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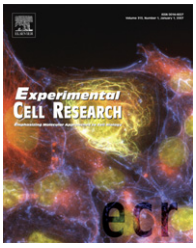

Research Article

Mechano-transduction mediated secretion and uptake of galectin-3 in breast carcinoma cells: Implications in the extracellular functions of the lectin

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ABSTRACT

In the following experiments, we sought to understand the triggering mechanism which propels galectin-3 to be secreted into the extracellular compartment from its intracellular stores in breast carcinoma cells. We also wanted to analyze in greater details the role of galectin-3 in cellular adhesion and spreading. To do this, we made use of two pairs of breast carcinoma cell lines where one of the pair has high expression of galectin-3 and the other low expression of the lectin. We determined that galectin-3 secreted into the conditioned medium of sub-confluent and spread cells in culture was quite low, almost negligible. However, once the cells were detached and rounded up, a mechano-sensing mechanism triggered the rapid secretion of galectin-3 into the conditioned medium. The secretion was constitutive as long as the cells remained detached. Galectin-3 was shown to be actively taken up from the conditioned medium by spreading cells. The cells which express and secrete high levels of galectin-3 adhered and spread much faster on plastic than those with reduced expression. The uptake of galectin-3 according to our data was important in cell spreading because if this process was compromised significantly, cells failed to spread. The data suggested that galectin-3 uptake modulates the adhesion plaques in that cells which express high levels of galectin-3 have thin-dot like plaques that may be suited for rapid adhesion and spreading while cells in which galectin-3 expression is reduced or knocked-down, have thick and elongated plaques which may be suited for a firmer adhesion to the substratum. Recombinant galectin-3 added exogenously reduced the thickness of the adhesion plaques of tumor cells with reduced galectin-3 expression. Taken together, the present data suggest that galectin-3 once externalized, is a powerful modulator of cellular adhesion and spreading in breast carcinoma cells.

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Introduction

Galectin-3 is a chimera type member of the galectin family of animal lectins, with an amino terminal domain, a collagen like domain and a carboxyl terminal domain [1]. Galectin-3 like other members of the family, lacks signal peptide and is largely located in the cytosolic compartment of the cell [2]. However, the protein is unique in that it has been located in

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the nucleus [3], mitochondria [4], and other intracellular organelles [5]. A nuclear localization signal in the molecule has been identified [6]. Despite its lack of the classical signal peptide for secretion, galectin-3 can be transported into the extracellular milieu, via a non-classical pathway [2,7]. Recently we demonstrated the ability of galectin-3 to cross the lipid bilayer of large unilamellar vesicles [8], suggesting that the lectin has yet an unknown novel sequence that enables it to traverse lipid membranes.

A number of studies have suggested that galectin-3 is preferentially secreted via the exosomal transport mechanism [5,9]. Exosomes are membrane vesicles that are secreted into the conditioned medium of cells upon the exocytic fusion of multivesicular bodies with the cell surface. However, the triggering event that externalizes galectin-3 in this pathway has been elusive [9]. Once in the extracellular milieu, galectin-3 being a sugar binding protein interacts with a myriad of partners including the extracellular matrix proteins laminin and fibronectin [10]. Galectin-3 also interacts with a number of cell surface proteins that are rich in polylactosamine residues, the preferred receptors of the lectin [10,11]. One of the key physiological functions that has been repeatedly attributed to galectin-3 is cellular adhesion [10,12]. Currently, the prevailing opinion is that the interaction of galectin-3 and other members of the galectin family with the well-established players of cellular adhesion such as integrins is responsible for the adhesive properties [13–16]. In other words, galectin-3 is more likely to play regulatory roles in the adhesive process rather than a direct role. Initial studies suggested that the interaction of galectin-3 with integrins on the cell surface reduced their adhesion to their extracellular matrix ligands [15]. Other studies further suggested that galectin-3 modulates the endocytic uptake of β1 integrins [17] in a manner similar to its role in the endocytosis of advanced glycation end products [18]. The endocytic uptake of the integrins would in theory down-regulate the avidity of the integrins.

In the present studies, we have demonstrated that galectin-3 is secreted and taken up by the cells using a mechanotransduction mechanism. Detached and spherical cells secrete galectin-3 in a constitutive manner while attached and spreading cells take up galectin-3 from the conditioned medium. Our data suggest that the secreted galectin-3 remodels the adhesion plaques such that the plaques formed in cells which express high levels of galectin-3 are thinner and dot like in appearance compared to the thick and elongated adhesion plaques in breast carcinoma cells with reduced expression of galectin-3.

Materials and methods

Materials

The anti-galectin-3 mAb producing hybridoma TIB 166 was purchased from ATCC (Manassas, VA). The rabbit polyclonal antibodies against galectin-3 were kindly provided by Dr. Avraham Raz of Karmanos Cancer Research Institute, Detroit, MI. Recombinant galectin-3 was kindly donated by Dr. Richard Cummings through the Consortium for Functional Glycomics. Fetuin-A was purchased from Calbiochem (San Diego, CA). All the other reagents used were purchased from Sigma (St. Louis, MO) unless otherwise stated.

Cell culture

Two sets of breast carcinoma cell lines kindly provided to us by Dr. Avraham Raz of Karmanos Cancer Research Institute, Detroit, MI, were used in the study. The first set comprised of two sub-lines of the breast carcinoma MDA-MB-435 transfected with either galectin-3 in the antisense orientation [19] resulting in MDA-MB-435-Gal-3As (435 AS) or with vector only (435 V) as control. The other set was obtained by introducing galectin-3 into the null-expressing non-tumorigenic BT-549 cells resulting in a sub-line named 549 Gal-3. As control, BT5-549 was transfected with the vector only to give the sub-line 549-PCN [20]. The cell lines were routinely maintained in complete medium (CM) consisting of DMEM/F12 supplemented with essential and non-essential amino acids, 100 μg/ml penicillin–streptomycin, 2.5 μg/ml Fungizone, 20 ng/ml epidermal growth factor, 10 μg/ml insulin, 0.5 μg/ml hydrocortisone, 98 ng/ml cholera toxin, and 10% heat inactivated Fetal bovine serum. Most of the experiments were done in serum free medium (SFM) either without or supplemented with the growth factor cocktail (hydrocortisone, cholera toxin, insulin, and epidermal growth factor). The LNCaP cells (purchased from ATCC) were maintained in RPMI-1640 medium with 10% serum. The cells adhere much more slowly to the substratum and do not spread well.

Secretion of galectin-3 by detached cells

The galectin-3 expressing 549-Gal-3 carcinoma cells were grown until approximately 70% confluent. The cells were then detached and plated in 12-well microtiter plates at 5×10^5cells/well and allowed to grow for 2 days at 37°C in the humidified incubator. After 48 h, the complete medium was replaced with 200 μl/well of serum free medium (SFM) without (control) or with 2.5 mM EDTA for 5, 10, 30, and 60 min. At the indicated time points, 100 μl of the conditioned medium from each well was centrifuged to pellet any suspended cell and the supernatant assayed for galectin-3 by Western blot as previously described [21]. At the end of the experiment, the wells were washed twice and the adherent cells fixed and stained with crystal violet. The dye was released from the cells with acetic acid and O.D. 570 nm determined as described [22]. The cells were also allowed to adhere in the 12-well microtiter plate (2.5×10^5 cells/well) in 400 μl of SFM in the presence or absence of 2.5 mM EDTA. After 6 h incubation, the conditioned medium (100 μl) was centrifuged to pellet any suspended cells and the supernatant assayed for galectin-3. The experiment was repeated with the 435 V/435 AS pair.

We were also interested in determining how much galectin-3 is secreted over a period of 3 h relative to the cytoplasmic galectin-3. To do this, 435 V cells were allowed to adhere and spread in complete medium in 12-well microtiter plate (2.5×10^5 cells/ml; 200 μl/well) in the absence or presence of 2.5 mM EDTA for 3 h. At the end of the incubation period, the conditioned medium was centrifuged to pellet floating cells and the relative concentration of galectin-3 in the conditioned medium determined by Western blot as described above. The same number of

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