Project background and aims

Perturbed cellular signalling and resistance to apoptotic cell death can lead to cancer development. My host lab is investigating whether lysosomal-mediated cell death can be used to kill breast cancer cells resistant to apoptosis. To support this goal, my summer project aimed to characterise the autophagic-lysosomal phenotype of different breast cancer subtype-specific cell lines.

Summary of work undertaken, results obtained and future directions

I compared the autophagic environments in 1 normal (MCF10A) and 4 breast cancer cell lines representing the main breast cancer subtypes: luminal A/B (MCF7), HER2+ (SKBR3), and Triple Negative Breast Cancer (TNBC: MDA-MB-231 and MDA-MB-468). To investigate autophagic activity (flux), I exposed the different cell lines to nutrient starvation and/or an autophagy inhibitor (bafilomycin A) and assessed autophagosome accumulation by performing immunofluorescence staining and confocal microscopy for the autophagosome marker, LC3B (see Figure 1A for example images). Under normal conditions, the number of LC3-positive puncta increases with starvation and bafilomycin A exposure. I evaluated autophagic flux by counting the number of LC3B puncta per cell using a modified image analysis pipeline (Schüssele et al., 2023). For MDA-MB-468 and MCF7 cells, LC3B puncta count increased with bafilomycin treatment under non-starved and starvation conditions (Figure 1B) – showing autophagic flux. MCF10A cells however possessed high basal autophagic activity, with few differences observed between conditions (Figure 1A-B). In SKBR3 cells, LC3B puncta number did not appear to change with bafilomycin A and/or starvation, suggesting low levels of autophagic flux. Overall, MDA-MB-231 had fewer LC3B puncta under basal conditions, which did not increase in response to combined starvation and bafilomycin treatment, suggesting potential defects in autophagic induction and/or lysosomal degradation (Figure 1B). In MDA-MB-468 cells, control levels were similar to starved, suggesting reliance on autophagy even under fed conditions. However, the close proximity of many autophagosomes makes accurate image quantification challenging, therefore these results should be interpreted with caution before additional independent repeat experiments.

To investigate this further, I transfected cell lines with a GFP-mCherry-LC3B construct which allows autophagosomes (GFP and mCherry) and autolysosomes (mCherry only) to be distinguished due to the pH sensitivity of GFP, thus allowing monitoring of autophagy activity by live fluorescence imaging. In SKBR3, MCF10A and MDA-MB-468 cell lines, there is a clear increase in yellow puncta and red puncta in starved cells compared to control (Figure 1C). This increase in autophagosomes and autolysosomes shows autophagy has been induced by starvation, which had been difficult to detect when immunostaining for LC3 in fixed cells. SKBR3 cells had the lowest basal level of autophagosomes and autolysosomes, followed by MCF10A and MDA-MB-468 cells, with starvation showing the same trend (Figure 1C). Unfortunately, transfection issues prevented the analysis of MDA-MB-231 and MCF7 cell lines, which will require optimisation in follow-up studies.

Finally, I treated cell lines with the lysosomotropic agent hydrochloroquine (HCQ) to explore their sensitivities to lysosomal permeabilisation. In MCF10A, MDA-MB-231, MDA-MB-468 and SKBR3 cell lines, HCQ treatment resulted in increased lipidated LC3B-II levels (Figure 1D), indicating their sensitivities to HCQ-induced autophagy inhibition. However, relative basal LC3B-II levels differed between these lines, with SKBR3 and TNBC lines possessing the lowest and highest levels respectively, further demonstrating their different autophagic phenotypes as suggested by my imaging experiments. Alongside, I assessed the protein levels of the key autophagy receptor p62, which is expected to accumulate in response to autophagy inhibition, as observed in the SKBR3 cell line. Changes in p62 levels in other cell lines, however, were subtler; perhaps due to transcriptional

regulation and/or potential proteasome degradation. Future studies should therefore assess p62 transcript levels.

In summary, I sought to characterise the autophagic phenotypes of distinct breast cancer cell lines. My initial findings suggest that HER2+ SKBR3 cells possess lower basal autophagy levels, and that these cells are relatively insensitive to lysosomotropic agents. My results also suggest that TNBC cell lines are more dependent on autophagy and sensitive to inhibition. In future work, my host lab will repeat these experiments and use other methods to induce lysosomal permeabilisation to assess the different sensitivities of each cell line to autophagy-lysosomal perturbations. This will further our understanding of the contribution of autophagic-lysosomal pathways in distinct breast cancer subtypes.

Project impact

Breast cancer is the most common female cancer, and multidrug resistance is a serious problem to patient prognosis. This work will help inform our understanding of the role of autophagy-lysosomal pathways in different breast cancer subtypes, and whether some subtypes might be susceptible to autophagy inhibition and lysosome dysfunction. Ultimately, this could help develop lysosome-targeting treatments for therapy-resistant cancers. This is especially beneficial for TNBC patients, who have a worse prognosis and no targeted therapy options.

This studentship helped the Biochemical Society achieve its strategy as I had the opportunity to share my results with my host department's Epithelial Tumour Biology group, and I gained knowledge from presentations during group meetings. As a future bioscientist, this experience has provided me with invaluable support for my career development.

Skills gained and contribution to future career plans/goals:

I have gained new laboratory skills and I can now independently perform techniques including mammalian cell culture, western blotting and fixed and live-cell confocal microscopy. Additionally, I assisted my fellow lab members with generating novel organoid cultures and preparing samples for flow cytometry.

Alongside lab-based skills, I have refined a variety of transferable skills. Analysing and interpreting experimental results has improved my data handling, and troubleshooting has developed my problem-solving abilities. Moreover, giving presentations in lab and multi-group meetings has improved my oral communication skills. Finally, performing background research prepared me for future literature reviews and research projects.

This experience has opened my eyes to the world of scientific research and the work – both successes and failures – behind academic papers. As a result of my studentship, I am inspired to pursue postgraduate studies and lab work. The connections I made in my six weeks will be a valuable resource for the next steps in my academic journey.

References:

Schüssele DS, Haller PK, Haas ML, Hunter C, Sporbeck K, Proikas-Cezanne T. Autophagy profiling in single cells with open source CellProfiler-based image analysis. *Autophagy*. 2023;19(1):338-351. doi:10.1080/15548627.2022.2065617

Figures:

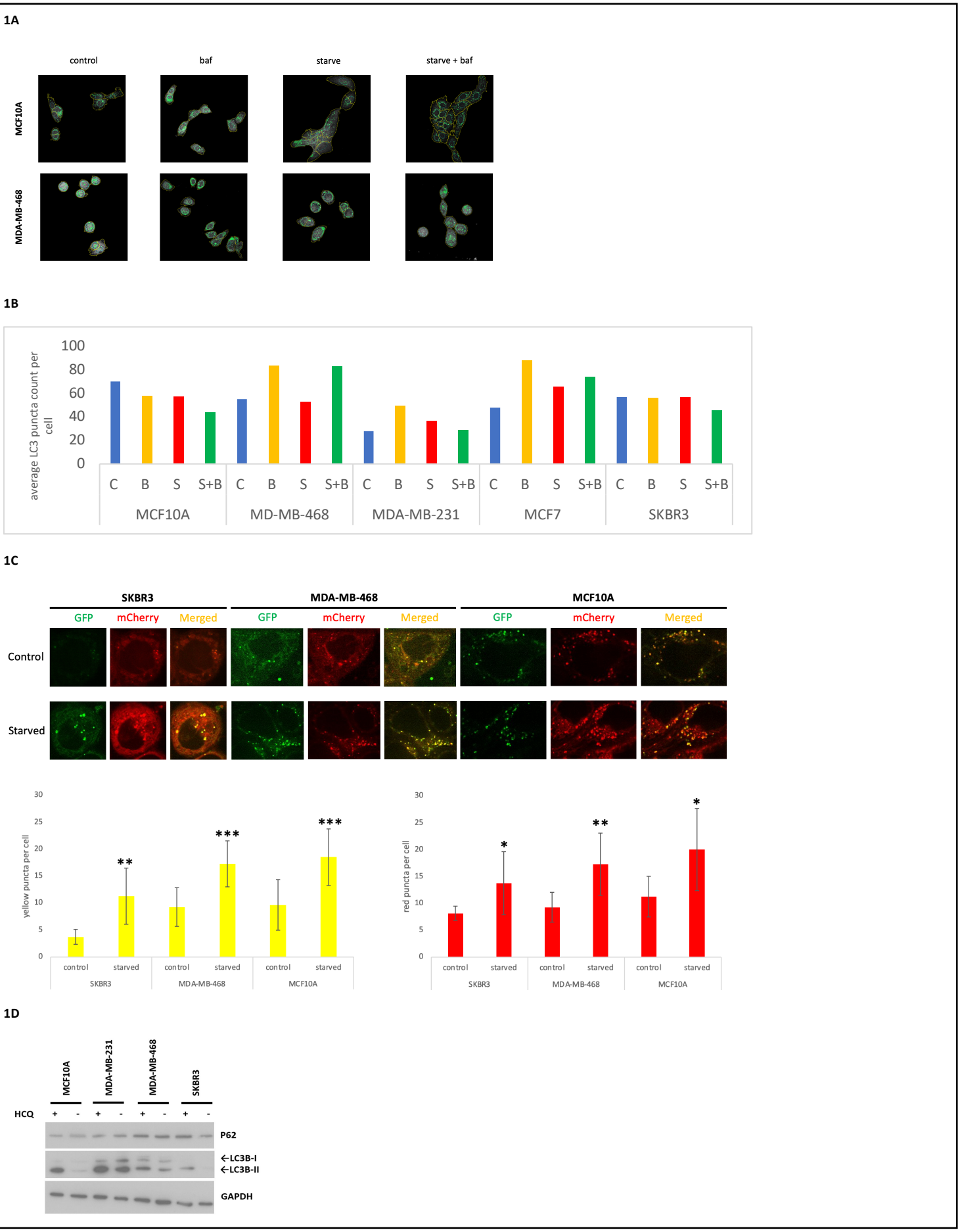

Figure 1: Breast cell lines have different levels of autophagic flux/different responses. A-B: *MCF10A, MDA-MB-468, MDA-MB-231, MCF7 and SKBR3 cells were subjected to the following treatments: control (complete media and buffer); baf (complete media and 50nM bafilomycin A); starve (HBSS media and buffer); starve + baf (HBSS media and 50nM bafilomycin A) for 2 hours before being fixed. Samples were stained using antibodies for LC3 and DAPI and imaged using confocal microscopy. A) Example images for MCF10A and MDA-MB-468 cells after CellProfiler analysis, LC3B puncta shown as green dots.* B) *Graph shows the average LC3 puncta count for each cell line under the different conditions. Image analysis was performed in CellProfiler using representative images to determine the number of LC3 puncta per cell. C= control, B = baf, S = starve, S+B = starve+ baf* C) *MCF10A, MDA-MB-468 and SKBR3 cells were transfected with tandem mCherry-GFP-LC3 construct using lipofectamine 2000 for 5 hours, then subjected to starvation (HBSS media) or control (complete medium) for 3 hours before live spinning disk confocal fluorescence microscopy. Representative images were taken of the cells. Red puncta represent autolysosomes and yellow (red and green) represent autophagosomes. Graphs show the average number of yellow and red puncta per cell for both conditions. Puncta were manually counted using ImageJ. * = p<0.05 ** = p<0.001 *** = p<0.0001 vs. control, independent samples t-test. Data are mean ± S.D.* D) *The effects of hydroxychloroquine on autophagy across representative breast cancer cell lines. Cells from MCF10A, MDA-MB-231, MDA-MB-468 and SKBR3 cell lines were treated with 10ug/ml HCQ (+) or DMSO (-) for 1 hour. LC3B and p62 protein expression levels were analysed by western blot to determine autophagic flux. GAPDH was used as a loading control.*

Figure 2: Group photo. Left to right – Dr Bethan Lloyd-Lewis, Eloise Lines, Neve Prowting (PhD student)