CrkI Adaptor Protein Modulates Cell Migration and Invasion in Glioblastoma

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Crk was originally isolated as an oncogene product of the CT10 chicken retrovirus, and it belongs to a group of adaptor proteins that are comprised of SH2 and SH3 domains, which interact with phosphotyrosine and proline-rich regions, respectively. The human *crk* gene is translated into two products, CrkI (28 kDa) and CrkII (42 kDa), by alternative splicing. CrkII, which is predominantly expressed in many cell lines, consists of one SH2 and two SH3 domains with a spacer region between the SH3 domains that includes a tyrosine phosphorylation site (Tyr221). Upon tyrosine phosphorylation, CrkII undergoes intramolecular binding, which results not only in blockade of CrkII SH2-mediated binding to phosphotyrosine residues in other molecules, but also in reduced affinity of the CrkII central SH3 domain. In contrast, CrkI consists of one SH2 and only one SH3 domain, and it lacks this tyrosine phosphorylation site. Since CrkI but not CrkII expression induces transformation in rat 3Y1 fibroblasts, CrkI appears to resemble the *v-crk* oncogene product not only in its structure but also in its function. CrkII has been implicated in FAK-induced cell migration by coupling with $p130^{cas}$; however, the expression and function of CrkI in tumor cells are poorly understood.

We have demonstrated specific expression of *crkI* in glioblastoma tissues and analyzed the role of CrkI in malignancy of glioblastoma. The *crkII* mRNA was detected both in normal brain and glioblastoma tissues, whereas *crkI* mRNA levels were quite low in normal brain and up-regulated in glioblastoma tissues. Expression of CrkI but not CrkII in glioblastoma U87MG cells induced transformation that stimulated cell migration concomitant with tyrosine phosphorylation of $p130^{cas}$. When mock and CrkI-transformed U87MG cells were cultured on Matrigel, Akt was phosphorylated to comparable levels. Whereas treatment of control U87MG cells with N-cadherin blocking antibody GC-4 preferentially inhibited PI 3-K/Akt activation, CrkI-transformed cells were resistant to it. Invasion by both mock- and CrkI-transfected cells was inhibited by the PI 3-K inhibitor, indicating that the PI 3-K/Akt pathway is essential for invasion. Consistent with its effects on Akt activation, GC-4 suppressed invasion of mock-transfected but not CrkI-transformed cells. These results indicate that expression of *crkI* but not *crkII* is specifically up-regulated in glioblastoma tissues, which contributes to malignancy of glioblastoma while activating $p130^{cas}$. CrkI is also involved in promoting the invasive phenotype by activating PI 3-K/Akt signaling without N-cadherin-mediated intercellular interactions. CrkI may be not only a diagnostic marker, but also a molecular target for drug development against glioblastoma.