

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

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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<sup>2+</sup> dependent way, we assessed if the binding was Ca<sup>2+</sup> dependent also for SH3BGRL3. The experiments showed that SH3BGRL3 binds the Myo1c neck in presence of Ca<sup>2+</sup>, differently from calmodulin that binds it in absence of Ca<sup>2+</sup>.

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<sup>2+</sup> dependent way and that this interaction is involved in cell migration in our model.

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### Keywords

Protein interaction, cell migration