Title: Study the molecular mechanism of a novel colon cancer therapy by microbial protease mediated apoptosis

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All developing countries are currently plagued by colon cancer, where early diagnostic systems are lacking and mortality rates continue to rise. Conventional cancer therapies are accompanied by severe, dose-limiting side effects. To improve the treatment and survival of patients at high risk of metastasis, a better molecular understanding of the early mechanisms leading to metastasis is required. Bacterial proteases have long been tested for cancer treatment as they can kill cells and alter cellular processes that are associated with carcinogenesis and may either stimulate cellular aberrations or inhibit normal cell controls. Hemagglutinin protease (HAP) is noteworthy in the virulence and pathogenesis of *V. cholerae*. In our present study, we intend to show the apoptotic response of hemagglutinin protease in colon cancer cells along with its role in tumor regression in mice models. Previous studies have shown PAR1 mediated apoptosis in breast cancer cells by *V. cholerae* HAP. Our data revealed that purified HAP induced apoptosis in human and mouse colon cancer cells. Results showed HAP to activate the intrinsic pathway of apoptosis in colon cancer cells. HAP treatment also increased the survival of intraperitoneally CT26 induced mice. Further results have shown HAP to cause activation of nuclear factor κB (NFκB) and MAP kinase pathways leading to elevated cellular ROS levels in colon cancer cells. Furthermore, HAP induced the overexpression and activation of PAR1 in colon cancer cells. Our results have further shown that HAP treatment enhances the binding probability of Sp1 transcription factor to PAR1 promoter regions in a PI-3K/PKCζ dependent pathway ultimately leading to PAR1 overexpression in colon cancer cells. Moreover, we constructed a peptide 'PFISED' upon the unique PAR1 cleavage patterns of HAP. The peptide showed apoptosis in both human and mouse colon cancer cells, was able to enhance survival of CT26 induced mice, and induced all the signaling pathways leading to cellular death similar to HAP. In conclusion, *V. cholerae* HAP is a potent apoptosis inducer of both human and mice colon cancer cells, and the novel apoptotic peptide 'PFISED' is a potential candidate for colorectal cancer therapy.

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