SIRT participates at DNA damage response

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1. Introduction

Sir2 maintains genomic stability in multiple ways in yeast. As a NAD^+-dependent histone deacetylase, Sir2 has been reported to control chromatin silencing. In both budding yeast and *Drosophila*, overexpression of Sir2 extends life span [1]. Previous reports have also demonstrated that Sir2 participate at DNA damage repair. A protein complex containing Sir2 has been reported to translocate to DNA double-strand breaks.

Following DNA damage response, SIRT1 deacetylates p53 protein and attenuates its ability as a transcription factor. Consequently, SIRT1 over-expression increases cell survival under DNA damage inducing conditions [2]. These previous observations mean a possibility that signals generated during the process of DNA repair are delivered through SIRT1 to acetylated p53. We present herein functional evidence for the involvement of SIRT1 in DNA repair response to radiation. In addition, this modulation of DNA repair activity may be connected to deacetylation of MRN proteins.

2. Methods and Results

U2OS, HT1080, 293T cells (ATCC, USA) were maintained in Dulbecco's modified eagle's medium (DMEM) supplemented with 5% fetal bovine serum. The cells were maintained in 37°C humidified atmosphere incubator with 5% CO_2.

Human SIRT1 was subcloned into the retroviral vector pMFG-puro. The retroviruses were produced by transient transfection of pMFG-puro, MFG-SIRT1 plasmids into H29D packaging cells. Q293A cells were infected with retrovirus containing 8 ㎍/㎖ of polybrene for 4 hrs. Twenty four hours after the infection, the cells were selected in 6 ㎍/㎖ puromycin (BD Biosciences, USA).

293T cells were transfected with SIRT1 and/or p300 plasmids for analysis of MRN acetylation assay or interaction between each others using Lipofectamin 2000 (Invitrogen, USA). The cells were collected 48 hrs after the transfection, and the cell extracts were used to confirm the effect of siRNA by Western blot analysis.

2.1 SIRT1 interacts with MRN

Zhigang Yuan reported that NBS1 is an acetylated protein and that the acetylation level is tightly regulated by the SIRT1 deacetylase [3]. SIRT1 associates with the MRE11-RAD50-NBS1 (MRN) complex and, importantly, maintains NBS1 in a hypoacetylated state, which is required for ionizing radiation-induced NBS1 Ser343 phosphorylation. In addition, we also found that MRN is one of SIRT1 substrates in yeast-two-hybrid result. Therefore, we examined whether SIRT1 could physically form complex with MRN and regulate its acetylation. To define this hypothesis, we infected HT1080 and U2OS cells with retrovirus carrying SIRT1 and then isolated mixed populations resistant to puromycine. And we then examined possible interactions between SIRT1 and MRN proteins in the lysate of these cells by immuno-precipitation. As shown in Figure 1A, SIRT1 co-precipitated with MRN in the lysates of SIRT1-overexpressed U2OS cells. This interaction was reciprocally further confirmed by immunoprecipitating the lysate with anit-MRN antibody and subsequently probing the blotted precipitate with SIRT1 antibody (Fig. 1A). These results were also reproduced in HT1080 cell (Fig. 1B). These findings indicate that SIRT1 and MRN can interact and physically form complex with one another.

2.2 SIRT1 regulates acetylation of MRN

To test which HAT protein regulates MRN acetylation, we transfected a variety of HAT genes, p300, PCAF and Tip60 to 293T cells in respectively. As shown Figure 2B, p300 increases acetylation of MRN, while the other HATs don't affect it. In agreement with the finding that SIRT1 binds MRN, acetylation of MRN was gradually decreased with SIRT1 trasfection volume (Data not shown). These result strongly indicate that p300 acetylates MRN and SIRT1 reversibly regulates acetylation of MRN

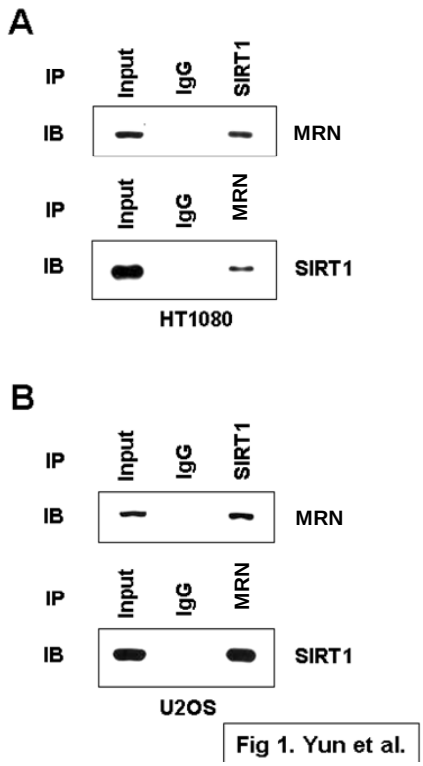

Fig. 1. SIRT1 interacts with MRN in HT1080 and U2OS.

3. Conclusions

MRE11-RAD50-NBS1 (MRN) have the nuclease activity that responses as a DNA damage sensor and is critical in regulating cellular responses to DNA double-strand breaks. MRN is modified with arginin-methylation by PRMT1, and mutation of the arginines severely disrupts its enzyme activity although the mutated protein still makes the MRN complex [4]. We report that MRN is an acetylated protein and that the acetylation level is tightly regulated by the SIRT1 deacetylase. SIRT1 associates with the MRN complex and, importantly, maintains MRN in a hypoacetylated state. Our results means that deacetylation of MRN by SIRT1 may plays a key role in the dynamic regulation of the DNA damage response and in the maintenance of chromosome stability.

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