Autophagy inhibition on PanNET models in vitro and in vivo

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NANETS

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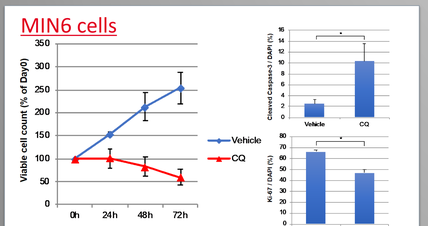
Abstract (Click to see the background)

- The aim of this study is to investigate whether autophagy inhibitors (CQ; chloroquine, HCQ; hydroxychloroquine) show inhibitory effect on PanNET models in vitro and in vivo.
- CQ showed inhibitory effect on two PanNET cell lines. Apoptosis was induced and ki-67 labeling index declined.
- In the spontaneous PanNET mouse model, all 18-month-old Men1+/- mice had pancreatic tumors, and HCQ treatment decreased mean tumor size, inducing apoptosis on tumor cells.
- Autophagy inhibition induced apoptosis on PanNET cell lines and tumor cells in Men1+/- mouse model.

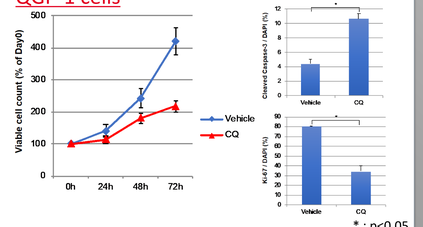
Conclusion

- Autophagy inhibition induced apoptosis on PanNET cell lines and Men1+/- mouse model.
- Inhibitory effect of HCQ might be beneficial on PanNET patients.

MIN6 cells



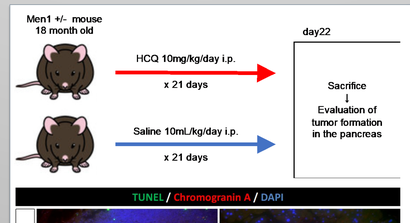
QGP-1 cells



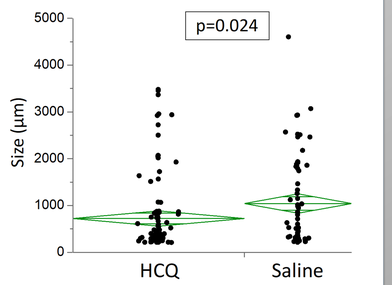
* : p<0.05

Results 1 (Click)

- MIN6 cells and QGP-1 cell were used as in vitro PanNET models.
- CQ showed inhibitory effect on both PanNET cell lines.
- Apoptosis was induced and ki-67 labeling index declined.
- ER stress-mediated apoptosis was induced.



Mean diameter of Islet (tumor) decreased



◀ TUNEL(+) apoptotic tumor cells
HCQ group : 22.5 cells/HPF
Saline group : 1.3 cells/HPF

Results 2 (Click)

- In 18 month-old Men1+/- mice, HCQ or saline were administered intraperitoneally for 21 days.
- HCQ administration decreased mean tumor size.
- Histological analyses showed more TUNEL positive apoptotic cells in HCQ group than in control group.

Click Headings to View More Information

Background

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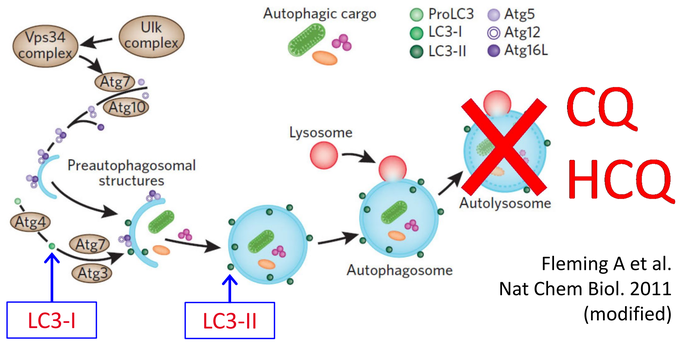
Background / Aim

- Autophagy is a “self-eating” degenerative pathway through which cells digest foreign proteins or organelles to supply amino acids.
- Autophagy inhibition has been shown inhibitory effect on cancer cells, inducing apoptosis on them, and clinical trials using autophagy inhibitor chloroquine (CQ) or hydroxychloroquine (HCQ) are ongoing in several types of cancer.
- The aim of this study is to investigate whether autophagy inhibitors show inhibitory effect on PanNET models in vitro and in vivo.

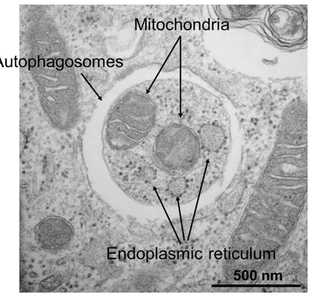
What is Autophagy? ---“self-eating” degenerative pathway



Fleming A et al.
Nat Chem Biol. 2011
(modified)

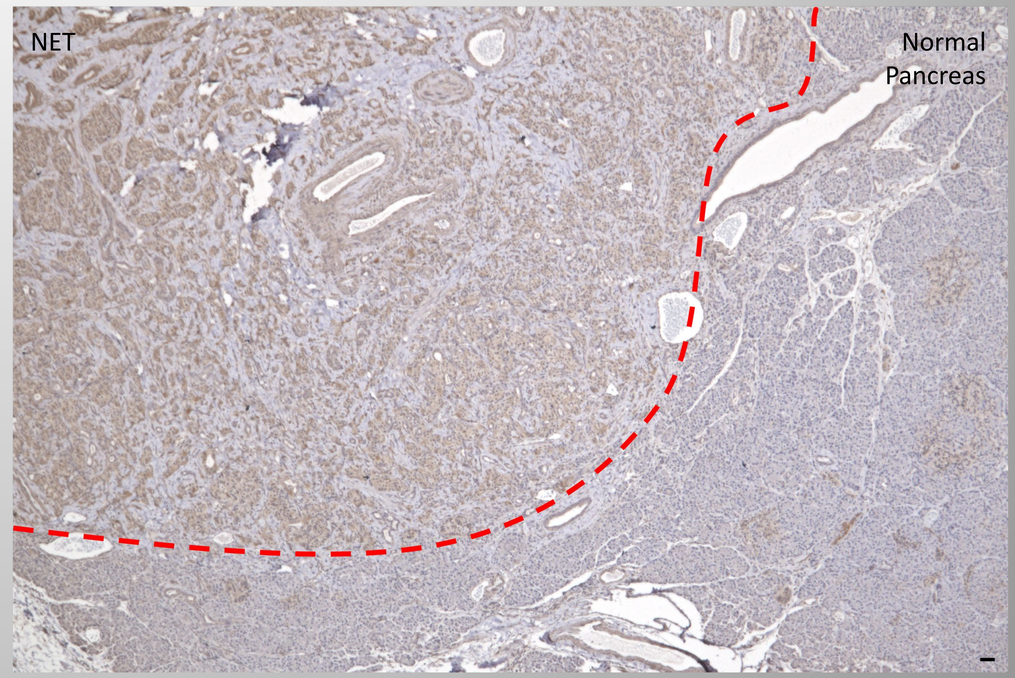
Membranous protein LC3 is converted from LC3-I to LC3-II when corporated into autophagosome.
CQ/HCQ blocks digestion by lysosomal enzymes.
→accumulation of autophagosome in the cytoplasm (Fig.2)

TEM (Transmission Electron Microscope) as a gold standard for autophagy evaluation



From HP of Mizushima Lab
(Univ. of Tokyo)

Figure 1.
IHC of LC3 indicated Autophagy upregulation in human PanNET



Immunohistochemistry of LC3 protein showed strongly positive stain in PanNET tumor cells. (Bar=50μm)
Negative in normal parenchyma. Weakly positive in normal islet.
↓
Indicative of autophagy flux upregulated in PanNET cells.

Results 1: Inhibitory effect of Autophagy inhibition on PanNET cell lines

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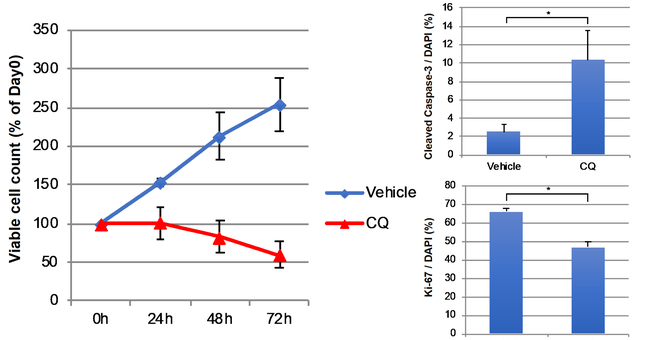
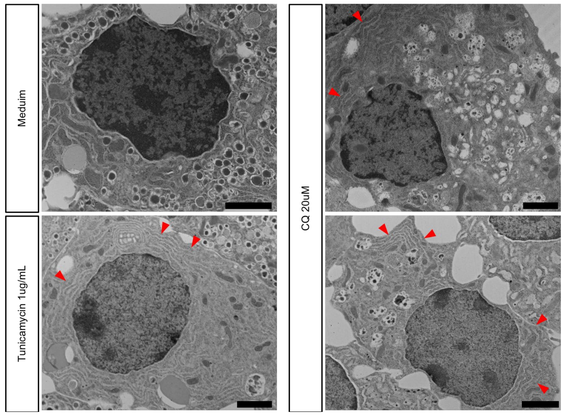
Figure 2. CQ exerted inhibitory effect on PanNET cell lines

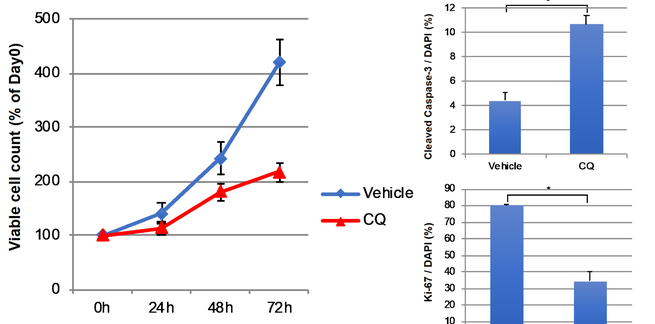
1. MIN6 cells: Mouse Insulinoma 6



* : p<0.05



2. QGP-1 cells:
cell line derived from human malignant somatostatinoma



* : p<0.05

Bar = 2μm
Red arrowheads: dilated ER
Yellow arrowheads: autophagosomes

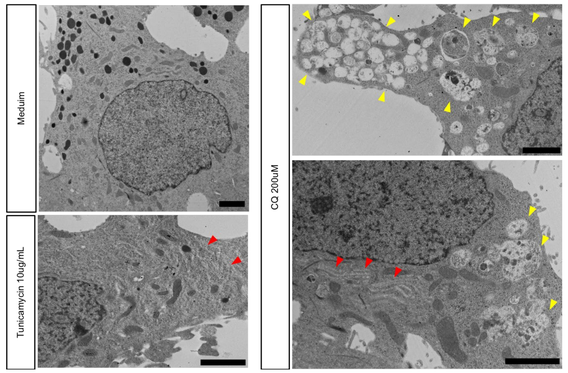
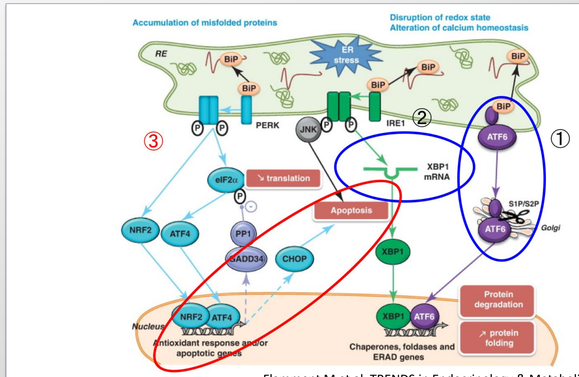


Figure3. Evaluation of ER stress



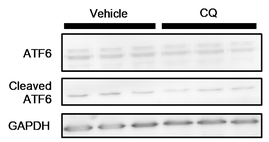
Flamment M et al. TRENDS in Endocrinology & Metabolism 2012 (modified)

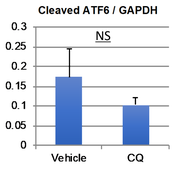
Response to ER stress
=Three major UPR pathways

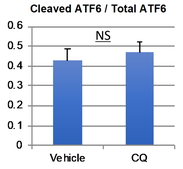
1. Cleavage of ATF6
2. Phosphorylation of IRE1
   →splicing of XBP1
3. Phosphorylation of PERK → p-eIF2α
   →ATF4 →CHOP

1. Cleavage of ATF6

< Western Blot of ATF6 >

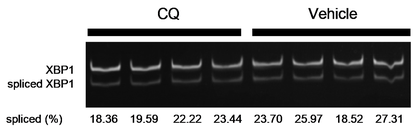


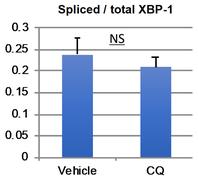




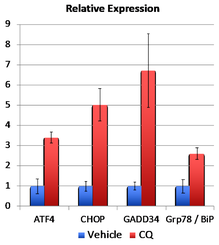
2. Splicing of XBP1

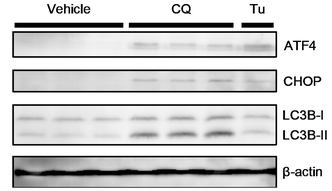
< qPCR of XBP1 >



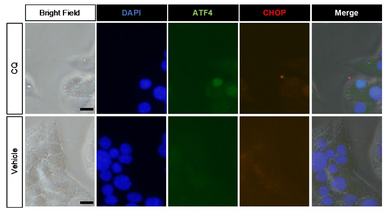


3. Induction of ATF4, CHOP





Tu: tunicamycin 1ug/mL
(= positive control of ER stress)



IF of MIN6 cells

upper: CQ 20μM
lower: vehicle
(24hr treated)

(Bar = 10μm)

- In both cell lines, CQ exerted inhibitory effect.
- CQ induced apoptosis and reduced proliferation.
- TEM analyses showed cumulation of autophagosomes and dilated ER.

- Upregulation of ATF4 and CHOP indicated the ER stress on MIN6 with CQ.
- Proapoptotic protein CHOP was significantly upregulated, suggesting the existence of ER stress-mediated apoptosis.

Results 2: Men1+/- mouse showed in vivo effects of Autophagy inhibition

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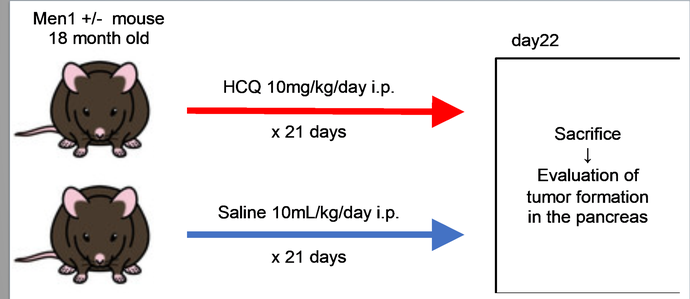
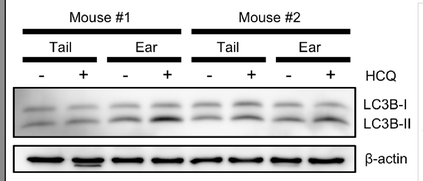
Figure 4. In vivo experimental design

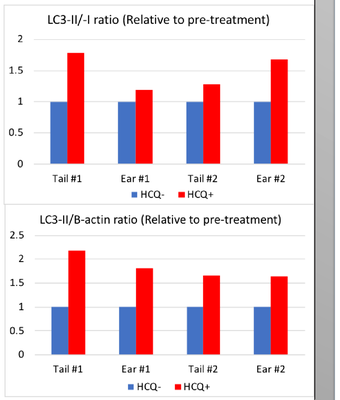


Men1+/- mouse:
129S(FVB)-*Men1*$^{tm1.2Ctre}$/J
Bought from Jackson Laboratory

Reported that 16month-old mice bore pancreatic islet tumors (5/6 penetrance)
(JS Crabtee *et al*. PNAS 2011)

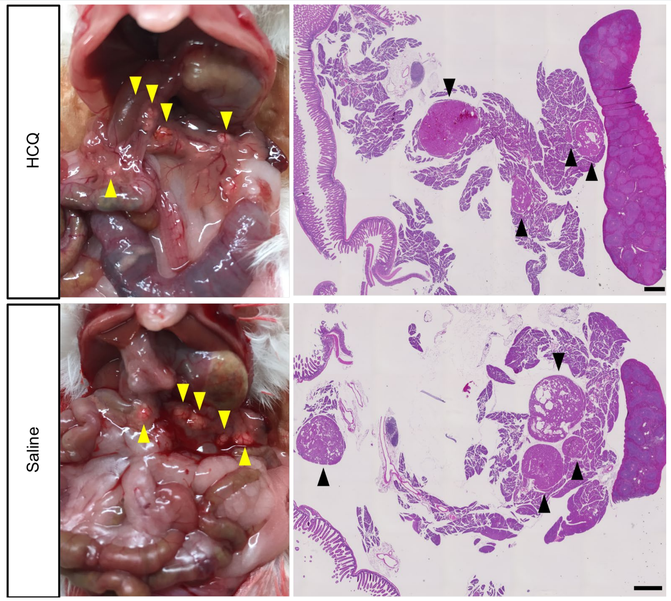
Intraperitoneal administration of HCQ causes systemic disturbance of autophagy



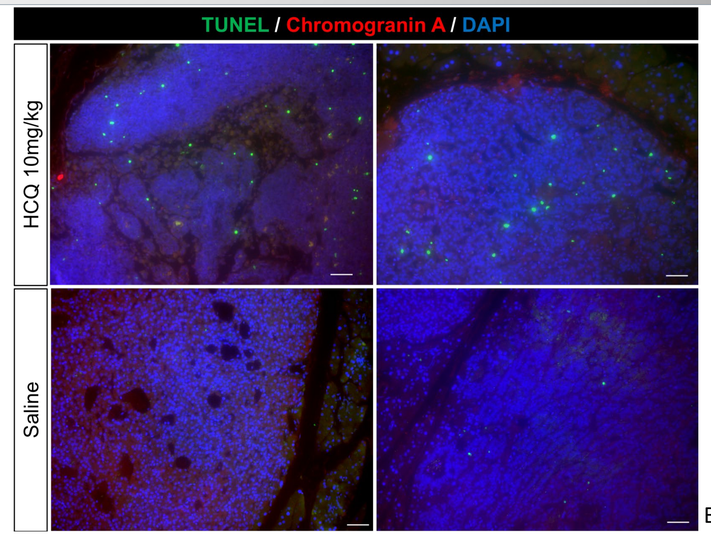


Western blot analysis of protein extracted from mouse before and after administration of HCQ 10mg/kg x7days.
↓
Accumulation of LC3-II was shown in peripheral tissues.

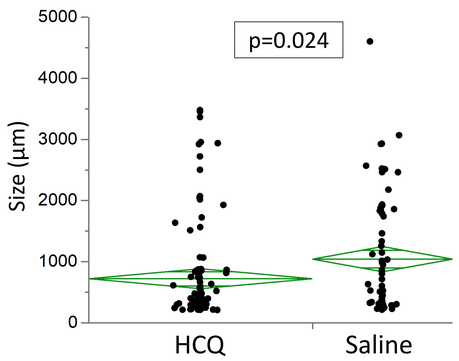
Figure 5. HCQ induced size reduction on islet tumors of Men1+/- mice



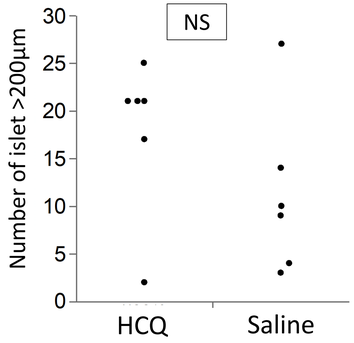
Macroscopic(left) and microscopic(right) images. Bar=1mm.



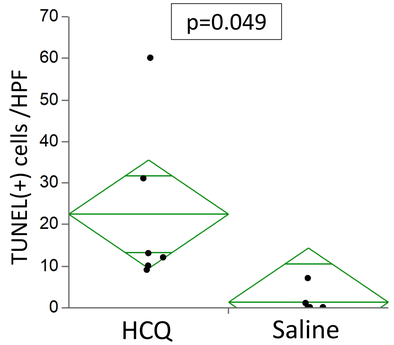
Mean diameter of Islet (tumor) decreased



Number of tumor per mouse has not changed

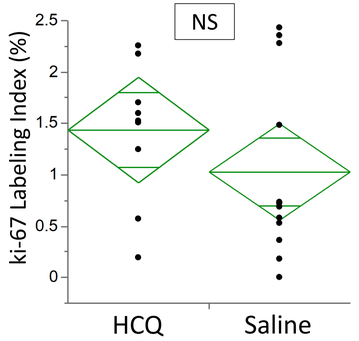


More TUNEL(+) apoptotic tumor cells in HCQ group



HCQ group : 22.5 cells/HPF
Saline group : 1.3 cells/HPF

Ki-67 index was not changed by HCQ treatment



In all tumors in this model, ki-67 index was less than 3%, comparable to NET G1