Molecular and Cellular Endocrinology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

The follicular thyroid cell line PCCL3 responds differently to laminin and to polylaminin, a polymer of laminin assembled in acidic pH

C.Y. Palmero [a,1], L. Miranda-Alves [a,1], M.M. Sant'Ana Barroso [b], E.C.L. Souza [c], D.E. Machado [a], A. Palumbo-Junior [a], C.A.N. Santos [a], D.M. Portilho [a], C.S. Mermelstein [a], C.M. Takiya [b], D.P. Carvalho [c], C. Hochman-Mendez [b], T. Coelho-Sampaio [b], L.E. Nasciutti [a,*]

[a] Laboratory of Cellular Interactions, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Brazil
[b] Laboratory of Extracellular Matrix Biochemistry, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Brazil
[c] Laboratory of Endocrine Physiology Doris Rosenthal, Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 14 January 2013
Accepted 23 May 2013
Available online xxxx

Keywords:
Thyroid
PCCL3 cells
Laminin
Polylaminin
Iodide uptake
Cell spreading

ABSTRACT

The extracellular-matrix protein laminin forms polymers both *in vivo* and *in vitro*. Acidification of pH leads to the formation of an artificial polymer with biomimetic properties, named polylaminin (polyLM). Follicle cells in the thyroid are in close contact with laminin, but their response to this important extracellular signal is still poorly understood. PCCL3 thyroid follicular cells cultured on glass, on regular laminin (LM) or on laminin previously polymerized in acidic pH (polyLM) showed different cell morphologies and propensities to proliferate, as well as differences in the organization of their actin cytoskeleton. On polyLM, cells displayed a typical epithelial morphology and radially organized actin fibers; whereas on LM, they spread irregularly on the substrate, lost cell contacts, and developed thick actin fibers extending through the entire cytoplasm. Iodide uptake decreased similarly in response to both laminin substrates, in comparison to glass. On both the LM and polyLM substrates, the expression of the sodium iodide symporter (NIS) decreased slightly but not significantly. NIS showed dotted immunostaining at the plasma membrane in the cells cultured on glass; on polyLM, NIS was observed mainly in the perinuclear region, and more diffusely throughout the cytoplasm on the LM substrate. Additionally, polyLM specifically favored the maintenance of cell polarity in culture. These findings indicate that PCCL3 cells can discriminate between LM and polyLM and that they respond to the latter by better preserving the phenotype observed in the thyroid tissue.

1. Introduction

The thyroid gland produces thyroid hormones (triiodothyronine, T3 and thyroxin, T4) and calcitonin in two different cell types, the thyroid follicle cells (TFCs) and the parafollicular or C cells, respectively (De Felice and Di Lauro, 2004). In the thyroid gland the structural and functional units, called follicles, are embedded in the interfollicular extracellular matrix (ECM) (Capen, 1991). Although thyroid-stimulating hormone (TSH) is the major regulator of thyroid cell proliferation, differentiation, and function, including iodide uptake (Vassart and Dumont, 1992), some evidence indicates that ECM components can also affect cell behavior during morphogenesis and malignant transformation (Toda et al., 2001). For instance, three-dimensional collagen matrices or alginate scaffolds have been shown to induce folliculogenesis (Garbi et al., 1984; Tognella et al., 1999) of normal follicle cells. Cell proliferation and survival have also been demonstrated to depend on contact with ECM proteins such as fibronectin and collagens (Vitale et al., 1997, 1998; Fragman et al., 2006). Tumorigenesis has been associated with the alteration in expression of ECM components such as fibrillin, thrombospondin-1 or their receptors (Tseleni-Balafouta et al., 2006; Ghoneim et al., 2008; Nucera et al., 2010).

In the thyroid gland, the basal side of the follicle cells is in close contact with a specific type of ECM, the basement membrane. The basement membrane consists mainly of laminin, type IV collagen, entactin/nidogen and heparan sulfate proteoglycans (Martin and Timpl, 1987; Yurchenco et al., 2004), whereas the former provides most of its signaling properties (Miner and Yurchenco, 2004). Laminins are composed of three different polypeptide chains, termed α, β and γ, which in combination will give rise to 18

* Corresponding author. Address: Programa de Pesquisa em Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Cidade Universitária – Ilha do Fundão – CEP 21941–590 Rio de Janeiro, RJ, Brazil. Tel.: +55 21 25626476.
E-mail addresses: luiz.nasciutti@icb.ufrj.br, luiz.nasciutti@histo.ufrj.br (L.E. Nasciutti).
[1] These authors contributed in the same manner to this article.

0303-7207/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.mce.2013.05.020

isoforms (Aumalley et al., 2005; Durbeej, 2010). The basement membrane of thyroid follicles can express two isoforms, laminin-111 and laminin-211, which are apparently co-localized (Andre et al., 1994). In an *in vitro* study, Toda et al. (1995) demonstrated that laminin stimulates the production of triiodothyronine more than other protein substrates such as collagen I, collagen IV and fibronectin. To our knowledge, this is the only study that examined the effect of laminin on normal thyroid cells, although the possibility that laminin plays a role in thyroid tumors has also been investigated. In a rat model of thyroid carcinogenesis, the expression of laminin proved to be inversely correlated with malignant progression (Lu et al., 2000). In addition, it has been reported that a progressive loss of laminin in the follicle basement membrane occurred upon tumor development in the Wag/Rij rat model of human medullary thyroid carcinoma (Lekmine et al., 1999).

Laminin forms sheet-like polymers within the basement membrane, and these polymers are thought to convey specific signaling to the contiguous cells (Miner and Yurchenco, 2004). Freire and Coelho-Sampaio (2000) showed that in acidic pH, laminin underwent polymerization in a cell-free environment independently of the protein concentration. Later, it was demonstrated that these laminin polymers formed in acidic pH (polyLM), were biomimetic in the sense that they exactly reproduced the structure of polymers that are naturally assembled by cells (Barroso et al., 2008). In the nervous system, polyLM has been shown to provide different cellular responses from those elicited by non-polymerized laminin, both *in vitro* (Freire et al., 2002) and *in vivo* (Menezes et al., 2010).

In view of the paucity of reports assessing the effects of laminin on normal follicle cells and the availability of a newly developed biomimetic tool to study cellular responses to laminin, in the present study we compared the overall morphology, actin fiber organization, cell proliferation and iodide uptake; and analyzed the expression and intracellular distribution of sodium iodide symporter (NIS) in PCCL3 follicle cells. These cells were seeded on uncoated glass coverslips, or on coverslips with laminin or polylaminin. Our data indicated that PCCL3 cells are capable of discriminating between the three substrates, which underscores the importance of the ECM environment for the biology of the thyroid gland.

2. Materials and methods

2.1. PCCL3 cell culture

The Fischer rat thyroid cell line, PCCL3, kindly provided by Dr. Roberto Di Lauro (Stazione Zoologica Anton Dohrn, Naples, Italy), was grown in Ham's F12 medium (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 5% FBS (Laborclin, Paraná, Brazil), 1 mM non-essential amino acids (Life Technologies, Carlsbad, CA, USA), 10 mM glutamine (Sigma–Aldrich), 100 U/mL penicillin/100 μg/mL streptomycin (Sigma–Aldrich) and a six-hormone mixture (6H: Sigma–Aldrich) containing insulin (1.3 μM), hydrocortisone (1 μM), transferrin (60 pM), l-glycyl-histidyl-lysine (2.5 μM), somatostatin (6.1 nM), and thyroid-stimulating hormone (TSH: 1 mU/mL), as reported previously (Ambesi-Impiombato et al., 1980; Weiss et al., 1984). Cells were cultured in a humidified atmosphere of 95% air 5% CO_2 at 37 °C. At confluence, cells were detached with 0.25% trypsin/EDTA (Sigma–Aldrich) and replated in new flasks after 1:3 dilution.

2.2. Laminin-coated coverslips

Matrices of laminin were prepared as previously described (Freire et al., 2002; Barroso et al., 2008). Briefly, laminin was diluted to a final concentration of 50 μg/mL in either 20 mM sodium acetate, pH 4 (polyLM) or 20 mM Tris–HCl, pH 7 (LM), both containing 1 mM $CaCl_2$. Aliquots of 100 μL were placed on pre-washed 13 mm diameter glass coverslips and incubated overnight at 37 °C. The coverslips were carefully washed three times with PBS, pH 7.0 and used as a substrate for cell plating.

2.3. PCCL3 cell culture on laminin substrates

PCCL3 cells were seeded at a density of 10^5 cells/well on either plain glass coverslips (GL) or coverslips previously coated with LM or polyLM, and placed on 24-well flat-bottom plates. Cells were grown in Ham's F-12 medium, following Coon's modification, supplemented with 5% FBS (Laborclin), and the six-hormone mixture. Cultures were maintained for 24, 48 and 72 h, and changes in cellular morphology on each substratum were observed by phase-contrast microscopy (Olympus CKX41 microscope) (40×/0.55 objective lens).

2.4. Immunocytochemistry

In order to observe actin distribution, cells grown for 24, 48 or 72 h on each substrate were fixed with 4% paraformaldehyde (PFA) (Sigma–Aldrich) plus 4% sucrose in Sorensen's phosphate buffer (0.1 M, pH 7.4) at room temperature for 15 min and washed in 0.01 M phosphate buffer saline (PBS) pH 7.5. After washing, cells were incubated in 50 nM NH_4Cl in PBS for 30 min, and then permeabilized in 0.1% Triton X-100 in PBS for 30 min. Cells were then incubated for 60 min with Cy3-conjugated phalloidin (1:50; Sigma–Aldrich) at room temperature.

To observe extracellular laminin, cells were seeded on glass coverslips for 3 or 4 days in the absence or in the presence of 1 mU/mL of bovine TSH. Cells were fixed with 4% PFA for 15 min and blocked for non-specific binding with 5% BSA and 10% normal goat serum for 1 h at 37 °C. The membrane permeabilization step was not performed. Cells were incubated overnight with anti-rabbit laminin antibody at 4 °C (LM; 1:100; Sigma–Aldrich). For visualization of NIS and ZO-1 proteins, PCCL3 cells cultured on the different substrates for 72 h were fixed with PFA for 15 min, washed with PBS, and incubated in 50 nM NH_4Cl. Then the cells were incubated for 30 min with a solution of 5% BSA plus 0.1% gelatin in PBS. After permeabilization, PCCL3 cells were incubated overnight with a rabbit anti-NIS polyclonal antibody at 4 °C (the anti-NIS antibody was a generous gift from Dr. Roberto Di Lauro) or with a rabbit anti-ZO-1 polyclonal antibody (Invitrogen). After the primary antibody reaction, cells were incubated with a fluorescence-conjugated secondary antibody for 2 h in a humidified chamber at room temperature. The secondary antibody was goat anti-rabbit IgG Alexa 546 (Life Technologies). Coverslips were mounted directly on N-propyl gallate and inspected by means of a Zeiss Axiovert 100 microscope. DAPI (4′,6-diamidino-2-phenylindole dilactate; Sigma–Aldrich) was used for nuclear staining. The images in Fig. 4A–I (40×/0.75 objective lens) were obtained on a Nikon Eclipse E800 microscope coupled to an Evolution VF cooled-color camera (Media Cybernectics), and processed using Q Capture software. Fig. 5A and B (60×/1.40 oil objective lens) were acquired in a Nikon Eclipse TE300 microscope coupled to a Cool SNAP-Pro color digital camera (Media Cybernetics). Finally, the images in Figs. 7B–D and 8C–K (63×/1.4 oil objective lens) were taken using a Leica TCS SP5 confocal microscope using LAS AF software. Fig. 8 was processed using the software Imaris (Bitplane AG Imaris 7.2.3, free version), through a deconvolution tool coupled with a surface analysis. In this figure, only nuclei (blue) of images F–H were deconvoluted, whereas in images I–K both stains (anti-ZO and nuclei) were processed by deconvolution. All other images were processed using Image J version 1.45s (Wayne Rasband, USA).

Please cite this article in press as: Palmero, C.Y., et al. The follicular thyroid cell line PCCL3 responds differently to laminin and to polylaminin, a polymer of laminin assembled in acidic pH. Molecular and Cellular Endocrinology (2013), http://dx.doi.org/10.1016/j.mce.2013.05.020