Critical functions of L-selectin-mediated lymphocyte homing and recruitment

Junya Mitoma
Tohoku Pharmaceutical University
Xingfeng Bao
Burnham Institute for Medical Research
Minoru Fukuda

Method Article

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Abstract

Introduction

To analyze glycan function, enzyme digestion is one of the easiest method. We successfully demonstrated the elimination of N-glycans, O-glycans and heparan sulfate on lymph node frozen section by N-glycanase \(N-glycosidase F), O-sialoglycopeptidase, and heparitinases, respectively. N-glycanase treatment usually needs denaturing condition such as SDS treatment, but this can be substituted with acetone treatment of the frozen section.

Reagents

N-glycosidase F, Calbiochem. O-sialoglycoprotein endopeptidase, Accurate Chemical and Scientific Corporation. Heparitinase I and II and heparinase, Seikagaku.

Procedure

Fixation 1. Fix lymph node frozen sections with acetone for 15 min at room temperature. 2. Wash the sections with PBS 3 times and treated with the following enzymes. **N-glycanase treatment** 3. Incubate the fixed lymph node frozen sections with 100 mU/ml N-glycosidase F in 10 mM HEPES-NaOH, pH 7.4, 0.1% Triton X-100, 0.1 M 2-mercaptoethanol and complete protease inhibitor \(Roche) at 37°C for 2 h. Proceed to 6. **_O_-sialoglycopeptidase treatment** 4. Incubate the sections with 0.1 ~ 1 mg/ml O-sialoglycoprotein endopeptidase[1] for in 10 mM HEPES-NaOH, pH 7.4 at 37°C for 2 h \(ref. 1). Proceed to 6. **Heparitinases** 5. Incubate the sections with 25 mU/ml Heparitinase I and II and heparinase in PBS containing 1 mg/ml BSA, 1 mM $CaCl_2$ and the protease inhibitor at 37 °C for 2 h. Proceed to 6. **Washing** 6. The digested sections were washed with PBS containing 0.1 mg/ml BSA at least 3 times and subjected to further staining. **Staining** 7. Stanadard immunostaining technique using antibodies specific for appropriate glycans can be done to evaluate the digestions. Lectin staining including L/E/P-selectin-IgM staining may be also applicable[2].

Timing

5 hrs

Critical Steps

The section should not be dried. Keep the sample in a moisture chamber

Troubleshooting

When the sections are treated with N-glycanase, weak L/E-selectin staining might persist. In that case, fix the section with 4% paraformaldehyde at room temperature for 15 min, denature proteins with 2% SDS and 5% 2-mercaptoethanol at 65 °C for 10 min. For O-sialoglycopeptidase treatment, it is very difficult to obtain the best results and trial and error may be necessary.

Anticipated Results

N-glycanse treatment removes all E/L-selectin-IgM binding in O-glycan deficient mice[2]. O-sialoglycopepeptidase digestion partially cleaves O-glycans but not always complete. Digestion with heparitinase I and II with heparinase eliminates all heparan sulfate as judged by staining with monoclonal antibody 10E4 \(ref. 2).

References

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