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Automated Imaging of Large Gold-Labelled Western Blots. How this is Helping Research in Type 2 Diabetes

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According to the World Health Organisation, there are around 150 million cases of type 2 diabetes world-wide and its prevalence is expected to double over the next 15 years. Therefore, finding new therapies to protect against this disease is becoming of increasing importance. Obesity and insulin resistance have been recognised as leading causes of type 2 diabetes1, although the molecular understanding of the link between obesity and type 2 diabetes has yet to be fully clarified with many avenues of research undertaken. One such direction has examined the impact of various secretory proteins from adipose tissue, such as adiponectin, which appears multifaceted, involved in the regulation of energy expenditure, lipid metabolism and insulin sensitivity and so may have protecting effects against the pathogenesis of type 2 diabetes [2].

At present our team in the Division of Clinical Sciences at the University of Warwick is studying adiponectin and other proteins as they could potentially offer interesting new areas of drug targeting for type 2 diabetes.

Adiponectin is a 30 kD protein, which can aggregate to form high molecular weight configurations. To visualise adiponectin, researchers in our team have in the past run mini-gels to separate out 30kDa protein, but this was inadequate to examine the higher order structures. As such we modified this to work with the large Western blots labelling our Western blots with an 125I secondary antibody and then analysing them using an automated phosphorimager [3]. This was not ideal as researchers in our team disliked working with radioactive label because it is expensive and requires stringent disposal protocols.

Therefore one solution was to continue labelling larger blots but this time with chemiluminescent substrates and expose them to X-ray film manually [4]. Again this has proved problematic in certain instances as it can involve a great deal of optimisation with use of expensive antibodies to ensure a constant exposure time with X-ray film for the protein investigated. Using an inaccurate exposure time can affect subsequent Western blots using the same antibody and may make generating an optimum result a costly process.

To overcome these problems our team decided to label larger blots with a secondary antibody conjugated to gold and take images of the blot as it developed. This method of labelling adiponectin on Western blots has not been previously described in the literature, as gold labelling has been more commonly used in microscopy applications [5], [6]. Using this method required a camera with a large enough focal length to accommodate the entire blot and it was also essential to generate a number of images of the blot to ensure the correct exposure. For this task our team assessed CCD camera based systems and chose to automate the imaging process with a ChemiGenius2 automated multi-purpose image analyser (Figure 1) from Syngene (Cambridge, UK). This system can image large blots because its darkroom can accommodate blots of up to 25 cm x 30 cm and its camera has a motorised zoom lens which can capture images of large areas. The system generates 16 bit images with 65,536 grey levels, resulting in a dynamic range which is more than double that of X-ray film. It also provides hands free automated capture of a series of images and means that researchers in our team can safely generate images of perfectly exposed blots, without the expense and time of developing many X-ray films or using radioactive labels.

Figure 1:Syngene's ChemiGenius2 chemiluminescent image analysis system

METHOD

To produce and label large Western blots our team used the following method. Human Serum was run out on a non-reducing, non-heated, denaturing PAGE, (4-12%) continuous gel for 3 hours. The proteins were transferred onto nylon membranes by standard Western blotting. The membranes were probed initially with a Ponceau-S solution (Sigma, Poole, UK) to visualise total protein. The blot was then washed and probed using a primary anti-adiponectin antibody raised against the human amino-terminal hypervariable region of adiponectin and it was then probed with a secondary antibody conjugated with 10nm gold particles (Amersham-Biosciences, Little Chalfont, UK). The gold bands on the blot were then developed using IntenSE™ BL (Amersham-Biosciences) a silver staining reagent, which intensifies small gold particles and allows visualisation of the higher molecular species on the membrane inside the darkroom. A series of images of the blot were captured using GeneSnap image acquistion software. When the image with the optimum exposure had been generated, the amount of adiponectin and its aggregate forms were analysed automatically with GeneTools software.

RESULTS

Using gold stained immunoblots of large protein gels, researchers in our team have been able to obtain better separation of proteins and have detected adiponectin proteins of over 200 kD (Figure 2).

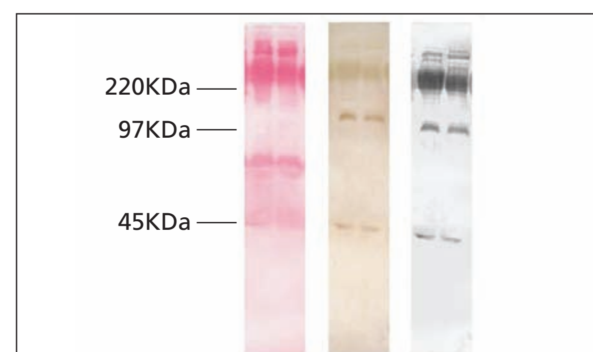

Figure 2: A Western blot of adiponectin and its high molecular weight forms stained with Ponceau S (left) and gold staining (middle) and an image of the blot generated with a ChemiGenius2 (right)

CONCLUSIONS

Using a ChemiGenius2 has provided a previously unattainable method for detecting large proteins labelled with gold because the system automatically detects and analyses the gold coloured bands on gels or blots of up to 25 cm x 30 cm. This allows researchers in our team the flexibility to run longer gels for the best separation of adiponectin aggregates of up to 200 kD. As the system's software can capture an image series, we can more easily determine when we have reached the top end of the dynamic range of our gold labelled image thus saving time, whilst generating one correctly exposed blot image. Additionally, since the images generated have a higher dynamic range than X-ray film and analysing them is fully automated we can produce accurate quantitative data, something, which is difficult to obtain with manual exposures of blots on X-ray film.

In summary, using a ChemiGenius2 to visualise and analyse large gold-labelled Western blots is helping our Group to produce more accurate information on protein aggregates, like adiponectin. By allowing us to determine the relationship of high molecular weight proteins associated with disease we may ultimately enhance our course for determining critical pathways in disease and discover new drug targets. This could in turn provide more rapid production of novel therapies for debilitating diseases such as type 2 diabetes.

References

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