JSPS REPORT

Title: DSTORM Imaging of Receptor Signalling in Epithelia and Immune Cells in Human Bowel Cancer.

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Introduction

This investigation aimed to determine the localisation of the Grb-2 and PLC-γ in lymph nodes from cancer tissue using immunohistochemistry, in combination with immunofluorescence, to perform confocal microscopy and direct stochastic optical reconstruction microscopy (dSTORM).

Colorectal cancer associated lymph nodes were used to understand the Grb2 and PLC-γ expression. There are a number of different cell types within lymph nodes as depicted in Figure 2. Determining the distribution of Grb2 and PLC-γ expression within lymph nodes could provide greater insight into proline-rich motif-SH3 domain driven signalling within these tissues.

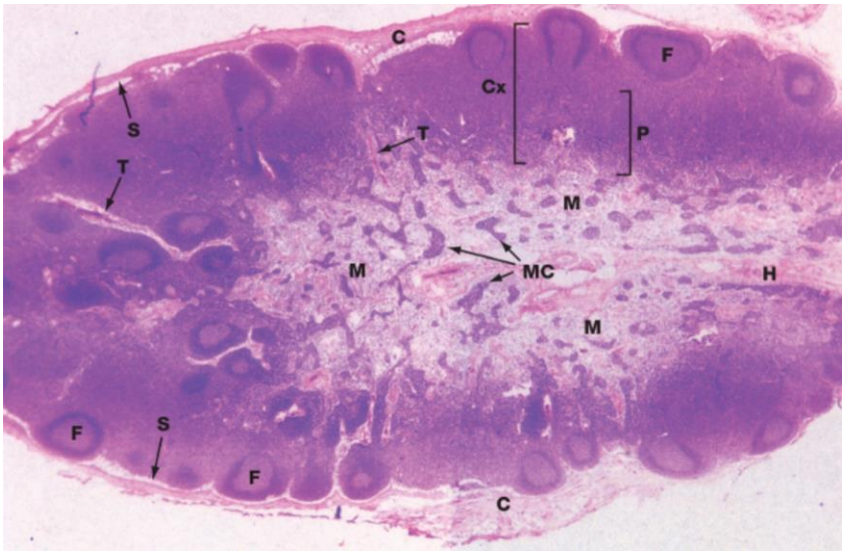

Figure 2. Taken from Young, 2006. The anatomy of a lymph node stained with H & E. Key: ***C*** *– capsule;* ***Cx*** *– cortex;* ***F*** *– follicle (with germinal centre);* ***H*** *– hilum;* ***M*** *– medulla;* ***MC*** *– medullary cords;* ***P*** *– paracortex;* ***S –*** *subscapular sinus;* ***T*** *– trabecula. The cortex of the lymph node contains the B cell follicles, while T cells are found within the paracortex (Colbeck, Ager, Gallimore and Jones, 2017).*

Methods

The tissue used in this experiment was colorectal, containing colorectal cancer associated lymph nodes, and was obtained from the Leeds Teaching Hospital NHS Trust. The expression of Grb-2 and PLC-γ was determined by immunoperoxidase staining and immunofluorescence. Table 1 details the antibodies used in each procedure and the respective microscopes used to analyse the samples.

Staining Technique	Primary Antibodies (dilution)	Secondary Antibody (dilution)	Microscope
Immunohistochemistry	-	-	Standard microscope
Immunoperoxidase	▪ Rabbit Anti-Grb2 (1:100µl) ▪ Rabbit Anti-PLC-y (1:25µl)	Horse Anti-Rabbit IgG Polymer reagent	Standard microscope
Immunofluorescence	▪ Rabbit Anti-Grb2 (1:100µl) ▪ Rabbit Anti-PLC-y (1:50µl)	Goat Anti-Rabbit (1:200µl)	Confocal microscope
Immunofluorescence	▪ Rabbit Anti-Grb2 (1:100µl) ▪ Rabbit Anti-PLC-y (1:25µl)	Donkey Anti-Rabbit (1:200µl)	Oni microscope

Table 1. Details of the procedures used during the investigation.

Results

The results from the immunohistochemistry and immunoperoxidase staining are presented in Figures 3-5. The immunoperoxidase staining conveys a differential localisation of Grb-2 and PLC-γ the lymph nodes cells.

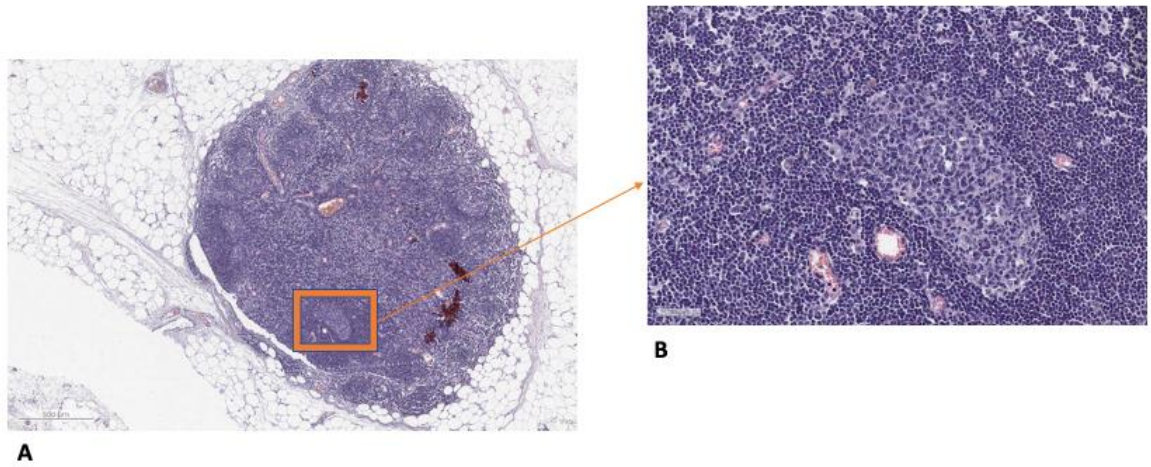

Figure 3. Immunohistochemistry staining was used initially to select the lymph node sections, and also demonstrates the appearance of the lymph node cells in the absence of Grb2 and PLC-y expression as the haematoxylin stains the nuclei of the cells purple and the eosin stains the cytoplasm of the cells pink. Fig. 3.A – shows the entire lymph node at 500 µm. Fig. 3.B – focuses on a follicle within the lymph node, at a higher magnification of 50 µm.

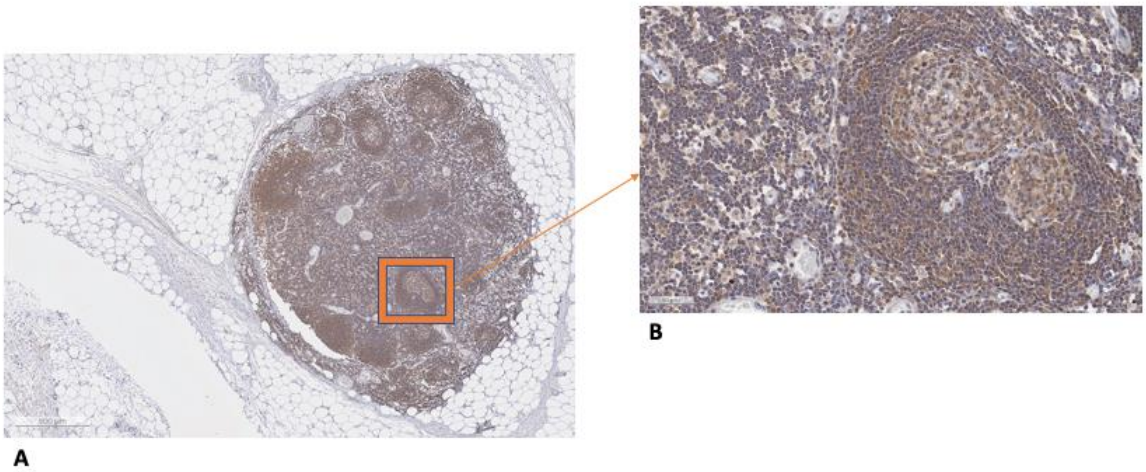

Figure 4. Immunoperoxidase staining of Grb2. Fig. 4.A – shows the entire lymph node at 500 µm. Fig. 4.B – focuses on a follicle at a higher magnification of 50 µm. The staining pattern of Grb2, conveys expression of this protein in the follicle germinal centre, as well as surrounding the follicle and in the cells out with the follicle.

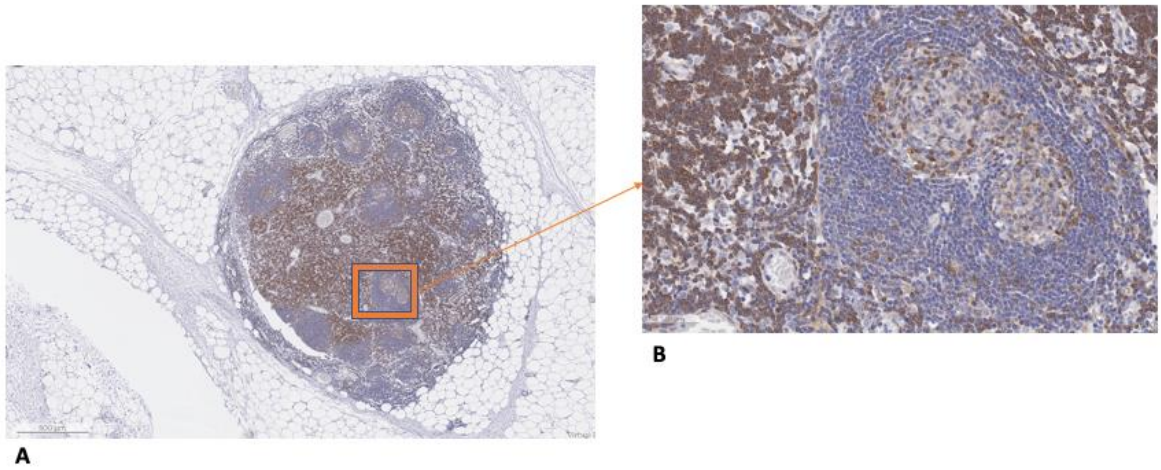

Figure 5. Immunoperoxidase staining of PLC-γ. Fig. 5.A – shows the entire lymph node at 500 µm. Fig. 5.B – focuses on a follicle at a higher magnification of 50 µm. The PLC-γ protein is shown to be expressed in cells outside the follicle and in the germinal centre, however there is markedly limited expression within the outer layer of the follicle and outer cortex of the lymph node.

The immunofluorescent staining was analysed using the confocal microscope and the following images obtained are presented in Figures 6 and 7.

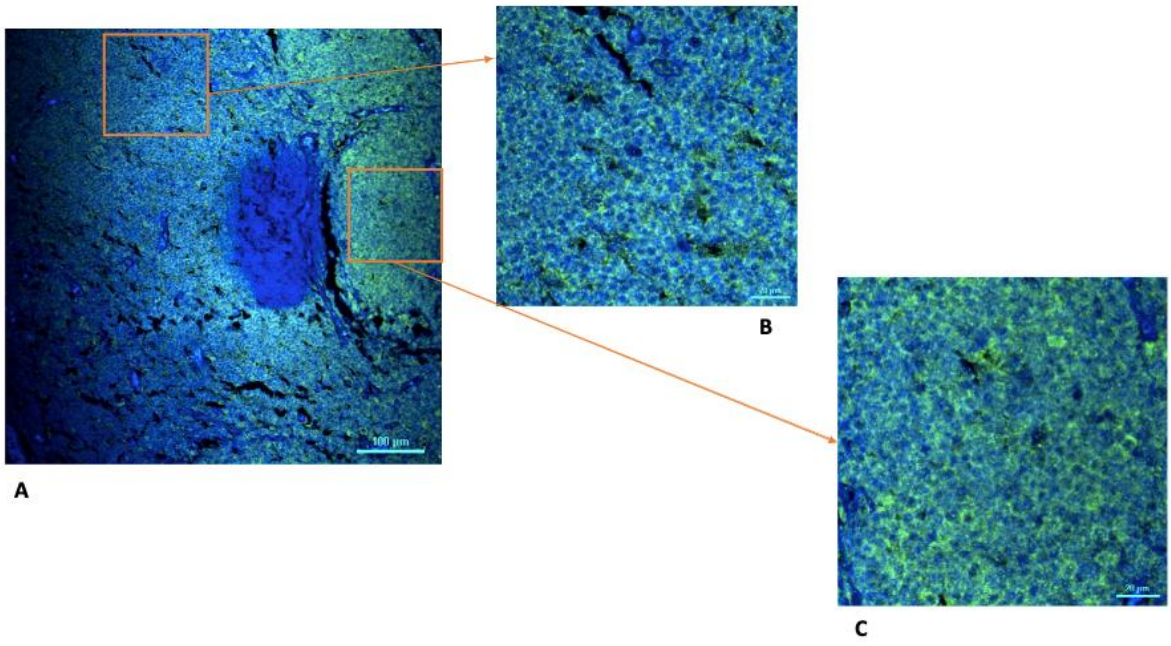

Figure 6. Confocal microscopy of Grb2. Fig. 6.A – provides larger context at 100µm, from which two areas were focused on at a magnification of 20µm in Fig.6.B and Fig. 6.C. These highlight expression of Grb2 in the T cells of the lymph node.

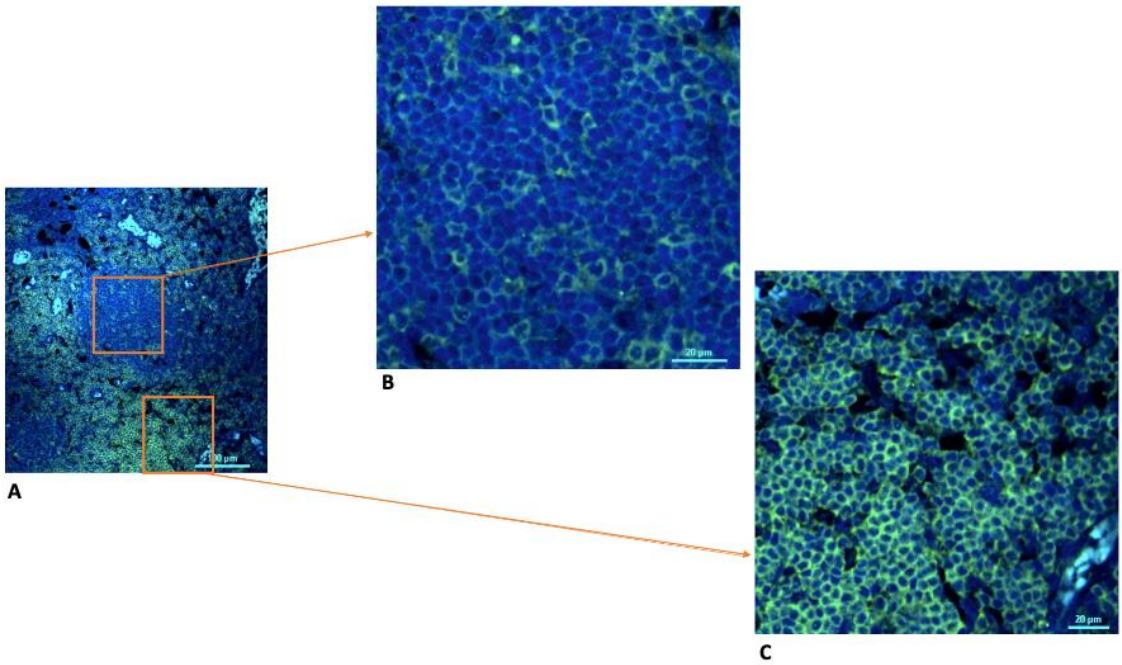

Figure 7. Confocal microscopy of PLC-γ. Fig. 7.A – magnification of 100µm. Fig. 7.B – presents the follicle area of the lymph node at a magnification of 20µm, where there is limited expression of PLC-γ. Fig. 7.C – at a magnification of 20µm, this demonstrates PLC-γ localisation within the T cells.

The dSTORM images present a more detailed analysis of the Grb2 and PLC-γ expression using the Oni microscope, presented in Figures 8 and 9.

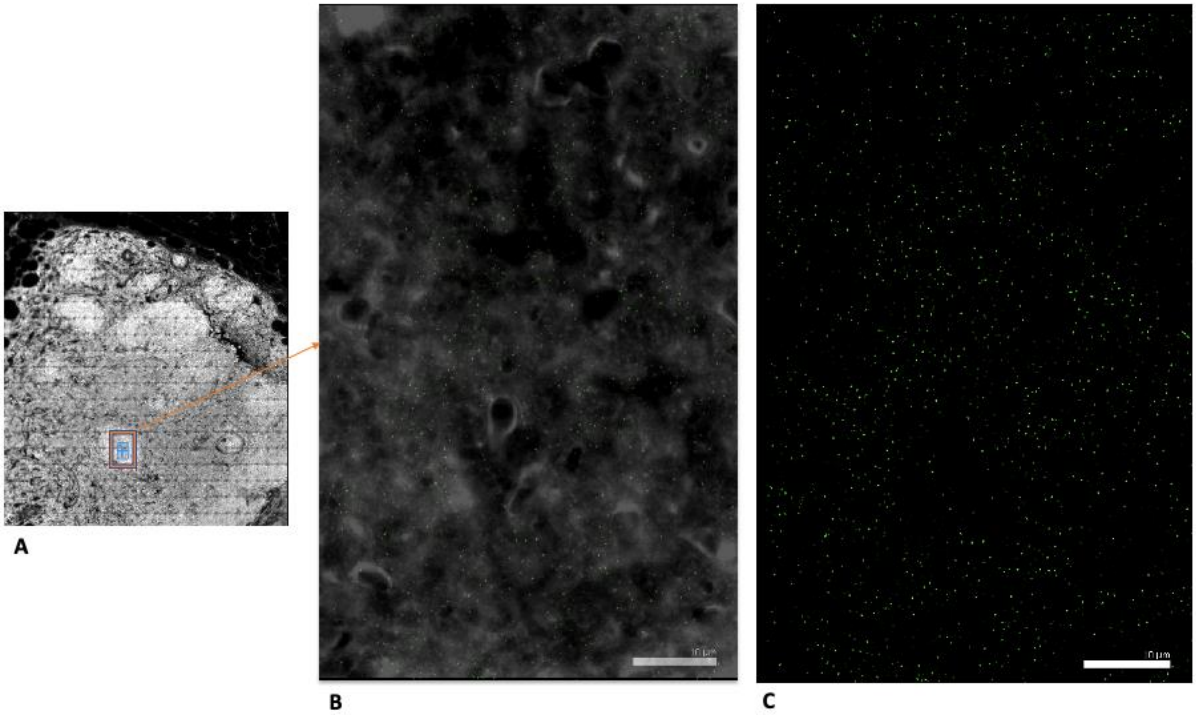

Figure 8. DSTORM imaging GrB2. Fig. 8.A – Multiple images of the lymph tissue complied to form a larger image providing context for are of the lymph node expressing the two SH3 domain proteins. Fig.

8.B – at a magnification of 10µm, this image consists of the underlying dSTORM image with an overlay of a transparent image of the cell structures, showing the expression of Grb2 within the cells. Fig. 8.C – presents the dSTORM image at a magnification of 10µm, with Grb2 expression distributed in clumps amongst the cells.

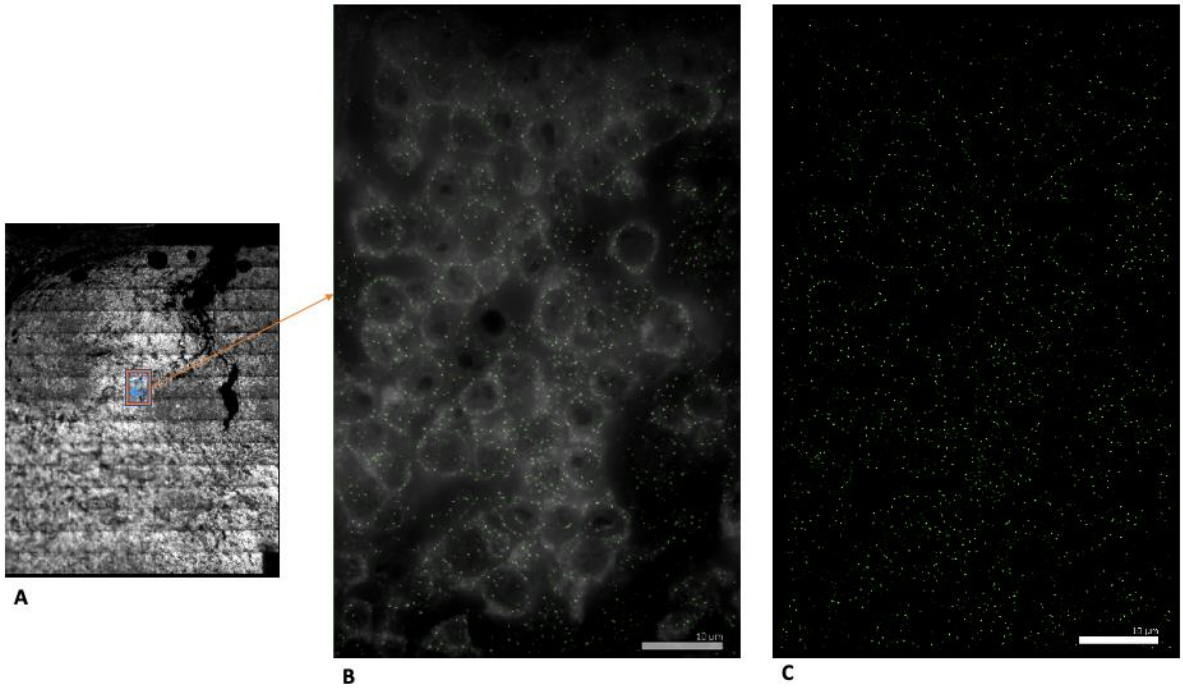

Figure 9. DSTORM imaging of PLC-γ. Fig. 9.A – shows a number of smaller images stitched together in order to orientate figures 9.B and 9.C. Fig. 9.B – shows the dSTORM expression, at a magnification of 10µm, with a transparent overlay of the cell outlines. Fig. 9.C – presents the dSTORM image of the PLC-γ expression of clump-like-structures distributed throughout the cells, at a magnification of 10µm.

Discussion

The two SH3 domain-containing proteins were found to be expressed within the immune cells of human lymph node tissue; therefore, suggesting that there may be proline-rich motif-SH3 domain driven signalling. The differential immunoperoxidase staining pattern of the conveyed that Grb2 was localised in the follicle and surrounding cells, while the PLC-γ was expressed in the germinal centre of the follicle and surrounding cells but not in the outer layer of the follicle. The confocal images demonstrate that there is in Grb2 and PLC-γ expression within the T cells and the follicle centre cells; however, with limited expression in the cells at the edge of the follicle. The dSTORM results demonstrated a linear expression of Grb2 and PLC-γ in clump-like formations, with greater expression of PLC-γ compared with Grb2.

In conclusion, this investigation acknowledged what has been found within the literature, that Grb2 and PLC-γ are expressed in cancer associated lymph nodes (Timsah, *et al.,* 2014 and Timsah, *et al.,* 2015). Furthermore, this study provided a novel insight to Grb2 and PLC-γ localisation using dSTORM.

Reflection

This project enabled me to assist with the entire tissue preparation procedures from tissue fixation to immunohistochemical and immunofluorescence staining with lab technician Mike Shires. In addition, I had the opportunity to observe confocal and Oni microscopy with lab technicians Haley Slaney and Aurora Bono. Participating in this project at Leeds, had the added benefit of providing me with an insight into one of the first digital pathology platforms, and I was shown the digitalisation process by Mike Hale. I thoroughly enjoyed working with the team at the Welcome Trust Lab and the experience has provoked a desire to participate in future research.

Figure 10. – Hayley Slaney working at the Oni microscope (left) and myself learning how to use the Oni microscope (right).

References

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