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SUMOylation of RhoGDIα is required for its repression of cyclin D1 expression and anchorage-independent growth of cancer cells

Zipeng Cao[a,b], *Xueyong Li*[a], *Jingxia Li*[a], *Beipei Kang*[b], *Jingyuan Chen*[b], *Wenjing Luo*[b], *Chuanshu Huang*[a,*]

[a]*Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987, USA*

[b]*Department of Occupational and Environmental Health, School of Public Health, Fourth Military Medical University, 169 Changlexi Road, Xi'an, Shannxi 710032, China*

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ABSTRACT

Selective activation of Rho GTPase cascade requires the release of Rho from RhoGDI (GDP-dissociation inhibitors) complexes. Our previous studies identified RhoGDIα SUMOylation at Lys-138 and its function in the regulation of cancer cell invasion. In the current study, we demonstrate that RhoGDIα SUMOylation has a crucial role in the suppression of cancer cell anchorage-independent growth as well as the molecular mechanisms underlying this suppression. We found that ectopic expression of RhoGDIα resulted in marked inhibition of an anchorage-independent growth with induction of G0/G1 cell cycle arrest, while point mutation of RhoGDIα SUMOylation at residue Lys-138 (K138R) abrogated this growth suppression and G0/G1 cell cycle arrest in cancer cells. Further studies showed that SUMOylation at Lys-138 was critical for RhoGDIα down-regulation of cyclin D1 protein expression and that MEK1/2-Erk was a specific downstream target of SUMOylated RhoGDIα for its inhibition of C-Jun/AP-1 cascade, *cyclin d1* transcription, and cell cycle progression. These results strongly demonstrate that SUMOylated RhoGDIα suppressed C-Jun/AP-1-dependent transactivation specifically *via* targeting MEK1/2-Erk, subsequently leading to the down-regulation of cyclin D1 expression and anti-cancer activity. Our results provide new mechanistic insights into the understanding of essential role of SUMOylation at Lys-138 in RhoGDIα's biological function.

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1. Introduction

Cell cycle progression is controlled by the expression of a family of proteins called cyclins (Sherr, 1994). Cyclin D1 is the major D type cyclin in many cell types and is usually the first cyclin to be induced when cells enter the G1 phase from quiescence (G0) (Stacey, 2003). Once expressed, cyclin D1 binds to CDK4 (cyclin-dependent kinase 4) or CDK6 to form an active holoenzyme that phosphorylates the retinoblastoma 1 (Rb1) protein. Phosphorylation of Rb1 results in its dissociation

Abbreviations: CDK, cyclin-dependent kinase; Erk, extracellular stimuli mitogen-activated protein kinase; GDIs, GDP-dissociation inhibitors; GEFs, guanine nucleotide exchanging factors; GTP, guanosine triphosphate; MEK, MAP kinase; SUMO, small ubiquitin-related modifier.

* *Corresponding author*. Tel.: +1 845 731 3519; fax: +1 845 351 2320.
E-mail addresses: Chuanshu.huang@nyumc.org, luowenj@fmmu.edu.cn (C. Huang).

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with E2Fs and then promotes transcription of E2F downstream targets, such as cyclin E and cyclin A, and further resulting in cell cycle progression (Stacey, 2003). Therefore, tight control of cyclin D1 gene expression is a crucial issue in the regulation of G1-phase progression. In non-tumor cells, the cyclin D1 gene senses the mitogenic potential of the microenvironment during cell-cycle entry from quiescence because its induction requires coordinated signaling from the extracellular matrix and soluble growth factors (Assoian and Klein, 2008). These controls can be lost during cellular transformation upon exposure to carcinogens or tumor promoters, resulting in the corresponding overexpression of cyclin D1 in a number of cancers, including those of the breast, liver, lung, and colon (Gillett et al., 1996; Yamamoto et al., 2006; Molenaar et al., 2008; Sanchez-Mora et al., 2008). Cyclin D1 levels can be regulated in a transcriptional and post-transcriptional manner (Zhang et al., 2012; Ouyang et al., 2005).

The Rho family contains 20 members (Vega and Ridley, 2008), and over 60 Rho effectors have been identified (Lu et al., 2009). The important role of the Rho family of small GTP (Guanosine Triphosphate) binding proteins in cancer development has been well established (Sahai and Marshall, 2002). The members of the Rho GTPase family are well known for their regulation of actin polymerization and cytoskeletal structures (Vega and Ridley, 2008) and they also contribute to the regulation of many different biological processes, including cell cycle progression with the best-studied members being RhoA, Rac1, and Cdc42 (Coleman et al., 2004). It has previously been shown that active Rho influences cell cycle progression via the regulation of a number of cell cycle regulatory proteins (Etienne-Manneville and Hall, 2002). One mechanism is through regulating expression and activity of CDK inhibitors, such as p27 (Hirai et al., 1997; Hu et al., 1999) and p21 (Liberto et al., 2002; Olson et al., 1998). In addition to the regulation of CDK inhibitors, Rho has been reported to influence cyclin levels. Rho and ROCK are necessary for Ras-GTP loading and lead to increased to increased cyclin D1 transcription following growth factor stimulation (Swant et al., 2005; Welsh et al., 2001). Inhibition of Rho or ROCK function inhibits cyclin A expression and blocks cell proliferation in atrial myofibroblasts (Porter et al., 2004; Croft and Olson, 2006). Moreover, Rho GTPase activity is necessary for cyclin E expression in rat astrocytes (Tanaka et al., 1998).

RhoGDIs have been identified as key regulators of Rho family GTPases as typified by their ability to prevent nucleotide exchange and membrane association (Garcia-Mata et al., 2011). In resting state, Rho family members are bound to RhoGDIs preventing the conversion from their inactive GDP bound state to the active GTP bound state (Faure and Dagher, 2001; DerMardirossian and Bokoch, 2005). There are three isoforms of RhoGDIs: RhoGDIα, β (also named D4/LyGDI), and γ. Among these RhoGDIs, RhoGDIα is ubiquitously expressed and binds to all of the Rho family proteins thus far examined (Dovas and Couchman, 2005), whereas RhoGDIβ and γ show unique tissue expression patterns, and their substrate specificities have not been precisely determined (Harding and Theodorescu, 2010). RhoGDIs regulate a multitude of cellular phenotypes including cell division, morphology, migration, vesicular trafficking and gene expression (Harding and Theodorescu, 2010). It is likely that they affect these diverse phenotypes principally by controlling the location and activity of members of the Rho family of small GTPases (Dovas and Couchman, 2005). Indeed, there is substantial biochemical and structural evidence showing that Rho GEFs (Guaninenucleotide Exchanging Factors) that cannot act on Rho GTPases form complex with RhoGDI. The GDI-GTPase complex is thus a major form of RhoGDI regulation of Rho GTPase activity and function (DerMardirossian and Bokoch, 2005).

SUMO (Small Ubiquitin-related Modifier) modification (SUMOylation) is an important post-translational protein modification that modulates the biological functions of proteins (Muller et al., 2004; Geiss-Friedlander and Melchior, 2007). SUMOylation is a highly dynamic process with a three-step reaction consisting of SUMO activation, transfer, and ligation that are catalyzed by E1 heterodimeric enzyme [SAE1 (SUMO Activating Enzyme E1)/SAE2], E2 enzyme (Ubc9), and E3 SUMO ligases (Melchior, 2000; Kotaja et al., 2002). Unlike ubiquitination, which usually facilitates protein degradation, SUMOylation results in pleiotropic functional consequences that include changes in subcellular localization, protein stability, alterations in DNA binding, and transcriptional activity (Johnson, 2004; Gill, 2005). Transcription factors, co-activators, and co-repressors are predominant targets of SUMOylation, which alters their activity and results in changes in gene expression and function (Johnson, 2004; Gill, 2005). Our most recently studies demonstrate that RhoGDIα can be SUMOylated specifically at residue Lys-138 and that this SUMOylation is crucial for RhoGDIα inhibition of cancer cell motility and invasion (Yu et al., 2012). In the current study, we aimed to investigate the potential biological role of RhoGDIα SUMOylation at Lys-138 in RhoGDIα regulation of cancer cell anchorage-independent growth and that molecular mechanisms that lead to this regulation. We found that loss of RhoGDIα SUMOylation by specific K138R point mutation of RhoGDIα at Lys138 impaired its ability to repress cyclin D1 expression and attenuated its induction of G0/G1 growth arrest and inhibition of anchorage-independent growth. Further studies demonstrated that the suppression of RhoGDIα SUMOylation at Lys-138 was mediated by its inhibition of MEK1/2/Erk/AP-1 cascade.

2. Materials and methods

2.1. *Cell culture and plasmids*

HCT116 cells and its transfectants, including HCT116 (GFP-vector), HCT116(RhoGDIα-WT), HCT116(RhoGDIα-K105R), and HCT116 (RhoGDIα-K138R) were established in our previous studies (Yu et al., 2012). The HCT116 cells were cultured in McCoy's 5A medium (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 2 mM L-glutamine (Life Technologies, Inc., Rockville, MD) at 37 °C in 5% CO_2 incubator. The constructs of -963 cyclin D1 promoter-driven luciferase reporter (-963 CD1 Luc) and its AP-1 binding site mutant reporter (-963 AP-1mut CD1 Luc) were gifts from Dr. Richard G Pestell, Thomas Jefferson University Jefferson Medical College. The plasmid TAM67, dominant negative C-Jun mutant, dominant negative Erk1 mutant (Erk1-K71R), *cyclin d1* promoter-driven luciferase reporter,

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