



Evaluation of anti-cancer activity of natural compounds and biological relationship between Autophagy and Ciliogenesis

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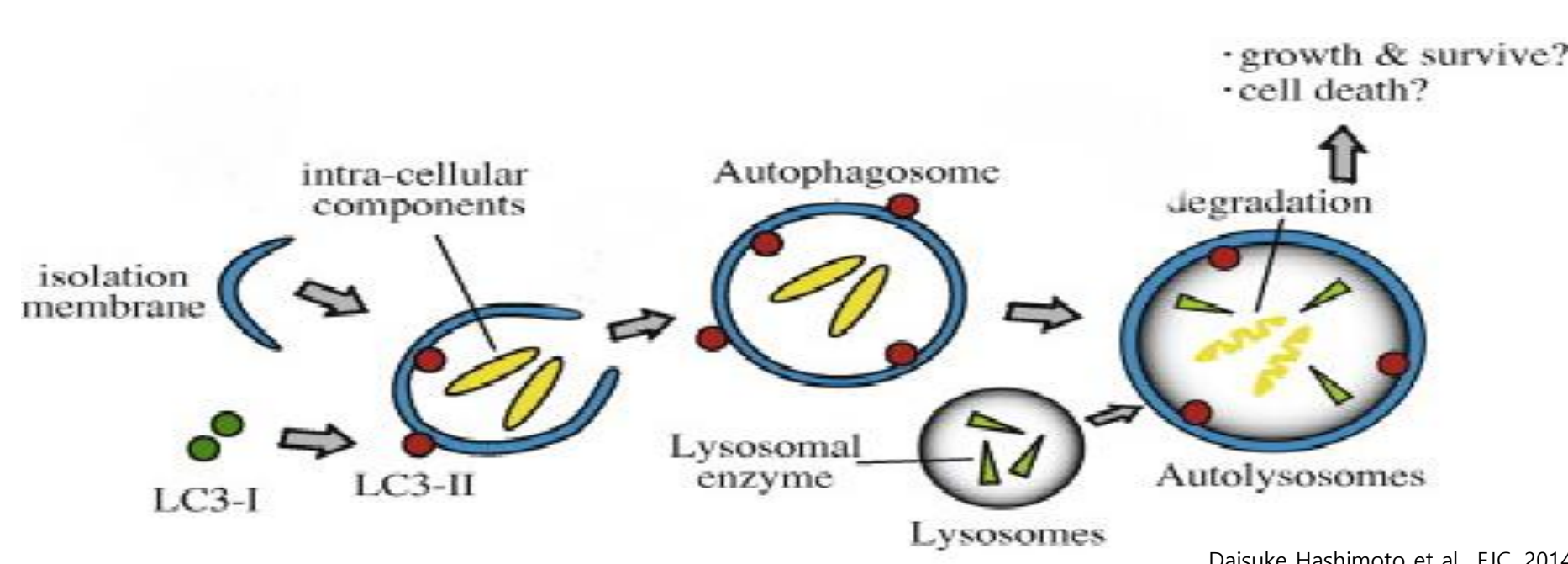
Abstract

Gastric cancer is the most common malignant tumor in Korea, and it ranks second in cancer. for gastric cancer treatment, we tested the activity of anticancer in three compounds derived from natural substance. we conducted a MTT assay to identify the cancer cell proliferation of the three compounds, and show the results. cilia-formation activity has been reported to play an important role in many cancer. primary cilium regulates the cancer progression to positive or negative in cell. to identify, we tested for cilia-formation in cancer cells that have processed the small molecules by using ICC (Immunocytochemistry). autophagy has potential as anti-cancer activity. additionally, we tested the autophagy activity of the three small molecules by checking the evaluation marker lysosome activity, LC3 conversion and p62. these findings demonstrate that the molecule is responsible for inducing autophagy and for anti-cancer activity. finally, we selected one of the most effective anti-cancer molecule and will identify target protein of the molecule by using DARTS and LC-MS/MS.

Introduction

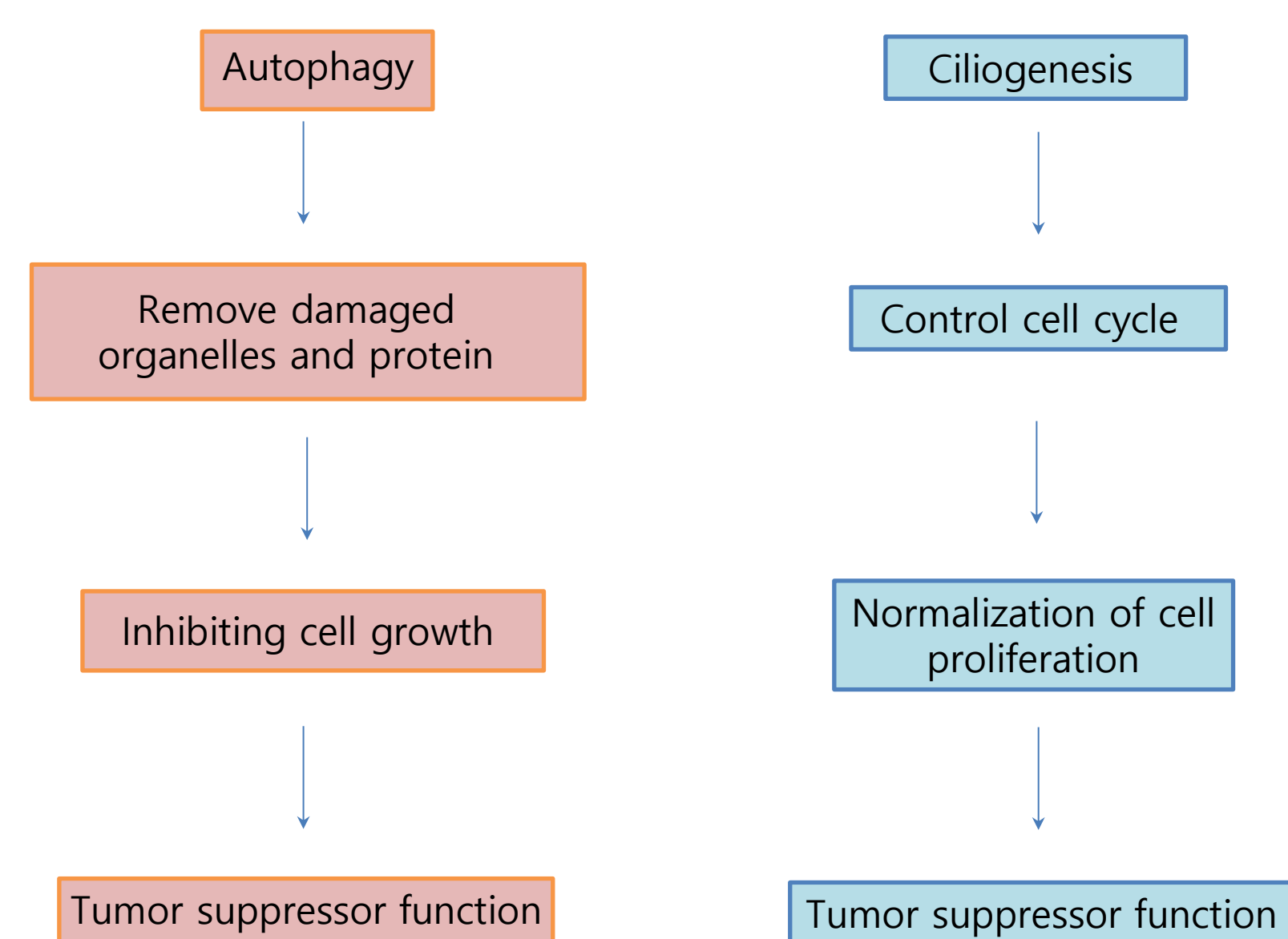
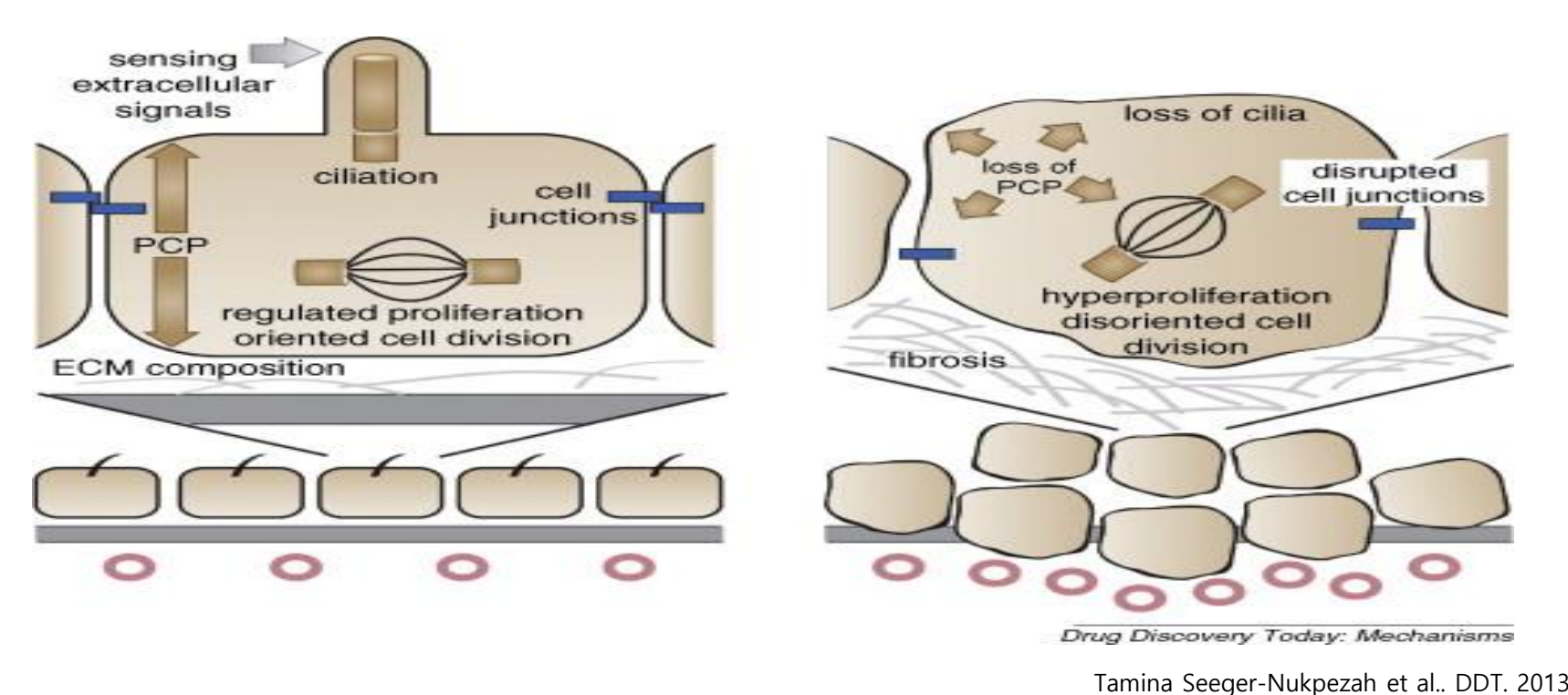
Autophagy

- Degradation process in cell
- Remove dysfunctional organelles and protein
- Recycling of cellular components
- Regulates cell growth



Ciliogenesis

- Related to cell signal pathways
- Assembly and disassembly occur during cell cycle
- Can cause many human disease related to non-functional cilia
- Cilia-formation affects cancer development

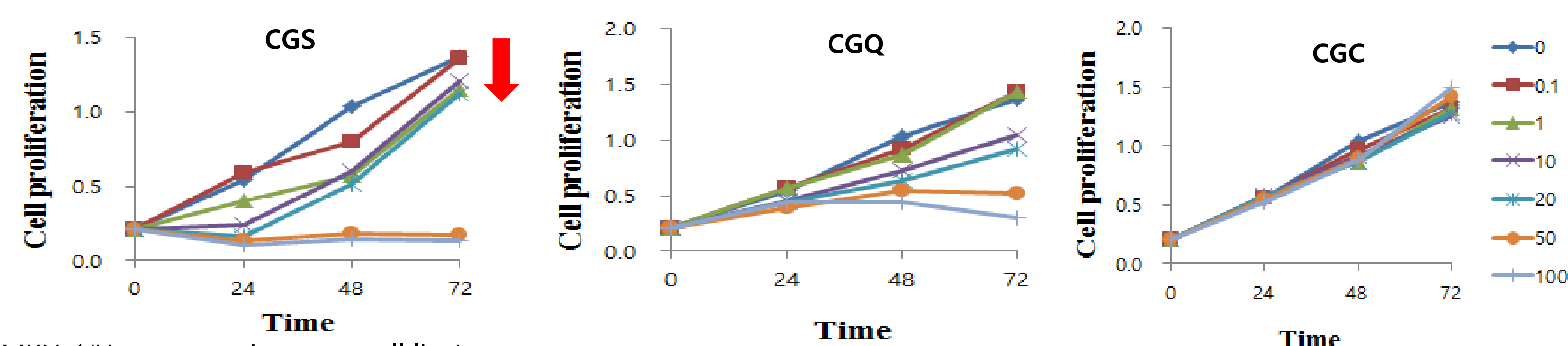


The illustrative model shows the relation between autophagy, cilia and cancer. Autophagy can regulate cilia-formation by decomposing cilia essential proteins IFT20, OFD1. conversely, cilia can enhance autophagy through Shh signal. Both ciliogenesis and autophagy affect cancer-progression.

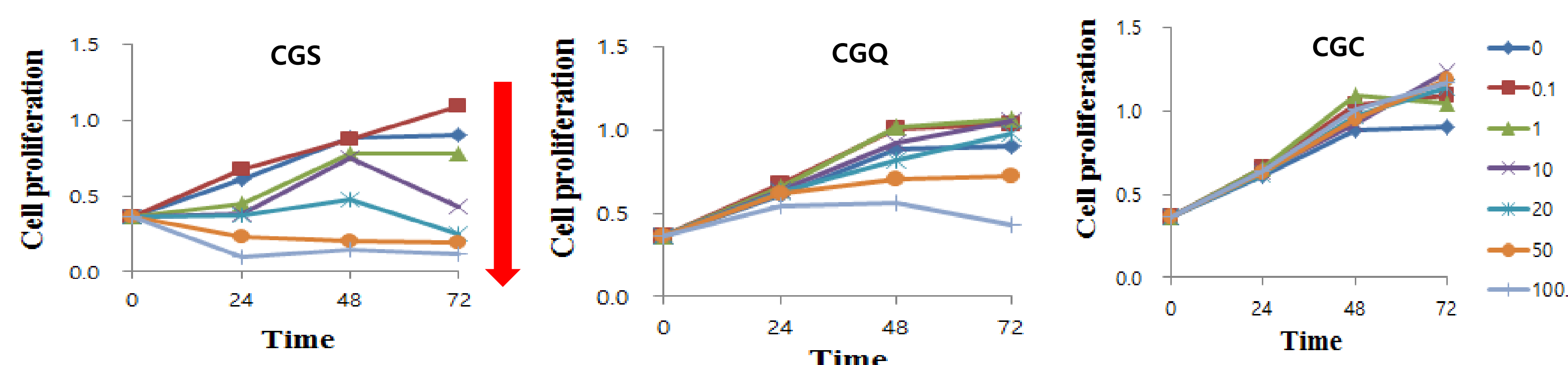
Results

CGS inhibits cell proliferation in gastric cancer cell line (MKN-1)

GES-1 (Human gastric epithelium cell line)



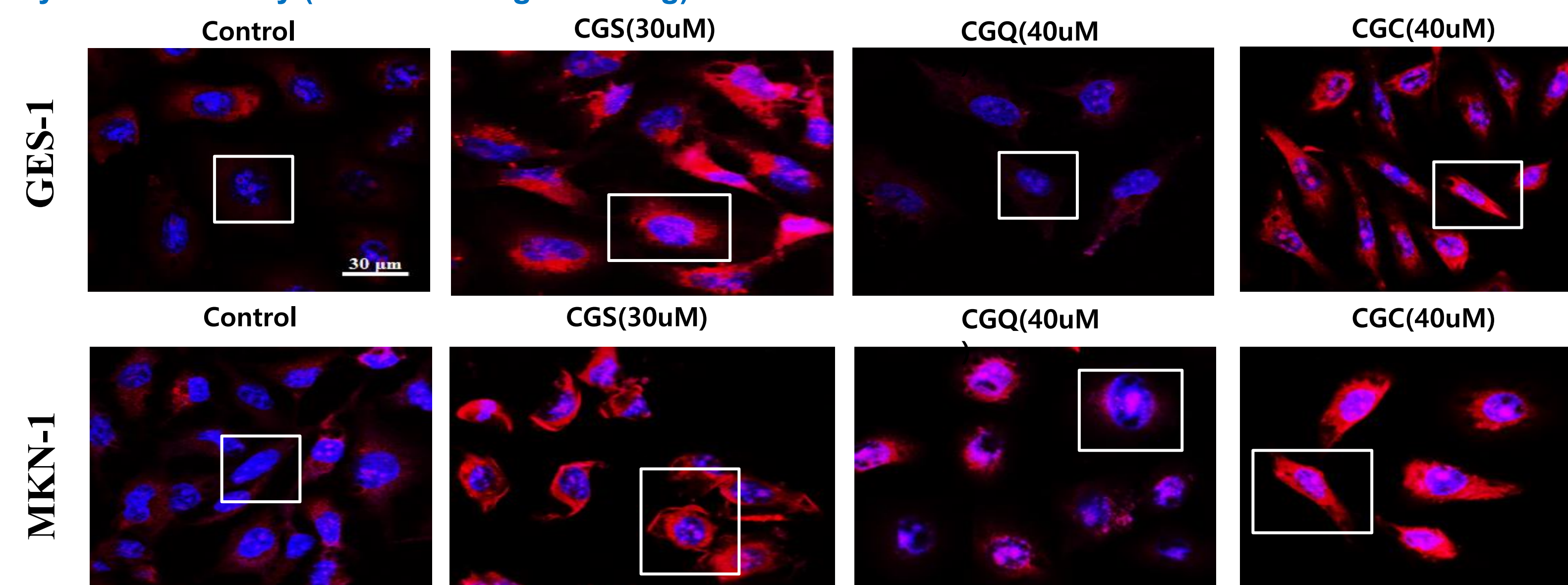
MKN-1 (Human gastric cancer cell line)



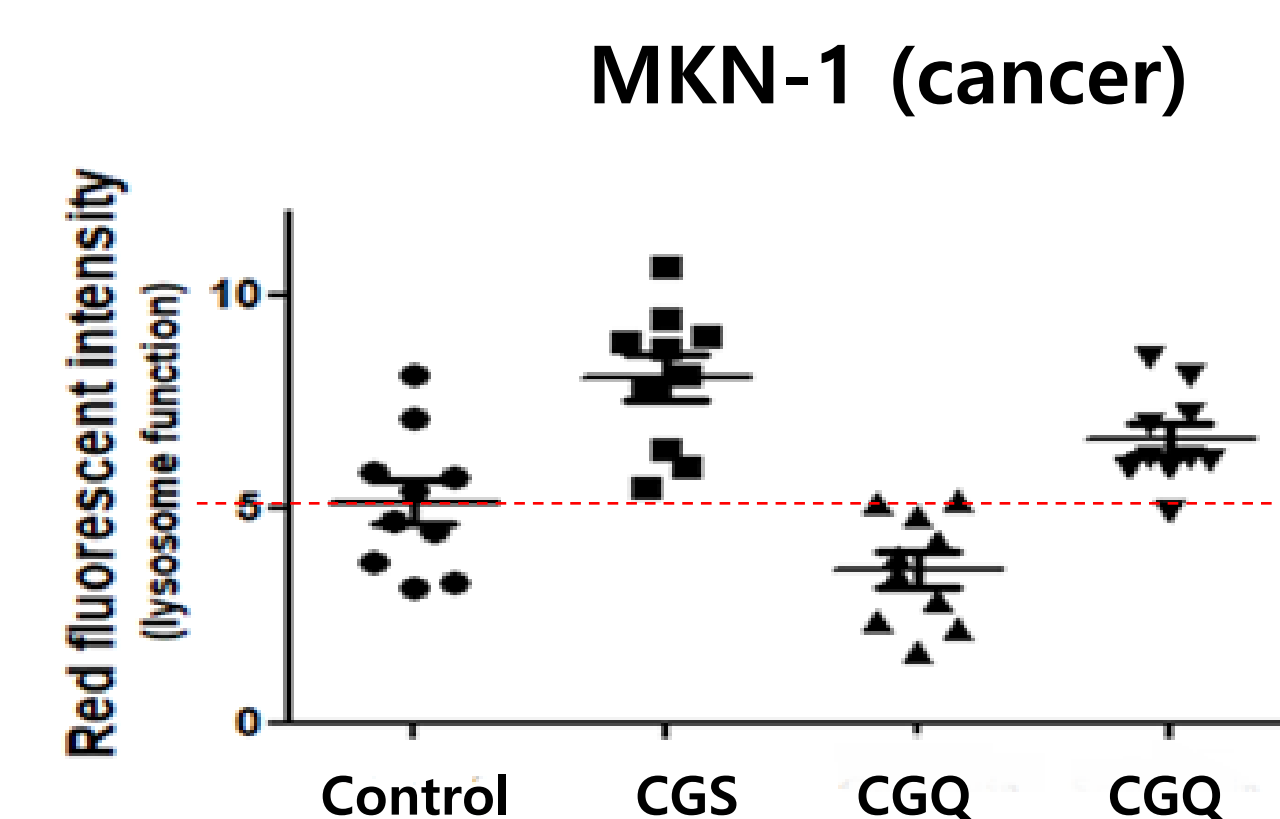
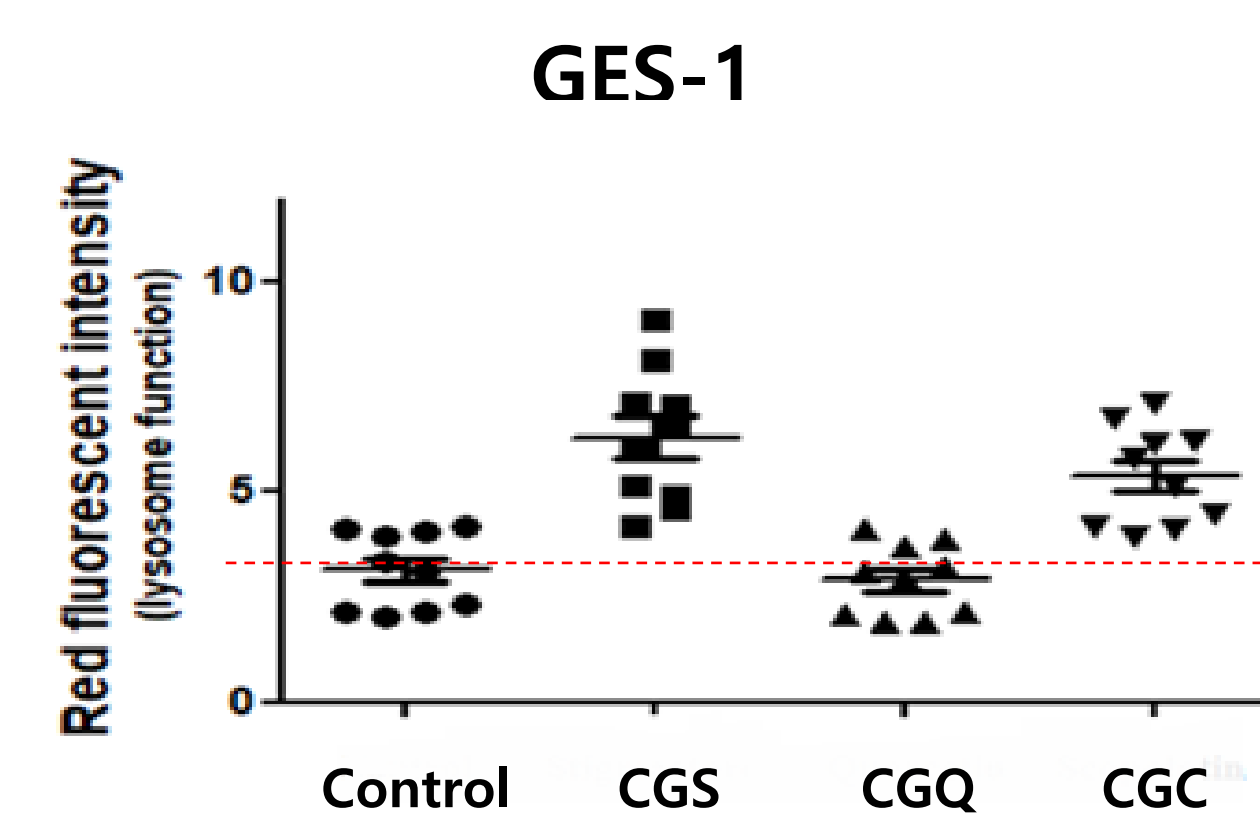
IC ₅₀ (μM)	CGS	CGQ	CGC
GES-1	44.7	53.8	-
MKN-1	30.2	98.9	-

Autophagy activity of three small molecules

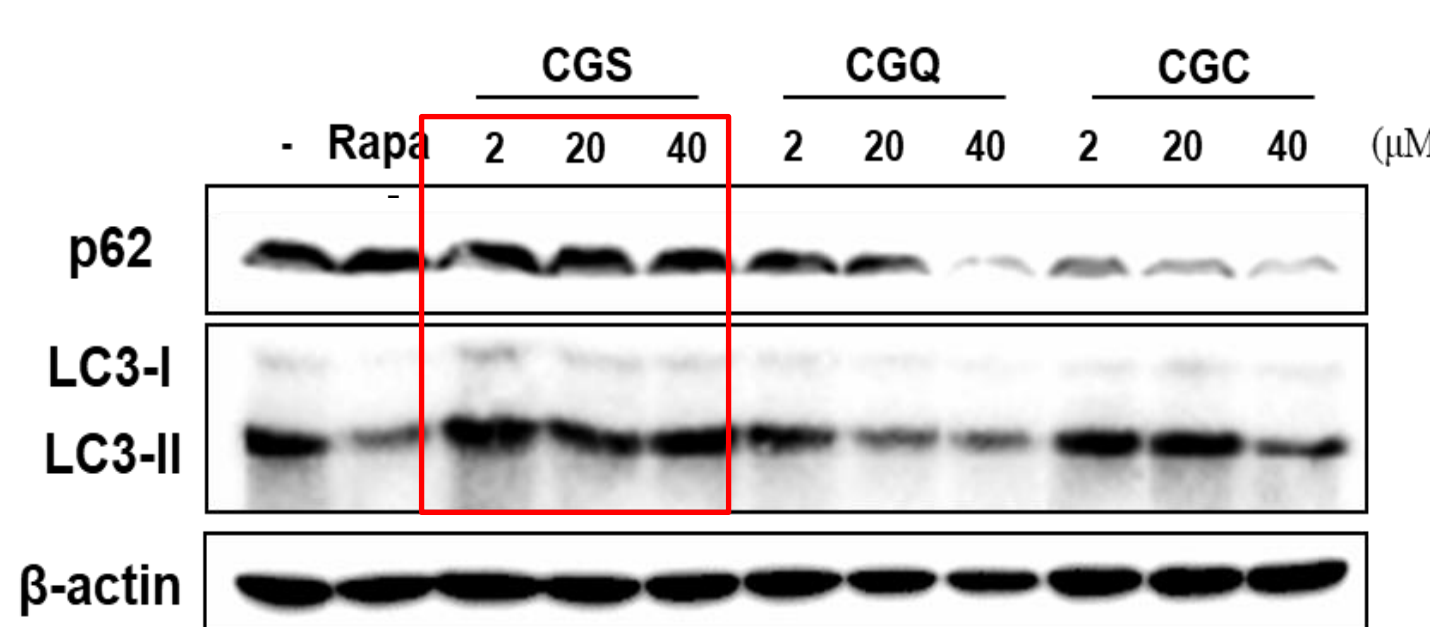
Lysosome activity (acridine orange staining)



Blue : DAPI(nucleus), Red : Acridine orange(functional lysosome)

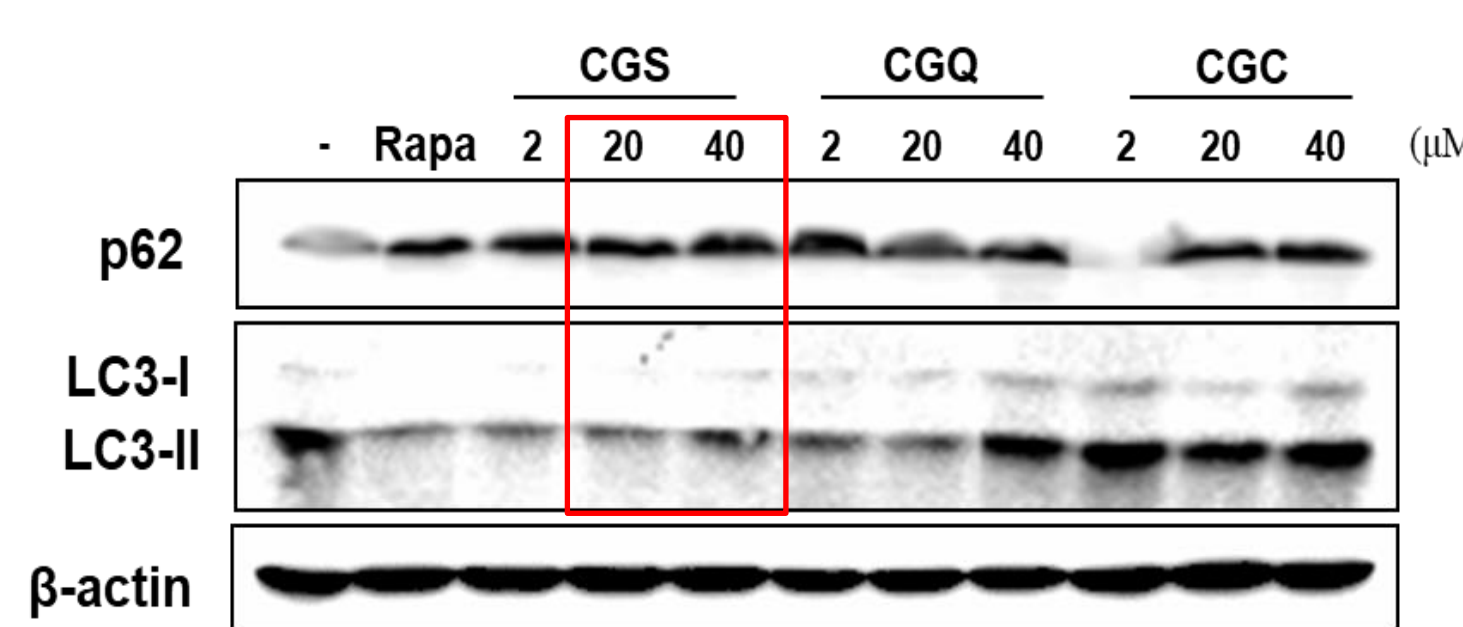


LC3-II & p62 levels (autophagy markers)



GES-1 (normal)

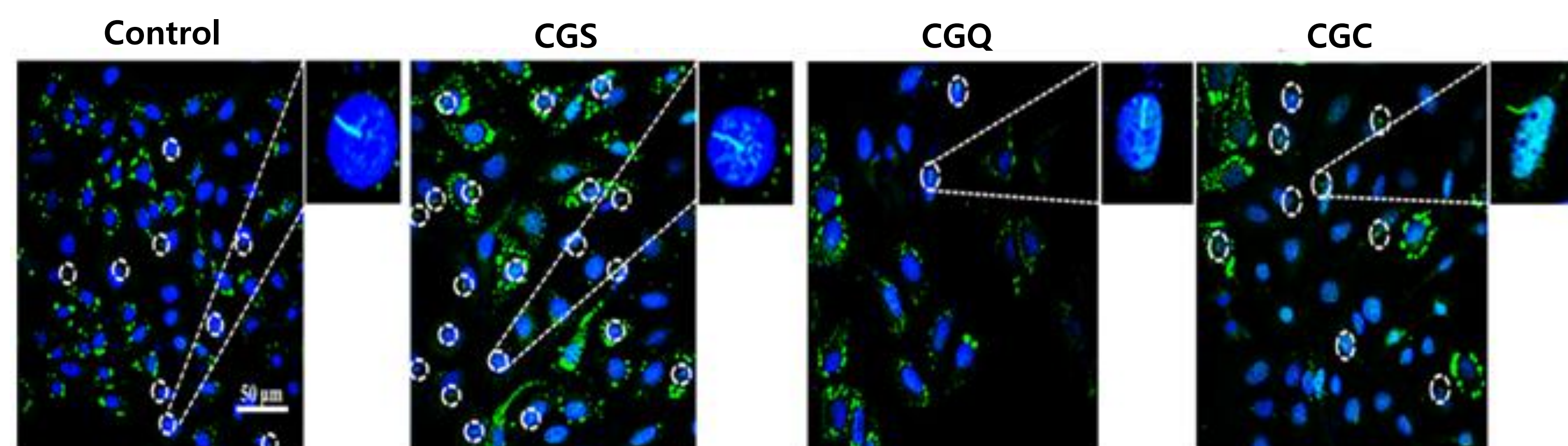
- CGS induced autophagy activity
- We proposed that there is a relationship between cell proliferation inhibition and autophagy



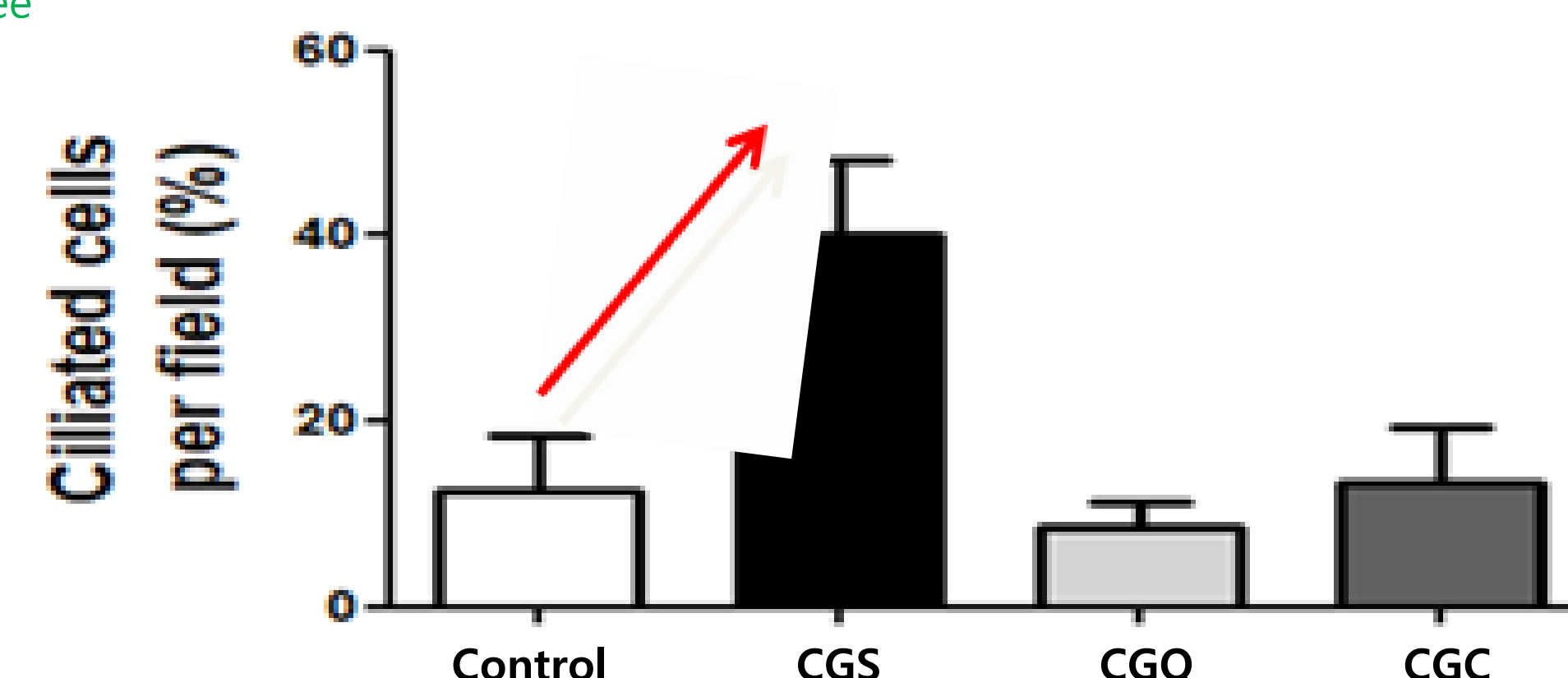
MKN-1 (cancer)

CGS-induced autophagy promotes ciliogenesis

GES-1 Confocal image by using ICC (ImmunoCytoChemistry)



Blue : DAPI(nucleus), Green



- In the GES-1 cell line, CGS effectively induced cilia-formation. In previous studies, CGS also most effectively inhibited cell proliferation of the two cell lines.
- We proposed that there is a biological relationship between cell proliferation regulation and cilia-formation

Summary & Discussion

- We identified that there is a cell proliferation inhibitory activity in CGS-CGQ-CGC order and CGS effectively shows as an anti-cancer activity through IC₅₀ in MKN-1 cell line
- CGS effectively induced the autophagy evaluation marker, lysosome activity in two cell lines. But LC3 conversion, p62, more specific marker, did not induce in MKN-1 cell, only induced in GES-1
- CGS effectively induced the cilia formation in GES-1 cell. We considering further experiments because the data of MKN-1 cell were not valid.

Our findings showed interaction between autophagy and cilia-formation with cell proliferation. CGS, the most inhibitory to cell proliferation, was observed to induce autophagy and cilia-formation in GES-1 cell. But in MKN-1, it was different. We also observed that growth of GES-1 cell line is 1.5 times faster than MKN-1 cell line during the experiment. We hypothesized that there would be an interaction between autophagy and cilia-formation in fast cell proliferation's view. We are planning to change cell line with other rapidly proliferating cancer cell to conduct the experiment, also to identify target protein of the CGS that show anti-cancer activity.

