Get yourself to eat your way out of cancer and neurodegenerative diseases

A cells health is maintained by numerous biological processes one of which termed autophagy (or self-eating) involves the breakdown of parts of the cell in an orderly manner, this is in order to generate energy and replace dysfunctional parts of the cell, termed organelles. The cell generates energy when deprived of nutrients in its external environment. Self-eating of the cell in a controlled manner thus maintains cell health which is termed homeostasis. Autophagy can be non-specific (termed macro-autophagy) or organelle specific termed micro-autophagy. The self-eating process involves the production of double membrane vesicles (derived from the membranes of the cell) termed autophagosomes (self-eating by internal phagocytosis) which then gobbles up or ingests parts of the cell, which could include the power units of the cell, mitochondria (mitophagy) or the protein processing organelle, the Endoplasmic Reticulum (ER phagy). The autophagosome then fuses with numerous lysosomes (to form an autophagolysosome) which contain enzymes that breakdown the contents of the autophagosome to release energy and maintain cell health. The main biological marker of autophagy is located in the autophagosome, is a protein termed LC3B which is a microtubule associated protein (the skeleton of the cell). The more LC3B in the cell the more autophagy is taking place within the cell. Lack of low level autophagy or maintenance of cell health has been implicated in cancer and a range of neurodegenerative diseases such as Parkinson's disease.

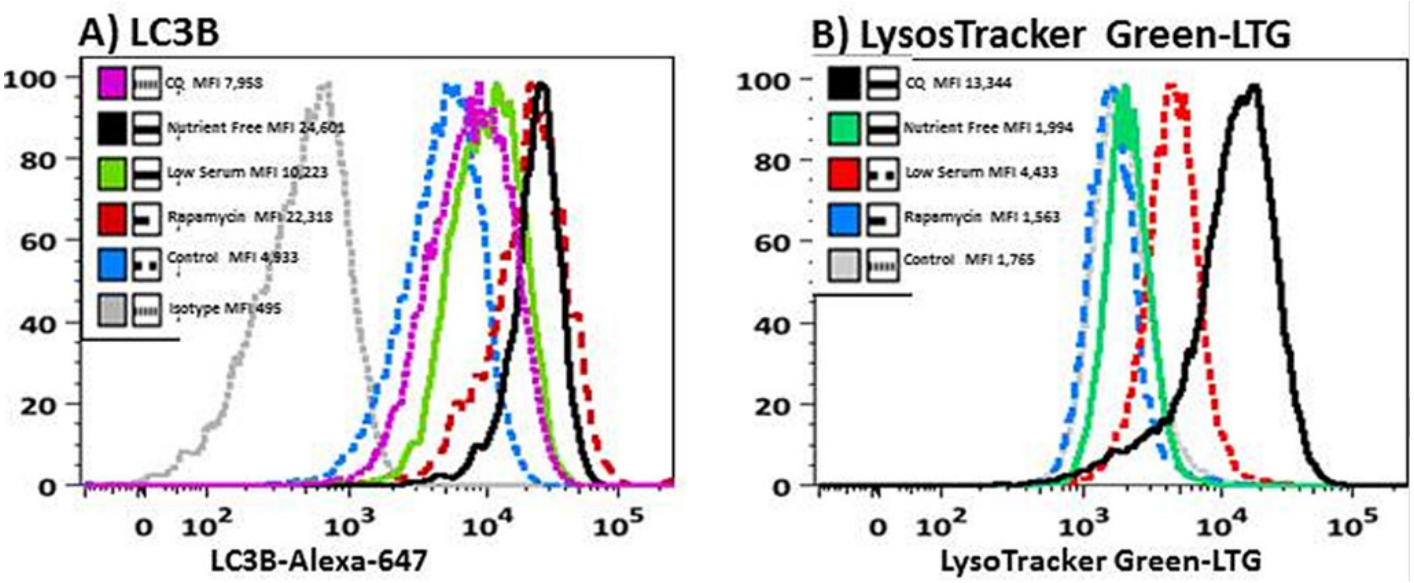

Fig. 1. Jurkat T-cells were untreated (control) or treated with CQ, rapamycin or starved for 24 h. Cells were then labelled with A) anti-LC3B-Alexa-647 (red) or B) LysoTracker Green and analysed flow cytometrically. Histograms show the red or green fluorescence increasing from left to right, with MFI (Median Fluorescence Intensity) values indicating the relative degree of fluorescence and hence the level of autophagy taking place in cells undergoing the different treatments.

Flow cytometry (cell measurements made under flowing conditions) analyses cells in suspension by the use of lasers and electronic detectors under flowing conditions rapidly (up to 30,000 cells/sec). The cells have previously been specifically labelled with fluorescently labelled antibodies or cell organelle specific probes which the lasers excite and are then detected. Each cell is represented as a dot on a graph. The fluorescent signal is proportional to the amount of antibody/organelle present in the cell giving a measurement of the amount of antibody/specific organelle present within the cell.

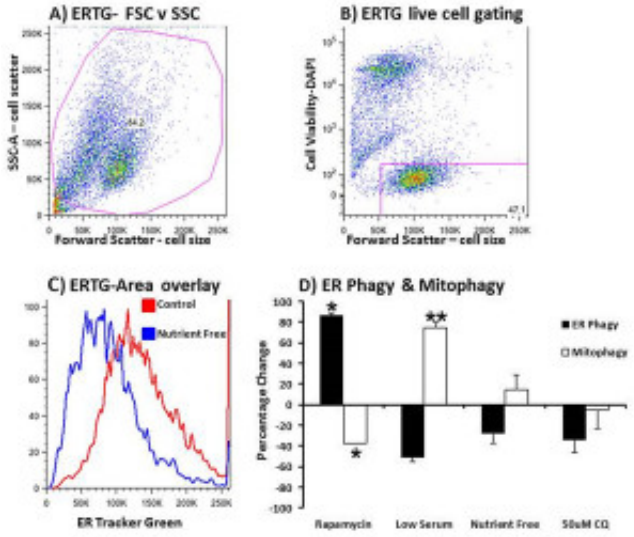

Fig. 2. Jurkat T-cells were untreated (control) or treated with CQ, rapamycin or starved for 24 h. Cells were located using the cell size detector A); then labelled with a cell viability dye, DAPI B); cells highlighted in the box (DAPI negative) are live cells. Live cells labelled with ER Tracker Green were compared to untreated or control cells in a histogram with increasing fluorescence from left to right indicting the difference in size of the ER C). Drug treatments and cell starvation protocols were then compared for differences in ER size (ER phagy) and cells undergoing mitophagy using the MitoTracker Green probe to show changes in cell mitochondria mass. Error bars denote SEM, n=3, * P

Here we show that the amount of macro-autophagy as indicated by increase in the amount of LC3B detected within the cell after depletion of cell nutrients after 24 h or treatment of autophagy inducing drugs such as by rapamycin (an antibiotic, used in the treatment of cancer) or anti-malarial drug, chloroquine (CQ), see Figure 1. Increasing fluorescence indicates increasing amounts of LC3B and thus the cells are undergoing a higher degree of macro-autophagy, see figure 1A. A fluorescent probe specific for the detection of lysosomes shows that the cell produced more lysosomes to fuse with the autophagosomes, see figure 1B.

Within the same cells the size or mass of the ER and mitochondria can be determined by flow cytometry when the cells are labelled with fluorescent probes specifically designed to measure the size of the ER (ER phagy) and mitochondria (mitophagy) for example when starved or treated with rapamycin and CQ, see figure 2. Under early conditions (less than 48 h) the ER size and mitochondria can increase (a cell coping mechanism) and finally decrease in size indicating the presence of micro-autophagy within each cell, see Figure 2.

Thus flow cytometry is an excellent technique to rapidly access the degree of macro or micro-autophagy taking place within the cell at anyone time. This approach enlightens us to the complex processes involved in autophagy and can help us understand how this process can be manipulated to develop new and better treatments for cancer and neurodegenerative diseases.

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Publication

Flow cytometric assays for the study of autophagy.
Warnes G
Methods. 2015 Jul 1