Title: "Anti-cancer activity of microbial protease isolated from environmental sources"

Abstract

The purpose of this study was to identify novel proteases that can induce apoptosis on cancer cells. In this study we have purified secretory protease subtilisin from *Bacillus amyloliquefaciens*. The protease was purified from DHS-96 an environmental microbial strain. 16s rRNA sequencing confirmed that DHS 96 belongs to *Bacillus amyloliquefaciens*. Subtilisin induced apoptosis in colon (HT29) and breast (MCF7) cancer cells but showed no effect on mouse peritoneal macrophage and on normal breast cells (MCF10A). Western blot analysis showed that Bax, Bcl-2 level remained unchanged but tubulin level decreased significantly. Subtilisin does not induce the intrinsic pathway of apoptosis; rather it induced tubulin degradation in MCF-7 cells, whereas in normal cells (MCF-10A) tubulin degradation was not observed. In depth analysis showed subtilisin activates ubiquitination and proteasomal mediated tubulin degradation which was completely restored when proteasome inhibitor MG132 was used. We further observed PARKIN, one of the known E3-ligase is overexpressed and interacts with tubulin in subtilisin treated cells. PARKIN activation and tubulin degradation leads to ER-stress which in turn activates caspase-7 and PARP cleavage, thus guiding the subtilisin treated cells towards apoptosis. To our knowledge this is the first report of subtilisin induced apoptosis in cancer cells by proteasomal degradation of tubulin.

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