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Regulation of Cell Migration and Anchorage-dependent Cell Signaling by Focal Adhesion Protein, vinexin

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Anchorage-independent cell proliferation is one of the most prominent features of tumor cells. Loss of anchorage to substratum inhibits cell proliferation of normal cells and sometimes induces the apoptosis. In contrast, tumor cells can proliferate and survive without anchorage to substratum. Loss of anchorage-dependency for cell proliferation well correlates with the malignancy of tumor cells. Increased evidences indicate that proteins localized at focal adhesions (cell-extracellular adhesion sites) are involved in this regulation. These proteins facilitate the activation of upstream activators of ERK, a kinase responsible for cell proliferation, in adherent cells. Thus, in adherent cells proteins localized at focal adhesion activate ERK synergistically with growth factor stimulation, leading to cell proliferation. However, little is known whether inactivating signals (i.e. phosphatases) for ERK are regulated in an anchorage-dependent manner. Here we show that phosphatase activity against ERK is enhanced in suspended cells significantly (Fig. 1). Furthermore, treatment of NIH3T3 cells with orthovanadate increased ERK activation in suspended cells, although it did not stimulate anupstream activating kinase for ERK. These observations suggest that phosphatase(s) against ERK play(s) a role for anchorage-dependence of ERK activation. We have previously shown that exogenous expression of a focal adhesion protein, vinexin β, allows the anchorage-independent activation of ERK. Thus, we examined the effect of vinexin β on activation and inactivation process of ERK. Interestingly, expression of vinexin β did not affect the upstream activating kinases for ERK, but delayed the inactivation of ERK. Furthermore, binding of vinexin β inhibited the inactivation of ERK in vitro. Overexpression of ERK-specific phosphatase MKP3 suppressed the delayed inactivation of ERK induced by vinexin β (Fig. 2).

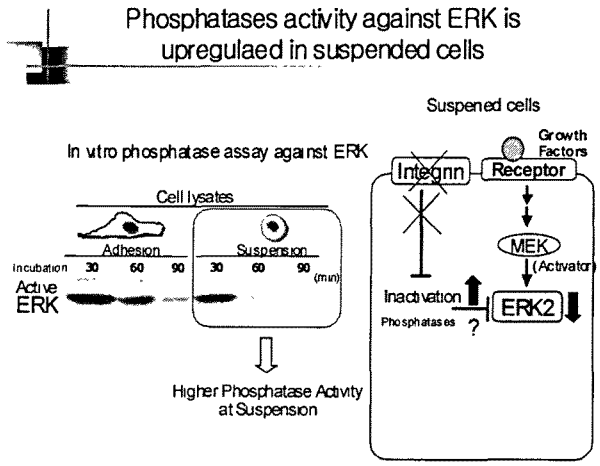

Fig. 1

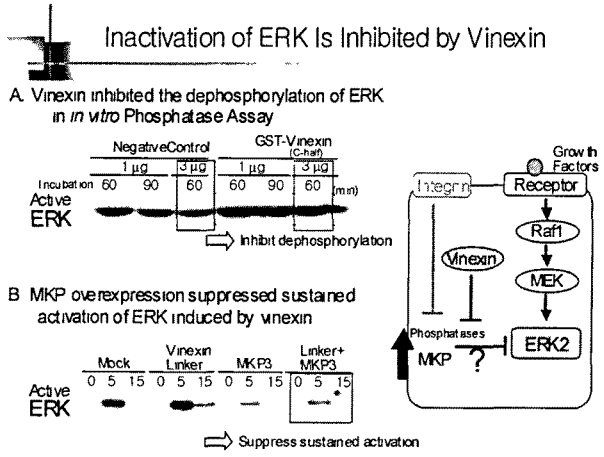

Fig. 2

Together, we propose a model that anchorage dependence of ERK activation is regulated by both activation and inactivation process and that vinexin β can induce the anchorage-independent activation of ERK by inhibiting the inactivation process (Fig. 3).

Tumor cell invasion and metastasis is the biggest problem for cancer therapy. If we can control the invasion and metastasis, we could overcome the cancer by surgery or other therapy. Cell invasion and metastasis is a complicated process but involves cell migration, which requires the regulated actin reorganization, the regulated turn over of cell adhesions,

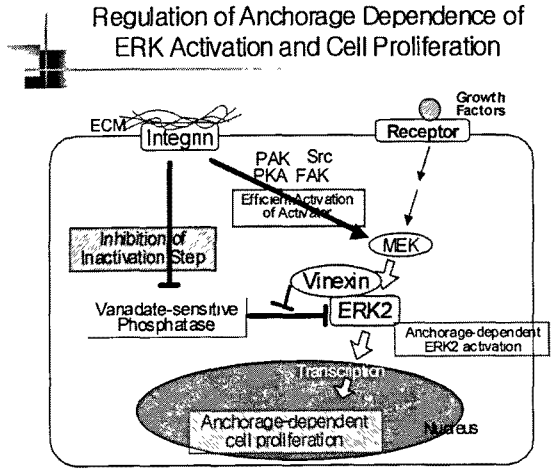

Fig. 3

and the coordination of these events. However, the mechanism of this coordination is largely unknown. To understand the mechanism, we focus on a focal adhesion protein, vinexin, again. Vinexin is known to be involved in regulation of actin cytoskeleton and cell adhesion as well as anchorage-independent signals. Furthermore, vinexin was reported to be decreased and increased in ovarian cancer and hepatocarcinoma, respectively. Although vinexin could be a candidate for a regulator of cell migration through the coordination of actin cytoskeletal reorganization and cell adhesions, how vinexin regulate actin cytoskeleton and whether vinexin is actually involved in cell migration are not known. Thus, we first examined the effect of vinexin on actin-regulating molecules. We found that one of actin-polymerization stimulator, WAVE2, was increased by expression of vinexin β. Furthermore, vinexin β bound to WAVE2 and increased the phosphorylation of WAVE2, suggesting the possibilitythat vinexin regulates actin reorganization through modulating WAVE2 activity. We nextexamined the effect of loss of vinexin expression on cell migration. Knockdown of vinexin expression using siRNA reduced the cell migration in *in vitro* wound healing assay. Furthermore, knockout of vinexin β gene induced the retarded wound closure at tail skin (Fig. 4).

Together, these observations suggest the possibility that vinexin bregulates the cell migration through the control of actin-regulatory protein WAVE2 and works as a linker between the regulation of cell adhesions and actin cytoskeletal reorganization (Fig. 5).

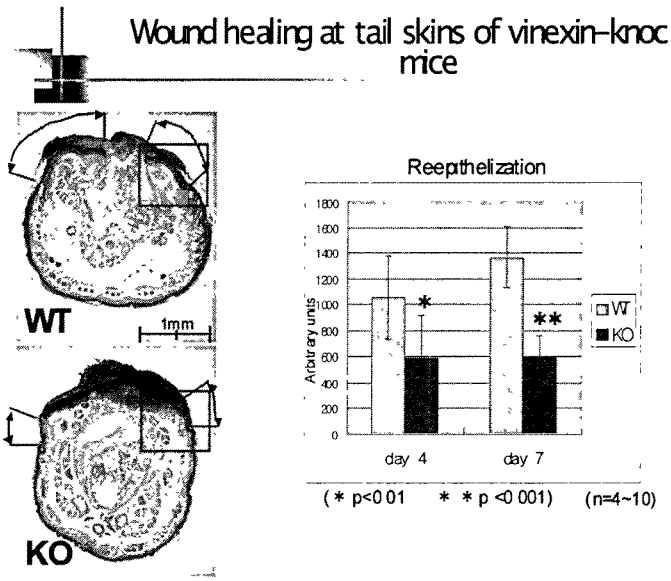
Wound healing at tail skins of vinexin-knockout mice
WT
1mm
KO
Reepthelization
Arbitrary units
1800
1600
1400
1200
1000
800
600
400
200
0
*
**
W
KO
day 4
day 7
(* p<0.01 * * p <0.001) (n=4~10)

Fig. 4

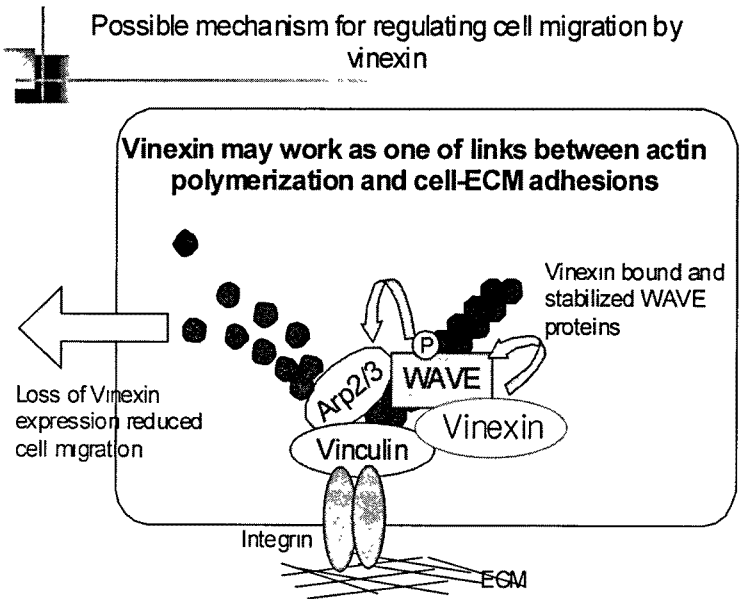
Possible mechanism for regulating cell migration by vinexin
Vinexin may work as one of links between actin polymerization and cell-ECM adhesions
Vinexin bound and stabilized WAVE proteins
P
Arp2/3
WAVE
Vinexin
Vinculin
Loss of Vinexin expression reduced cell migration
Integrin
ECM

Fig. 5