Glycogen synthase kinase3 beta phosphorylates serine 33 of p53 and activates p53's transcriptional activity

ABSTRACT

GSK3β can regulate p53's transcriptional activity by phosphorylating serine 33. However, GSK3β does not appear to be part of the p53-DNA damage response pathway. Instead, GSK3β may provide the link between p53 and non-DNA damage mechanisms for p53 activation.

INTRODUCTION

Background The p53 tumor suppressor gene is activated during several cellular processes. These include DNA damage caused by Ionizing Radiation and genotoxic agents, by expression of activated oncogenes such as ras or myc, or during progression of primary cells to senescence. The activation of p53 by these diverse stimuli can initiate either growth arrest or apoptosis depending on the cellular context. p53 posses sequence-specific DNA binding activity and functions in the cell as a transcriptional regulator. Many p53 regulated genes have been identified, and the majority of the cellular effects of p53 activation can be attributed to the activation of these p53 target genes. The mechanism of p53 activation in response to either DNA damage or oncogene expression occurs through stabilization of the p53 protein. In unstimulated cells, the mdm2 protein binds to the N-terminal transactivation domain of p53 and targets it for ubiquitin-dependent degradation. Activation of p53 requires disruption of the mdm2-p53 interaction to allow p53 accumulation in the cell. 2 distinct mechanisms for p53 activation have so far been elucidated. The expression of oncogenes such as ras in untransformed cells stimulates transcription of the p14Arf gene. p14Arf binds to and sequesters mdm2, allowing free p53 protein to accumulate in the cells. Activation of p53 by DNA damage is also brought about by inhibition of p53-mdm2 interaction. The product of the Ataxia Telangiectasia gene, the ATM protein kinase, directly phosphorylates serine 15 of the p53 protein in response to Ionizing Radiation. In addition, ATM phosphorylates and activates chk2 kinase. Activated chk2 can then directly phosphorylate serine 20 of p53. ATM therefore controls the phosphorylation of serines 15 and 20 of p53. In addition, DNA damage increases the phosphorylation of serines 33 and 37 of p53 through an ATM-independent mechanism. These DNA damage-induced phosphorylations of p53 block the binding of mdm2 to the N-terminal of the p53 protein. Thus phosphorylation of the p53 protein in response to DNA damage or expression of p14Arf prevents mdm2 binding and p53 protein then accumulates in the cell. Although stabilization of the p53 protein is the initial step in p53 activation, subsequent steps, including activation of p53's DNA binding activity and changes in p53's transcriptional activity, are also involved. For example, p53's DNA binding activity is increased by the DNA damage-induced acetylation of the C-terminal of p53, and this acetylation requires the prior phosphorylation of the N-terminal of p53. In addition, phosphorylation of the N-terminal transactivation domain of p53 may be required to stimulate transcriptional activation of p53 target genes. Multiple phosphorylation sites have been detected in the N-terminal of p53, including serines 6, 9, 15, 20, 33, 37 and 46. While phosphorylation of serines 15 and 20 of p53 are clearly dependent on the ATM and chk2 protein kinases, the kinases responsible for phosphorylation of the remaining serine residues in vivo is not clear. The activation of p53 by DNA damage or oncogenes such as ras results in either growth arrest or apoptosis of the affected cell. In this study, we have examined how Glycogen Synthase Kinase 3β (GSK3β), a protein kinase

involved in tumorigenesis, differentiation and apoptosis, regulates the function of p53. GSK3β phosphorylates several transcription factors, including NFATc and HSF1. GSK3β is constitutively active in resting cells but is inhibited when cells are exposed to growth factors. GSK3 inhibition occurs when the p110-PI 3-kinase/Protein Kinase B (PKB) pathway is activated by growth factors. Activated PKB then phosphorylates GSK3β, inhibiting GSK3 kinase activity. This activation of the p110-PI 3-kinase/PKB pathway, and inhibition of GSK3, delivers a strong anti-apoptotic signal to the cell. Given the well characterized role of p53 in apoptosis, we examined if GSK3p participates in the regulation of the p53 protein. GSK3 phosphorylates the consensus sequence Ser-X-X-X-Ser(P), where the C-terminal serine residue is already phosphorylated. Thus GSK3 only phosphorylates target proteins which have already been phosphorylated by a separate, priming kinase. p53 contains 5 potential GSK3 phosphorylation sites, 3 in the N-terminal transactivation domain and 2 in the C-terminal regulatory domain. Here, we show that GSK3β, but not GSK3α, can phosphorylate serine 33 of p53 in vitro when serine 37 is already phosphorylated. Further, GSK3β can increase p53's transcriptional activity in vivo, and this activation is lost when serine 33 is mutated to alanine.

CONCLUSION

Conclusions This study demonstrates that GSK3β, but not GSK3α, can directly phosphorylate serine 33 of p53 when serine 37 of p53 is already phosphorylated. GSK3β can increase p53's transcriptional activity in vivo, and this activation requires serines 33 and 37 of the p53 protein. Thus GSK3β may phosphorylate and activate p53 in vivo. However, GSK3β is not part of the p53-DNA damage response pathway. Instead, GSK3β may provide the link between p53 and non-DNA damage mechanisms for p53 activation, such as oncogene activation.