p53-Bad: A Novel Mitochondrially Targeted Gene Therapy for Ovarian Cancer

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With an ~70% fatality rate, ovarian cancer is classified as the deadliest gynecological malignancy. Recent gene therapy using p53 failed. An alternative approach to wild type (wt) p53 is mitochondrially targeted p53. Previous work has shown that mitochondrially targeted p53 can kill cancer cells in vitro via the extrinsic apoptotic pathway. This proposal tested a novel tumor suppressor-proapoptotic hybrid (called p53-Bad) for gene therapy of ovarian cancer. p53 tumor suppressor was fused to Bad (proapoptotic protein) to trigger “apoptotic collapse” at the mitochondria. However, Bad normally gets sequestered by 14-3-3 protein in the cytoplasm, and is unable to localize to the mitochondria, hence rendering Bad unable to induce apoptosis. Pro-apoptotic activity and mitochondrial localization of Bad are regulated via phosphorylation serine residues 112, 136, and 155, and Bad can only activate apoptosis when is unphosphorylated. Therefore, I hypothesized that if serine residues 112, 136, and 155 in Bad are mutated to alanines to prevent phosphorylation, then the apoptotic potential of p53-Bad would be increased to create a more potent tumor suppressor for ovarian cancer gene therapy. I tested the effect of mutations in Bad on the localization and apoptotic activity of p53-Bad. Flow cytometry was used to test levels of apoptosis were done. The double mutant p53-Bad (S112A/S136A) was is more efficient in cell death compared to unmutated p53-Bad. However, the addition of the third mutation S155A did not appear to further increase apoptosis. These results may aid in developing more efficient gene therapy for ovarian cancer.

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