IJAE
Vol. 122, n. 1 (Supplement): 78, 2017
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

SH3BGRL3 binds myosin 1c and is involved in MDA-MB-231 cell migration

Filippo Di Pisa[1], Elisa Pesenti[1], Maria Bono[1], Claudya Tenca[1], Andrea Scaloni[3], Silvia Bruno[1], Franco Fais[1,2], Paolo Scartezzini[1], Fabio Ghiotto[1,2]

[1] Department of Experimental Medicine (DIMES), University of Genoa, Genoa, Italy
[2] Molecular Pathology Unit, IRCCS Policlinico S. Martino, Genoa, Italy
[3] Institute for the Animal Production System in the Mediterranean Environment (ISPAAM) and CNR, Naples, Italy

SH3BGRL3 is a gene belonging to SH3BGR family, it is ubiquitously expressed and encodes for a 93 AA thiorerodoxin-like protein evolutionarily conserved. A proteomic study reported that SH3BGRL3 binds the cytoplasmatic domain of ERBB2 receptor. On this basis we performed immuno-staining experiments in FLAG-SH3BGRL3 transfected SKBR3 cell line that showed SH3BGRL3 and ERBB2 co-localization. Nonetheless, co-immunoprecipitation (Co-IP) of ERBB2 using FLAG-SH3BGRL3 as bait and vice versa was not achievable. Therefore, to investigate SH3BGRL3 potential interactors we performed Co-IP experiments from SKBR3 lysates transfected with FLAG-SH3BGRL3 followed by mass spectrometry analysis. The results revealed myosin 1c (Myo1c) as a candidate interactor. Subsequent Co-IP experiments followed by WB analysis validated the interaction between the two proteins. To map the interaction site we performed Co-IP experiments using SKBR3 cells co-transfected with FLAG-SH3BGRL3 and HA tagged deletion mutants of Myo1c that showed SH3BGRL3 binding to the neck region of Myo1c. Since Myo1c neck region binds calmodulin in a Ca^{2+} dependent way, we assessed if the binding was Ca^{2+} dependent also for SH3BGRL3. The experiments showed that SH3BGRL3 binds the Myo1c neck in presence of Ca^{2+}, differently from calmodulin that binds it in absence of Ca^{2+}.

Myo1c is a motor protein involved, among its different functions, in cell membrane dynamics. Thus we investigated SH3BGRL3 involvement in cell migration using MDA-MB-231 cell line. We transfected MDA-MB-231 cells with FLAG-SH3BGRL3 and performed immuno-staining and Co-IP experiments that showed co-localization and interaction of Myo1c and SH3BGRL3. Accordingly, we performed migration assays using boyden chambers after silencing or not SH3BGRL3 expression by means siRNAs. The results showed a statistically significant decrease in migration capacity of silenced cells respect to controls. Our data show that SH3BGRL3 binds Myo1c neck region in a Ca^{2+} dependent way and that this interaction is involved in cell migration in our model.

Keywords
Protein interaction, cell migration