Detection of sialic acids on the cell surface using flow cytometry

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Running Head: Sialic acids staining of monkey mesenchymal stem cells (54 character)

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Abstract

We described a method to detect α2-3 linked and α2-6 linked sialic acids on the cell surface with using flow cytometry. Cells were fixed with 4% paraformaldehyde, and then α2-3 and α2-6 sialic acids were stained with biotinylated MAACKIA AMURENSIS LECTIN II (MALII) and biotinylated ELDERBERRY BARK LECTIN (SNA), respectively. Sialic acids on the cell surface were cleaved by sialidase in acetate buffer at pH 5.5 to confirm the specificity of staining. Streptavidin conjugated with Alexa flour 488 was used to detect biotinylated lectins. Thus, the α2-3 linked and α2-6 linked sialic acids on the cell surface were semi-quantitatively detected by flow cytometry.

Key words: Sialic acid, α2-3 linkage, α2-6 linkage, flow cytometry, MALII, SNA

1 Introduction

Sialic acids are neuraminic acid derivatives consisting of a nine-carbon sugar neuraminic acid. More than 50 sialic acid forms have been found in nature [1,2]. The sialic acids in the sugar chain of proteins are mainly expressed on the surface of cells and play important roles as functional receptors in physiological and pathological processes, including cellular recognition, cellular development, bacterial and viral infections, and immunological functions [1-8]. Here, we described a method to detect α2-3 linked and α2-6 linked sialic acids on the cell surface using flow cytometry. The differential distribution of the α2-3 linked and α2-6 linked sialic acids is reported [9,10]. Therefore, the distinction of linkages of sialic acids on the cell surface using flow cytometry may help to distinguish cell types and examine cell function in vitro and ex vivo.

2 Materials

2.1 Cell preparation

1. Cells: In the present protocol, monkey bone marrow-derived mesenchymal stem cells (BM-MSCs) were used for sialic acid staining.

2. PBS: Dulbecco's Phosphate Buffered Saline without calcium ion.

3. 4% paraformaldehyde (4% PFA).

4. 1% BSA/ PBS: 1% w/v bovine serum albumin (BSA) in PBS with adding 0.1% w/v volume sodium azide.

5. 50 mL sterile tubes.

6. Water bath.

7. Centrifuge.

8. Cell counter.

9. 10% DMSO/FBS: 10% v/v Dimethyl sulfoxide (DMSO) in fetal bovine serum (FBS). For cell stock, if necessary. (Note 1)

2.2 Cell staining and analysis

1. Biotinylated MAACKIA AMURENSIS LECTIN II (MALII): specifically recognize α2-3 sialic acids [10]. (Note 2)

2. Biotinylated ELDERBERRY BARK LECTIN (SNA): specifically recognize α2-6 sialic acids [11]. (Note 2)

3. Sialidase from *Vibrio cholerae*: for cleaving α2-3 sialic acids. (Note 3)

4. Sialidase from *Arthobacter ureafaciens*: for cleaving α2-6 sialic acids. (Note 3)

5. Acetate buffer pH 5.5: 3.4 g sodium acetate trihydrate in 500 mL distilled water adjust to pH 5.5 with acetic acid. (Note 4)

6. Streptavidin conjugated with Alexa flour 488 (SA-Alexa 488).

7. PBS.

8. 5 mL polystyrene round-bottom tubes.

9. Paraffin film: for sealing the top of 5 mL polystyrene round-bottom tube.

10. 37 °C incubator.

11. Centrifuge.

12. Flow cytometer and analyzing software.

3 Method

Overview of the experiment is shown in Figure 1.

3.1 Cell preparation

1. Prepare at least 5×10^6 cells in a 50 mL tube.

2. Add the appropriate volume of PBS into the tube containing the cells.

3. Centrifuge to collect the cells at 1400 rpm at 4 ^{o}C and discard supernatant, then tap or vortex the tube to break the cell pellet.

4. Add 0.5 mL of 4% PFA into the tube to fix the cells then place in a water bath previously warmed at 37 ^{o}C for 10 min.

5. Add enough volume of 1% BSA/ PBS and mix well to neutralize 4% PFA.

6. Centrifuge and discard supernatant, then tap or vortex the tube to break the cell pellet.

7. Add enough volume of PBS. Then, centrifuge and discard supernatant again.

8. Option: if you would like to stop the experiment at this point, add 10% DMSO/FCS and store the cells at -80 ^{o}C. (Note 1)

3.2 Cell staining and analysis

1. Count the number of the fixed cells and dilute the cell concentration to 5×10^6 cells/mL by acetate buffer pH 5.5.

2. Aliquot 1×10^6 (= 1 mL) of the cells to five 5 mL polystyrene round-bottom tubes.

3. Add 1/100 volume (10 μL) of appropriate sialidase to negative control tubes. (Note 5)

4. After sealing the top of the tubes with paraffin films, place the tubes in an incubator warmed at 37 °C for over 20 h.

5. Add 2 mL of PBS to each tube. Then, centrifuge and discard supernatant, then tap or vortex the tube to break the cell pellet.

6. Add 5 μL of MALII, SNA or PBS to each tube.

7. Incubate the tubes for 2 h at room temperature.

8. Add 2 mL of PBS to each tube. Then, centrifuge and discard supernatant, then tap or vortex the tube to break the cell pellet.

9. Add 1 μL of SA-Alexa 488 to all tubes.

10. Incubate for 30 min on ice.

11. Add 2 mL of PBS to each tube. Then, centrifuge and discard supernatant. The procedure is repeated once more.

12. Add 250 μL of PBS and suspended cells in solution.

13. Fluorescence intensity of the stained cells is analyzed by flow cytometry. The results are shown in Figure 2.

4. Notes

1. When stopping the experiment after fixing cells by 4 % PFA, add 10 % DMSO/FCS into cell pellets and store the cells at -80 °C. When resuming, thaw cell stocks with a suitable warmed medium.
2. Biotinylated MAACKIA AMURENSIS LECTIN II (MALII) and biotinylated ELDERBERRY BARK LECTIN (SNA) should be freshly prepared for sialic acid staining.
3. Sialidase should be freshly prepared.
4. Acetate buffer pH 5.5 should be made each time.
5. Add sialidase from *Vibrio cholerae* to one tube as a negative control of MALII, and add sialidase from *Arthobacter ureafacien*s to one tube as a negative control of SNA.

Reference

1. Varki A (1992) Diversity in the sialic acids. Glycobiology 2:25-40
2. Schauer R, Kamerling JP (2018) Exploration of the Sialic Acid World. Adv Carbohydr Chem Biochem 75:1-213
3. Zhang C, Chen J, Liu Y, Xu D (2019) Sialic acid metabolism as a potential therapeutic target of atherosclerosis. Lipids Health Dis 18:173
4. Pearce OM, Läubli H (2016) Sialic acids in cancer biology and immunity. Glycobiology 26:111-128
5. Bi S, Baum LG (2009) Sialic acids in T cell development and function. Biochim Biophys Acta 1790:1599-1610
6. Anderson GG, Goller CC, Justice S, Hultgren SJ et al (2010) Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. Infect Immun 78:963-975
7. Matrosovich M, Herrler G, Klenk HD (2015) Sialic Acid Receptors of Viruses. Top Curr Chem 367:1-28
8. Lübbers J, Rodríguez E, van Kooyk Y (2018) Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. Front Immunol 9:2807

9. Sata T, Lackie PM, Taatjes DJ et al (1989) Detection of the Neu5 Ac (α 2,3) Gal (β 1,4) GlcNAc sequence with the leukoagglutinin from Maackia amurensis: light and electron microscopic demonstration of differential tissue expression of terminal sialic acid in α 2,3- and α 2,6-linkage. J Histochem Cytochem 37:1577-1588

10. Geisler C, Jarvis DL (2011) Effective glycoanalysis with *Maackia amurensis* lectins requires a clear understanding of their binding specificities. Glycobiology 21:988-993

11. Shibuya N, Goldstein IJ, Broekaert WF et al (1987) The elderberry (Sambucus nigra L.) bark lectin recognizes the Neu5Ac(α 2-6)Gal/GalNAc sequence. J Biol Chem 262:1596-1601

Figure legends

Figure 1. Overview of the procedure

(A) Cell preparation: cells are collected and fixed in 4%PFA. After fixing cells, the experiment could be suspended if necessary, and the cells could be stocked in 10% DMSO/ FBS. (B) Cell staining: the cells were divided into 5 tubes, and two out of 5 tubes are treated with sialidase for making the sialic acid-removing control (sialidase control). Then, biotinylated lectins are added two tubes including sialidase control tubes. PBS are added the remaining tube for control. After staining each sialic acid with the specific lectins, streptavidin-Alexa flour 488 is added into all tubes to detect the biotin.

Figure 2. Sialic acid staining of monkey mesenchymal stem cells (MSCs)

The monkey MSCs expressed both α2-3 linked and α2-6 linked sialic acids on the surface. The filled histograms indicate cells as a control without addition of lectins. The lines indicate cells stained with specific lectins, and the broken lines indicate cells stained with specific lectins after treatment with sialidase to remove sialic acids on the surface of the cells. The cells treated with sialidase revealed a lower fluorescence intensity than did those without treatment with sialidase, which indicates that each lectin specifically recognize sialic acids on the cell surface.