Abstract

Small-Inhibitory RNA (siRNA)-mediated Silencing of DNA-PK Leads to Decreased Protein Levels and Increased Cell Kill in CRL-1743 breast cancer cells, A Potential Technique for Radiation-Sensitization of Metastatic Breast Cancer

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Objective: When eukaryotic cells are unable to repair DNA damage, such as double-stranded breaks that are sustained commonly with ionizing radiation, apoptosis results. DNA protein kinase (DNA-PK) is an endogenously expressed protein that facilitates the repair of DNA double-stranded breaks. By using short-inhibitory RNA (siRNA) to DNA-PK in CRL-1743 cancer cells, we have sought to create a possible molecular radiation sensitizer for metastatic breast cancer to the spine.

Methods: CRL-1743 breast cancer cells were cultured in vitro. Cells were transfected with either siRNA to DNA-PK (via a 5 Kb plasmid provided by T.L. DeWeese), green fluorescent protein (GFP; to test the efficacy of transfection), or not transfected at all. Forty-eight hours after transfection, GFP transfected cells were observed for level of transfection, siRNA transfected cells were re-suspended and plated for radiation (5Gy dose using Gammacell 40 irradiator); nine to ten days after radiation, cells were stained with cresyl violet and the colonies were counted and analyzed. The remaining cells were lysed and proteins were run on a Western blot assay. Protein concentration was compared between transfected and non-transfected cell lines, using the endogenously expressed beta-actin as a baseline control for protein expression.

Results: Transfected CRL-1743 breast cancer cells showed a 20% decrease in the expression of DNA-PK using Western blot analysis; following *in vitro* radiation. In addition, cell death in cancer cells was increased following successful transfection of siRNA to DNA-PK.

Conclusion: CRL-1743 cancer cells transfected with a plasmid-based siRNA to DNA-PK yielded a modest decrease in endogenous expression of DNA-PK, with concomitant increased cell-kill due to ionizing radiation. Because DNA-PK is a key protein involved in the repair of DNA double stranded breaks, these results suggest that siRNA-mediated silencing of this molecule may permit the increased sensitization of metastatic breast cancer to the spine with ionizing radiation.

Figure 1. GFP Transfection at 40% Confluency

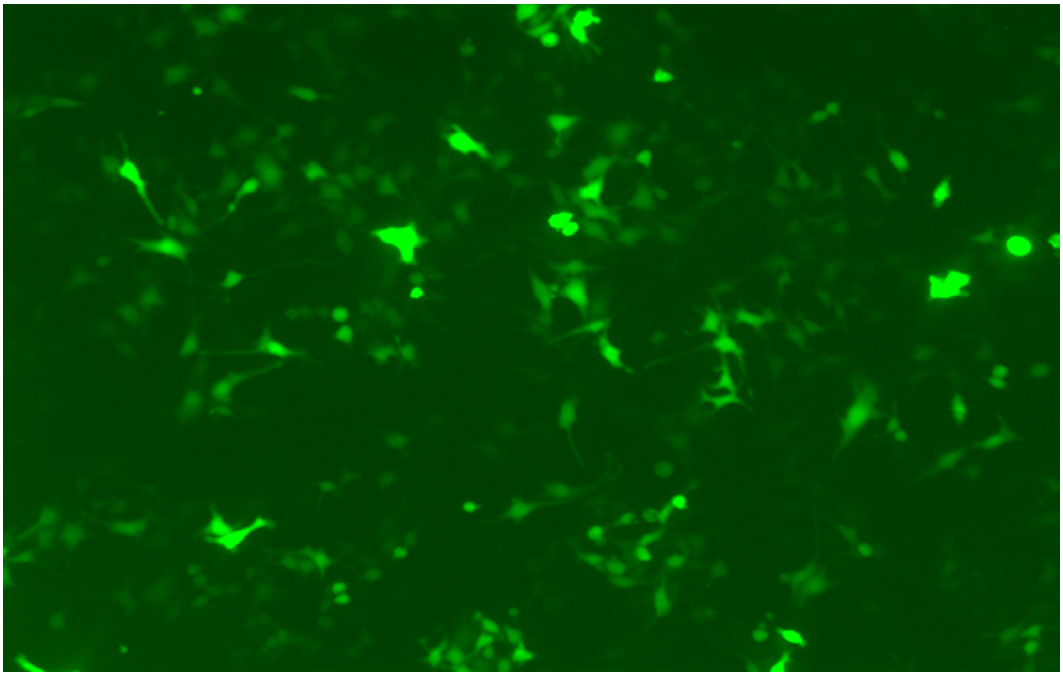
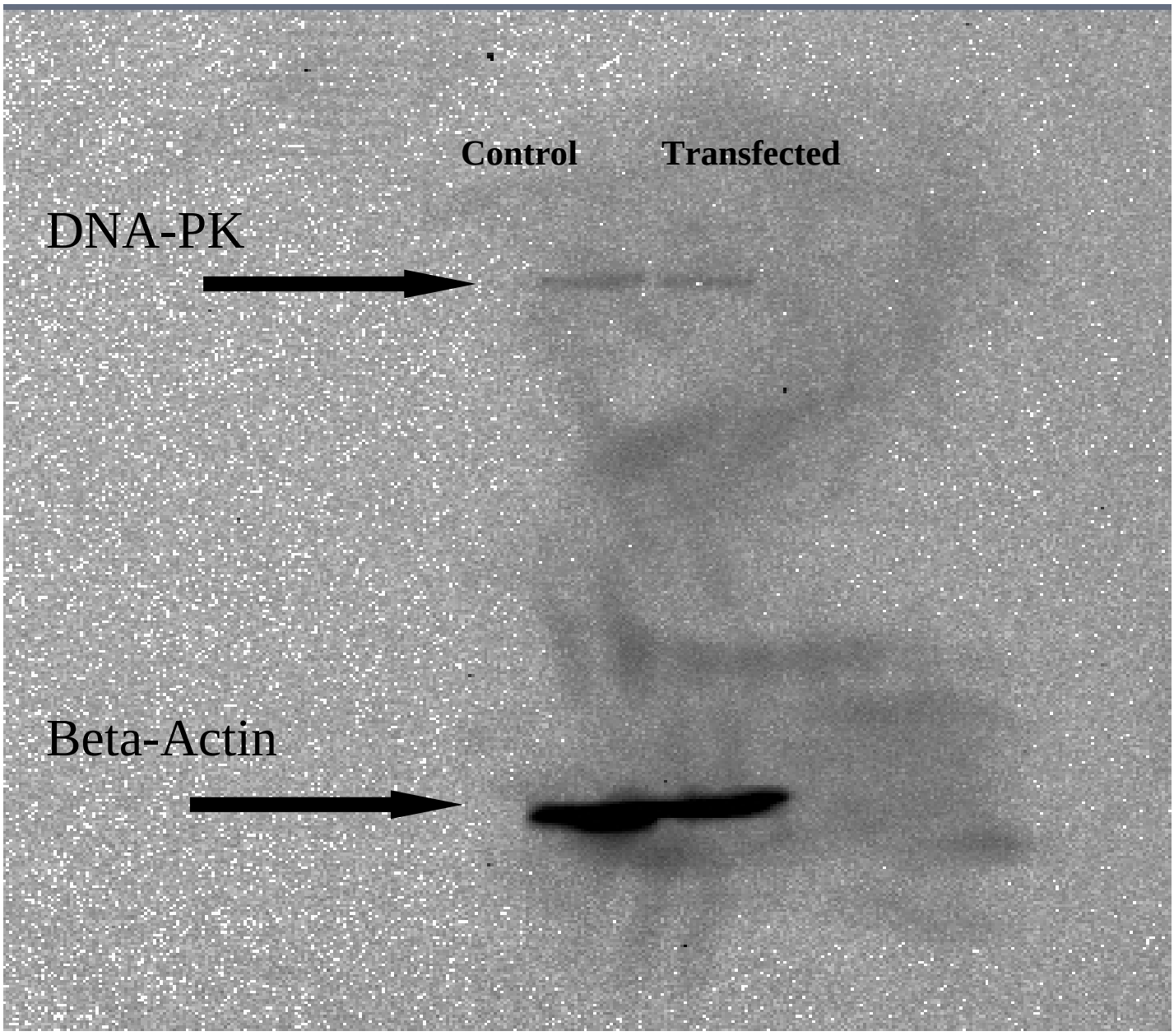
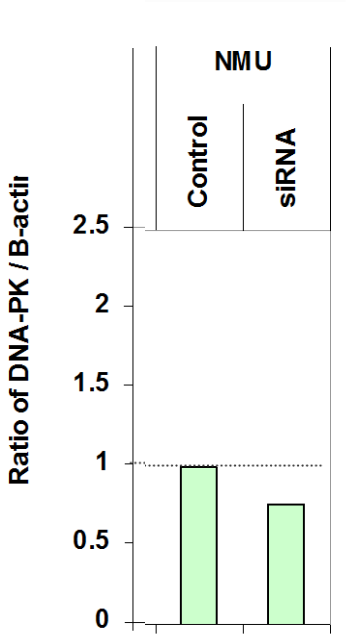


Figure 2. Western blot analysis demonstrating 20% downregulation of DNA-PK and graphical representation of reduced DNA-PK rxpression when compared to Beta-Actin baseline.




NMU
Control
siRNA
Ratio of DNA-PK / B-actin
2.5
2
1.5
1
0.5
0