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RAC1 SUMOylation is required for cell invasion in triple negative breast cancer cells.

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Introduction

Posttranslational modifications are one of the most important mechanisms of cellular protein regulation. To date, more than 450 protein modifications have been described, including phosphorylation, glycosylation, acetylation, Ubiquitination and SUMOylation, which can alter the activity of the target protein through the change in cell localization, protein-protein interaction or protein stability, ensuring a rapid and dynamic response of cells to intra and extra-cellular stimuli [1].

SUMOylation has become one of research hotspots in recent years and it has been show to play essential roles in various biological functions. The imbalance of SUMOylation and deSUMOylation is highly associated with the occurrence and various diseases, including cancers. Interestingly, it has been reported that SUMOylation regulates the epithelial-mesenchymal transition (EMT), which is an important program for cancer cell dissemination.

The Rho family of small G-proteins are members of the Ras superfamily that play an important role in metastasis by enhancing tumor cell motility and invasion. Specifically, RAC1 and CDC42 are RHO family members that regulates the cytoskeleton-dependent processes during the cell migration. Activated RAC1 can regulate multiple cellular events including cytoskeleton dynamics to maintain cellular morphology, polarity, adhesion and migration, cell cycle, gene expression and apoptosis [2]. All these functions are of great importance in the pathogenesis and tumor evolution, as well as in the processes of cell dissemination or metastasis[3,4].

Previous work by the group identified Ubiquitin-like (Ubl)-type modifications of RAC, including ubiquitylation and SUMOylation, in response to cell migration. RAC1 is SUMOylated by SUMO1 and this modification is required for the maintaining of the active form during cell migration required for EMT initiation of epithelial cells [5].

Objectives

Our main goal is to study the role of RAC1-SUMO1 on cancer dissemination using breast cancer as model.

Methodology

The triple negative breast cancer cell line MDA-MB-231 was treated with an inhibitor of SUMOylation machinery: the monomer C15:1 of the Ginkgolic acid (GA). Specifically, this compound is able to inhibit SUMOylation through its binding to E1 activating enzyme, blocking the SUMO conjugation cascade in the first step.

Results

It has been suggested that the inhibition of the SUMO machinery reduced cell invasion. Thus, we treated the triple negative breast cancer cell line MDA-MB-231 with 10 µM of GA (a concentration that does not affect cell viability) and analysed the implication on cell invasion. We observed that impairing 50% the total SUMOylation was enough to reduce 50% its invasive capacity. Furthermore, treatment with GA for 24 hours reduce dramatically the activation of RAC1 (RAC1-GTP). Moreover, confocal microscopy images revealed a non-migratory phenotype in cells treated with GA, similar to the one observed when cytoskeleton organization is inhibited through RAC1 deactivation.

In order to confirm if these effects were due to the GA-induced inhibition of the SUMOylated form of RAC, MDA-MB-231 cells were transfected with plasmids expressing GFP, GFP-RAC1 or a RAC1 chimaera that mimics constitutive RAC1 SUMOylation (GFP-SUMO1-RAC1) prior to treatment with GA. Interestingly, whereas the transfection with the wilt type form of RAC1 did not rescue the invasive phenotype, GFP-SUMO1-RAC1 transfected cells rescued the invasiveness of untreated cells. Furthermore, whereas transfection with wild-type RAC1 did not

restore the migratory phenotype, SUMO1-RAC1 rescued around 60% of the lamellipodia-ruffle formation that was lost by GA treatment alone. Interestingly, the SUMO1-RAC1 chimaera was not able to rescue the proliferative phenotype observed in response to GA.

Conclusions

We demonstrate that SUMOylation of the GTPase RAC1 is necessary to promote the invasion of breast cancer cells. Furthermore, we observed that this modification does not affect other essential processes in the cell such as cell proliferation. These results establish the bases to address studies on its potential as a therapeutic target.

References

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