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

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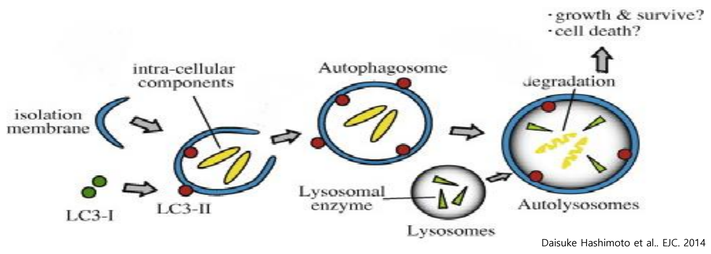
Abstract

Gastric cancer is the most common malignant tumor in Korea and a leading cause of cancer-related deaths. To identify novel therapeutic agents, we investigated the anticancer activity of three compounds derived from natural substances. We first confirmed that all three compounds inhibit cancer cell proliferation via the MTT assay. To elucidate the underlying mechanisms, we then examined their effects on two key cellular processes: primary cilia formation and autophagy. Using immunocytochemistry (ICC), we assessed changes in cilia formation, a known regulator of cancer progression. Concurrently, we evaluated the induction of autophagy—a potential anti-cancer pathway—by measuring lysosome activity and the levels of LC3 and p62. These findings demonstrate that the compounds' anticancer activity is associated with the induction of autophagy. Based on these results, we have selected the most potent compound for subsequent target protein identification using DARTS and LC-MS/MS.

Introduction

Autophagy

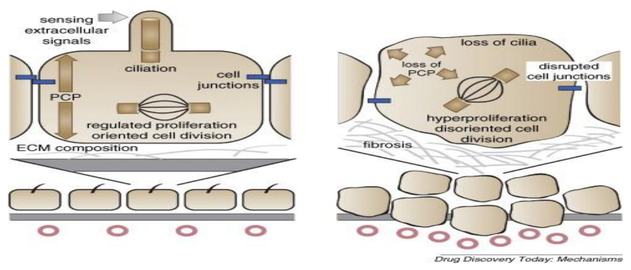
- Degrades intracellular constituents
- Removes dysfunctional organelles and proteins
- Recycles cellular components
- Regulates cell growth



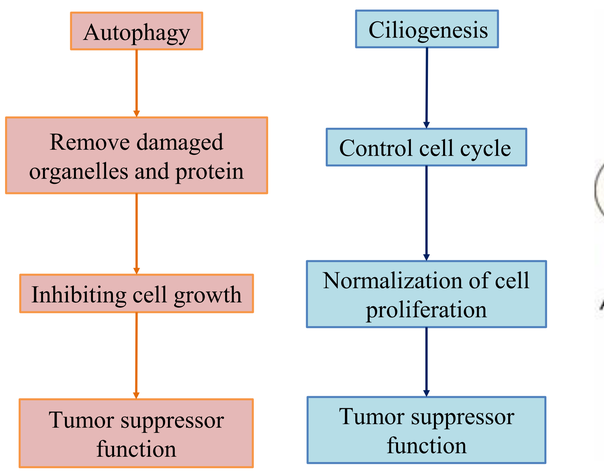
Daisuke Hashimoto et al.. EJC. 2014

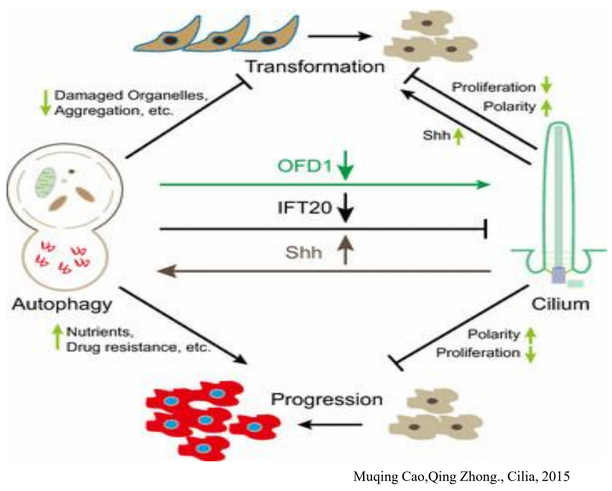
Ciliogenesis

- Relates to multiple cell-signaling pathways
- Assembles and disassembles in coordination with the cell cycle.
- Induces numerous human diseases when cilia are non-functional
- Affects cancer development through altered cilia formation



Tamina Seeger-Nukpezah et al.. DDT. 2013





Muqing Cao,Qing Zhong., Cilia, 2015

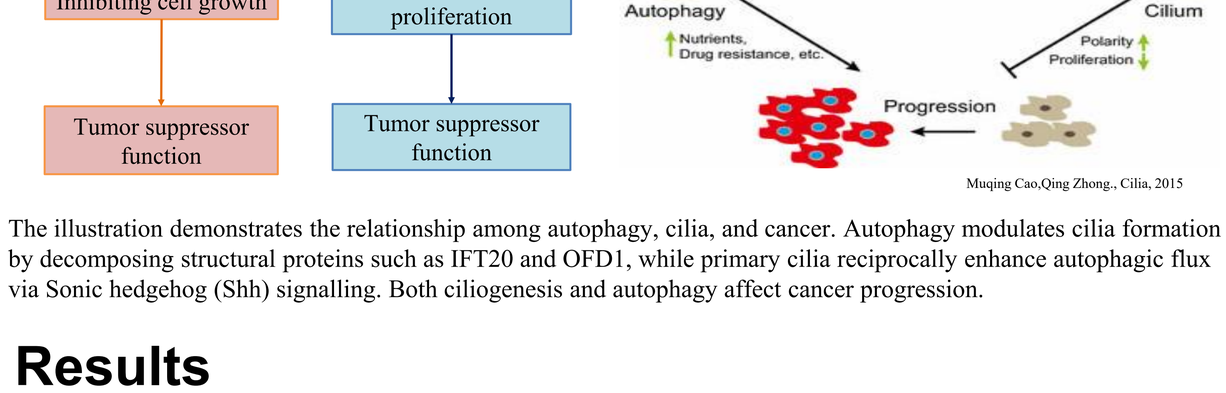
The illustration demonstrates the relationship among autophagy, cilia, and cancer. Autophagy modulates cilia formation by decomposing structural proteins such as IFT20 and OFD1, while primary cilia reciprocally enhance autophagic flux via Sonic hedgehog (Shh) signalling. Both ciliogenesis and autophagy 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	-

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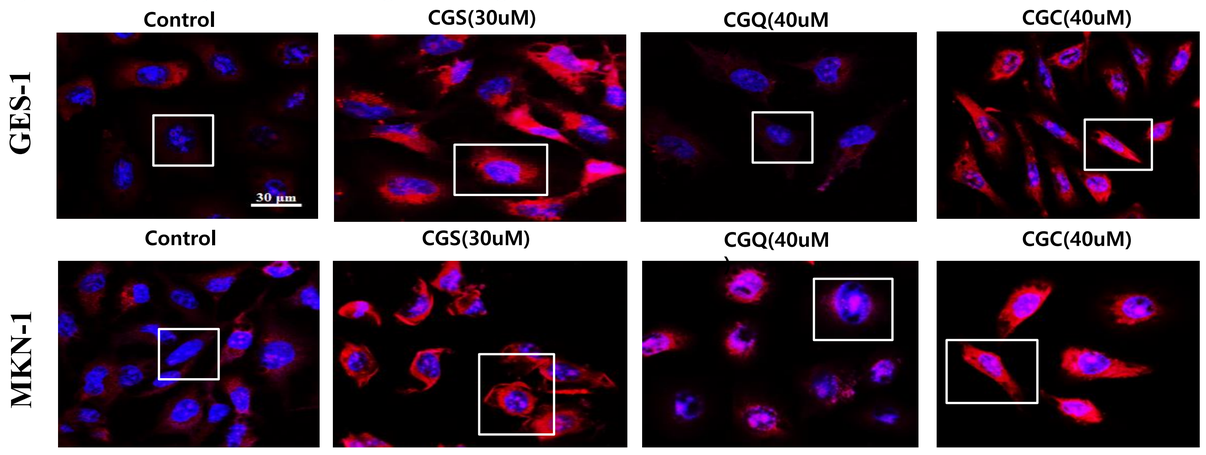
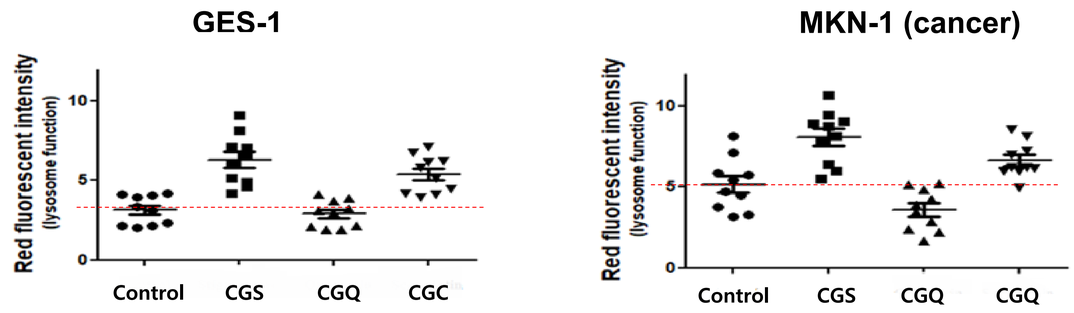
Autophagy activity of three small molecules

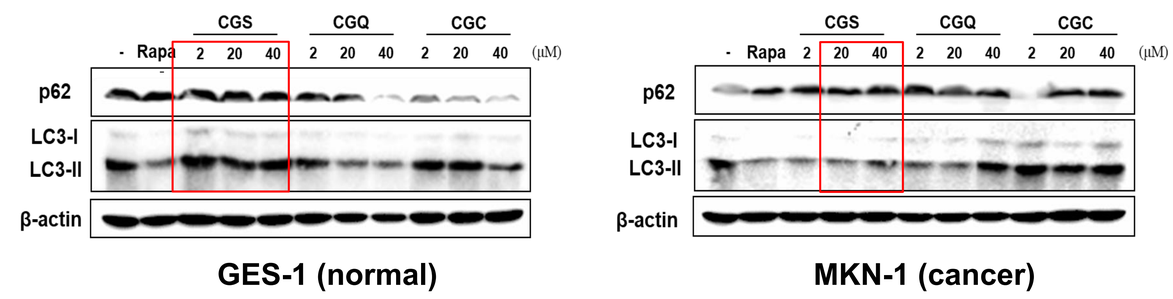
Lysosome activity (acridine orange staining)



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



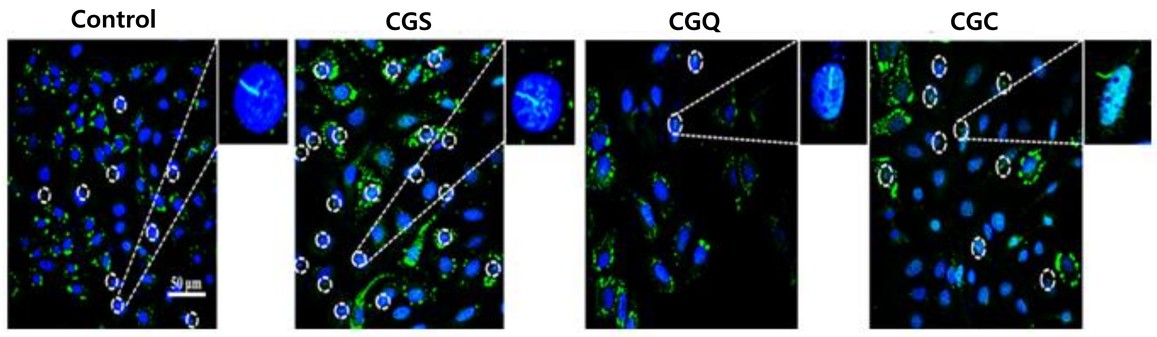
LC3-II & p62 levels (autophagy markers)



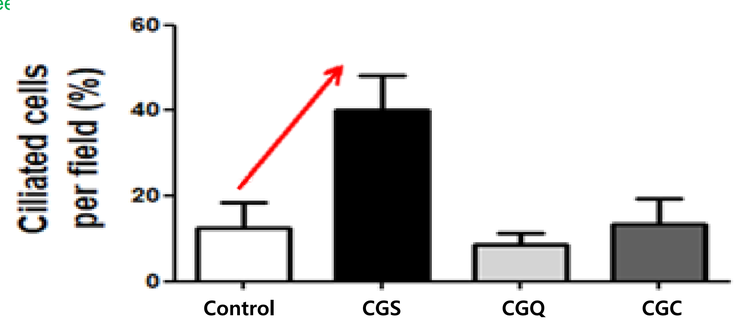
- We observed that CGS treatment resulted in the induction of autophagy.
- We propose a potential relationship between cell proliferation inhibition and autophagy.

CGS-induced autophagy promotes ciliogenesis

GES-1 Confocal image by using ICC(ImmunoCytoChemistry)



Blue : DAPI(nucleus), Gree



- We observed effective cilia formation induced by CGS in GES-1 cells. This is consistent with our prior studies, which demonstrated that CGS was the most effective inhibitor of cell proliferation across the two cell lines.
- Thus, we suggest that the mechanisms governing cell proliferation and cilia formation are biologically interconnected.

Summary & Discussion

- We found that cell-proliferation inhibition followed the order CGS > CGQ > CGC, with CGS showing the strongest anticancer activity (IC_{50}) in the MKN-1 cell line.
- CGS also increased general autophagy markers and lysosomal activity in both cell lines; however, the more specific markers LC3 conversion and p62 were elevated only in GES-1 cells, not in MKN-1 cells.
- CGS markedly promoted cilia formation in GES-1 cells. Because the MKN-1 data were inconclusive, we plan to repeat the experiments in a different rapidly proliferating cancer cell line and further clarify CGS's target protein.

Our findings revealed an interaction between autophagy and cilia formation in relation to cell proliferation. CGS, the compound most inhibitory to proliferation, induced both autophagy and cilia formation in GES-1 cells; however, MKN-1 cells showed a different response. We also observed that the growth rate of the GES-1 line was 1.5 times faster than that of MKN-1 during the experiment. These observations led us to hypothesize that rapidly proliferating cells display stronger crosstalk between autophagy and cilia formation. To test this idea, we will switch to another fast-growing cancer cell line for further experiments and identify the CGS target protein responsible for its anticancer activity.