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 synergistically** 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).

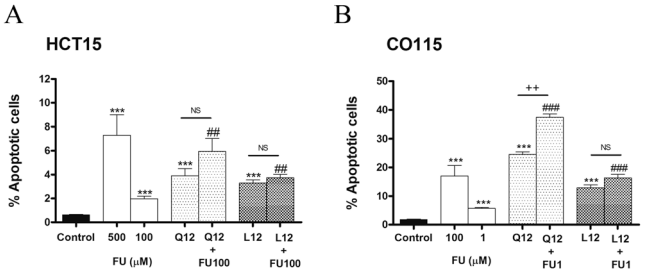

Fig. 1 - Effect on apoptosis by 5-fluorouracil (FU) 500, 100 and 1μM, quercetin 12μM (Q12) and luteolin 12μM (L12) alone, as well as the natural compounds co-incubated with FU for 48h, in HCT15 (**A**) and CO115 (**B**) cells, using TUNEL assay. Results are presented as mean ± SEM of at least 3 independent experiments. *** P≤ 0.001, when compared with control; ++ P≤ 0.01, when compared with the respective natural compound alone; ## P≤ 0.01 and ### P≤ 0.001, when compared with FU alone; NS, not significant differences observed between each other.

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

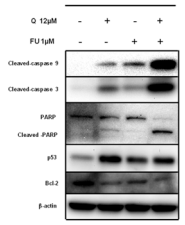

Fig. 3 - Effects on caspase-9, caspase-3, PARP-1, p53 and Bcl-2 expressions, for 48h, of co-incubation of quercetin 12μM (Q12) and 5-fluorouracil 1μM (FU1) in CO115 cells, by western blot. Images are representative of at least 3 independent experiments.

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

Q demonstrated to induce apoptosis dependent on p53 status

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

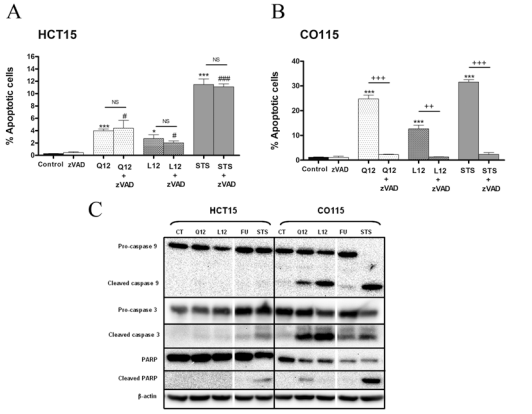

Fig. 2 - Effect of a caspase inhibitor zVAD-FMK (zVAD) 20μM on the apoptosis induction by quercetin 12μM (Q12), luteolin 12μM (L12) and staurosporine (STS) 0.25μM, for 48h, in HCT15 (**A**) and CO115 (**B**) cells, using TUNEL assay. Results are presented as mean ± SEM of at least 3 independent experiments. * P≤ 0.05, *** P≤ 0.001, when compared to control; ++ P≤ 0.01 and +++ P≤ 0.001, when compared with the respective compound alone; ## P≤ 0.01 and ### P≤ 0.001, when compared with zVAD alone; NS, not significant differences observed between each other. Effects on caspase-9, caspase-3 and PARP-1 expressions, for 48h, of Q, L, 5-FU (FU) and STS alone, in HCT15 and CO115 cells (**C**), by western blot. Images are representative of at least 3 independent experiments.

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).

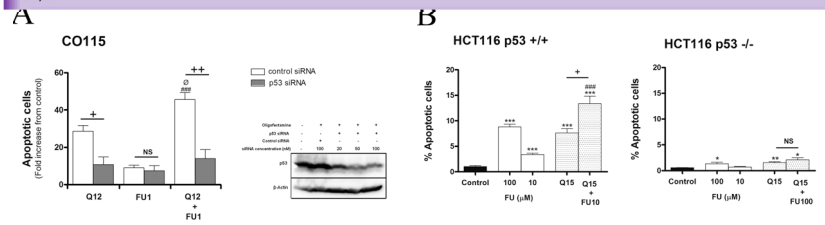

Fig. 4 – Dependence on p53 for apoptosis induction by quercetin (Q) and 5-fluorouracil (FU). (**A**) CO115 cells knockdown for p53 by siRNA (100nM) were incubated with FU 1μM (FU1) or Q 12μM (Q12) alone, and with both in combination for 48h and apoptosis assessed by TUNEL assay. Control cells were transfected with control siRNA. Compounds were added 24h after transfection. p53 knockdown efficiency was monitored by western blot (inset). (**B**) HCT116 p53-wt (p53 +/+) and HCT116 p53-null (p53 -/-) cells were used to observe effects of FU, Q and both in combination on apoptosis after 48h of incubation, as assessed by TUNEL assay. FU10: FU 10μM; FU100: FU 100μM; Q15: Q 15μM. (**A; B**) Results are presented as mean ± SEM of at least 3 independent experiments. * P≤ 0.05, ** P≤ 0.01 and *** P≤ 0.001, when compared with control; + P≤ 0.05, when compared with each other; ### P≤ 0.001, when compared with respective FU alone; Ø P≤ 0.05, when compared with respective Q alone; NS, not significant differences observed between each other.

Material and Methods

• Apoptosis by TUNEL assay

TUNEL (TdT mediated dUTP Nick End Labelling) 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, z-VAD-fmk (zVAD) alone and zVAD in combination with Q, L and staurosporine (STS). Cells were collected, fixed and cytospined to a polylysine treated slide. Then, cells were permeabilised with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. TUNEL assay was performed following the manufacture's instructions (Roche). The percentage of apoptotic cells was calculated from the ratio between TUNEL positive cells and total number of cells (nuclei staining with Hoechst) using a fluorescent microscope.

• p53 knockdown in CO115 cells

CO115 cells were transiently transfected with Oligofectamine and 100nM of p53 siRNA in OPTI-MEN according to the manufacturer's instructions. The p53 siRNA target sequence was guggaguauuuggaugaca (Invitrogen). Control of siRNA experiments were included by using a siRNA against GFP. Confirmation of p53 knockdown was done by western blot analysis. 24h after transfection, cells were incubated with 5-FU or Q alone and with both in combination, for 48h, and apoptosis measured by the TUNEL assay.

• p53 knockout in HCT116 cells

Two isogenic HCT116 colon carcinoma cells, p53-wild type and p53 complete knockout for p53, kindly provided by Vogelstein, were used.

• Apoptosis-related-Protein Expression by Western Blotting

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 measured by western blot. After 24h of treatment with test compounds, cells were lysed and then separated by SDS gel electrophoresis and electroblotted to a PVDF membrane. Membranes were blocked and incubated with primary antibody and secondary antibody. Immunoreactive bands were detected using the Immobilon solutions under a chemiluminescence detection system. β-actin was used as a loading control.

Acknowledgments

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