# Modulation of Radio-response by the Modification and Stabilization of c-Myc by IKKγ

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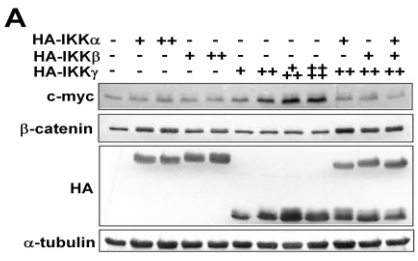
## 1. Introduction

The transcription factor c-Myc plays a critical role in multiple cellular processes including cell cycle progression, proliferation, differentiation, and apoptosis. The expression of c-Myc is generally upregulated in most of human cancers during tumor progression [1]. Correct regulation of c-Myc accumulation is thus essential, and achieved by multiple mechanisms acting at different stages of protein expression including the regulation of transcription, mRNA stability, translation, and protein stability [2]. Various mechanisms have been reported to regulate the stability of c-Myc. IKKγ is a critical component for the activity of IKK complex, which is essential for NF-κB activation in response to a variety of stress stimuli. Besides the cytoplasmic role of IKKγ in regulating the activity of IKK complex, IKKγ was shown to shuttle between cytoplasm and nucleus and play a nuclear role in transcriptional repression of the NF-κB pathway [3]. Here, we report that IKKγ stabilizes c-Myc protein through direct interaction and the stabilization and possible modification of c-Myc by IKKγ modulates the cellular response to ionizing radiation.

## 2. Methods and Results

### *2.1 IKKγ overexpression results in the increase of endogenous c-Myc protein level.*

When IKKγ was overexpressed in HEK293T cells, endogenous c-Myc protein level was significantly increased in dose-dependent manner (Fig. 1 A). Additional transfection of IKKα or IKKβ blocked the IKKγ-mediated c-Myc increase, while the overexpression of IKKα or IKKβ alone did not cause any change in c-Myc protein level. The effect of IKKγ overexpression on c-Myc accumulation is also shown by immunofluorescence staining (Fig 1 B).



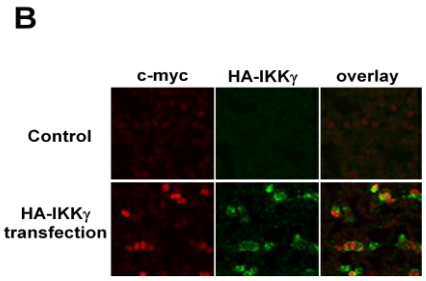




Fig. 1. IKKγ overexpression causes the increase of c-Myc protein level.

### *2.2 IKKγ causes c-Myc protein increase by enhancing protein stability.*

To study how IKKγ induces c-myc protein increase, we compared the mRNA and protein level of c-myc after IKKγ overexpression. mRNA level of c-Myc was not changed by IKKγ overexpression, while c-Myc protein was highly increased (Fig. 2 A). IKKγ overexpression does not activate the transcription from c-Myc promoter (Fig. 2 B). Next, we tested whether IKKγ overexpression affects the protein stability of c-Myc. As expected, IKKγ protects c-Myc protein from degradation and the ubiquitination of c-Myc is reduced in the presence of IKKγ (Fig. 2 C,D). These results clearly show that IKKγ induces c-Myc protein accumulation by stabilizing c-Myc protein, not by transcriptional induction.

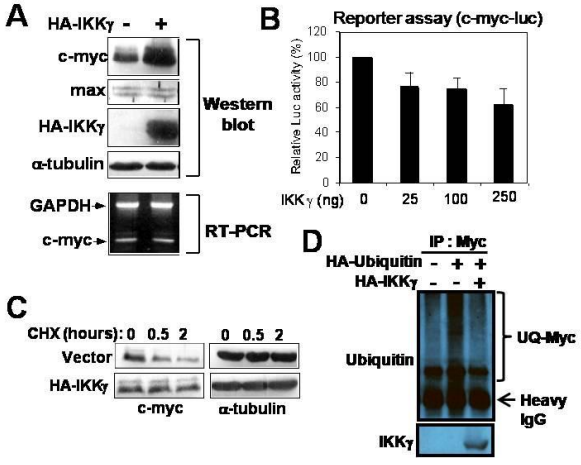



Fig. 2. IKKγ causes c-myc protein increase by enhancing protein stability.

### *2.3 IKKγ-mediated c-Myc stabilization takes place in nucleus by direct interaction.*

Overexpressed IKKγ is enriched in the discrete region in nucleus, where c-Myc is highly accumulated and colocalized with IKKγ (Fig. 3A). c-Myc protein was readily co-precipitated with IKKγ in coimmunoprecipitation experiment (Fig. 3 B). These observations strongly suggest that IKKγ stabilizes c-Myc in the discrete region of nucleus through direct interaction.

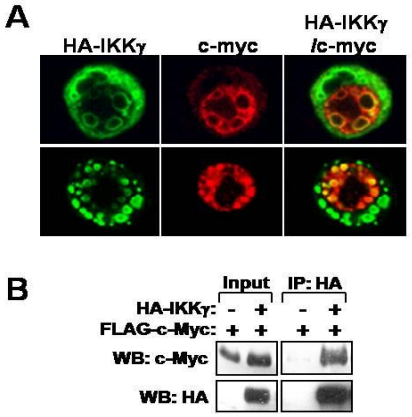
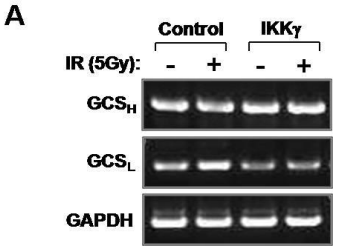



Fig. 3. IKKγ-mediated c-myc stabilization takes place in nucleus by direct interaction. (

### *2.4 c-Myc stabilized by IKKγ modulates the cellular response to ionizing radiation.*

We next investigated whether IKKγ can regulate the c-Myc transcriptional activity. When IKKγ is expressed, the induction of γ−$GCS_L$, which is one of the radiation-responsive c-Myc downstream genes, by ionizing radiation is inhibited (Fig. 4 A). This suggests that IKKγ induces the modification c-Myc to the form unable to activate the transcriptional induction of specific target genes in response to ionizing radiation.

Growth inhibition by ionizing radiation is significantly reduced in IKKγ -overexpressing cells (Fig. 4 B), suggesting that the modification and stabilization of c-Myc by IKKγ makes cells resistant to ionizing radiation.



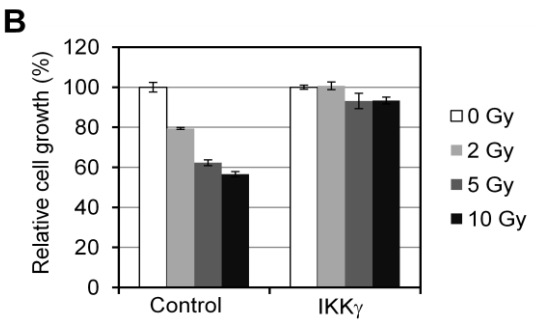




Fig. 4. Overexpression of IKKγ modulates the cellular response to ionizing radiation.

### 3. Conclusions

IKKγ is demonstrated here to modify and stabilize c-Myc protein. Compared to the classical role as an essential scaffold protein for the activity of IKK complex, the stabilization of c-Myc is a unique function of IKKγ that has never been reported. And this comprises the new novel mechanism to regulate c-Myc function. Our evidences indicate that c-Myc, when modified and stabilized by IKKγ, renders cells more resistant to ionizing radiation. Thus, the interaction between IKKγ and c-Myc can serve as the good target for the development of the therapy to overcome radio-resistance.

### REFERENCES

[1] C.E. Nesbit, J.M. Tersak, and E.V. Prochownik, Myc oncogenes and human neoplastic disease, Oncogene, Vol. 18, p. 3004, 1999
[2] Spencer CA, Groudine M. Control of c-myc regulation in normal and neoplastic cells. Adv Cancer Res. Vol. 56, p. 1, 1991.
[3] Verma UN, Yamamoto Y, Prajapati S, Gaynor RB. Nuclear role of I kappa B kinase-gamma/NF-kappa B essential modulator (IKK gamma/NEMO) in NF-kappa B-dependent gene expression. J Biol. Chem. Vol. 279(5), p. 3509, 2004.