Quercetin synergistically induces sensitivity to 5-Fluorouracil through p53 modulation in colorectal cancer cells

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Introduction
Colorectal tumors (CRC) with microsatellite instability (MSI) and mutations in p53 show resistance to chemotherapy with 5-fluorouracil (5-FU), the most widely pharmacological drug used for CRC treatment. In a previous study, we showed that two flavonoids quercetin (Q) and luteolin (L) have antiproliferative and proapoptotic effects on two human MSI CRC derived cell lines: CO115 (wild-type for p53) and HCT15 (harbors a p53 mutation). The present study aims to find if the combination of Q or L with 5-FU increases sensitivity of CRC cells to 5-FU and characterize the dependence of the compounds on the p53 status. The sensitivity of the cells to 5-FU was evaluated by TUNEL assay and the effects on apoptosis induction of co-incubation of the flavonoids, quercetin (Q) or luteolin (L), with 5-FU were characterized. The mechanisms of apoptosis induction were assessed by western blot, and p53 mediated effects confirmed by small interference RNA (siRNA) in CO115 cells and using HCT116 wild-type and p53 knockout cells. This study suggests the potential applicability of these phytochemicals for enhancement of 5-FU efficiency in CRC therapy, especially Q in p53 wild-type tumors.

Results and Discussion

**CO115 cells are more sensitive to 5-FU than the p53 mutated HCT15 cells. Q synergically induced apoptosis with 5-FU in CO115 cells.**

**Additive effects on apoptosis were observed for L + 5-FU (in both cell lines) and Q + 5-FU (in HCT15).**

![Figure 1](image1.png)

**Molecular markers of apoptotic mitochondrial pathway were also synergistically induced by Q and 5-FU.**

**Apoptosis induced by Q and L was caspase dependent in CO115 cells but not in HCT15.**

**Q remarkably increased p53 expression in CO115 cells and knockdown of p53 by siRNA in these cells abrogated the induction of apoptosis by Q and 5-FU (Fig. 4A). p53 knockout in HCT116 cells totally abrogated apoptosis induction by Q and 5-FU (Fig. 4B).**

**Potential applicability of Q and L for enhancement of 5-FU efficiency in CRC therapy**

**Q demonstrated to induce apoptosis dependent on p53 status**

Material and Methods

- **Apoptosis by TUNEL assay**
  TUNEL (TdT mediated dUTP Nick End Labeling) staining was performed to estimate the percentage of apoptotic cells of 5-FU alone and 5-FU in combination with Q or L, as well as, 2'-deoxyadenosine (dA); cells were collected, fixed and cytospined to a polylysine Immobilon solutions under a chemiluminescence detection system. Secondary antibody. Immunoreactive bands were detected using the membranes were blocked and incubated with primary antibody and electrophoresis and electroblotted to a PVDF membrane. The abundance of p53 (DO-1), cleaved caspase-9 (Asp 315), caspase-3 (H-277), PARP-1 (F-2), Bcl-2 (C21) and β-actin were used as loading controls.

- **Apoptosis-related Protein Expression by Western Blotting**
  HCT15 cells were transiently transfected with Oligofectamine. 24h after transfection, cells were incubated with 5-FU (500, 100 and 1 M), quercetin (Q) and luteolin (L) (12 M) alone, and with both in combination for 48h and apoptosis assessed by TUNEL assay. Control cells were transfected with control siRNA.

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