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Essential Overlapping Functions Of Crk And Crkl In Glioblastoma And Diffuse Intrinsic Pontine Glioma Cells

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ESSENTIAL OVERLAPPING FUNCTIONS OF CRK AND CRKL IN GLIOBLASTOMA AND DIFFUSE INTRINSIC PONTINE GLIOMA CELLS

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ABSTRACT

Summary: Some glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG) patients show elevated protein levels of Crk and CrkL, but the contribution of Crk and CrkL to the brain tumors is less clear. Our results implicate that the two proteins may be important for glioma cell invasion into healthy brain tissues and by reducing the protein levels, we demonstrate that Crk and CrkL play important roles in tumor cell migration.

Background: The expression levels of CT10 regulator of kinase (Crk) and Crk-like (CrkL) are elevated in many human cancers, including glioblastoma (GBM) and diffuse intrinsic pontine glioma (DIPG). GBM and DIPG are both highly aggressive brain tumors derived from glial cells. Elevation of Crk and CrkL contributes to poor prognosis, and they have been proposed as therapeutic targets for GBM. We recently demonstrated that Crk and CrkL play essential overlapping roles in GBM cell migration. Here we have investigated if Crk and CrkL play similar roles in GBM and DIPG cells.

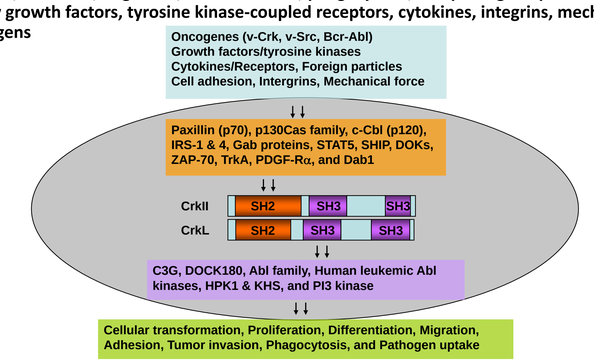
Methods: We induced gene knockdown of Crk, CrkL, or both in vitro in a human GBM cell line, U-118MG, and a human DIPG cell line, SF8628 by electroporating with small interfering RNAs (siRNAs). Then we determined the respective, quantitative contributions of Crk and CrkL to cellular phenotypes. Impedance-based, real-time measurements of tumor cell adhesion, migration, and invasion were performed using the xCELLigence Real-Time Cell Analyzer (Agilent).

Results: The combined use of specific and potent Crk and CrkL siRNAs induced effective knockdown of CrkII, CrkI, and CrkL in GBM and DIPG cells. Crk knockdown did not affect cell morphology or proliferation in both GBM and DIPG cells. On the other hand, CrkL knockdown caused shrinkage of cells and inhibition of cell migration and adhesion in both cell lines. In both GBM and DIPG cells, Crk/CrkL double knockdown resulted in more pronounced morphological alterations and robust inhibition of proliferation and adhesion. Furthermore, Crk/CrkL double knockdown completely blocked cell migration and invasion in both cell lines.

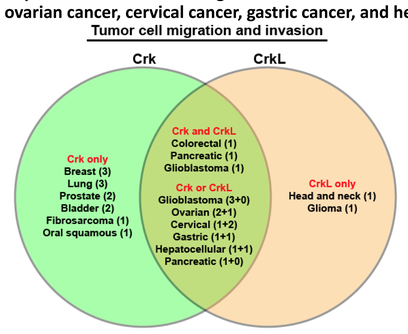
Conclusion/Significances: These results demonstrate both the predominant role of CrkL and the essential overlapping functions of Crk and CrkL in GBM and DIPG cells. Our study indicates that migration and invasion of GBM and DIPG cells depends entirely on Crk and CrkL. Our results suggest that inhibition of Crk and CrkL activity may suppress invasion of glioma into healthy brain tissues. Impedance-based, real-time measurement of glioma cell migration represents a robust assay for monitoring Crk and CrkL activities.

INTRODUCTION

By mediating protein-protein interactions through their SH2 and SH3 domains, Crk and CrkL play essential roles in signal transduction pathways. Crk and CrkL are expressed ubiquitously, and they have been implicated in diverse biological processes. Crk and CrkL mediate cytoskeletal changes, cell proliferation, adhesion, migration, differentiation, phagocytosis, and pathogen uptake that are induced by growth factors, tyrosine kinase-coupled receptors, cytokines, integrins, mechanical force, and pathogens



Crk and CrkL play critical roles in tumor cell migration and invasion in a variety of cancers. In most of these studies, loss of either Crk or CrkL resulted in decreases in tumor cell migration and invasion, suggesting that tumor cell migration and invasion are highly demanding cellular processes that require both Crk and CrkL to reorganize the cellular cytoskeletal network. In particular, both Crk and CrkL have been demonstrated to be important for tumor cell migration and invasion in colorectal cancer, glioblastoma, ovarian cancer, cervical cancer, gastric cancer, and hepatocellular carcinoma.



Higher expression of Crk and CrkL in cancer tissues has been reported to contribute to poor prognosis and lower survival of patients in many cancer types.

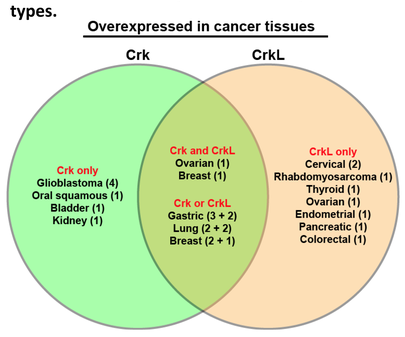


Table 1. Cell Line Comparison

	GBM (U118MG)			DIPG (SF8628)		
	Crk KD	CrkL KD	dKD	Crk KD	CrkL KD	dKD
Morphology	No effect	Shrinkage	Rounded	No effect	Shrinkage	Rounded
Cell Proliferation	No effect	Inhibition	Inhibition	Increase	Increase	Inhibition
Migration	Delayed	Inhibition	Blocked	Delayed	Inhibition	Blocked
Invasion	No effect	Inhibition	Blocked	Delayed	No effect	Blocked
Adhesion	Increase	Inhibition	Inhibition	Inhibition	Inhibition	Blocked

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2) Park T. Crk and CrkL as Therapeutic Targets for Cancer Treatment. Cells. 2021 Mar 27;10(4):739. doi: 10.3390/cells10040739.

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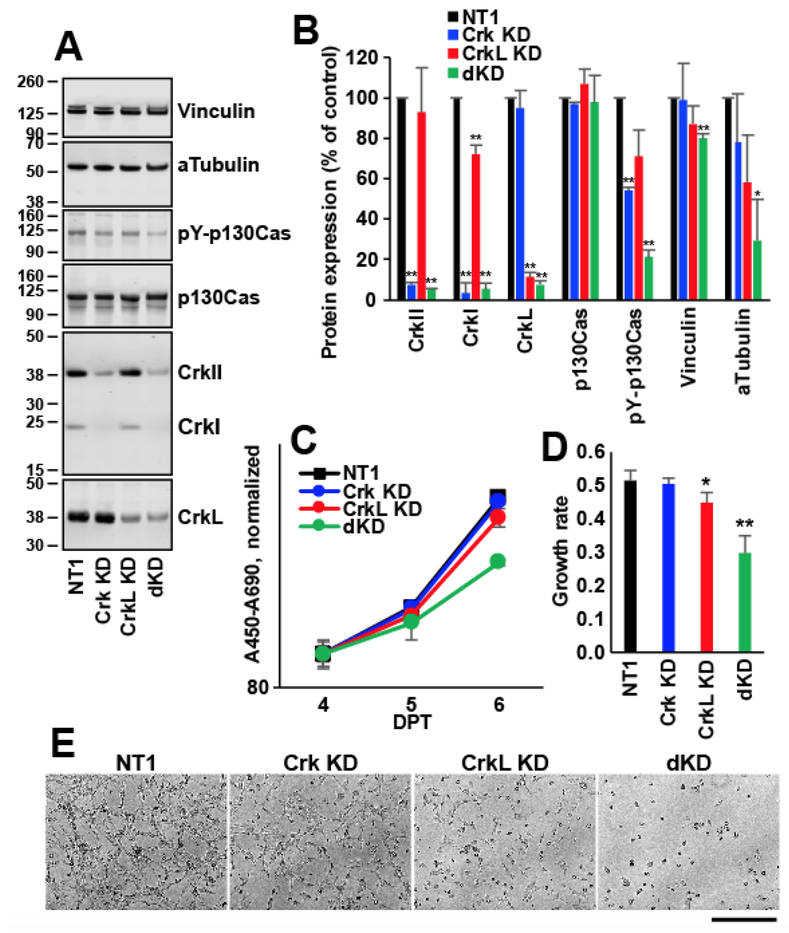
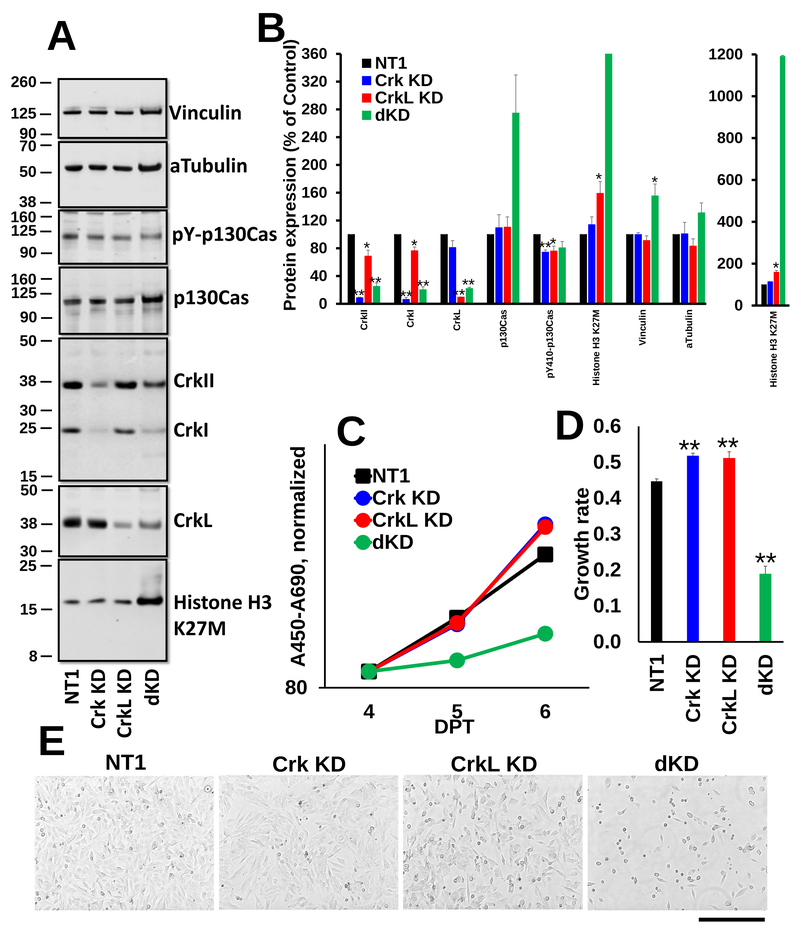
Result 1. Single and double knockdown of Crk and CrkL using siRNAs in human GBM cells



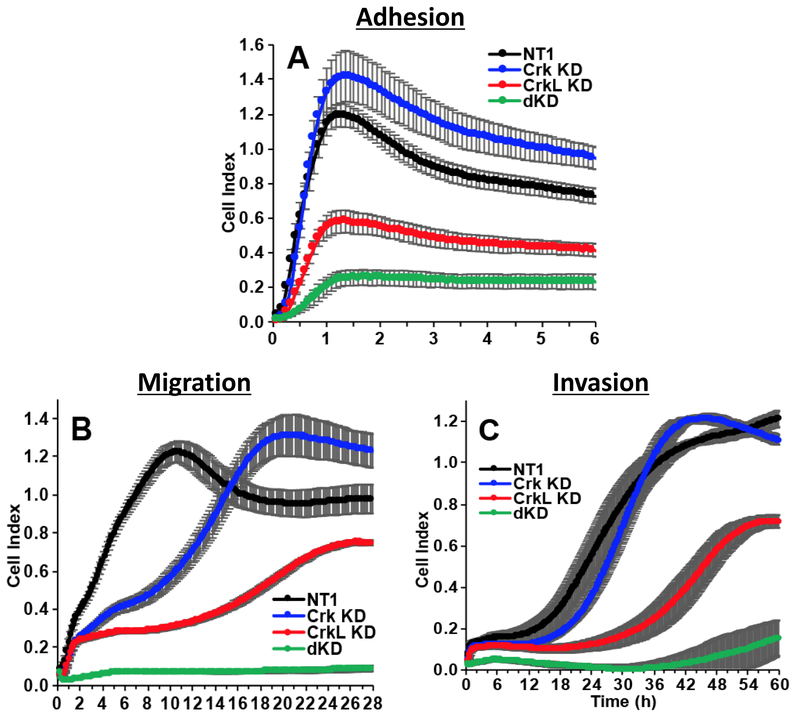
A. U-118MG cells were electroporated with non-targeting siRNA (NT1) (80 pmol), Crk siRNA 10 (40 pmol), CrkL siRNAs 22 (20 pmol) plus 24 (20 pmol), or with Crk and CrkL siRNAs together. The cells were harvested and re-plated at 3 days post transfection (DPT), and total cell lysates were prepared at 4 DPT for Western blot analyses. Protein levels upon siRNA transfection were compared with NT1. Alpha-tubulin and vinculin levels were measured as controls. B. Protein bands were quantified using the Odyssey system, calculated as percentages of the control (NT1), and their mean ± SD values are shown. *p < 0.05, **p < 0.01, compared with NT1. C. Proliferation of U-118MG cells electroporated with Crk and CrkL siRNAs after re-plating at 3 DPT was quantitatively measured using WST-1. The A450-690 values are presented in the logarithmic scales. D. Exponential trendlines for the WST-1 assay graphs were drawn and their slopes, the coefficients of x, are presented as the rates for exponential cell growth. E. After cells were transfected with Crk and CrkL siRNAs followed by re-plating at 3 DPT, phase-contrast images of live cells were taken at 4 DPT using the EVOS system. Representative images are shown. Scale bar: 400 µm.

Result 3. Single and double knockdown of Crk and CrkL using siRNAs in human DIPG cells



A. SF8628 cells were electroporated with non-targeting siRNA (NT1) (80 pmol), Crk siRNA 10 (40 pmol), CrkL siRNAs 22 (20 pmol) plus 24 (20 pmol), or with Crk and CrkL siRNAs together. The cells were harvested and re-plated at 3 days post transfection (DPT), and total cell lysates were prepared at 4 DPT for Western blot analyses. Protein levels upon siRNA transfection were compared with NT1. Alpha-tubulin and vinculin levels were measured as controls. B. Protein bands were quantified using the Odyssey system, calculated as percentages of the control (NT1), and their mean ± SD values are shown. *p < 0.05, **p < 0.01, compared with NT1. C. Proliferation of SF8628 cells electroporated with Crk and CrkL siRNAs after re-plating at 3 DPT was quantitatively measured using WST-1. The A450-690 values are presented in the logarithmic scales. D. Exponential trendlines for the WST-1 assay graphs were drawn and their slopes, the coefficients of x, are presented as the rates for exponential cell growth. E. After cells were transfected with Crk and CrkL siRNAs followed by re-plating at 3 DPT, phase-contrast images of live cells were taken at 4 DPT using the EVOS system. Representative images are shown. Scale bar: 400 µm.

Result 2. Inhibition of cell adhesion, migration, and invasion by Crk and CrkL knockdown



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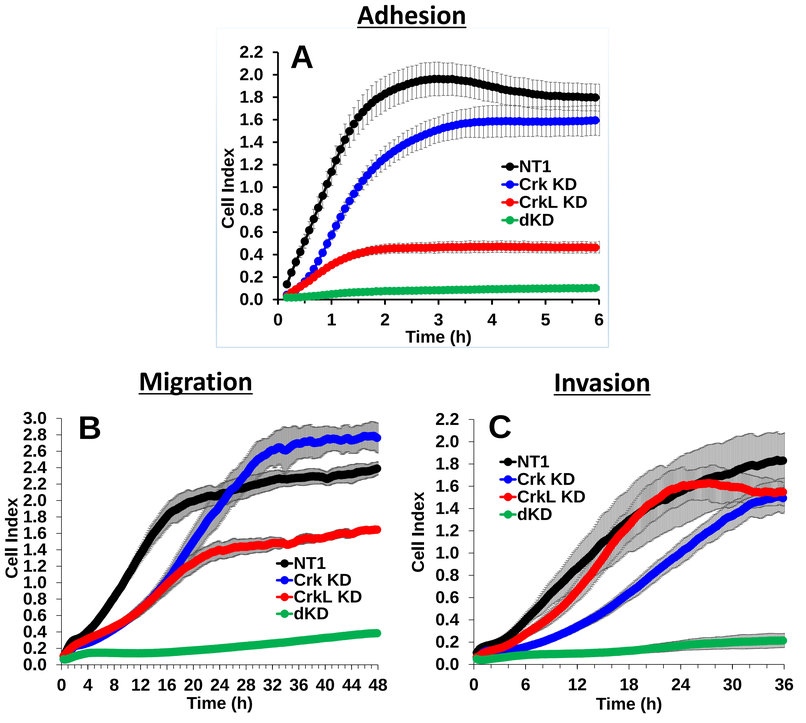
Result 4. Inhibition of cell adhesion, migration, and invasion by Crk and CrkL knockdown



SF8628 cells were electroporated with NT1 (80 pmol), Crk siRNA 10 (40 pmol), CrkL siRNAs 22 (20 pmol) plus 24 (20 pmol), or with Crk and CrkL siRNAs together. At 3 DPT, the cells were harvested and plated onto an E-Plate 16, and two CIM-Plates without and with Matrigel coating for cell adhesion (A), migration (B), and invasion (C) using the xCELLigence system according to the Experimental Procedures. Cell index values were obtained from 4 wells for each sample, and their mean ± SD values are shown. Three independent experiments were carried out, and the results were reproducible.

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