Autophagy inhibition on PanNET models in vitro and in vivo

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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.

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.

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**

### 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.

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**What is Autophagy?** --- "self-eating" degenerative pathway

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)

**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.


From HP of Mizushima Lab (Univ. of Tokyo)
Results 1: Inhibitory effect of Autophagy inhibition on PanNET cell lines

Figure 2. CQ exerted inhibitory effect on PanNET cell lines

1. MIN6 cells: Mouse Insulinoma 6
   - In both cell lines, CQ exerted inhibitory effect.
   - CQ induced apoptosis and reduced proliferation.
   - TEM analyses showed cumulation of autophagosomes and dilated ER.

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

Figure 3. Evaluation of ER stress

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

Flamment M et al. Trends in Endocrinology & Metabolism 2012 (modified)
Results 2: Men1+/- mouse showed in vivo effects of Autophagy inhibition

Figure 4. In vivo experimental design

Men1+/- mouse: 16 month old

- HCQ 10mg/kg/day i.p. for 21 days
- Saline 10mL/kg/day i.p. for 21 days

Men1+/- mouse:
129S(FVB)-Men1tm1.2Ctre/J
Bought from Jackson Laboratory

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

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

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 x 7days.

Accumulation of LC3-II was shown in peripheral tissues.

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
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

<table>
<thead>
<tr>
<th>Size (μm)</th>
<th>HCQ</th>
<th>Saline</th>
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<tbody>
<tr>
<td>Mean</td>
<td>3000</td>
<td>2500</td>
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<tr>
<td>p-value</td>
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<table>
<thead>
<tr>
<th>Number of islet &gt;200μm</th>
<th>HCQ</th>
<th>Saline</th>
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<tbody>
<tr>
<td>Number of tumor per mouse</td>
<td>ns</td>
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<table>
<thead>
<tr>
<th>TUNEL+ cells/HPF</th>
<th>HCQ</th>
<th>Saline</th>
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<tbody>
<tr>
<td>HCQ group: 22.5 cells/HPF</td>
<td>Saline group: 1.3 cells/HPF</td>
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<table>
<thead>
<tr>
<th>Ki-67 Labeling Index (%)</th>
<th>HCQ</th>
<th>Saline</th>
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<tbody>
<tr>
<td>p=0.049</td>
<td>ns</td>
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TUNEL / Chromogranin A / DAPI

Bar=50μm