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Laminin-1 is phosphorylated by ecto-protein kinases of monocytes

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Abstract

Monocytes encounter basement membranes and interact with laminins while crossing the vascular barrier. It is known that these cells possess ecto-protein kinase activity on their surface. Several proteins of the extracellular matrix can be phosphorylated by ectokinases. Therefore, it has been hypothesized that monocyte ectokinases could phosphorylate laminins and influence their biological properties.

In order to test the above hypothesis, we used intact human monocytes and adenosine triphosphate labeled with radioactive phosphate at the third phosphate ([γ-^{32}P]-ATP) to phosphorylate laminin-1. Autoradiography after sodium dodecyl sulphate polyacrylamyde gel electrophoresis (SDS–PAGE) electrophoresis indicated phosphorylation of laminin-1 on the beta and/or gamma chains. After phosphorylation, phosphoserine could be detected on Western blots by a specific monoclonal antibody. Phosphorylation was not detected when monocytes were pre-treated with trypsin and was inhibited by a specific ecto-protein kinase inhibitor (K252b). Laminin phosphorylation was also inhibited by heparin, a known inhibitor of casein kinase II and by pretreatment of monocytes by a monoclonal anti-casein kinase II antibody. Heparin binding, cell attachment and proliferation, and monocyte migration were enhanced on the phosphorylated laminin-1 as compared to the non-phosphorylated controls.

These data indicate that laminin-1 can be phosphorylated by monocyte casein kinase II type ectokinase. This phosphorylation influences important functions of laminin and therefore could provide an additional means for the interaction of monocytes with basement membranes.

Keywords: Basement membranes; Laminin-1; Extracellular phosphorylation; Casein kinase II; Heparin

1. Introduction

Protein phosphorylation catalyzed by protein kinases is an important mechanism regulating numerous cellular processes. While the majority of the research has focused on understanding the role of intracellular protein kinases, several studies have provided evidence for the existence of ecto-protein kinase activity on the surface of several different types of cells (Chiang, Kang, & Kang, 1979; Dusenbery, Mendiola, & Skubitz, 1988; Ehrlich, Davis, Bock, Kornecki, & Lenox, 1986; Haldar & Majumder, 1986; Kubler,

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Pyerin, & Kinzel, 1982; Remold-O'Donnell, 1978). Ecto-protein kinases or ectokinases, have been defined as plasma membrane-associated protein kinases that have their catalytic site outside the cell and utilize extracellular adenosine triphosphate (ATP) (Imada, Sugiyama, & Imada, 1988). Considering that the surface of the cells is directly involved in cell–cell and cell–matrix interactions, the existence of protein kinase activities at the membrane surface could provide a novel means for cells to communicate and interact with their environment.

In fact, phosphorylation of ecto-domains of cell-surface proteins by ectokinases have been demonstrated to play a role in many cellular responses including regulation of cell growth and ligand-binding properties of cell-surface receptors (Babinska, Ehrlich, & Kornecki, 1996; Hogan, Pawlowska, Yang, Kornecki, & Ehrlich, 1995; Redegeld, Caldwell, & Sitkovsky, 1999). In addition, studies have shown that molecules of the extracellular matrix, such as fibronectin, vitronectin and collagen type IV, could also serve as substrates of ecto-protein kinases (Imada et al., 1988; Revert et al., 1995; Seger, Gechtman, & Shaltiel, 1998). Interestingly, this ecto-phosphorylation can influence the molecule's major properties, such as cell adhesion and migration, as shown for vitronectin (Stepanova et al., 2002). It has been reported that vitronectin phosphorylation by casein kinase II of the surface of blood cells, actually converts vitronectin from a cellular 'glue' to a cellular "super glue" (Seger et al., 1998).

Previous studies performed in our laboratory have shown that laminin-1 can be phosphorylated by protein kinase A (PKA) as well as protein kinase C (PKC) (Koliakos et al., 2000; Koliakos, Trachana, Gaitatzi, & Dimitriadou, 2001).

Laminins, are multidomain and multifunctional cross-shaped glycoproteins that consist of three distinct disulfide linked polypeptide chains (alpha, beta, and gamma). The prototype laminin, named laminin-1 (alpha1-beta1-gamma1) (Burgeson et al., 1994), was isolated from the matrix of the Engelbreth–Holm–Swarm (EHS) tumor (Timpl et al., 1979). Laminins are structural components of the basement membrane and play a key role in cell adhesion, migration, differentiation and proliferation of several cell types (Aumailley & Smyth, 1998; Castronovo, 1993; Chang et al., 1995; De Arcangelis et al., 1996; Fukushima, Ohnishi, Arita, Hayakawa, & Sekiguchi, 1998; Goldfinger, Stack, & Jones, 1998; Iwamoto et al., 1987; Jiang, Cram, DeAizpurua, & Harrison, 1999; Kadoya et al., 1995).

Laminin-1 also interacts with heparin and its heparin binding sites are known (Kouzi-Koliakos, Koliakos, Tsilibary, Furcht, & Charonis, 1989; Yurchenco, Cheng, & Schittny, 1990). Since heparan sulfate side chains are present in both basement membrane proteoglycans and cell surface molecules (Battaglia, Mayer, Aumailley, & Timpl, 1992), this interaction is considered to be rather important.

Monocytes are bone marrow derived leukocytes that infiltrate almost every tissue of the body. Upon extravasation into sites of infection or into inflammatory loci, monocytes encounter basement membrane and interact with laminins to cross the vascular barrier. White blood cells have been studied in the past for the expression of ectokinases (Amano, Kitagawa, & Akamatsu, 1984; Apasov, Smith, Jelonek, Margulies, & Sitkovsky, 1996; Geberhiwot & Skoglund, 1995; Skubitz & Goueli, 1991). It has been reported that, ectokinase activity is present on the surface of monocytes (Geberhiwot & Skoglund, 1997). In addition, it has been demonstrated, that a number of molecules of the extracellular matrix, laminin included, undergo modifications during the process of monocyte extravasation. Secretion of monokines, chemokines and degradative enzymes are the main mechanisms suggested to mediate these modifications (Madri, Graesser, & Haas, 1996; Owen & Campbell, 1999). Laminin phosphorylation could serve as an additional mechanism to modify the molecule and therefore alter the signals fostering cell adhesion, proliferation and migration. However, laminin phosphorylation by monocyte ectokinases was hitherto not studied. Therefore, in the present study, we investigated if laminin can be phosphorylated by monocyte ectokinases and if this phosphorylation can influence the functional properties of the molecule.

2. Materials and methods

2.1. Laminin and monocyte preparation

Engelbreth–Holm–Swarm tumor laminin-1 was purchased from Sigma and was kept in aliquots in liquid nitrogen until use. Molecular weight determinations, and verification of the integrity and purity of laminin-1,