HDAC regulates eIF2-α phosphorylation in stress condition

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1. Introduction

Histone deacetylase plays a role in a wide range of cellular processes including cell proliferation, senescence and apoptosis, thereby regulating cellular stress response and lifespan. HDAC is believed to delay the aging process by regulating metabolism and endocrine signaling and protecting against age related diseases [1].

The eukaryotic initiation factor 2-alpha (eIF2) is central to translation control in response to cellular stress. Translation is controlled by different extra and intra-cellular stimuli, such as nutrients, growth factors, hormones and stress signals. The inhibition of protein synthesis, as a result of eIF2 phosphorylation occurs very rapidly following exposure to stress [3].

In this paper, our results show that depletion of HDAC remarkably increases eIF2 phosphorlyation in stress condition.

2. Methods and Results

Western blot analysis

For harvesting the Whole cell lysates, the samples were washed with PBS (–) and lysed with lysis buffer and Cells were lysed for 20 min on ice and lysate was separated from cell debris by centrifugation for 10 min at 15000 rpm in a micro-centrifuge at 4 ^{o}C. The resulting samples (40 ug of protein) were used for immunoblot analysis.

Small interfering RNA–mediated silencing of SIRT1.

Small interfering RNA (siRNA)-mediated silencing of HDAC was performed as previously described [4]. After silencing, cells were incubated as indicated, ATF4 expression and eIF-2α phosphorylation examined by immunoblotting. The efficiency of gene silencing was verified in each experiment by immunoblotting.

2.1. HDAC regulates eIF2α phosphorylation

By our finding that HDAC interacts with eIF2 , we tried to investigate the possible regulatory overlap between these two proteins in eIF2 mediated stress response. For this, we generated HDAC depleted HeLa cells made by si-RNA against HDAC. These cells were treated for 1 or 3hr with different stress conditions, such as serum starvation and ER stress (MG132). Under all different stress conditions, levels of phosphorylated eIF2 were dramatically increased in the HDAC depleted cells than the control cells, indicating HDAC's role in stress dependent regulation of eIF2 phosphorylation (Fig. 1). Deacetylase plays a role in a wide range of cellular processes including cell proliferation, senescence and apoptosis, thereby regulating cellular stress response and lifespan. HDAC is believed to delay the aging process by regulating metabolism and endocrine signaling and protecting against age related diseases [1].

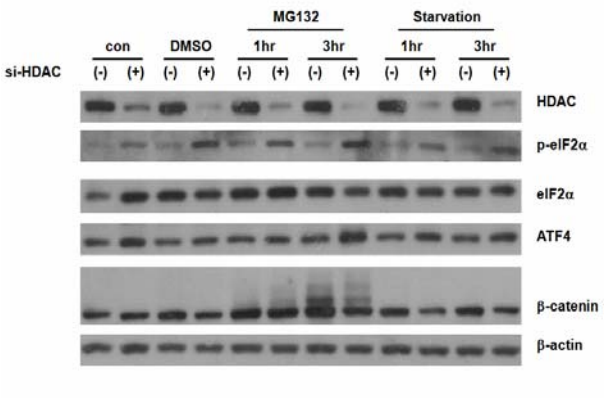

Fig. 1. HeLa cells depleted for HDAC show higher eIF2 phosphorylation in response to stress.

2.2 Depletion of HDAC induces ATF4 accumulation and increases stress sensitivity.

Previous reports have presented that HDAC plays a role in stress resistance. Dwonstream to phosphorylation of eIF2a in response to cellular stress is the expression of stress response proteins such as ATF4. To find proof which HDAC and eIF2a shares same resistant response against stress, we investigated expression of ATF4 between two type of cells; depleted HDAC and the control in variety of stress signals. These cells were treated for 1 or 3hr with different stress conditions, such as serum starvation and ER stress (MG132). Under all different stress conditions, we detected more increased level of ATF4 than control. In contrast of accumulated ATF4 protein, level of other protein, β-catenin, was decreased. eIF-2 plays an important role in the protein synthesis by phosphorylating and down-regulating global protein synthesis. These results indicate that depletion of HDAC up-regulate ATF4 by eIF-2 phosphorylation (Fig. 1). Interestingly, the transcription of ATF4 mRNA

was not observed to be decreased in the HDAC depletion (Data not shown).

3. Conclusions

Phosphorylation of eIF2α is the first step towards shutting down global protein synthesis in response to stress stimuli, thereby facilitating preferential expression of stress related genes for effective functioning of cells under stress condition. We demonstrate that under various different stress conditions, HDAC depleted HeLa cells show higher phosphorylation of eIF2α. Cells affected by stress which must adapt if they are to survive. HDAC play an stress protection for cell survive under stress condition. Depletion of HDAC, cell affected by stress, induced eIF2α phosphorylation, attenuated protein synthesis and down-regulated ATF4 protein expression.

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