1. **No.**

   FP01802040

2. **Title**

   The Role of nAChR in the Regulation of Intestinal Barrier Function

3. **Period**

   2016-2018

4. **Summary**

We employed the monolayer cultures of HT-29/B6 cell, a human colon cancer cell line, as a model of colonic epithelial tissue. Immunoblot analyses revealed that nAChR stimulation caused the phosphorylation of three MAP kinases, ERK, JNK and p38, which were suppressed by the each kinase-specific inhibitor, U0126（U0）/ERK, SP600125（SP）/JNK, SB203580（SB）/p38. These three inhibitors blocked nAChR-induced FAK activation. JNK inhibition by SP resulted in an enhancement of the nAChR-induced secretion by the colonic epithelial cells. These results suggest that three MAP kinases are involved in the nAChR-induced FAK activation and that JNK negatively regulates the nAChR-induced secretion.

The epithelial barrier function in monolayer cultures of HT-29/B6 cell is estimated by measuring trans-epithelial electrical resistance (TER) in a tumor necrosis factor alpha (TNF)-induced inflammation model of colonic epithelial cells. TNF induced the reduction of TER which was ameliorated by the stimulation of nAChR. The amelioration was canceled by the addition of SB. It has been suggested that nAChR induces p38 activation which further activates TNF-α converting enzyme（TACE）to cleave TNFR, leading to the downregulation of TNF signaling and the amelioration of barrier damage.

The nAChR-induced phosphorylation of ERK was suppressed by the addition of TACE inhibitor TAPI-0, or SB, or EGFR-specific inhibitor AG1478. The stimulation of nAChR leads to p38 activation which further activates TACE to liberate cell surface EGFR ligands. EGFR, then, induce the phosphorylation of ERK.

These results suggest that nicotinic signaling system may enforce intestinal epithelial barrier function against inflammatory barrier disruption and may prevent inflammatory expansion at the same time, and also might contribute to the maintenance and regeneration of the colonic epithelial tissue via EGFR signaling.