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Adaptor Protein Regulation of T-Lymphocyte Migration

Background
T-lymphocyte migration is central to the adaptive immune response, which is dependent mainly on the integrin LFA-1-mediated signalling. LFA-1 interacts with its ligand ICAM-1 (expressed on endothelium) and this interaction triggers cytoskeletal rearrangement and T-cell motility.

Aim
To investigate the role of CG-NAP in T-lymphocyte migration.

Methodology
1. LFA-1/ICAM-1-induced migration-triggering model system using the human T-cell line HuT78
2. Phase-contrast microscopy
3. Immunofluorescence staining and confocal microscopy
4. Flow-cytometry
5. SDS-PAGE and Western Immunoblotting
6. siRNA-mediated gene knockdown by nucleofection

Results
1. The adaptor protein CG-NAP is expressed in HuT78 T-cells
Cellular lysates obtained from 0.1, 0.5 or 1 million HuT78 cells were resolved by SDS-PAGE and Western immunoblotted with anti-CG-NAP or anti-GAPDH (loading control).

2. CG-NAP is localized to the centrosomal region in T-cells
Both resting and LFA-1-stimulated HuT78 cells were examined for cellular localization of CG-NAP by confocal microscopy. CG-NAP was found to be co-localized with the microtubule-organizing centre (MTOC) in the centrosomal region.

3. Delivery of siRNA into T-cells by nucleofection
Nucleofection method was optimized to deliver siRNA into T-cells. HuT78 cells (1x10^6) were nucleofected with 100 nM DY-547-labelled non-specific siRNA, incubated for 6, 24, 48 and 72 h and subsequently transfection efficiency was analysed by flow-cytometry.

4. siRNA-mediated knockdown of CG-NAP in T-cells
HuT78 cells were nucleofected with non-specific (NS) or CG-NAP-targeted siRNA and the expression levels of CG-NAP, α-Tubulin and GAPDH were analysed after 24 h (a) and 48 h (b) by Western immunoblotting.

5. Knockdown of CG-NAP inhibits T-cell migration
siRNA-mediated knockdown of CG-NAP expression in HuT78 T-cells inhibited LFA-1-induced migratory phenotypes.

6. Knockdown of CG-NAP interferes with α-Tubulin polymerization
siRNA-mediated knockdown of CG-NAP interfered with LFA-1-induced cytoskeletal remodelling in HuT78 cells. Shown below are representative confocal images of cells immunostained for α-Tubulin and actin.

Conclusion
1. CG-NAP is expressed in HuT78 T-cells and is localized to the centrosomal region in both resting and migrating cells
2. siRNA-mediated CG-NAP knockdown inhibits LFA-1-induced cytoskeletal remodelling and migration of T-cells.

Future works
1. Further mechanistic study using human primary T-lymphocytes
2. Investigate detail molecular mechanism involved in the CG-NAP functions.