Serine / threonine protein phosphatase 5 (PP5) participates in the regulation of glucocorticoid receptor nucleocytoplasmic shuttling

ABSTRACT

These studies suggest that PP5 participates in the regulation of glucocorticoid receptor nucleocytoplasmic shuttling, and that the GR-induced transcriptional activity observed when the expression of PP5 is suppressed by treatment with ISIS 15534 results from the nuclear accumulation of GR in a form that is capable of binding DNA yet still requires agonist to elicit maximal transcriptional activation.

INTRODUCTION

Background Glucocorticoids influence a wide spectrum of cellular functions through their action on soluble intracellular receptors. In most cells, unliganded glucocorticoid receptors (GR) reside predominately in the cytoplasm, where they exist as a heteromeric complex comprised minimally of GR, 90-kDa and 70-kDa heat shock proteins (hsp90 and hsp70). Other proteins (i.e. p60/Hop, p23, hsp40, FKBP52, and FKBP51) have been implicated in the assembly/stabilization of the GR-hsp90-hsp70-complex in a form that has high affinity for agonist [for review, see Ref.]. Upon agonist binding, the complex undergoes a transformation, and the ligand bound GR translocates into to the nucleus in a manner that is determined by a nuclear localization sequence (NLS) contained in the receptor. There the GR acts as a ligand-activated transcriptional stimulator or repressor of primary response genes by binding to glucocorticoid hormone-responsive elements (GRE) contained in the promoter regions of steroid-responsive genes and either facilitating or repressing the formation of an active transcriptional complex. Although little is known about the molecular machinery that regulates steroid receptor movement through the cytoplasm and into the nucleus, several studies suggest that movement is influenced by reversible phosphorylation. Evidence for this originated from the studies of Qi et al., which revealed that the hormone insensitivity produced by cellular transformation with v-mos (a serine/threonine protein kinase that acts as an oncogene) results from both a decrease in the nuclear retention of liganded receptor and a decrease in the reutilization of GR protein that cycles back into the cytoplasm. Subsequently, DeFranco et al. reported that treatment with okadaic acid, a potent ser/thr protein phosphatase inhibitor, also results in inefficient nuclear retention of agonist-bound GR and the cytoplasmic "trapping" of GR in a form that is unable to "recycle". Recent studies with okadaic acid suggest phosphorylation alters the high affinity binding of GR to hsp-90, and that an intact cytoskeleton is required for ligand-activated GR translocation through the cytoplasm to the nucleus. The ability of okadaic acid to influence the intracellular partitioning of GR suggests that an okadaic acid sensitive ser/thr protein phosphatase (PPase) participates in the regulation of GR movement. In vitro, okadaic acid acts as a potent inhibitor of serine/threonine protein phosphatases type 1 (PP1) and 2A (PP2A). Accordingly, many of the effects produced by the treatment of cells with okadaic acid have been attributed to the inhibition of these two enzymes. However, due to toxicity and solubility constraints, in living cells it is difficult to distinguish the actions of PP2A from those of PP1 using okadaic acid. Furthermore, in humans, it is now clear that there are four isoforms of PP1 [PP1α, PP1δ, PP1γ1 and PP1γ2, two isoforms of PP2A (PP2A α and PP2Aβ) and four structurally related phosphatases, PP4, PP5, PP6 and PP7. Although detailed dose-response studies have not been reported for native PP5, PP6 and PP7, studies with PP4 and

recombinant PP5 indicate they are also sensitive to okadaic acid. Like calcineurin (PP2B) and PP2C, PP7 is apparently insensitive to inhibition by okadaic acid. Recent studies indicate that PP5 associates with the GR-hsp90 complex suggesting that PP5 may influence the actions of GRs. However, studying the cellular roles of PP5 has proven difficult, in part, because no physiological substrates for PP5 have been identified. In addition, in crude cell homogenates PP5 resides predominately in an inactive state that represents <1% of the measurable PPase activity. To characterize the cellular roles of PP5 we have, therefore, developed chimeric antisense 2'-O-(2-methoxy) ethylphosphothioate oligonucleotides capable of inhibiting the expression of human PP5 at nanomolar concentrations. Because the lead compound targeting PP5 (ISIS 15534) acts via RNAase H mediated degradation, studies with ISIS 15534 do not allow us to assess how rapid changes in PP5 activity affect cellular functions (Northern analysis indicate that it takes ~ 6 for the mRNA degradation to occur and, due to the half-life of the preexisting protein, it takes ~ 24 hours for the protein levels to fall. Nonetheless, ISIS 15534 potently inhibits the expression of PP5 in cultured cells for ~ 48-72 hours (IC50 of <75 nm), which affords a ~ 24-48 hour window in which the expression of PP5 is essentially ablated. More importantly, because ISIS 15534 has no effect on the structurally related PPases, it can be employed to specifically inhibit the actions of PP5 by suppressing PP5 protein levels in cultured human cells. To assess the role of PP5 in the regulation of GR-mediated events, binding studies were conducted with [3H]dexamethasone before and after ISIS 15534-mediated suppression of PP5 expression. These studies revealed that the suppression of PP5 expression had no apparent effect on dexamethasone binding, suggesting that PP5 does not affect the formation of the high-affinity ligand binding complex or hormone binding to the GR. In contrast, mobility gel-shift analysis revealed that treatment with ISIS 15534 produces a marked increase in the association of GR with GRE-containing DNA, and transient transfection studies employing a GR-responsive reporter plasmid revealed that the suppression of PP5 expression activates GR-dependent transcription in the absence of hormone. When A549 cells were treated with ISIS 15534 and then dexamethasone, the effect was additive, with maximal dexamethasone induced luciferase activity ~ 10 times greater than the maximal dexamethasone-induced response attainable in the presence of PP5. Together, these studies indicate that PP5 acts as a suppressor of GR-induced transcription. To further characterize the mechanism by which PP5 affects GR function, in the present study we employed a GR-GFP fusion protein and fluorescent microscopy to follow the movement of GR in cells treated with dexamethasone and ISIS 15534. These studies indicate that PP5 mediated suppression of GR-function arises from the ability of PP5 to suppress the nuclear accumulation of GRs.

CONCLUSION

Conclusions These studies indicate that PP5 participates in the regulation of GR nucleocytoplasmic shuttling, and that the suppression of PP5 expression results in the nuclear accumulation of GR in the absence of hormone. Therefore, the previously reported increase in GR-induced transcriptional activity that occurs after ISIS 15534 induced suppression of PP5 expression likely results from the nuclear accumulation of GR in a form that is capable of binding DNA yet still requires agonist to elicit maximal transcriptional activation. Still, it is not yet clear if PP5 acts to suppress the nuclear accumulation or to facilitate the nuclear export of GRs. Thus, the precise molecular mechanism by which PP5 suppresses the nuclear accumulation of GR remains to be elucidated.