RNA interference during taxol treatment promotes apoptosis in a highly invasive human glioblastoma cell line

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The RNA interference (RNAi) is an incredibly powerful tool to specifically knockdown a gene's message, and subsequently the protein level of the targeted gene. Induction of apoptosis offers a novel and potentially useful method to improve patient responses during conventional chemotherapy. Taxol (paclitaxel) is a potent anti-neoplastic drug, which strongly binds to the β-tubulin and prevents tumor cell division. The aim of the present investigation was to induce apoptosis during low dose taxol treatment in brain tumor cells through the knockdown of Bcl-2, which is upregulated in malignant gliomas and protects tumor cells from apoptosis. In the present study we used Bcl-2 siRNA to knockdown the cognate mRNA and subsequent protein levels in a highly invasive glioblastoma cell line, U-251MG. The cells in culture were treated with Taxol (100 nM) and Bcl-2 siRNA (100 nM) for 72 h in reduced serum media. Bcl-2 siRNA treatment resulted in about 70% knockdown of Bcl-2 mRNA and the related protein level. FACS analysis demonstrated apoptosis of more than 50% cells treated with Taxol and Bcl-2 siRNA. Caspase-3 and calpain activity assay showed increased activity indicating enhanced apoptosis. Immunofluorescence for cleaved caspase-3 and calpain depicted increased levels and co-localization of both molecules. Western blot analysis demonstrated increased activities of cleaved caspases, TRADD, BAD, BAX, PARP and DFF40. Taxol treatment of U-251 cells without Bcl-2 siRNA did not results significant amount of apoptosis when compared with the combined therapy. The treatment with Bcl-2 siRNA alone causes about 30-40% apoptosis. The results of the present study demonstrated that combined treatment of Taxol and Bcl-2 siRNA induces intrinsic caspase mediated signaling pathway and apoptosis in U-251 glioblastoma cells. Furthermore, the study suggests that the combined treatment of Taxol and Bcl-2 siRNA offers a novel and potential tool for cancer therapy.