Tumor angiogenesis is closely regulated by oxygen-dependent proteolysis

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We previously showed that exposure of human HepG2 hepatoblastoma cells to hypoxic conditions results in the overexpression of 6.0 kb IGF-II mRNA, suggesting that hypoxia may be a strong stimulus for the induction of IGF-II expression in the process of hepatocarcinogenesis. The 6.0-kb mRNA of IGF-II transcripts is transcribed under the control of human IGF-II P3 promoter. We have then discovered that hypoxia increases the expression of Egr-1 but not that of SP-1. These results suggest that Egr-1 may play a role in the activation of P3 promoter by hypoxia. Electrophoretic mobility shift assays and supershift assays demonstrate the increased DNA binding activity of Egr-1 protein by hypoxia. Moreover, cotransfection of HepG2 cells with an expression vector encoding Egr-1 protein and IGF-II P3 promoter-luciferase reporter plasmid shows that the activity of P3 promoter can be activated by Egr-1 in a dose-dependent manner.

To delineate Egr-1 signaling pathway, we performed yeast two-hybrid screening and identified three positive clones (mouse proteasome C8 subunit, human ubiquitin conjugating enzyme (hUBC9), protein inhibitor of activated STATy) interacting Egr-1 protein. We then confirmed their Egr-1 interacting activities by in vitro binding assay. Furthermore, Western blot analysis demonstrated that inhibition of proteasome activity by proteasome inhibitors blocks Egr-1 degradation in HepG2 cells. In addition, cotreatment of cycloheximide and proteasome inhibitors also showed unchanged Egr-1 expression. These findings indicate that proteolysis of Egr-1 in vivo is mediated by the ubiquitin-proteasome pathway.

On the other hand, the transcriptional regulator hypoxia-inducible factor 1 (HIF-1) is an essential mediator of hypoxia-induced tumor angiogenesis. A central mode of regulation of HIF-1 is through oxygen-regulated proteolysis of HIF-1α subunits involving the ubiquitin-proteasome pathway. Recent studies suggested that pVHL regulates HIF-1α proteolysis by acting as the recognition component of a ubiquitin ligase complex. There is also evidence that the stimulation of angiogenesis is one of the consequences of VHL gene inactivation. We therefore investigated whether histone deacetylase 1 (HDAC1) interacts with VHL tumor suppressor protein, and the interaction regulates expression of VEGF. We found that the two proteins indeed interact both in vitro and in vivo, and the extent of its association in changed in hypoxic condition. Moreover, VEGF and HIF-1α expressions were increased in HDAC1 transfected cells, but decreased in cotransfectants of HDAC1 and pVHL. However, cotransfection of HDAC1 and VHL recovered the decrease of VEGF and HIF-1α expression by VHL in VHL−/− cells. In addition, overexpression of VHL decreased the HDAC1-induced VEGF promoter activity. Taken together, these results suggest that the interaction of HDAC1 and pVHL may reduce pVHL function against HIF-1α and VEGF, result in increase of VEGF activity.

Taken together, these data delineate a novel insight for hypoxia-induced tumor angiogenesis, in which oxygen-dependent proteolysis of Egr-1 and HIF-1α/pVHL in response to hypoxia might have a significant role in the regulation of tumor angiogenesis.