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Analysis of mechanism of adaptor protein Crk-induced epithelial-mesenchymal transition (EMT) and its implication to human cancer metastasis

(アダプター分子 Crk による上皮間葉移行機構とヒト癌転移誘導におけるその役割の解明)

Abstract

(Background and Objectives) Lung cancer is a major leading cause of death in both men and women worldwide, significantly because of early aggressive metastasis. The process of epithelial-to-mesenchymal transition (EMT) and Crk adaptor protein family share several pivotal properties including tumor invasiveness and metastasis, however; the integration or crosstalk of Crk-mediated signaling in EMT induction have remained obscure.

(Methods) Clinical samples from 111 NSCLC patients evaluated by Crk/E-Cadherin immunohistochemistry. Crk luciferase promoter activity was examined under the effect of cytokines and growth factors. Using lentiviral overexpression of CrkI or CrkII in A549 lung adenocarcinoma cells was done, qRT-PCR and immunoblotting and co-immunoprecipitation were utilized to determine the expression of downstream mRNA and proteins and binding affinity to Crk, Pulldown assay was done to examine activity of Rac1 and RhoA. Migration and metastatic assays, *in vitro* and *in vivo* were used to demonstrate increased metastatic ability in Crk overexpressing cells. Snail Knockdown A549 Cells were used to determine the molecular effect of Snail on other related signals.

(Results and discussion) In this study, immunohistochemical analysis revealed an increased expression of Crk in lung adenocarcinomas especially at the invasive front correlating with a poor prognosis. In *in vitro* experimental setting mimics tumor microenvironment, Crk promoter activity was promoted after stimulation with several growth factors and cytokines, along with enhancement of CrkI protein by TGF- β 1. To reveal the distinct functions of Crk family of proteins, CrkI and CrkII were separately overexpressed in A549 lung adenocarcinoma cells. Although either forced expression of CrkI and CrkII was sufficient to induce mesenchymal features with increased motility and invasion, tumor metastatic ability in *in vivo* model was

remarkably greater in CrkI overexpressing cells than CrkII, with marked phosphorylations of c-MET, Gab1, and p130^{Cas}. Of note, expression of EMT-related molecules was strictly and exclusively regulated *via* Crk/Rac1/Snail and Crk/RhoA/Slug signalings. TGF- β 1 and TGF- β R1 expression was also increased by forced Crk expression, and exogenous TGF- β 1 synergistically enhanced Crk-induced EMT signatures. Inhibition of TGF- β R significantly declined Crk-evoked expression of EMT-related markers.

(Conclusion) Here, we unveiled novel mechanisms where CrkI especially provokes invasiveness and metastasis of lung adenocarcinoma cells *via* Rac1- and RhoA-dependent regulation of EMT markers and the feedback activation loop of TGF- β 1 signaling.