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The Discusion and Optimization Research on HSD17B13 Protein Separation and Purification Technology

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Abstract

Background/Objectives: The Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease that affects nearly a quarter of the world population. Recent studies have shown that there is a close relationship between liver lipid-titrated 17β Hydroxysteroid Dehydrogenase Type 13 (HSD17B13) protein and NAFLD. At the same time, the purification of HSD has been trying to find better conditions and methods, at present, the HSD protein separation and purification technology is incomplete, the cost is high, the purity is low, the purification quantity is small and so on. This paper first reviewed the research progress of Steatosis and some basic properties about HSD, such as HSD expression site, Lipid titration function, in vitro catalytic substrates, and the relationship between HSD and liver diseases.. **Methods/Statistical analysis**: The optimization of HSD purification method is considered be of great help to the further study of its structure and function, so this research put forward two hypotheses to explore its effect on the purification of HSD. The first is to change the kind of tag when expressing plasmid in E. coli, so as to change the adsorption degree of protein with corresponding group and beads, and the second is to change the concentration of different components in Wash Buffer, the aim is to find the best concentration for removing foreign protein and maintaining the adsorption capacity of HSD. **Findings**: The purity of HSD is closely related to tag species and Wash Buffer concentration in different experimental groups, his-Binding Buffer with 0.5% sodium deoxycholate powder added and Wash Buffer with Tris-HCl 50 mM, NACL 300 mM, Imidazole 40 mM for each component are used. **Improvements/Applications**: After the better purification effect, the higher purity HSD can pave the way for the future study of its potential substrate and mechanism of action.

Index Terms

Protein Purification, HSD17B13, His tag, Gst tag, Wash Buffer.

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I. RESEARCH BACKGROUND

A. Nonalcoholic fatty liver disease

Fatty liver is divided into alcoholic fatty liver and non-alcoholic fatty liver. Alcoholic fatty liver is mainly caused by long-term drinking or a large number of alcoholics, while non-alcoholic fatty liver is mainly affected by obesity, genetic factors and some metabolic neutralization diseases. Non-alcoholic NAFLD is a metabolic chronic liver disease in which fat accumulates in at least 5% of the liver cells in the absence of excessive alcohol consumption. NAFLD can range from simple fatty liver disease to non-alcoholic steatohepatitis (NASH) with inflammatory responses, which, if left untreated, can progress to irreversible fibrosis, cirrhosis and even liver cancer.

Liver is an important metabolic organ in human body, and liver damage caused by NAFLD often leads to systemic metabolic disorders, such as increased risk of cardiovascular disease, diabetes and cancer. NAFLD poses an extremely serious threat to human health with a high incidence rate. About one quarter of the world's population suffers from NAFLD. In terms of different regions, there are about 173-338 million people with NAFLD in China, 76 million in the US and 120 million in Europe.

What is more worrying is that there is no effective medical treatment for NAFLD. Therefore, research on NAFLD is of great significance for improving human health.

B. *HSD17B13 protein introduction*

The full name of HSD17B13 protein is member 13 of the 17β Hydroxysteroid Dehydrogenase family . The gene encoding human HSD17B13 is located in band 2, region 2 of the long arm of chromosome 4. Human HSD17B13 protein consists of 300 amino acids.

A number of literatures have detected THE RNA level of HSD17B13 in different tissues by qPCR, and confirmed that HSD17B13 is expressed abundantly in liver [1,2], as shown in Fig.1.

HSD17B13 was first cloned in 2007 by Kiyoto Motojima research group in Japan, who found that HSD17B13 could locate on the surface of lipid droplet, and its n-terminal amino acid 135 was sufficient to mediate its lipid titration capacity.

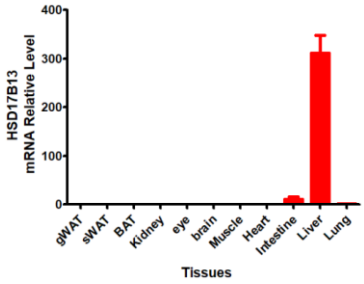

Fig. 1. mRNA levels of HSD17B13 in different tissues

HSD17B13 is a member of the 17βHSD family of proteins with lipid dehydrogenase activity. In general, the reactions catalyzed by them take NADP+ or NAD+ as coenzymes to dehydrogenate hydroxyl groups of lipid substrates into carbonyl groups, as shown in Fig.2.

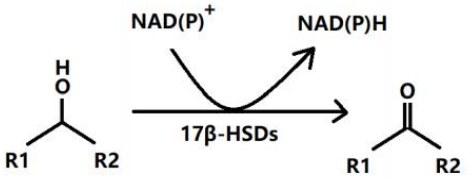

Fig.2. Dehydrogenation catalyzed by members of 17βHSD family

17βHSD family has a total of 14 members, most of which are involved in the metabolism of sex hormones, such as HSD17B5. Some members are also involved in the oxidation process of fatty acid β in peroxisome, and HSD17B9 is involved in retinol metabolism [3].

C. Association between HSD17B13 and liver disease

Results from recent years have demonstrated a strong association between HSD17B13 protein and liver diseases such as NAFLD, Nash, liver fibrosis, cirrhosis, and liver cancer.

Previous literature indicate that the amount of HSD17B13 protein in the liver of patients with NAFLD and Nash is significantly increased compared with normal.

Su W（2014) [4] conducted a comparative analysis of three liver samples from patients with simple fatty liver and three normal liver samples, and they separated the lipid droplets from these samples for proteomics research. The research data showed that HSD17B13 was one of the proteins with the most abundant content in liver lipid droplets. The content of lipid droplets in liver of patients with fatty liver was significantly increased. Ma Y (2019) [5] study also showed that HSD17B13 content was significantly increased in the livers of patients with NASH.

It has also been experimentally demonstrated that the SNP variant rs72613567ta of HSD17B13 reduces the risk of cirrhosis and liver cancer in an alcohol abuse population [6], suggesting that HSD17B13 itself may contribute to the development of liver cancer.

D. Significance of HSD17B13 purification technology

To sum up, the study of HSD protein is important

in the field of now treating liver diseases [7-10]. However, becausethe HSD protein separation and purification technology is incomplete, high cost, low purity, and low purification volume, the optimal purification method still remains unexplored in the laboratory until now. Additional advances in the development of more advanced techniques for targeting this protein will also help liver medicine. Based on this, the research started on the purification of HSD.

E. Raise Question

The laboratory in which we did our experiments had tried purification experiments in eukaryotic systems and found little to do so, so this experiment used a prokaryotic system and intended to explore three key influential factors in the method that the laboratory had tried - different tag, no detergent dosage and different wash buffer ratio brought to the purification impact, and to optimize the purification method.

II. EXPERIMENTAL METHODS

A. Total protein extraction

1. RNA extraction and E. coli treatment

In this study, mRNA was extracted from liver cells, reverse transcribed to cDNA with the corresponding primers to find the corresponding HSD sequence, and this DNA was amplified by PCR, cloned into the pet-28 (a) vector, resulting in the pet-28 (a) - HSD plasmid, and transferred into dh-5 α Coli are amplified, and dh-5 α Coli plasmids transfection into BL21 E. coli.

2 LB medium preparation and E. coli culture

Wash Erlenmeyer flasks 2-3 times, weigh 10 g NaCl, 10 g yeast extract , 5 g tryptone with an electronic balance, pour Erlenmeyer flasks to make up to 1L of solution, repeat the procedure, make up 6 bottles of medium, close. The transfected E. coli are plated, and after incubation for a period of time, the E. coli colonies are transferred to LB medium for one night, and IPTG is added when the bacteria grew vigorously.

3. Protein extraction from broken E. coli

The LB broth supplemented with IPTG was removed and centrifuged at 4000 rpm for 15 min to pellet the bacteria, which was prepared by adding his binding buffer containing sodium deoxycholate powder at a concentration of 0.5% with protease inhibitors (PMSF), added to the bacterial pellet, mixed with a vortex mixer, and decanted the supernatant of the broth after centrifugation to allow the pellet and detergent to mix, adding 250μL per flask PMSF, inhibit most of the proteases, so that after ultrasonic breaking, proteases can not degrade HSD. Wash the probe, put the test tube into ice water to prevent the high temperature produced by ultrasound from affecting the protein activity of HSD, and put into the ultrasonic cell disintegrator to break the bacterial wall for 30 min. Sampling: take 20μL loading buffer with 80μL of bacteria liquid was mixed and put into a metal bath pot at 95 ℃ for 15-20 min, and removed.

B. Protein isolation

1. Remove insoluble component

Prepare a clean Ni column: pour off the last remaining elution buffer and wash the column with his binding buffer, allowing the HSD to bind better to the column. After centrifugation at 20000 rpm for 1 h at 4℃, 50 ml tube after removing centrifugation mix the solution and beads, combine in 4℃ cold room for 1h, pour the liquid into the column, collect the flow through.

2. Change detergent dosage

The amount of antifouling agent added in step B.a was reduced, and the three experimental groups are controlled as follows, respectively: without the addition of deoxycholate, with the addition of 0.25% deoxycholate, and with the addition of 0.5% deoxycholate, continuing to operate according to the process described above.

3. Change the Wash Buffer ratio

The three sets are set up to dispense different wash buffer solutions in 0.5% detergent, other operations and conditions are as follows:

① Control: Tris HCl 50mM, NaCl 300mM, imidazole 20mM adjusted pH = 8.0

② Experimental group 1: Tris HCl 50mM, NaCl 500mM, imidazole 20mM adjusted pH = 8.0

③ Experimental group 2: Tris HCl 50mM, NaCl 300mM, imidazole 40mM adjusted pH = 8.0

Wash three times with wash buffer from each of the three flow to wash away weakly bound proteins, and add elution buffer to wash down HSD.

C. Detection of protein

1. Polyacrylamide gel electrophoresis

Blue coloured 3-well solutions are taken in 96 well plates with a total of six samples. Mix in all three wells for 8 μL protein sample and 2μL of loading buffer, intermediate process sample mix 1 μL sample and 9μL loading buffer. The solution is added to the well of the gel with a pipette, the protein is run through the concentrate using a low voltage of 80 V, electrophoresed using a voltage of 200 V for

40 min and the gel is removed.

2. Coomassie bright blue staining

The removed gel is put into the petri dish and poured into coomash bright blue solution until the gel is covered. The gel is dyed overnight and decolorized with DD water. The results are observed and analyzed in scanning imaging.

3. Western Blot analysis

After the samples are added with the appropriate amount of SDS loading buffer, they are processed by heating at 95 ° C for 15 min, or samples stored frozen at - 20 ° C are heated at 55 ° C and centrifuged at 12000 rpm for 1 min. Appropriate amounts of sample are added to SDS-PAGE protein gel loading wells, and the gel was run using a bio rad electrophoresis apparatus, 60V under low pressure conditions until bromophenol blue became a line, and then pressurized to 100V and continued running for 1-2 hours until the protein ran to the appropriate position.

The running gel is stopped, the gel plate is removed, and the protein glue is transferred to a bio rad transmembrane reader at a constant flow of 300 Ma for 60-90 min, and the protein is transferred to a PVDF membrane. At the end of the trans membrane, the trans membrane clip is removed, and the desired band of interest is cut out and placed into PBST containing 5% non fat milk and blocked for 1 h at room temperature.

Wash the membrane three times with 1 X PBST solution, add the corresponding primary antibody solution and incubate overnight at 4 ℃. Primary antibodies are retrieved and washed three times for 5 min each with 1 X PBST. Appropriate ratio diluted secondary antibodies are added and incubated for 1 h at room temperature. Recover or pour off secondary antibody and wash three times for 5 minutes each with 1 X PBST solution.

Equal volumes of ecl-a and ecl-b solution are mixed and added to PVDF membrane in the dark room, after 1 min, excess luminescent solution was blotted off, placed in transparent cellophane, exposed in the dark room with X-ray film, and sequentially developed in developing solution, water, fixative.

III. RESEARCH RESULTS

A. Effect test of changing the dosage of detergent

Since the mass of his tag is so small that it can be almost ignored, the band expected in this experiment should be about 34kD. After changing the dosage of detergent, the target protein of about 34kD in the protein of Elution buffer increased, but the protein from Elute still had many heterobands.

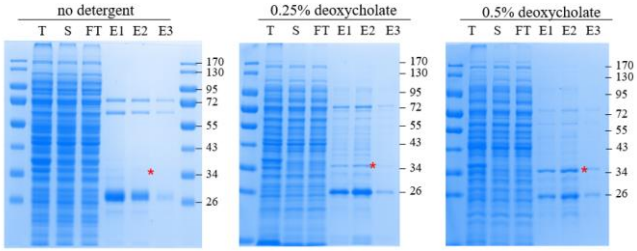

Fig.3. Comparison of dye test images by changing detergent dosage (T:total protein S: supernatant F: Flow through, E: elute sample)

B. Effect detection of changing the ratio of wash buffer

The control group of wash buffer is 50mM/L Tris-HCl , 300mM/L NaCl and 20mM/L Imidazole (left) the experimental group 1 is 50mM/L Tris-HCl 500mM/L NaCl and 20mM/L Imidazole (middle) the experimental group 2 is 50mM/L Tris-HCl 300mM/L NaCl and 40mM/L Imidazole(right).The test results are shown in the following Fig. 4:

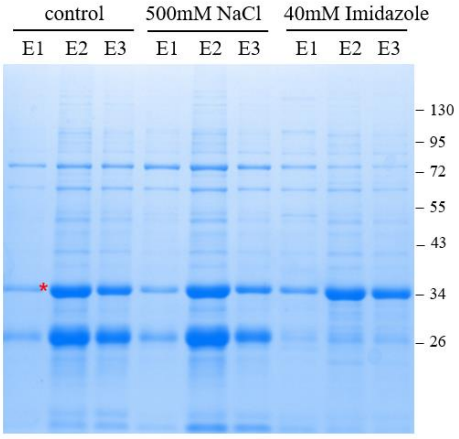

Fig. 4. Comparison of dye test images by changing wash buffer ratio (E：Elute)

It is obvious that with the increase of NaCl dosage, the depth of 34kD zone in experimental group 1 is similar to that in control group, and there is no significant change in other zones. When the dosage of imidazole is increased, the 34kD band depth of experimental group 2 is similar to that of control group, but the 26kD and 70-80kD bands are significantly shallower, and the impurity bands are significantly reduced. The target protein bands in the second eluent of the three groups are deeper than those in the first and third eluents.

IV. ANALYSIS AND DISCUSSION

A. Eukaryotic system

At the beginning of this study, it is intended to study the purification in eukaryotic system and prokaryotic system. However, Professor Lipeng's laboratory tried to use eukaryotic system last year, but the results were not ideal, the purity was not high, and the purification amount was very low. It may be that the eukaryotic system is more complex, the expression of HSD protein is very small, and serum DMEM is required. The cost is expensive, so it is not suitable to extract a large amount of HSD protein by this experimental method, Therefore, this experiment first focused on the prokaryotic system attempt.

B. GST tag

The same experiment was done with GST tag before this study. Coomassie brilliant blue method and Western blot are used to detect GST tag treated with 0.5% sodium deoxycholate in prokaryotic system. GST is about 26kD and HSD protein is about 34kD, so gst-hsd is about 60KD. The results are shown in Fig.5 and Fig.6. The staining image showed that HSD protein is more in total, but very little in supernatant, flow through solution and two elution buffers; Western blot showed that GST-HSD is highly expressed, but there is no target size protein in elusion. It may be that the GST tag in HSD band is not easy to dissolve and easy to form inclusion bodies, or it may be that the detergent used in this experiment is not appropriate to separate the GST tag labeled HSD from other substances in the cell after wall breaking.

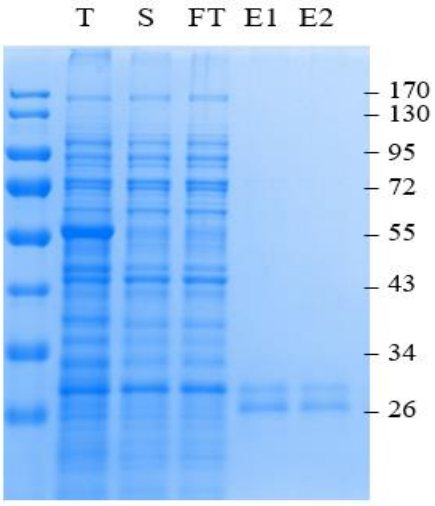

Fig. 5. Staining image of GST tag labeled protein treated with 0.5% sodium deoxycholate (T:Total ，S: Supernatant，F: Flow Through，E：Elute)

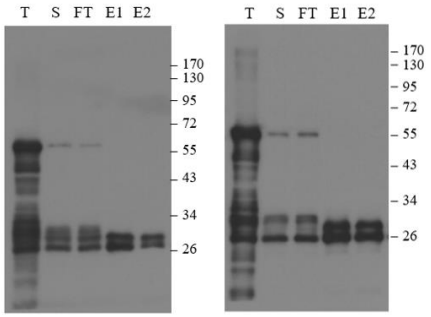

Fig. 6. Western blot results using GST antibody (left) and HSD antibody (right) (T:Total ， S: Supernatant，F: Flow Through，E：Elute)

C. Impact of the dosage of detergent

In this experiment, it is found that the purification effect of 0.5% detergent is significantly better than that of 0 and 0.25%, which indicates that the increase of detergent concentration within this concentration range is conducive to the purification of HSD, possibly because detergent helps to change the surface tension and help wash out the target protein by wash buffer.

D. Impact of the ratio of wash buffer

The experimental results show that the addition of sodium chloride in the wash buffer does not significantly affect the purification purity, but the increase of the dosage of imidazole has a significant effect. The reason may be that the imidazole can helps wash buffer better specifically bind tag and wash away other miscellaneous proteins.

The reason why the protein content of the three eluents is different is that the combination of his and beads is good at the first time, and the degree of protein elution of the eluent is low. In the third time, because the protein content of the eluent is less, and the previous two times have been eluted more, the protein content in elution is less.

V. RESEARCH CONCLUSION

Through investigation and experiment, it is found that prokaryotic system can express higher amount of HSD protein than eukaryotic system, and the purification process is more practical. Because GST tag may form inclusion bodies, the purification effect is poor, but its tag can better combine with HSD and improve the extraction amount of HSD. In addition, it was found that changing the concentration of some salts could effectively improve the purity of protein. Increasing the dosage of detergent in the binding buffer within the concentration range of 0-0.5% can dissolve more proteins in the sediment. Increasing

the concentration of sodium chloride in the wash buffer does not play a significant role. Increasing the imidazole concentration in the wash buffer can remove more miscellaneous proteins and improve the purification purity, but it will reduce the adsorption capacity of the target protein. Based on this, this experiment sets the imidazole gradient concentration to obtain a better salt concentration. In conclusion, it is found that the purification purity of HSD17B13 is closely related to the type of tag and the concentration of wash buffer. In each experimental group, the optimal purification condition is to use his tag and wash buffer with the concentration of Tris HCl 50mM/l, NaCl 300mM/l and imidazole 40mM/l. In the future, we will continue to try to find the optimal purification conditions for HSD, so as to prepare for the future study of its potential substrate and mechanism of action.

VI. FUTURE PROSPECTS

HSD17B13 is 17 βHSD family of enzymes, its enzyme activity is the key to its function and associated with NAFLD. Therefore, the most important thing about HSD research is actually the activity of HSDenzyme. Although some literatures [5] have speculated about its potential substrates, such as retinol, the evidence is not sufficient, and the real substrate of HSD will be further investigated in the future.

We also plan to verify these possible substrates in vitro, that is, to react with the purified HSD in vitro, and map the substrate concentration of HSD on different substrates Initial velocity curve, to calculate the Km and $Kcat$ of HSD for different substrates, so as to confirm which substrate HSD has the strongest binding ability and catalytic ability. After confirming the most likely substrate of HSD we still hope to analyze the structure of HSD bound to the substrate, so as to confirm that HSD can bind to this molecule at the structural level.

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REFERENCES

[1] Horiguchi Y, Araki M, Motojima K. (2008). 17β-hydroxysteroid dehydrogenase type 13 is a live r-specific lipid drop letassociated protein. *Biochem Biophys Res ComMun*, 370(2): 235-238.

[2] Zhao J, Zhang M, Liu J. (2019). AKR1C3 expression in primary lesion rebiopsy at the time of metastatic castration-resistant prostate cancer is strongly associated with poor efficacy of abiraterone as a first-line therapy. *Prostate*, 79(6):1553-1562.

[3] Wen B, Li S, Li H. (2016). Microphthal mia-associated transcription factor regulates the visual cycle genes rlbp1 and rdh5 in the retinal pigment epithelium. *Sci Rep*, 6(5):212-215.

[4] Su W, Wang Y, Jia X. (2014). Comparative proteomic study reveals 17βHSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA*, 111(7):11437-11442.

[5] Ma Y, Belyaeva O V, Brown P M. (2019). 17 beta hydroxysteroid dehydrogenase 13 is a hepatic retinol dehydrogenase associated with histological features of nonalcoholic fatty liver disease. *Hepatology*, 69(7):1504-1519.

[6] Yang J, Trépo E, Nahon P. (2019). A 17beta-hydroxysteroid dehydrogenase 13 variant protects from hepatocellular carcinoma development in alcoholic liver disease. *Hepatology*, 70(5):231-240.

[7] Metallo CM, Gameiro PA, Bell EL.(2012). Reductive glu-tamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature*. 481(4):380–384.

[8] Furuhashi M, Hotamisligil GS.(2008). Fatty acid-binding proteins:role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov* 7(2):489–503

[9] Horton JD, Goldstein JL, Brown MS.(2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Clin Invest* 109(7):1125–1131.

[10] Walther TC, Farese RV Jr. (2012). Lipid droplets and cellular lipid metabolism. *Annu Rev Biochem* 81(3):687–714.