The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Studying composition of autophagosomes in human tumours
Short Project Title: (If main project title is >120 characters including spaces)	Studying composition of autophagosomes in human tumours *This title will be used on the main section of the website and on the iSAP shortlisting application.*

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Vladimir Kirkin
Other members of the supervisory team:	Prof Pascal Meier Ms Sharon Gowan

DIVISIONAL AFFILIATION	
Primary Division:	Division of Cancer Therapeutics
Primary Team:	Cancer Pharmacology and Stress Response

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

The goal of this project is to characterize the protective role of selective autophagy in human cancers, with the potential to develop novel therapeutic strategies in oncology. Defects in selective autophagy have been linked to tumorigenesis. The underlying mechanism has been proposed to be accumulation of aberrant proteins and organelles that drive reactive oxygen species (ROS) production and result in persistent DNA damage. On the other hand, it is expected that cancer cells are dependent on selective autophagy for their survival due to progressive accumulation of genetic aberrations that drive production of misfolded proteins, protein aggregates and damaged organelles. Inability to clear cell junk should eventually result in cell demise, especially if a damage-inducing second drug already affects the cell. For therapies targeting autophagy, it is thus essential to assess the status of selective autophagy in cancer cells and patient-derived tumour material. The successful candidate will establish methods for purification of autophagosomes from cells and tissues and use these preparations to measure the levels of selective autophagy receptors (such as p62/SQSTM1, NBR1, etc.), ubiquitin and mitochondria before and after experimental manipulation of the selective autophagy, using small molecule or genetic tools. A proteomic approach will be employed to assess what additional cargo proteins are upregulated under the conditions when selective autophagy is inhibited. It is expected that multiple cancer-related, mutant proteins will be detected. Eventually, the effect of selective autophagy inhibition on cancer cell proliferation, migration and other hallmarks will be tested. Once the therapeutic concept has been demonstrated in cell culture, the candidate will perform in vivo experiments in xenograft models.

PROJECT AIMS

- Establish robust methods for purification of autophagosomes
- Characterise composition of autophagosomes in tumour vs. normal cells
- Study changes in autophagosome composition following autophagy modulation
- Use preclinical cancer models to validate findings in vivo

RESEARCH PROPOSAL

Project description

1. Establishing the protocol for isolating autophagosomes

A number of protocols for isolation of autophagosomes have been published. They typically involve use of density gradients or alternatively immunopreciptations using GFP-LC3 anchored in the autophagic membranes. Use of special treatments, such as vinblastine to block microtubular movement and thus fusion between the autophagosomes and the lysosomes can be an additional modification to the protocols aiming to enrich the autophagosomal fraction. Established cancer cell lines, such as HCT-116 and A549, will initially be used to establish the optimal conditions for isolation of autophagosomes. Cells will be transfected with mCherry-EYFP-LC3B (Gly-mutant will be used as a negative control) to monitor autophagosome formation by microscopy or FACS. Autophagy will be induced in the cells by using mTORi and Beclin1-TAT peptide. For inhibiting autophagy, inducible ATG7 and Beclin1 CRISPR constructs will be used. Autophagosomes will be purified from the cells under conditions fostering autophagosome formation (e.g., using Beclin1 peptide with vinblastine treatment). Purification will be performed using density gradients and by immunoisolation using anti-EYFP antibodies. Purified material will be assessed microscopically (mCherry-EYFP signal, electron microscopy) and biochemically (the presence of LC3, p62, NBR1 and other markers). Isolation of autophagosomes from mouse cells and tissues will be performed in the subsequent step. Here at the ICR, KPC mice that develop PDAC are available. They will be mated with mice expressing mCherry-GFP-LC3B (kind gift from Ian Ganley, University of Dundee) to derive PDAC-prone mice that express mCherry-GFP-LC3B throughout their body. Isolation of autophagosomes from tumour, stromal and immune cells of the mice will be practiced using both density gradient and immunoisolation techniques. Finally, isolation of autophagosomoes from patient-derived material will be attempted to obtain clinically relevant insights in the composition of autophagosomes in human tumours.

2. Profiling autophagosomal constituents in tumour and stromal cells

Successful isolation of high quality autophagosomes will enable analysis of their constituents. Comparative proteomics (SILAC and TMT) will be employed (in collaboration with the ICR-based proteomics unit) to assess autophagosomal preparations in cells undergoing autophagy (in comparison with those in which autophagy was disrupted by KO of key autophagy genes). In addition, autophagosomes derived from cancer cells will be compared with those from closely matched tissues in KPC mice (and ultimately in patient-derived tumour tissue). These experiments will help to identify proteins that are enriched in autophagosomes of cancer cells. Using the KPC mice, stromal cells will be separated from PDAC tumour cells to analyse their autophagosome composition. PDAC tumour cells will serve as a control for defining stromal-specific components. It will then be assessed if ubiquitination of substrates was involved in directing the proteins to the autophagosomes. So far, ubiquitin-dependent autophagosomal targeting has primarily been described. For most interesting candidates, protein and genomic sequencing will be undertaken to determine mutations that affected protein stability. This study has the potential to identify novel autophagy receptors in addition to cancer-specific substrate proteins and suggest vulnerabilities that could be exploited therapeutically.

3. Profiling autophagosomal constituents in APCs

Use of immunocompetent mouse models of cancer has the advantage that one can study the involvement of the immune system. The KPC:mCherry-GFP-LC3B mice will express mCherry-GFP-LC3B also in the immune cells.

Autophagosomes from APCs will be isolated once mice have developed PDAC and compared to autophagosomes derived from the same mice prior to PDAC. This will help to determine whether tumour-derived antigens are present in the autophagosomes of APCs specifically during PDAC formation. This is an important part of the project as, so far, no hard evidence for autophagy mediating antigen presentation by MHCII compartments in the AAPCs exists.

4. Studying the autophagy-dependent secretion of IGFBP7 as a model for unconventional functions of autophagy

Autophagy plays an ill-defined role in the unconventional protein secretion. It may stimulate fusion of various membranes resulting in 1) increased formation of multivesicular bodies (MVBs) and exosome production linked to that or 2) direct fusion of autophagosomes with the cell membrane, releasing free proteins (among other possibilities). IGFBP7 has a signal peptide and should be subject to conventional secretion via the "ER-Golgi-secretory vesicles" pathway. Interestingly, however, IGFBP7 possesses a *bona fide* LIR motif which interacts with GABARAP in an in vitro assay Therefore, this classically secreted protein is predicted to also be regulated by autophagy. It can thus be a good model to study the role of autophagy in conventional vs. unconventional secretion. We will characterize the interaction between IGFBP7 and different members of the LC3/GABARAP family (collaboration with Terje Johansen, University of Tromso, Norway). We will then construct a LIRless form of IGFBP7 to test if, like the wild-type counterpart this can be secreted by cells. By modulating autophagy, we will then test whether autophagy affects secretion of this protein in the absence of IGFBP7:LC3/GABARAP binding. By analysing medium supernatants, we will determine if the protein is secreted in exosomes or as a free protein, and whether autophagy provides a sort of switch between these two forms of secretion (conventional secretion should result in the free form of protein, while autophagy-mediated unconventional secretion might proceed via exosomes). Finally, we will determine expression of IGFBP7 by murine and human tumours (analysis will also be performed in stromal cells) and manipulate its expression levels to study the outcome on tumour progression in in vivo models. It is also possible that IGFBP7 secretion (in any of the possible forms) could serve as a marker for the autophagy status in cells.

LITERATURE REFERENCES

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KIRKIN, V., LAMARK, T., SOU, Y.S., BJORKOY, G., NUNN, J.L., BRUUN, J.A., SHVETS, E., MCEWAN, D.G., CLAUSEN, T.H., WILD, P., et al. (2009b). A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell 33, 505-516.

MANCIAS, J.D., WANG, X., GYGI, S.P., HARPER, J.W., and KIMMELMAN, A.C. (2014). Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature*, 509, 105-109.

ROGOV, V., DOTSCH, V., JOHANSEN, T. & KIRKIN, V. 2014. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol Cell,* 53**,** 167-78.

SEGLEN, P.O., and BRINCHMANN, M.F. (2010). Purification of autophagosomes from rat hepatocytes. *Autophagy,* 6, 542-547.

YAMANAKA, Y., WILSON, E.M., ROSENFELD, R.G., and OH, Y. (1997). Inhibition of insulin receptor activation by insulin-like growth factor binding proteins. J Biol Chem 272, 30729-30734.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc in Biology
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project.	• Project design and execution • Hands-on experience in vitro and in vivo • Cell and molecular biology techniques (work with nucleic acids and proteins) • Specialized biochemistry techniques (e.g., SILAC) • Transgenic mouse technology • In vivo experience