Biochemical Society Summer Vacation Studentship 2023

Analysis of the interaction between dematin and the adaptor protein 14-3-3

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Background

Obesity and diabetes are associated with high blood sugar, high fat levels, and inflammation; these conditions impact around 4.3 million individuals who are diagnosed in the UK and modulate a range of complex cellular signalling pathways inside the cell (Diabetes UK, 2023). The dematin protein has been originally found within red blood cells, but is also present in skeletal muscle, a key tissue involved in the regulation of blood sugar levels.

Dematin binds to a specific glucose transporter as well as to the structural elements of the cell, i.e., the cytoskeleton. Mice in which dematin cannot bind to the cytoskeleton are obese, implying that functional dematin may protect mice (and possibly humans) from obesity and its complications. However, the molecular mechanisms that regulate the association of dematin with the cytoskeleton and sugar metabolism are currently unknown.

Aims and Objectives

The overall aim of this project was to analyse whether previously identified dematin modifications regulate its function in terms of protein:protein interactions and actin binding. This project will specifically investigate the binding of dematin to the small adaptor molecule 14-3-3 and its localisation inside of the cell.

Methods

Dematin proteins as well as the adaptor protein 14-3-3 will be expressed in HEK393 cells. The expression of the proteins will be evaluated by western blotting and their interaction using co-immunoprecipitation and western blotting. In addition, dematin proteins will be expressed in C2C12-muscle cells and the localisation of dematin will be analysed in relation to the cellular actin network using immunocytochemistry and microscopic analysis.

Results and Analysis

Binding of 14-3-3 to dematin: Dematin and 14-3-3 were transfected into HEK293 cells and their expression was validated using western blotting with specific antibodies. Figure 1A shows that the proteins are well expressed. Furthermore, co-immunoprecipitation analyses were performed using a specific antibody against dematin. Co-purified 14-3-3 was detected using western blotting with an anti-14-3-3 antibody, confirming the interaction of both proteins (Figure 1B).

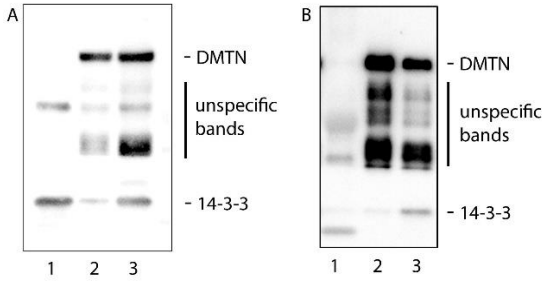

Figure 1: Co-immunoprecipitation of dematin with 14-3-3. Dematin and 14-3-3 proteins were transfected into HEK293 cells. Co-immunoprecipitation was carried out using an antibody against dematin. Western blots were performed to confirm protein expression (A) and the co-purification of 14-3-3 (B).

Localisation of dematin in muscle cells: C2C12 cells from the mouse myoblast cell line were cultured and

transfected with dematin. The dematin protein was labelled using a specific antibody. In addition, the actin cytoskeleton was labelled with an antibody against β-actin. The nucleus was stained with DAPI. The results demonstrate efficient expression of dematin in the transfected cells, which exhibits a similar staining pattern as cellular actin.

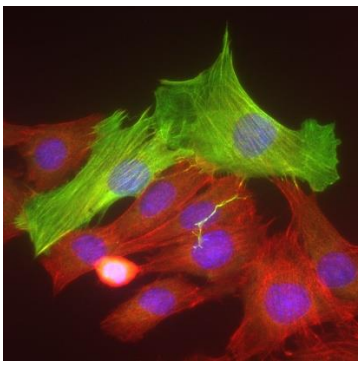

Figure 2: Expression of dematin in muscle cells.
Dematin was transfected into C2C12 cells. Dematin and actin were detected using specific antibodies and secondary antibodies coupled to Alexa488 (green) and Alexa568 (red), respectively. Cell nuclei were also stained using DAPI (blue).

Future Directions

The binding of dematin could be analysed with mutations in specific posttranslational modification sites (amino acids) to further understand the binding and function of 14-3-3 binding to dematin. Furthermore, the role of this interaction could be assessed in actin function and obesity.

Value of Studentship to Student

The studentship was an enjoyable experience that provided me with invaluable lab experience which would have otherwise been inaccessible, and further sparked my interests in pursuing a future career within biomedical research. The opportunity allowed me to develop crucial transferable skills such as time management, problem-solving, accurate record-keeping, and data collection, which I can apply not only as a student but also within my future career. Additionally, I gained proficiency in various techniques including cell culture, cell transfection, and cell lysis, western blotting, among others, and was able to independently conduct experiments by the end of the project.

Overall, this experience has had a significant impact on my personal and professional growth, fully immersing me in the life of a researcher.

Value of Studentship to Lab

It was great to have Areeba in the laboratory over the summer. She learned the techniques well and contributed significant data to the research project that will be used in future studies. Areeba was very enthusiastic and a great member of the laboratory team.

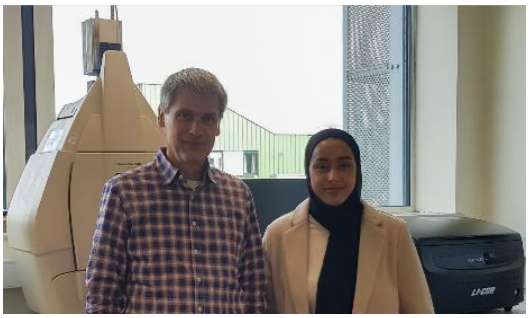

Figure 3: Areeba Tariq and Dr Jürgen Müller in the Lab.

References:

Husain-Chishti, A., Faquin, et al (1989). Purification of erythrocyte dematin (protein 4.9) reveals an endogenous protein kinase that modulates actin-bundling activity. *The Journal of Biological Chemistry*, [online] 264(15), pp.8985–8991. Available at: https://pubmed.ncbi.nlm.nih.gov/2722813/.

Khan, A.U., Hanada, T., et al (2008). Dematin and Adducin Provide a Novel Link between the Spectrin Cytoskeleton and Human Erythrocyte Membrane by Directly Interacting with Glucose Transporter-1. *Journal of Biological Chemistry*, 283(21), pp.14600–14609.

Lalle, M., Currà, C., et al (2011). Dematin, a Component of the Erythrocyte Membrane Skeleton, Is Internalized by the Malaria Parasite and Associates with Plasmodium 14-3-3. *Journal of Biological Chemistry*, [online] 286(2), pp.1227–1236.