

# Elucidating the Mechanisms Involved in the Cytotoxicity Induced by Marine Cyanobacteria Strains against the RKO Colon Carcinoma Cell Line

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Cyanobacteria are known to synthesize secondary metabolites that may have potential as drugs for the treatment of human diseases such as cancer. Previous studies on marine cyanobacteria isolated from the Portuguese coast revealed strains of the picoplanktonic genera *Cyanobium* and *Synechocystis* as potential sources of anticancer compounds. The ethyl acetate fraction of the strains *Cyanobium* sp. LEGE06113 and the *Synechocystis* salina LEGE06155 was found to reduce cell viability of cancer cell lines. This work aimed to elucidate the mechanisms involved in the cytotoxicity of these strains in the colon adenocarcinoma cell line RKO by employing real-time PCR (RT-PCR) for genes involved in cell cycle and apoptosis, by flow cytometry for cell cycle and by two-dimensional gel electrophoresis for protein expression. RT-PCR results revealed differences in mRNA expression of genes *CCNB1* (cell cycle) and *BCL-2* (apoptosis). Flow cytometry results revealed a decrease in the G0/G1 and S phase and increased its number in the G2/M phase, which is in accordance with the lower expression of *CCNB1*. The proteomic results demonstrated different protein patterns comparing treatment groups with control. Proteins differentially regulated in exposed RKO cells were involved in cell cycle regulation, apoptosis, cell structure, protein regulation and cell metabolism. Although several of these proteins were identified in cells exposed to both cyanobacterial extracts, the data provides an indication that the cytotoxicity is induced by different toxic mechanisms in LEGE06113 and LEGE06155 fractions.

Keywords: marine cyanobacteria; anticancer potential; cell cycle; apoptosis; proteomics