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# INTEGRIN β1 REQUIRES FOR ADHESION, SPREADING AND MIGRATION OF Hela CELLS

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Cell surface receptor integrin 1 plays important roles in signal transduction, focal adhesion formation, cell spreading and migration. This study shows that knockdown of integrin 1 in HeLa cells results disorganized actin cytoskeleton, impaired cell adhesion, spreading and migration. Outside-in activation experiment demonstrates that without integrin 1, cells can attach and spread to fibronactin but have a smaller shape and lacking protrusions, moreover attachment to laminin is poor and cells did not spread at all.

### Integrin 1 – cell adhesion – fibronactin – laminin

Բջջի մակերևույթի ռեցեպտոր ինտեգրին β1-ը կարևոր դեր է խաղում ազդակի տրանսդուկցիայի, կենտրոնական ադհեզիայի կազմավորման, ինչպես նաև բջիջների տարածման և միգրացիայի ժամանակ։ Տվյալ հետազոտությունը ցույց է տալիս, որ HeLa բջիջներում ինտեգրին β1-ի նոկդաունը բերում է ակտինային ցիտոսկելետոնի կառուցվածքի խախտմանը, թուլացնում է բջիջների ադհեզիան, տարածումը եւ միգրացիան։ Դրսից-ներս ակտիվացիայի փորձը ցույց է տալիս որ HeLa բջիջները առանց ինտեգրին β1-ի կարող են կանել և տարածվել ֆիբրոնեկտինի վրա սակայն ունեն ավելի փոքր ձևավորում և ելուստների բացակայում, դեռ ավելին նրանց ադհեզիան լամինինի հետ բավականին թույլ է, և բջիջները բոլորովին չեն տարածվում։

# Ինտեգրին β1 – ադհեզիա – ֆիբրոնեկտին – լամինին

Рецептор поверхности клеток интегрин β1 играет важную роль в трансдукции сигнала, формировании фокальной адгезии, распластывании и миграции клеток. В работе показано, что нок-даун интегрина β1 в клетках HeLa приводит к дезорганизации актинового цитоскелетона, к уменьшению клеточной адгезии, распластывании и миграции клеток. Эксперименты по активизации "снаружи-внутрь" показали, что без интегрина β1 клетки могут прикрепляться и распространяться на фибронектин, но в этом случае они имеют малую форму и отсутствие выступов, более того, прикрепление к ламинину очень слабое, и клетки ничуть не распластываются.

## Интегрин β1 – адгезия – фибронектин – ламинин

Integrins are a large family of cell surface receptors composed of 18 different and  $8\beta$  subunits [7]. Integrin  $\beta$ 1 is known to associate with multiple subunits including 1-11 and V and is widely distributed virtually in all mammalian cell types [7, 8, 10]. Through interaction with the extracellular matrices, integrin  $\beta$ 1 regulates multiple aspects of cell biology, involves in many intracellular transduction activation pathways [8], They regulate both outside-in and inside-out signaling to maintain important cell functions related to cell adhesion, migration and survival [1-3] all of which are important processes for actin cytoskeleton organization [8, 12]

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Besides, studies have been reported that integrins are invol-ved in the invasion of many pathogens, viruses and bacteria. In our resent study by using mouse embryonic fibroblast lacking integrin  $\beta$ 1 or si RNA knockdown approach in human cell line HeLa, we demonstrated that integrin  $\beta$ 1 mediates Vaccinia virus endocytoses through Akt activation signaling, in both MEF and HeLa cells [6]. When we looked integrin  $\beta$ 1 knockdown HeLa cells under microscopy we notice cell shape different compared to control knockdown HeLa cells. Phenomenon raised an interest and further investigated. Results demonstrate here that integrin  $\beta$ 1 requires for actin organization, adhesion, spreading and migration in HeLa cells.

*Materials and methods. Cells, antibodies and reagents.* HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 2% penicillin/streptomycin (Gibco) in a 5% CO<sub>2</sub> incubator at 37°C. The anti-integrin  $\beta$ 1 rat mAb, Mab13 was purchased from BD Pharmingen. Alexa Fluor 647 phalloidin and DAPI (4',6-diamidino-2-phenylindole) were purchased from Invitrogen. Anti- $\beta$  actin antibody, fibronectin (FN) and Laminin-1 (LN) were purchased from Sigma-Aldrich. The integrin  $\beta$ 1 small interfering RNA (siRNA) duplex (AAUGUAACCAACCGUAGCAUU) were purchased from Dharmacon Inc..

Confocal immunofluorescence microscopy. The KD HeLa cells (si-control or si-ITG $\beta$ 1) were seeded on glass cover slips (7x10<sup>4</sup>) in 12-well plates. The next day after being washed once with PBS cells were fixed using 4% paraformaldehyde, permeabilized (0,2% BSA/Saponin) and stained with Alexa Fluor 647 phalloidin for 1 hr at room temperature, cells were then washed 3 times with PBS stained with DAPI for 5 min. Cells were mounted in Vectashield medium (Vector Laboratories, Burlingame, CA) and images were collected with an LSM510 Meta Confocal Laser Scanning Microscope (Carl Zeiss) using a 63x objective lens.

Integrin  $\beta 1$  siRNA. HeLa cells were either mock-transfected (si-control) or transfected with siRNA duplexes (20 nM) targeting integrin  $\beta 1$  (si-ITG $\beta 1$ ) using the Lipofectamine 2000 reagent (Invitrogen) as described previously (5, 6)

*Cell spreading and adhesion assays.* Spreading and adhesion assays were performed as described previously (2, 11). In brief, non-tissue culture grade 96-well plates (FALCON) were coated with fibronectin (FN) or laminin-1 (LN-1) at 20  $\mu$ g/ml in 50 $\mu$ l volume overnight at 4°C, the next day coated wells were washed once with PBS and blocked with PBS containing 1% BSA at 37°C for 1 to 2hrs. While blocking the coated substrates cell suspension were prepared. HeLa KD cells (si-control and si-ITG $\beta$ 1) that were grown to confluency and serum starved for 2hrs were detached from the culture dish by trypsin/EDTA spin, washed once, suspended in serum free DMEM 2x10<sup>4</sup> cells per well was added to the substrates and incubated at 37°C for various time. Non adhered cells were washed out and attached cells were fixed with 95% ethanol for 10 min at RT stained with crystal violet for 30 min, after being washed with water images were collected with Nikon inverted microscope, or for colorimetric analyses 0.2% Triton X-100 was added to the stained cells for 5 min at RT then OD in 550 nm was measured in an ELISA reader.

*Wound healing assay.* Indicated cells were seeded in tissue culture dish and grown for  $\sim 100\%$  confluency then cell monolayer were wounded using pipette tip, cells were washed once with PBS to remove wounded cell debris and incubated with complete medium at 37°C. Images were collected with Nikon inverted microscope at 0 hrs (right after wounding) or 24 hrs of incubation.

**Results and Discussion.** Numerous studies have been described the important role and the function of integrin  $\beta$ 1 on cellular morphological changes in mouse model [1, 2]. To test weather integrin  $\beta$ 1 requires for actin cytoskeleton organization in HeLa cells, small interfering RNA experiments were conducted to knockdown  $\beta$ 1 from HeLa cells. To check siRNA quality first, control siRNA (si-control) or siRNA targeting integrin  $\beta$ 1 (si-ITG $\beta$ 1) was transfected into HeLa cells, followed by harvesting for immunoblot analyses (fig. 2B) and FACS analyses (data not shown). Total amount of integrin  $\beta$ 1 was knocked down (KD) in si-ITG $\beta$ 1 but not in si-control HeLa cells, conforming specificity of si-ITG $\beta$ 1 (fig. 2B). These HeLa KD cells were grown in glass cover slips in compleat medium over night fixed for immunofluorescence microscopy analyses (fig.1A) as determine by phalloidin staining

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si-ITG $\beta$ 1 KD HeLa cells showed rounded shape, disorganized actin staining lacking pro trusions compare to si-control KD cells. Indicating integrin  $\beta$ 1 is important for HeLa cell morphological organization.



Fig. 1. Altered cell morphology upon integrin  $\beta$ 1 knockdown in HeLa cells.(A) Immunofluorescence analysis of actin in KD HeLa cells. The KD HeLa cells mock-KD (si-Control) and si-ITG $\beta$ 1 KD cells were fixed permeabilized and stained with Alexa Fluor 647-phalloidin (white) to mark the cell body and DNA was visualized by DAPI staining (blue). (B) Immunoblots of lysates prepared from mock-KD (si-Cont.1 and 2) and si-ITG $\beta$ 1 KD HeLa cells using anti-ITG $\beta$ 1 (Mab13) or anti- $\beta$ -actin antibodies as loading control.

Integrins mediate dynamic interactions between the extracellular matrix and the actin cytoskeleton. The ability of integrins to mediate multiple inside-out and outside-in signaling pathways is essential to maintain the normal cell functioning: viability, adhesion, migration and growth. The Integrin 1 plays a crucial role in cell-to-cell and cell-to-matrix communications [4, 9]. Previously we performed outside-in activation experiments to turn-on the integrin dependent signaling pathway in inactive HeLa cells and we showed that upon activation on fibronactin and Laminin several integrin 1 downstream signaling like FAK, Akt and Erk are phosphorylated [6].

To address if integrin 1 is essential to mediating HeLa cell activation on extracellular matrix proteins were performed adhesion and spreading experiments from HeLa KD cells. Serum starved HeLa si-control and si-ITG 1 KD cells were seeded overnight in the complete medium. The next day cells were serum starved detached from culture dish with trypsin/EDTA and plated onto substrates coated with fibronactin (FN) and Laminin (LN) for 60 min.

Although both, si-control and si-ITG 1 KD cells adhere and spread to FN (fig. 2A-ab) however, si-ITG 1 KD cells have smaller shape as was seen in fig. 1A. In contrast, si-ITG 1 KD cells attached to Laminin poorly and those attached cells did not spread at all (fig. 2A-cd).

This difference was still obvious when cells were incubated even longer 120 min. (data not shown). Indicating integrin 1 binding to laminin is crucial for HeLa cells attachment and spreading. As a negative control cells were plated onto dishes coated with PBS/BSA (fig. 2A-ef). Consistent result was obtained when adhesion assay was repeated using GD25 cells, MEF lacking integrin 1 [2, 6] (data not shown). Earlier studies with GD25 cells showed that without integrin 1 these cells attachment to fibronactin is through v 3 receptor, whether it is also true for HeLa cells remain to identify.



**Fig. 2.** Adhesion and spreading of the si-control and si-ITGβ1 KD HeLa cells seeded on substrate coated with fibronactin or Laminin-1. (A) Morphology of the cells. Non tissue culture plates were coated with 20 g/ml fibronactin (FN) or laminin-1 (LN) blocked with 1% BSA cells were incubated at 37°C for 60 min, fixed stained and photographed. (see Material and Methods) (B). Quantification of adhered cells. (C) Quantification of si-ITGβ1 KD HeLa cells spread on LN. Graph analyses were performed using Prism software (GraphPad).



Fig. 3. Migration of si-control and si-ITG $\beta$ 1 KD HeLa cells. Confluent cell monolayer was wounded and photographed ether right after wounding 0 or 24 hrs post incubation

Adhesion is a complex mechanism and involves variety processes like cell migration, invasion, wound healing and tissue remodeling. Effect of integrin 1 was examined in migration assay. Confluent monolayer of HeLa si-control and si-ITG 1 KD cells were wounded and incubated for indicated time as shown in fig. 3 only in the presence of integrin 1 cells were able to migrate and repair the wound (fig. 3ab) in contrast to si-ITG 1 cells (fig. 3cd). Resent years HeLa cells are commonly used and important cell line for the researchers in molecular and cellular biology field which makes an interest to know more about these cell biology. More works can be done to investigate and dissect which downstream signaling and receptors are directly involved for integrin 1 function in HeLa cells.

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