

Localization of Integrins in Ehrlich Ascites Tumor Cells

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Introduction

Cell membranes are a mosaic of lipids and proteins. Diverse proteins with diverse functions are found at the surface of all cells. Integrins are a group of cell membrane proteins known to be very important since they are responsible for cell adhesion, a critical element in cell growth and survival.

Integrin stimulation always leads to a change in cytoskeletal actin and ion transport. Therefore integrins take part in an “outside in” signal cascade. This cascade induces a phosphorylation reaction involving focal adhesion kinase (FAK), a protein which binds to the cytoplasmic side of the integrins.

Cells exposed to hypotonic medium swell but then recover their volume by secreting ions. However, the signal that stimulates the ion secretion is not known. Disruption of actin filaments in swollen cells prevents cell volume recovery. This observation indicates that actin is involved in the signaling mechanism for increased ion transport that leads to regulatory volume decrease (RVD) volume recovery.

Recent studies show that the inhibition of FAK prevents volume recovery (1). This indicates that the stimulation of integrins, along with their link to actin, play a role in the signaling mechanism for volume recovery.

In our laboratory we use a well defined model for cell volume studies, Ehrlich ascites tumor (EAT) cells (2). These cells grow in suspension and are non-adherent. Therefore, we first wanted to determine if the adhesion protein, the integrins, are present on these cells. A fluorescent immuno-cytochemical marking technique was used for this detection.

Methods

50ul of the EATC cells and control cells were treated with 150ul of Pipe saponin for 2 minutes. The cells were then treated with 150ul of blocking buffer for 15 minutes.

After removal of the blocking buffer, the cells were exposed to the primary antibody against human integrin [monoclonal antibody, AIIB2, Developmental Studies Hybridoma Bank (3)] in a Tris- Buffered Saline containing Tween-100 (TBST) solution, concentration 1:50. Incubation was at room temperature for 1 hour. The primary antibody was then removed and the cells washed twice with a 100ul of Tris-Buffered Saline containing Azide (TBSA).

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A solution containing a 1:200 concentration of the secondary antibody and TBSA was prepared. This solution also contained fluorescein phalloidin (Fl-ph) in a 5:200 proportion in TBSA. Incubation was at room temperature for 30 minutes and the cells were washed twice with 100ul TBSA.

50ul of mounting media was added to the cells and they were mounted on slides for viewing in a Zeiz IM35 epi-fluorescent microscope.

Results:

Integrin localization showed that these proteins were randomly distributed on the cell membranes of many EATcells. However, some cells appeared to have no immuno-proteins staining. Therefore, it appears that expression of integrins is not a uniform characteristic of EAT cells.

Conclusion:

These results show that integrins may not play a primary role in the signaling mechanism stimulated by changes in cell volume.

References:

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