24)
T24 cell lines are transitional cancers cell line of human urine bladder. T24 cells were adherent cells form into monolayers when cultured. T24 cells were used in studies of studies on human bladder cancer. T24 cells were cultured in (Roswell Park Memorial Institute) RMPI medium supplemented with 1% Penicillin-streptomycin antibiotic solution. The cells from the second passage can be used for the assay. These cells were maintained by in the incubator at 37ºc under 5% CO₂.

2.2. siRNA reverse-Transfection
The siRNA reverse transfection (siRNA) assay is performed to down regulate the particular gene of interest with the predesigned siRNA with the help of Lipofectamine 2000 kit. The cells are transfected when they reach 80% confluence. The reagents are added followed by the addition of the cells in the 6 or 12 well plate at the same time. Using opti-MEM the lipofectamine and siRNA were diluted for the experiment. The diluted lipofectamine and diluted siRNA were combined and left for 20 minutes incubation at room temperature. Initially the reagents were added followed by the addition of cells at the same time. The specific targeting siRNA and scrambled siRNA were added in parallel wells as different controls. This plate was tilted slowly to ensure that the transfection reagents mix well with the cells. Then they were incubated for 24 hours in a 37ºc incubator with 5% CO₂ air flow. After 24 hours the medium in the wells were removed and replaced by the antibiotic medium.
and kept for incubation. This transfection is a transient mechanism, so the cells were taken out after 48 hours for the further analysis. The incubated cells were trypsinized and harvested on superfrost slides by cytopsin.

2.3. Optimization of siRNA reverse transfection
The cells were cultured in the 12 or 6 well plate with different volumes of the lipofectamine. This was done to check the sensitivity of the cells towards the transfection reagent. The volume of this reagent was optimized by analyzing the viability of the cells. After checking the cell sensitivity towards lipofectamine and optimizing the volume, different concentrations of siRNA were tried. The siRNA were transfected in different concentrations in order to check the perfect conditions for protein down regulation. The cells were then harvested on the slides and stained for the IHC. Different strategies of IHC were tried. After confirming that, the fixation was not showing any effect, they were stained normally by blocking unspecific binding. After looking at the protein expression on IHC the down regulation of the knocked protein was analyzed. Further, with the optimized conditions the transfection was repeated to replicate the results. Different strategies were tried for protein extraction. Three wells were pooled for 12 well plate for each sample of protein for the WB and the 1 well for 6 well plate. The transfection was confirmed by the IHC and its results were validated by performing a WB from the same experiment.

2.4. Preparation of Lipofectamine
Lipofectamine 2000 was diluted with opti-MEM and kept for incubation in room temperature for 5 minutes. 3.75µl of lipofectamine was diluted into 250µl of opti-MEM per each well for CACO-2 cells and T24 cells. The volume of lipofectamine was prepared according to the wells used for reverse transfection.

2.5. Preparation of siRNA
A 5 nmole siRNA ACAT1, STMN1, GAPDH which were in powder form were diluted with nuclease free water of 100µl and 50µl respectively to make them 50µM and 100µM respectively. The scrambled siRNA of human, rat, mouse was 40nmole in powder form and it was diluted to 40µM by adding 1ml of nuclease free water.

2.6. The transfection of CACO-2 cells in the 12 wells plate
The CACO-2 cells were transfected with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) siRNA initially to optimize the reverse siRNA transfection procedure. The concentration of Acetyl-Coenzyme A acetyltransferase 1 (ACAT1) (siRNA) was optimized to 50nM. The ACAT1 siRNA and scrambled siRNA of 50nM concentration were diluted in 750µl of opti-MEM. Then 750µl of the diluted Lipofectamine was added and mixed the solution by inverting and vortexing the tube. The reagent complex was incubated for 20 minutes at room temperature. After incubation 500µl of reagents were added to the each well of 12 well plate.

2.7. The transfection of T24 cells in the 6 well plate
The T24 cells were transfected with 100nM and 80nM of stathmin 1 (STMN1) siRNA was diluted in the 500µl of opti-MEM respectively in different tubes for duplicates and scrambled siRNA respectively. Both STMN1 and scrambled siRNA were mixed with the 500µl of
diluted lipofectamine respectively. The reagent complex was incubated for 20 min at room temperature. After the incubation 500µl of reagents were added to each well of 6 well plate followed by the cell suspension.

2.8. Preparation and cell suspension in the wells
The cells after reaching 70 to 80 percent of confluence were used for the Reverse transfection. Depending upon the cell growth and the proliferation of cells the start point of cell suspension into reverse transfection was estimated. The cells which were more than 8 passages were not used for reverse transfection. The cells were washed with Phosphate buffered saline (PBS) and were trypsinised with trypsin. The non-antibiotic medium was added to the cells. Approximately 500µl of cell medium having 400,000 CACO-2 cells were added in the 12 well plate with ACAT1 siRNA complex. 500µl of cell medium containing 300,000 T24 cells were added per each well in 6 well plate. After adding the cells the plates were tilted slowly to mix the cells and reagents and were kept for incubation at 37ºc incubator with 5% carbon air flow.

The non-antibiotic medium was aspirated with the suction after 24 hours and antibiotic medium was added. For 6 well plate 2 ml of total medium was added and for 12 well plate 1 ml of total medium was added. After 48 hours of the total transfection the cells were ready to protein extraction and IHC staining.

2.9. Immunohistochemistry and cytospin
After 48 hours of transfection the cells were trypsinized and washed with PBS buffer centrifuged at 85 Relative centrifugal force (RCF) for 5 minutes. They were then fixed in 80 %PBS and 20% FBS (Fetal bovine serum) solution. Then the cells were harvested on a Plus slides by the cytospin at 110 RCF for 3 minutes. Then the cell slides of both Specific siRNA and scrambled siRNA were stained by using the HPA004428 antibody as primary and HRP conjugated antibody as secondary. Diaminobenzidine (DAB) was used for visualizing the conjugation of antibodies. The transfection efficiency can be observed under microscope.

2.10. Extraction of protein using Radio-Immunoprecipitation Assay (RIPA) buffer for western blot
RIPA buffer and protein inhibitors were available commercially from SIGMA Aldrich Company, Stockholm. This kit was used to extract the protein from lysing the cells. The protease inhibitor was added at 1: 100 ratio with RIPA buffer. The mixture was kept on ice while working. The cells were washed with 1 ml of PBS twice by removing with the suction. Then 50µl - 60ul of the RIPA buffer was added into each well. The cells in the well were scraped with rubber policemen and the cells were suspended in the RIPA buffer. The cell suspension was transferred into a centrifuge tube and was incubated at 4ºc cold room on a gentle agitator for 20 minutes. After incubation, the eppendoff tubes were centrifuged at 14.8g at 4ºc for 20 min. Later the supernatant was transfered into a new centrifuge tube.

2.11. Estimation of protein concentration using the Bicinchoninic Acid (BCA) assay
This assay was performed in 96 well plate. Protein standard is commercially available was concentrated with 6 different concentrations from 1000µg/ml to 200µg/ml along with a blank. The protein standard was added in the wells as triplicates. The siRNA samples were diluted at
1:5 ratio with milliQ water and made into duplicates. BCA reagents A containing the
bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 N
NaOH and reagent B contains copper (II) sulfate pentahydrate 4% were added in 50A:1B
ratio. Total 200µl of the BCA reagents were prepared for each well. The protein standard and
the protein sample were added into the wells and with a micropipette, the BCA reagents were
also added into each well. This plate was incubated at 37ºc oven for 30 min. After the
incubation, the protein was estimated using Wallac 1420 workstation machine. The protein
concentration is measured at the 490nm. The results were tabularized and the amount of
protein was adjusted using Microsoft excel sheet and 15 to 25µg and was loaded according
to the concentration of protein.

2.12. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western
blot
Western blot is a technique used to detect a specific protein of interest in the complex of many
proteins with the help of specific antibodies. Chemiluminence detection system is used to
visualize the antibody bound specific protein. In this thesis, we used this technique to check
the down regulation of protein done by the siRNA reverse transfection.

2.13. Buffers
Blocking buffer, Wash buffer (TBST 0.05% Tween), Running buffer, Transfer buffer, 5x Red
were used in western blot.

2.14. Preparation of sample
The samples were taken out of freezer and thawed in room temperature. Adjusted volumes of
87% glycerol and 5xRed and H2o were added. After mixing with the sample it was heated for
5 minutes on incubator at 90ºc. Then immediately the sample was cooled on ice for 2 minutes
and vortexed well.

2.15. SDS-PAGE
The Criterion precast SDS-PAGE gels from Bio-Rad were taken out from the cold room and
set on the gel stand. Running buffer was added to the chambers to the top of the chambers.
Then 20µl of each sample was loaded to the each well and 5 µl Fermentas page ruler
prestained ladder was loaded per every 3 wells. After loading the samples the apparatus was
kept in 4ºc cold room and the running buffer was added to the chambers. A magnetic stirrer
was placed in the tank and Lid was closed. The PowerPac was set to 180 volts and run for 70
minutes until the marker front reaches the bottom of the Criterion gel.

2.16. Western blot Transfer
The PowerPac was switched off after the marker reaches the bottom of the gel. The gel was
carefully removed from the chamber. The Trans Blot Plus tank and filter papers, fiber pad,
PVDF membranes were prepared. The gel was transferred carefully to the apparatus and the
air bubbles were avoided by using tweezers to get a perfect transfer. The gel was placed in
the cassette and moved to the Trans Blot tank. The complete apparatus was placed in an ice
box and the tank was moved to the cold room. The tank was connected with electrodes and
run for 90 minutes at 85 volts. After finishing the transfer, the sandwich was carefully
removed and the membrane was ready to the antibody conjugation.

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2.17. Western-blot transfer detection with HRP-conjugated antibody

The primary antibody (HPA004428) for ACAT1 was prepared by diluting in 1:3000 ratio in total in 5ml of blocking buffer. After the incubation of the membrane in blocking buffer for 45 minutes the blocking buffer was discarded and the primary antibody was added to the 50 ml tube. This was incubated on a rock n roll for overnight. After incubation, washing was done with wash buffer for 4 times, changing the wash buffer for every 5 minutes. Later the HRP conjugated antibody for western blots was diluted in 1:3000(anti rabbit) for ACAT1 in 5ml of blocking solution. This secondary antibody was added into the 50ml tube and incubated on rock n roll for 1 hour. After incubation the secondary antibody was removed from the 50ml tube and washed 4 minutes with wash buffer for 5 times. The super signal Dura-kit was used for producing a signal on the membrane. The reagents A and B from the kit were mixed in 1:1 ratio making them for 4 ml. The membrane was removed carefully from the tube and was placed in a tray using tweezers. The reagent mix was added to the tray on the membrane carefully and incubated in dark for 3 minutes. After incubation, the chemiluminence was detected by the Chemidoc Instrument by using the Quantity one software. The pictures were taken CCD camera and the result was analysed.

3. RESULTS

3.1. Transfection of (Glyceraldehyde 3-phosphate dehydrogenase) GAPDH\(^+\) siRNA

For optimizing the reverse transfection initially, the GAPDH\(^+\) siRNA was transfected to the CACO-2 cells in parallel with the scrambled siRNA as control in 6 well plates. After the 48 hours of transfection hours they were harvested on slides and protein downregulation was analyzed by immunohistochemical-staining.

![GAPDH transfected cells](image1)

![Scrambled siRNA transfected cells](image2)

**Figure (4)** (a) GAPDH (housekeeping gene) which were knocked through predesigned GAPDH siRNA. (b) scrambled siRNA transfected cells which has no effect on these genes can be seen in the figure.

Comparing the expression of protein in the Figure (4.a) and (4.b) the protein down regulation brought down by the reverse transfection can be seen. The brown color indicates the antibody binding to the antigen of the protein in scrambled siRNA transfected cells.
3.2. Transfection of CACO-2 cells by Acetyl-Coenzyme A acetyltransferase 1 (ACAT1) siRNA

Two different siRNA ACAT1 from 5’ and 3’ end were transfected in CACO-2 cell lines to confirm the antibody specificity by down the target protein.

Figure (5) (a) Immunohistochemical staining of transfected cells with ACAT1 siRNA which show the protein down regulation. (b) Immunohistochemical staining of transfected cells with scrambled siRNA which were stained and show no effect of protein down regulation.

The transfection effect can be seen between the ACAT1 transfected cells and scrambled siRNA transfected cells from the Figure (5) (a) and Figure (5) (b). The difference seen in the color confirms the effect of reverse transfection and downregulation of proteins in transfected cells.

Figure (6) (a) Immunohistochemical staining of transfected cells with ACAT1 siRNA 3-5 end which show the protein down regulation. (b) Immunohistochemical staining of transfected cells with scrambled siRNA.

To confirm the transfection efficiency of the specific protein down regulation, the other end ACAT1 siRNA was also transfected. From the Figure (6) (a) it showed the same level of transfection efficiency shown as in the Figure (5) (a). The specific protein concentration was
not affected by the scrambled Rat, Human, and Mouse siRNA Figure (6) (b) conforming our transfection efficiency.

3.3 Western blot analysis
Further, to support the results of immunohistochemistry the proteins were extracted from the parallel wells from the same experiment. Western blot was performed to check the specific protein knock down to confirm the immunohistochemistry.

**Figure (7) (a)**

**Figure (7) (b)**

**Figure (7) (a) and (b)** ACAT1- siRNA used for knock down, scsiRNA scrambled siRNA used as control, 35KDA size of protein showed by the marker. (a) and (b) validation result of the western blot for two different siRNA of same specific siRNA from 3´and 5´ends.

From the Figure (7) (a) the difference between the specific siRNA and scrambled siRNA can be clearly seen. The ACAT1 siRNA transfected cells show a lighter band compared to the scrambled siRNA transfected cells. The beta actin shows the same band size confirming that the same amount of protein was loaded can be seen in Figure 7(a). This western blot confirms the down regulation of the protein by this siRNA which showed a similar effect of down regulation in immunohistochemistry from Figure (6) (a) and (b).

From the Figure 7(b) comparing the protein expression of immunohistochemistry Figure (5) (a) and (b) and western blot, Figure 7(b) the down regulation of protein can be verified.
3.4. Western Blot Analysis of STMN1 siRNA transfected in T24 cells

![Image of western blot](image)

**Figure (8)** This figure shows the western blot of T24 cells transfected with STMN1 siRNA and scrambled siRNA. (1) and (2) wells contain 100nM and 80nM concentration of scrambled siRNA respectively, (3) and (4) were duplicates of 100nM STMN1 siRNA vice versa, (5) and (6) were duplicates of 80nM STMN1 siRNA, (7) was a normal protein sample of non-transfected T24 cell line.

From the Figure (8) the transfection efficiency can be seen clearly as the bands in the (1) and (2) wells show no difference to the normal cell line protein. The down regulation of protein can be seen in the 3, 4, 5 and 6 wells form the Figure (8).

4. DISCUSSION

The usage of the validated antibodies is very important in better understanding particular biomarkers of interest. Identifying potential biomarkers leads to a new level in dealing with the diseases and can be great importance in clinical field. The biomarkers were used in the identification and diagnosis of the diseases like cancer. The functional studies of these particular biomarkers of interest give better understanding. Protein expression levels can be studied and their proliferation in the presence of drugs can be studied, which can be used in the clinical field. The antibody specificity of highly interesting antibodies are routinely analyzed by performing protein arrays and western blots. From these antibodies, some of the specific antibodies can be further validated through siRNA transfection.

For the optimization of reverse siRNA transfection, the CACO-2 cell lines were transfected with GAPDH siRNA along with scrambled siRNA. Although we initially had an efficient transfection as seen in the Figure (4) We could not manage to reproduce the results. We then decided to optimize this procedure with one of our target siRNA. CACO-2 cells were transfected with ACAT1 siRNA in different concentrations. After a series of transfections, we could down regulate our target protein as seen in the Figure (5). WB had shown the same result confirming our antibody specificity which can be seen in the the Figure (7) (a). The same result was obtained from the other prime end siRNA which had a good IHC and WB as seen in Figure (6) and (7) (b) respectively. From the data obtained the antibody specificity of the ACAT1 can be confirmed.
T24 cells were transfected with the STMN1 siRNA the cells couldn’t be stained but the western blot has showed a promising result which can be seen in Figure (8). Although the IHC has not worked, the WB can be believed. With the bands which can be seen in the two scrambled siRNA, from Figure (8) and a band from the non-transfected cell line of T24 prove our antibody specificity. The antibody specificity of ACAT1 and STMN1 was validated using the siRNA reverse transfection. The method was set up in the HPA group, in order to make it available as one of the validation mechanisms for several antibodies of interest.

In the further approach towards the research reverse transfection using ACAT1 siRNA can be done on the other cell lines for making its antibody specificity sure. Different strategies of IHC can be tried on the T24 cells. Different strategies like fixation of the cells in formalin, and endogenous peroxide blocking by using hydrogen peroxide H₂O₂ can be tried and the transfection can be done in the small chambers.
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6. REFERENCES


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