Cyclin-dependent kinase 5 negatively regulates LPS-induced inflammation in Human Peritoneal Mesothelial Cells

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OBJECTIVES

- Peritoneal mesothelial cells (PMCs) play an important role in peritoneal inflammatory and immune response.
- Cyclin-dependent protein kinase-5 (Cdk5), an unusual member of the Cdk family, does not regulate cell cycle. CDK5 has been reported to be involved in the regulation of inflammation response, but its expression and function in the PMCs has been rarely reported.
- This study was aimed to investigate the expressional level of Cdk5 in human PMCs and the effect of Cdk5 on the lipopolysaccharide (LPS)-induced inflammation through evaluating the production of IP-10, IL-1β and TNFα.

METHODS

- Human PMCs were maintained under defined *in vitro* conditions.
- Expression of Cdk5 in human PMCs was determined Western Blot and immunofluorescence assay.
- To observe the effect of LPS on the expression of Cdk5, the PMCs were exposed to LPS for 6 hours at different concentrations (0.01, 0.1, 1, 10 and 100 μg/ml) or treated with LPS at concentration of 1 μg/ml for different timescales (0, 0.5, 1, 2, 3, 6, 12, 24, 36h), respectively. Western Blot was used to detect the expression of Cdk5.
- In addition, PMCs were treated with LPS combined with different concentrations of 1NMPP1 (0.1, 1.0 and 10 μmol/L), an inhibitor of Cdk5. ELISA was used to detect the expressions of inflammatory factors TNFα、IL-1β and IP-10.

RESULTS

Figure 1. **Western Blot showed that human PMCs could express Cdk5 and its co-activated protein, p25/p35(A). Cdk5 mainly localized in the cytoplasm and its expression was significantly increased after LPS treatment determined by immunofluorescence assay (B and C).**

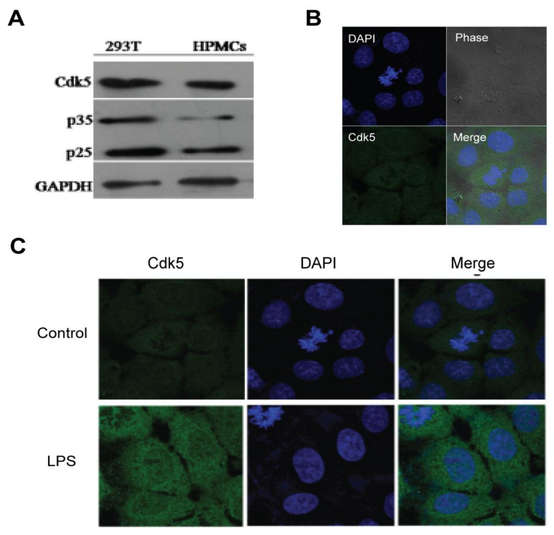

Figure 2. **LPS treatment resulted in the increase of Cdk5 expression in a concentration-dependent manner with the peak at 1 μg/ml (A), and in a time-dependent manner with the peak at 3～12 h(B) (including p25/p35 protein, figure not showed here).**

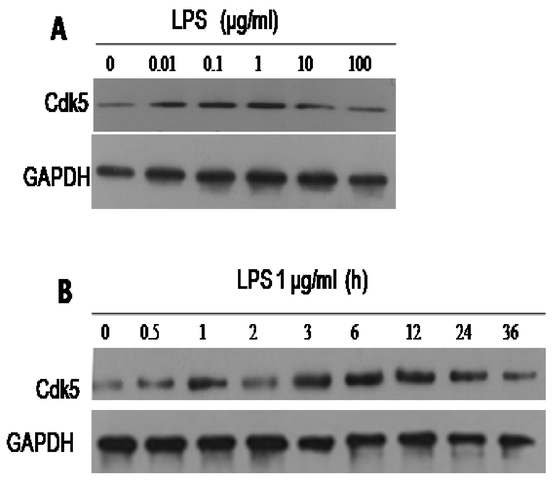

Table. **The secretions of inflammatory cytokines including TNFα, IL-1β and IP-10 after LPS (1μg/ml) treatment was significantly elevated using ELISA assay, and 1NMPP1 through inhibiting Cdk5 strongly suppressed these secretions.** (*compared to Control group, p<0.05; **compared to LPS group, p<0.05)

Factors Groups	TNFα (pg/ml)	IL-1β (pg/m)	IP-10 (pg/ml)
Control	30.64±6.51	7.18±3.29	25.82±5.54
LPS	319.01±22.09*	74.61±6.18*	288.61±29.58*
1NMPP1-0.1uM	215.97±18.95**	55.76±2.86**	183.67±7.63**
1NMPP1-1.0uM	174.93±14.52**	38.84±2.85**	102.50±7.73**
1NMPP1-10uM	76.63±8.20**	24.09±3.35**	28.53±4.23**

CONCLUSIONS

Cdk5 and its co-activated protein, p25/p35 are constitutively expressed in human PMCs cells. LPS stimulation could up-regulate the expression of Cdk5 and promote inflammation. Inhibition of Cdk5 down-regulates the secretion of inflammatory cytokines. These results suggested that Cdk5 might take part in the local defense of the peritoneal cavity by up-regulating inflammatory mediators, and that Cdk5 might play a potential role in peritoneal fibrosis induced by peritonitis.

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