Sustained Activation of β1-Integrins induces Proliferative Arrest or Apoptosis in Fibroblasts

Masaki Matsumura*, Mayu Eguchi, Toshiyuki Owaki, Fumio Fukai
Department of Molecular Patho-Physiology, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba 278-8510, Japan
*Contact author: j3109805@ed.noda.tus.ac.jp

Objectives: Cells require not only a signal from growth factor receptor but also an additional signal from a family of adhesion receptor, integrin, for their survival and proliferation. We previously found that a peptide derived from tenasin (TN)-C, termed TNIIIA2, strongly activates β1-integrins [1]. TNIIIA2 is capable of protecting normal mouse fibroblast NIH3T3 from anoikis-like cell death, but of inducing apoptosis in human sarcoma-like WI38VA13 cells. It is interesting to verify whether TNIIIA2 can induce apoptosis preferentially in malignant tumor cell types. In this study, we investigate the cellular responses of human fibrosarcoma-like cell line WI38VA13 and its parental normal cell line WI38 to β1-integrin activation.

Methods Results and Conclusions: TNIIIA2 induced β1-integrin activation also in WI38 normal cells. When WI38 normal cells were stimulated with TNIIIA2 on the fibronectin (FN)-coated culture plate, the proportion of cells spreading was increased. This spreading on the FN-substratum was retained with TNIIIA2 for a long time, resulting in proliferative arrest in WI38 normal cells, as evaluated by the BrdU assay. Cell cycle inhibitor proteins, p21waf1 and p16ink4a, became expressed in WI38 normal cells after treatment with TNIIIA2. Under the same conditions, WI38VA13 malignant cells underwent apoptosis, as judged by DNA fragmentation, cleavages of caspase-9 and -3 and PARP. The small G-protein Ras was spontaneously activated in WI38VA13 malignant cells, but not in WI38 normal cells. The status of Ras activation might be one of the determinants as to whether β1-integrin activation leads cells to proliferative arrest or apoptosis.

References

Promotion of PDGF-dependent cell proliferation through β1-integrin activation

Tatsuya Takai*, Toshiyuki Owaki and Fumio Fukai
Department of Molecular Patho-Physiology, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba 278-8510, Japan
*Contact author: j3109672@ed.noda.tus.ac.jp

Objective: Cross-talk between integrin and receptor tyrosine kinase (RTK) is essential for the anchorage-dependent regulation of cell proliferation. We recently found that NIH3T3 cell proliferation stimulated with PDGF is markedly promoted through β1-integrin activation by a peptide derived from tenasin-C, termed TNIIIA2 [1]. Integrin-mediated adhesion is generally considered to activate synergistically the Ras/MAP-kinase pathway in concert with the RTK signaling. As expected, the TNIIIA2-induced activation of β1-integrin induces a synergistic activation in not only the MAPK (ERK1/2) but also the small G protein Ras. Surprisingly, β1-integrin activation by TNIIIA2 also generates a conspicuous increase in the autophosphorylation of PDGF receptor (PDGFR) stimulated with PDGF. In this study, we investigate the signaling pathway relevant for promotion of the PDGF-dependent cell proliferation through β1-integrin activation by TNIIIA2.

Methods and Results: NIH3T3 cells were seeded on a culture plate coated with increasing concentrations of FN, stimulated with PDGF, and then examined for detection of tyrosine phosphorylation of PDGFR. Autophosphorylation of PDGFR was remarkably increased depending on the coated concentration of FN. To clarify the signaling route responsible for TNIIIA2-induced promotion of PDGFR autophosphorylation, we established the stable transfectants of NIH3T3 cells (NIHdnfak-shc) expressing dominant negative FAK and Shc, both of which are known to transduce a signal from β1-integrin to the Ras. Although the signal pathway from β1-integrin to the Ras was partially blocked in NIHdnfak-shc cells, neither autophosphorylation of PDGFR nor cell proliferation were influenced by TNIIIA2 treatment. Co-immunoprecipitation of β1-integrin with RTKs is an important approach to identify biochemical interaction between those receptors. Results showed that physical association of β1-integrin with PDGFR became detectable by treating cells with TNIIIA2.

Conclusions: These results suggest that TNIIIA2 promotes PDGF-dependent cell proliferation by inducing the direct association of β1-integrin with PDGFR, independent of the intracellular signaling pathways, such as the FAK-Src and caveolin.

References