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SIRT1 promotes DNA repair activity in response to radiation

Jaemin Jeong, Kee-Ho Lee
Laboratory of Molecular Oncology, Korea Institute of Radiological & Medical Sciences, Seoul, 139-706, jjmttl@nate.com

1. Introduction

Human SIRT1 controls various physiological responses including cell fate, stress, and aging, through deacetylation of its specific substrate protein. In processing DNA damage signaling, SIRT1 attenuates a cellular apoptotic response by deacetylation of p53 tumor suppressor. Ectopically over-expressed SIRT1 resulted in the increase of repair of DNA strand breakages produced by radiation. On the other hand, repression of endogenous SIRT1 expression by SIRT1 siRNA led to the decrease of this repair activity, indicating that SIRT1 can regulate DNA repair capacity of cells with DNA strand breaks.

2. Methods and Results

2.1 Enhancement of DNA repair capacity by SIRT1

Recent studies have provided evidences that SIRT1 increases cell survival upon stress response including hydrogen peroxide, anticancer drugs [1], and ionizing radiation [2]. To further evaluate the mechanistic basis of SIRT1 in increasing cell survival under DNA damage inducing condition, we examined whether SIRT1 could modulate DNA repair capacity which could critically affect the sensitivity to anticancer agents including radiation. To monitor DNA repair activity, we analyzed cell's ability to repair plasmid DNAs with strand breaks. Introduction of SIRT1 resulted in the increase of relative luciferase activity compared to that of empty control vector, indicating the increase of DNA repair activity by ectopically over-expressed SIRT1 (data not shown).

The above result was obtained by ectopically over-expressed SIRT1. Therefore, it was felt necessary to monitor DNA repair activity, when endogenous level of SIRT1 was lowered. Therefore, we applied a siRNA technology to reduce endogenous SIRT1. In order to get the most efficient siRNA to SIRT1, we designed 6 different siRNAs within the expression loci of SIRT1 gene and then co-transfected them into Q293A cells with SIRT1 expression plasmid. Figure 2A shows that all these siRNA efficiently reduced the level of exogenously overexpressed SIRT1 protein. Therefore, we selected one SIRT1 siRNA which lowered the SIRT1 protein level most effectively (Figure 1A). The selected SIRT1 siRNA also led to the decrease of endogenous as well as exogenous SIRT1 protein (Figure 1B). Q293A cells transfected with SIRT1 siRNA exhibited a marked reduction of DNA repair capacity, compared to those transfected with wild-type SIRT1. In contrast to wild type SIRT1, dominant-negative SIRT1, SIRT1-HY which expresses catalytically inactive form mutated at residue 363 histidine of SIRT1 protein by replacing with tyrosine [2], failed to enhance DNA repair capacity. These findings indicate that SIRT1 protein can enhance DNA repair capacity *in vivo* following DNA damage.

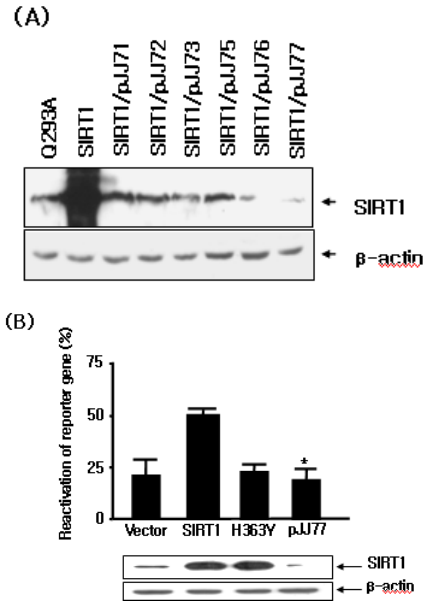

Figure 1. Reduction of DNA repair activity by SIRT1-SiRNA.

3. Conclusion

These observations suggest that SIRT1 modulates DNA repair activity. SIRT1, therefore, has been suggested to be a suppressor of apoptotic responses (3, 4). Related to the DNA damage pathway, our present study demonstrated that, when exposed to radiation, SIRT1 enhanced DNA repair activity

and physically formed complexes with repair protein Ku70, and subsequently deacetylated the latter, This scenario could be one plausible mechanistic basis of the promotion of cell survival.

REFERENCES

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