

Role of WASP in EMT

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EMT is crucial in both tumour progression and processes like development. EMT is defined as the process by which epithelial cells lose their cell: cell adhesion to become more mesenchymal in character. This process is reversible, and often incomplete so EMT is a spectrum. Despite this, key regulators have been identified in the past such as SNAIL1 (SNAIL) and TWIST1. WASP is a gene that is usually only present in haematopoietic cells but in cancer can be expressed in other cell types. When characterising parental MCF7 and MDA-MB-231 cell lines and analysing knock down cell lines of genes such as ILK, WASP etc we produced data relating to the movement and motility of various cells in response to different drugs or presence of these regulator proteins.

The aim of the project was to highlight the key proteins involved in EMT across MDA-MB-231, DLD1 and MCF7 cell lines and how these are affected by the WASP gene. This included analysing co-expression of junction proteins, cytoskeletal proteins, transcription factors and their localisation in cells. This was addressed using cell lines which we passaged, seeded and cultured before they were fixed to cover slips. Using these fixed cells we carried out multiple rounds of immunostaining with various murine and rabbit antibodies. These cells were analysed using a fluorescence microscope and Fiji editing software to increase clarity of fluorescence and produce composites. Also, we carried out multiple western blot experiments to outline expression of genes, check the quality of knock down cell lines and investigate intensity of proteins. Additionally, I used Trackmate software to track AML cells and create graphs including total displacement, distance and speed of cell movement to analyse the effect of different drugs on random movement of these cells either alone or in co-culture.

In this project I learned key lab skills such as handling, harvesting and seeding cell lines, working in aseptic conditions, immunostaining, fluorescence microscopy, gel electrophoresis, western blot experiments as well as image analysis. Most importantly I learned how enjoyable and fulfilling research is and had the privilege of working with Masters students and other interns like me who strive for making a meaningful contribution in this field. This project has solidified my desire to work in laboratory research / obtain a PHD in the future.



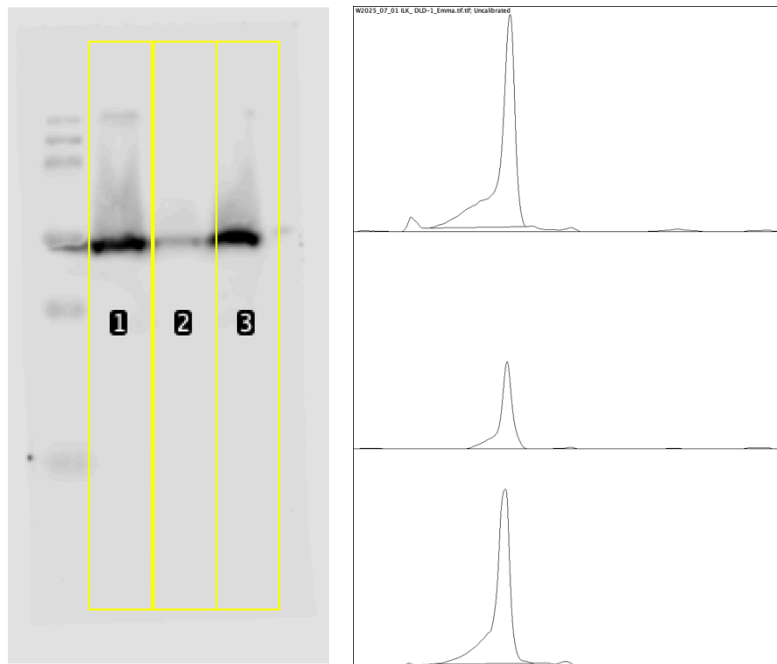


Figure1. Western Blot of whole cell lysates (1) = DLD1 parental (2) = DLD1 ILK Knock Down (3)= DLD1 Vimentin Knock Down. These were probed with an anti-ILK primary antibody followed by a secondary antibody with conjugated HRP. Signal intensity in lane 2 is much lower than that of lanes 1 and 3 (quantified on right panel) due to the cells being ILK-knock down.

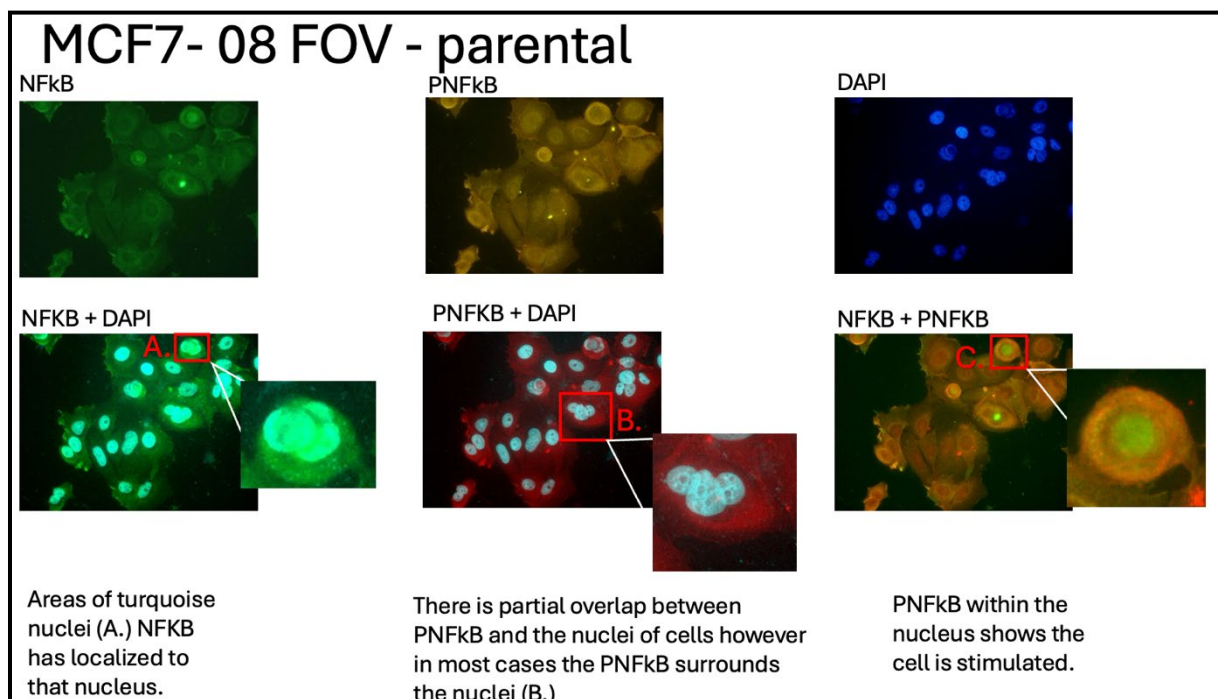


Figure 2. Fluorescence microscope images of MCF7 cells. Composite images were produced from immunostaining and subsequent fluorescence microscopy displaying phosphorylated nuclear factor kappa B (pNFkB), nuclear factor kappa B (NFkB) and nuclei. pNFkB was stained red with an anti-rabbit secondary antibody conjugated with Alexa 568, NFkB was stained green with an anti-mouse secondary antibody conjugated with Alexa 488 and nuclei were stained blue with DAPI.

My fluorescence images, western blots and various results combined with that of our team discovered a difference between the key EMT protein localisations/levels in parental breast cancer cells and those which were WASP knock-downs. This contributed to the understanding of the role of the WASP gene in EMT and further cancer cell motility.