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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Breast cancer is often accomplished with gene amplification of the chromosome 11q13, including EMS1 or cortactin, whose gene product is featured by a repeated domain and a SH3 domain at its C-terminus. Our recent study has demonstrated that overexpression of cortactin can promote metastasis based on an animal model in a tyrosine phosphorylation dependent manner. We also demonstrated that the primary biochemical function of cortactin is to activate Arp2/3 complex for the formation of a branched actin network at the cell front edge and to promote cell migration and invasion. A cortactin mutant with deletion of the Arp2/3 binding domain resulted in decrease in cell motility and disturbance of actin polymerization at cell edges. Since actin dynamics is also known to be regulated by phospholipids, we examined the possibility for the regulation of cortactin by phosphatidylinositides. By a direct binding assay, we demonstrated that recombinant cortactin binds specifically to a subset of phosphatidylinositides. Current work is to determine the structural domain of cortactin that is responsible for the binding and the effect of the binding on the activity of cortactin.

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Introduction

Breast cancer is frequently associated with gene amplification of the chromosome 11q13, resulting in overexpression of cortactin, a cortical actin-associated protein and a prominent substrate of protein tyrosine kinase Src. Cortactin is accumulated in peripheral structures of cells including lamellipodia and membrane ruffles where cortical actin is enriched(1). In MDA-MB-231 breast cancer cells plated on extracellular matrix cortactin is enriched in invadopodia, a type of membrane protrusions that participates in degradation of and invasion into the matrix. While the precise role of cortactin in tumor progression remains unclear, amplification and overexpression of cortactin appear to be intimately associated with patients with poor prognosis or relapse(2), indicating that overexpression of cortactin may contribute to a late stage of tumor progression. This notion is further strengthened by our recent study showing that MDA-MB-231 cells overexpressing wild-type cortactin acquired higher potential for cell migration in vitro and tumor invasion and metastasis in vivo(3). The protein sequence of cortactin is featured by six and half tandem copies of a unique 37-amino-acid repeat and a Src homology 3 (SH3) domain at the carboxyl terminus. Our recent study also demonstrated that cortactin binds via its N-terminus to Arp2/3 complex, a primary machinery for actin polymerization (4). We hypothesize that the association of cortactin with Arp2/3 complex and its regulation may be important for the dynamics of actin at cell leading edge and play an important role for tumor invasion and metastasis. Therefore, we may be able to compromise metastasis by targeting at the function of cortactin and its regulated actin polymerization.

Body

Task 1: Study the effects of phosphatidylinositides on the activity of cortactin-mediated actin polymerization and structural elements essential for the association of cortactin with phospholipids.

We have performed several experiments to examine the interaction between cortactin with several phosphoinositides. Our initial analysis was done with a membrane array spotted with various phosphatidylinositides and demonstrated that cortactin preferentially binds to PI-3-P, PI-5-P, PI-3,5P and PI-4-P (Figure 1). It appears that cortactin binds to the phosphatidylinositides with phosphate at either 3 or 5 group. However, other assays also show cortactin binds to PI-4,5P as well (data not shown). Further analysis is required to confirm this. To locate the binding domain of cortactin to bind to phospholipids, we analyzed several cortactin mutants and demonstrated that the binding domain is located within the repeat motif (data not shown)

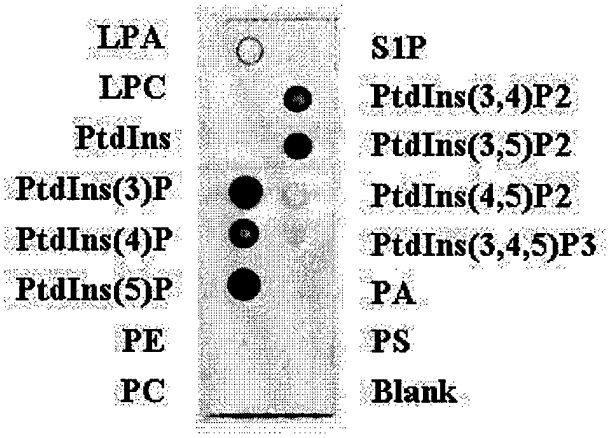

Figure 1. A membrane array (PIP-Strips) spotted with 100 pmole of phospholipids was obtained from Echelon Research Laboratories Inc. The membrane was blocked with 3% fatty acid-free bovine serum albumin in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% (v/v) Tween-20) for 1 hr. The blocked membrane was incubated for 2 hr at room temperature in the presence of recombinant cortactin at the concentration of 16 nM. After incubation, the membrane was washed three times with TBST plus 3% fatty acid-free bovine serum albumin for 10 min, then incubated with anti-cortactin antibody (4F11) for 2 hr at the concentration of 100 ng/ml followed by washing and incubation with horseradish peroxidase conjugated anti-mouse IgG antibody (Bio-Rad) with 1:10000 dilution. The immunocomplex of cortactin, phospholipids and antibody was finally visualized by chemiluminescence using ECL Kit (Amersham).

In addition, we also examined intracellular function of the association of cortactin with Arp2/3 by analyzing a cortactin mutant with deletion of the Arp2/3 binding domain. The expression of the mutant was driven by a viral promoter and was introduced into breast tumor cells MDA-MB-231 cells via infection. The population of the infected cells was analyzed for cell migration. As shown in Figure 2, the cells overexpressing the mutant had reduced motility by nearly 50% compared to the control cells while cells expressing wild-type cortactin had enhanced motility by nearly 2-fold.

Thus, the interaction of cortactin with Arp2/3 complex is essential for the cortactin-mediated cell motility.

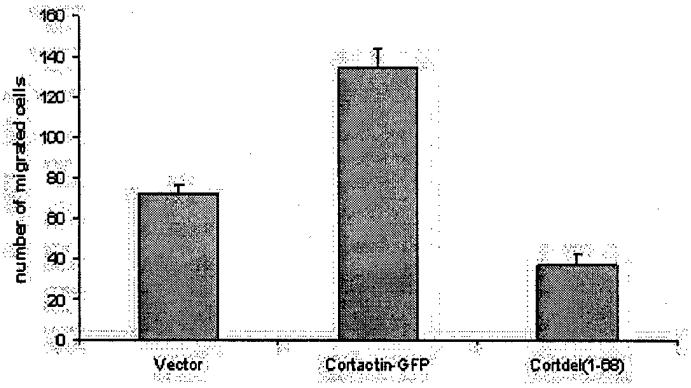

Figure 2. Analysis of migration of MDA-MB-231 cells expressing cortactin mutants. 3x104 of infected MDA-MB-231 cells were plated onto fibronectin-coated membranes in the cell culture insert of Transwell. After five hours of incubation, the number of MDA-MB-231 cells in microscopic 200x field that were migrated to the lower surface of filters was counted microscopically. Data shown are the representative of three experiments (mean ±SD).

Reportable outcomes

1. Li, Y., M. Tondravi, J. Liu, E. Smith, C. C. Haudenschild, M. Kaczmarek, and X. Zhan. 2001. Cortactin potentiates bone metastasis of breast cancer cells. *Cancer Res. 61:6906.*

Conclusion:

1. Wild-type cortactin binds preferentially to phosphatidylinositides with phosphates at either 3 or 5 position. The binding motif is located in the repeat domain.

2. Association of cortactin with Arp2/3 complex is essential for effective migration of tumor cells. Introduction of a cortactin mutant deficient in Arp2/3 binding can effectively inhibit tumor migration in vitro.

Reference

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3. Li, Y., M. Tondravi, J. Liu, E. Smith, C. C. Haudenschild, M. Kaczmarek, and X. Zhan. 2001. Cortactin potentiates bone metastasis of breast cancer cells. *Cancer Res. 61:6906.*

4. Uruno, T., J. Liu, P. Zhang, Y. Fan, C. Egile, R. Li, S. C. Mueller, and X. Zhan. 2001. Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nat. Cell Biol. 3:259.*