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Regulation of Redd1 Expression by Hypoxia

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1. INTRODUCTION

Redd1, a recently discovered stress-response gene, is regulated by hypoxia via hypoxia-inducible factor 1 (HIF-1) and by DNA damage via p53/p63; however, the signaling pathway by which its expression is induced by hypoxia has not been elucidated. We demonstrated that the up-regulation of Redd1 transcription by hypoxia and high cell density (HCD) depends on cooperation between Sp1 and HIF-1α downstream of the PI3K/Akt pathway.

2. METHODS and RESULTS

Expression of Redd1 was assessed by RT-PCR, and the levels of HIF-1α Sp1 proteins were determined by Western blot analysis.

2.1 Hypoxia and HCD induces Redd1 expression via HIF-1α

Hypoxia activates a transcription factor known as hypoxia inducible factor 1 (HIF-1) (1). Increasing the cell density of monolayer cultures can induce pericellular hypoxia (2). Sp1 is a ubiquitously expressed transcription factor that appears to participate in the regulation of HIF-1α expression.

Hypoxic response by $CoCl_2$ and HCD induced a sharp increase in the levels of HIF-1α and Sp1 proteins as well as in the level of Redd1 mRNA. To determine whether hypoxia-induced Redd1 expression requires the function of HIF-1α and Sp1, we suppressed the HIF-1α and Sp1 by siRNAs. SiRNA of HIF-1α and Sp1 blocked the expression of hypoxia induced Redd1 mRNA. These results demonstrate that the HIF-1α and Sp1 plays a critical role in hypoxia-induced Redd1 expression.

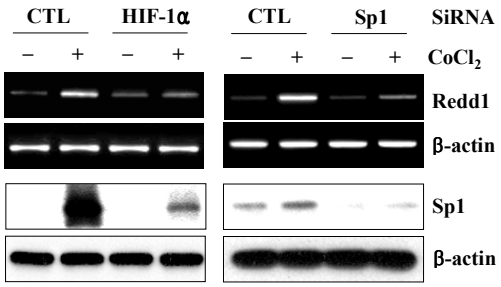

Figure 1. Hypoxia induces Redd1 expression by activating HIF-1α and Sp1.

2. PI3K and Akt participates in the regulation of Redd1 expression by HIF-1α and Sp1

To more fully elucidate the signaling pathways involved in the regulation of Redd1 expression, we examined the effect of hypoxia and HCD on PI3K activity. Hypoxia and HCD induced a significant increase in PI3K activity. Suppression of PI3K by PI3K inhibitor, LY294002 or dominant-negative p85 blocked the expression of hypoxia induced Redd1 mRNA.

PI3K phosphorylate and activate the serine/threonine kinase Akt (3). We examined the effect of hypoxia on the phosphorylation of Akt. Hypoxia enhanced the level of Akt phosphorylation. As shown in Fig. 2, the induction of Redd1 mRNA expression by hypoxia was significantly decreased in DN-Akt-transfected cells. Similarly, DN-Akt reduced the increase in HIF-1α and Sp1 by hypoxia.

These results indicate that activation of Akt downstream of PI3K participates in the induction of Redd1 expression by hypoxia and HCD.

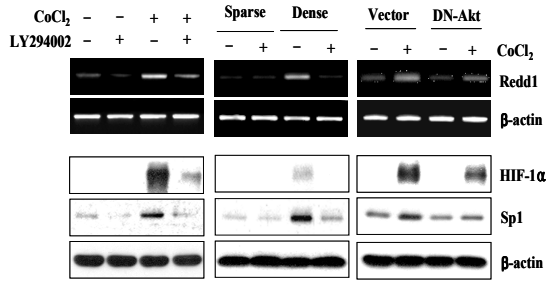

Figure 2. The PI3K/Akt pathway participates in regulation of Redd1 expression by HIF-1 α and Sp1.

3. CONCLUSION

We found that the induction of Redd1 expression by hypoxia and HCD requires the activation of HIF-1α and Sp1 downstream of the PI3K/Akt pathway.

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