Optimization of semi-dry blotting techniques to improve the transfer of granulins

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1. Abstract

Due to the increased incidence of obesity worldwide, nonalcoholic fatty liver disease (NAFLD) and its progressed version nonalcoholic steatohepatitis (NASH) are becoming a major burden for the health care system. Currently, a liver biopsy is the only method to determine the stadium of the disease [1]. Therefore, it is being researched whether progranulin is suited to be a biomarker in the serum to replace or lower the need for the invasive liver biopsy. To further study progranulin as well as its cleaved products, granulins, an efficient way to quantify these proteins is needed. Therefore, Western Blot method was chosen, even though the optimal molecular detection range for this method is 30-120 Kilodalton (kDa) [2] which is not ideal for the small sized granulins with a molecular size of 10 kDa. To overcome this problem, adaptations to the standard Western Blot protocol need to be tested to be able to successfully show granulins but also progranulin.

2. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease which is characterized by liver steatosis [3]. Fat depositions in the liver that occur without excessive alcohol consumption characterize this disease [4]. Risk factors are obesity, elevated blood lipids and diabetes mellitus [5]. In 20-30% of cases the disease progresses to the more severe form called Non-alcoholic steatohepatitis (NASH) [6]. NASH presents itself in various severity degrees depending on the state of fibrosis and cirrhosis and can progress to hepatocellular carcinoma [4]. NAFLD and NASH are an increasing burden on the global health-care systems. Not only the treatment but also methods for diagnosis and monitoring those diseases are expensive and invasive for patients, with liver biopsy being the only way so far to determine in which stadium the patient resides in [1].

Progranulin is a protein that is under investigation to be a possible new biomarker for NAFLD since it has been shown that patients with NAFLD display significantly elevated serum progranulin concentrations compared to healthy control groups [7]. Its molecular weight is 75 Kilodalton (kDa). Mouse GRN is an orthologous gene encoding for a protein with 79% conformability to the human granulin [8]. Granulins are the proteolytic cleavage products from progranulin. They have a molecular size of 10kDa. Progranulin has many different functions of which not all are well known or researched yet. It is center of many different studies and is being linked to an increasing amount of different health conditions such as the metabolic syndrome, cancer, or rheumatoid arthritis [9]. To dissect out the role that progranulin and granulins play in the disease progression of NAFLD towards NASH, it is necessary to further study these proteins in detail. A well-established method to investigate proteins is the Western Blot method.

3. Aim of thesis

Adapting the current standard Western Blot method so that it is best suited to achieve most small kDa protein saturation after the transfer with an iBLOT 2.

4. Material and Methods

Western Blot was performed according to internal protocol, trying different adaptations in voltage, time of transfer and type of membrane. For semi-dry transfer the iBLOT2 from BioRad was used together with its corresponding transfer-kit.
As primary antibody anti-mProgranulin, purified sheep IgG from R&D Systems were used. As secondary antibody Anti-sheep immunoglobulins with horseradish peroxidase by DAKO were used. Using a peroxide containing substrate solution a chemiluminescent reaction was catalyzed and the signal was detected using a high sensitivity reading camera.

5. Results

After deciding on the best settings for all the different steps in Western Blotting, the process was repeated using two control samples, two NAFLD samples and two NASH samples. The results showed a continual increase in PGRN from the control samples to NAFLD and NASH samples. Vice versa a steady decrease in granulin from the control sample to NAFLD and NASH became visible as shown in figure 1:

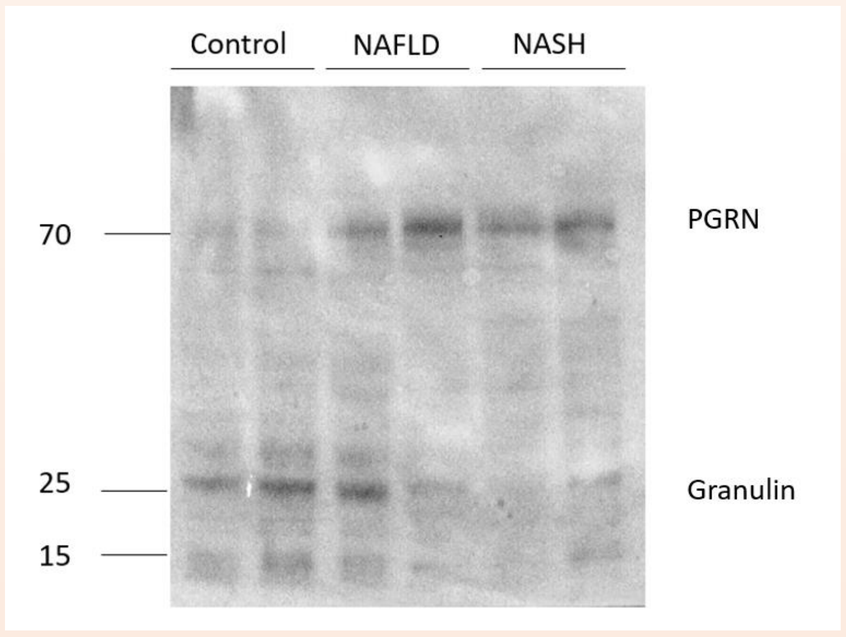

Fig. 1: Results Western Blot ECL, using control, NAFLD and NASH samples

6. Discussion

To summarize the results, to blot granulins as well as progranulin, semi-dry transfer with the adapted standard program 3 (20V, 5min), in addition to exchanging the nitrocellulose membrane for a PVDF membrane, turned out to be the best solution. The aim of the thesis was successfully reached.

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Figures

Fig. 1 Krummenacher, C. (2023f). Results Western Blot. In: medi.