Kawasaki Disease on PDGF Expression and VSMC Proliferation

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Summary: The effect of serum of patients with Kawasaki disease (KD) on expression of platelet-derived growth factor (PDGF) B chain protein in vascular endothelial cells (VEC) was studied by immunocytochemical method. Meanwhile, the effects of the endothelial cell conditioned media (ECM) on expression of PDGF receptor mRNA in vascular smooth muscle cells (VSMC) and on cell cycle of VSMC were investigated by the methods of nucleic acid hybridization and flow cytometry (FCM). The results showed that the serum of patients with KD induced the expression of PDGF-B chain protein significantly. ECM significantly promoted the expression of PDGF receptor mRNA and induced the proliferation of VSMC. These data suggest that the activation of PDGF-PDGF receptor may play a role in the pathogenesis of coronary complication of KD.

Key words: Kawasaki disease; platelet-derived growth factor; platelet-derived growth factor receptor; smooth muscle cell proliferation

It has been established that the platelet-derived growth factor (PDGF) in the body is involved in the process of growth, inflammatory reaction and injurious repair. PDGF and PDGF-receptor (PDGF-R) participates in pathological process changes of some auto-immune disease\(^\text{[12]}\). In this study, the effect of serum of patients with Kawasaki disease (KD) on expression of PDGF-B chain protein in vascular endothelial cell (VEC) was studied with immunocytochemical method. Meanwhile, the effects of the endothelial cell conditioned media (ECM) on expression of PDGF-R mRNA in vascular smooth muscle cell (VSMC) and on cell cycle of VSMC were investigated by using the methods of nucleic acid hybridization and flow cytometry (FCM). The purpose of this work is to further understand pathogenesis of coronary complication of KD at molecular level.

1 MATERIALS AND METHODS

1.1 Reagents

Serum-free medium (DME/F12), M199

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medium, infantile bovine serum and fetal bovine serum were bought from Sigma. PDGF-B monoclonal antibody was kindly provided by Dr. Sugihara, Japanese Mochida Pharmaceutical Inc., Japan. LSAB Immunocytochemical Detection Kit was procured from Zymed Corporation. PDGF-R 1 subtype gene probe (cDNA, 4.7 kp) was purchased from Oncogene Inc.

1.2 Preparation of KD Serum Sample

For this study, blood samples were collected from 3 patients with KD of acute stage, and 3 healthy children. 4 ml of venous blood was separated regularly and stored at \(-40\) C. Prior to assay, the specimens were melted and divided into two groups, which were KD serum group and normal serum group. All samples were isolated by micropore filter in order to remove bacteria.

1.3 Culture of Umbilical Vein Endothelial Cells and Grouping

The primary EC culture was obtained from the healthy newborn’s umbilical cord within 4 h after delivery\(^\text{[2]}\). The EC were identified by the presence of VII factor with immunofluorescence method. They were then classified into two groups: control group (M199 medium, 10 % infantile bovine
serum, 10 % normal serum) and experimental group (M_{19} medium, 10 % infantile bovine serum, 10 % KD serum).

A cover-slip was placed into the each bottle of cell culture for the purpose of bringing EC to grow on the surface of the cover-slip. After EC reached submerge, the cover-slips were taken out. Then, the specimens were fixed by cold acetone for 15 min and stored at −20 °C for assay.

1.4 Preparation of ECM

The prior media were shifted into DME/F_{12} after EC reached submerge and EC were cultured for another 24 h. The media collected were ECM.

1.5 Culture of Rabbit's VSMC

The primary cell culture and subculture were performed on Japanese big ear white rabbit's aortic median VSMC[3]. The media were removed after the well-grown fourth generation EC reached submerge and the cells were cultured in DME/F_{12} for another 24 h. The cell cycle was synchronized thereby. The cells were then subdivided into normal serum plus EMC group and KD serum plus EMC group. ECM was individually added to each cultured bottle at the same time. The cells were cultured for another 10 h, and then collected for FCM analysis and nucleic acid hybridization test.

1.6 Immunocytochemical Staining

The EC culture slide was stained with immunocytochemical technique according to LSAB kit instruction. The titer of work solution of PDGF-B monoclonal antibody was 1:500. PBS was used for primary antibody negative control. The mean optic density (OD) of PDGF-B expressive intensity in each group was estimated by using TJTY-300 image analyzer.

1.7 Northern Blotting Hybridization and Slit Hybridization\[4\]

Total RNA of each group was extracted from cells according to guanidine thiocyanate one-stage assay, and then subjected to agarose electrophoresis. The gel was transferred to nitrocellulose filter. The RNA extracted was applied to nitrocellulose by using slit suction sample application apparatus. The PDGF-R B nucleic acid probe was labeled with μ-\(^{32}\)P-dCTP by random primer. The Northern blotting hybridization were performed and then, the integral OD was measured for PDGF-R B mRNA positive expression in each group by using TJTY-300 image analyzer.

1.8 Flow Cytometric Measurement of Proliferation Cycle of VSMC

The fourth generation EC were collected and fixed by 70 % alcohol. The cellular density was adjusted to \(4 \times 10^7/\text{ml}\). 1 ml of the resultant cell suspension was taken, to which 0.5 ml of 0.5 % propidii iodide (PI) was added. The cells were stained in the refrigerator for 30 min with immunocytochemical technique in order to determine cellular differentiation. The DNA content of VSMC was measured with 6 duplications by using EPICS-751 FCM apparatus. The specimens of each group were counted for 1000 cells each time. The results were expressed by cell number in each phase of cell cycle.

1.9 Statistical Analysis

All values were expressed as \(\bar{x}\pm s\). Difference between the experimental group and control group were assessed by student's t test.

2 RESULTS

Staining of the umbilical venous EC PDGF-B was positive for cultures of each group. The positive signal was shown as yellow brown fine and dense particles. In the control group, PDGF-B located at the cytoplasm around the nucleus and mean OD was 0.0710±0.0091 (n=30); in the experimental group, the PDGF-B expression was increased significantly, as shown by diffuse staining within the whole cytoplasm, and the nucleus was also stained positively, but to a less extent. The mean OD was 0.2467±0.0340 (n=30) in experimental group. The mean OD in experimental group was much higher than that in control group (\(P<0.001\), fig. 1-2, table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>PDGF OD</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>30</td>
<td>0.0710±0.0091</td>
</tr>
<tr>
<td>KD group</td>
<td>30</td>
<td>0.2467±0.0340</td>
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</tbody>
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\(\ast P<0.01\) as compared with control group