Aspirin and regorafenib synergistically inhibit proliferation of colon carcinoma cells in vitro

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Background and Aim

Regorafenib is an oral multi-kinase inhibitor approved for metastatic refractory colorectal cancer. Recent studies have suggested that combining kinase inhibitors with aspirin may improve patient outcomes. We aimed to determine if aspirin and regorafenib combination treatment inhibits proliferation and induces apoptosis of colon carcinoma cells in vitro.

Methods

RKO, SW480 and LIM1215 colon carcinoma cells were treated with aspirin and regorafenib. The MTS assay was used to measure overall cell viability after 72 hours. Proliferation was measured by Ki67 staining, and cytotoxicity and apoptosis were measured by Annexin V and 7-AAD staining. AMPK phosphorylation was assessed by Western blotting.

Results

Aspirin and regorafenib combination treatment inhibits proliferation to a greater extent than either aspirin or regorafenib treatment alone, but does not induce cytotoxicity or apoptosis.

Ki67 median fluorescence intensity within the viable cell population was significantly lower following aspirin and regorafenib combination treatment compared to the untreated control (p <0.0001) or either treatment alone (p <0.01). The percentages of non-viable (7-AAD-positive) and early apoptotic (Annexin V-positive and 7-AAD-negative) cells did not change with 1 mM aspirin and 4 µM regorafenib combination treatment compared to the untreated control (p >0.05).

Increased AMPK activation may contribute to the observed anti-proliferative effect. Aspirin and regorafenib combination treatment induced AMPK phosphorylation to a greater extent than either treatment alone.

Bliss scores were positive for all combinations of physiological concentrations of aspirin (250 µM to 2 mM) and regorafenib (1 µM to 8 µM), indicating synergy.

Conclusions

Aspirin and regorafenib synergistically inhibit proliferation of colon carcinoma cells in vitro, without inducing cytotoxicity. This effect is associated with increased AMPK activation. Our findings suggest that the novel combination of aspirin and regorafenib may be an improved treatment strategy for metastatic colorectal cancer, and provide a rationale for further in vivo and mechanistic studies.