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Invited Mini Review

The diverse roles of RNA polymerase II C-terminal domain phosphatase SCP1

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RNA polymerase II carboxyl-terminal domain (pol II CTD) phosphatases are a newly emerging family of phosphatases that are members of DXDX (T/V). The subfamily includes Small CTD phosphatases, like SCP1, SCP2, SCP3, TIMM50, HSPC129 and UBLCP. Extensive study of SCP1 has elicited the diversified roles of the small C terminal domain phosphatase. The SCP1 plays a vital role in various biological activities, like neuronal gene silencing and preferential Ser5 dephosphorylation, acts as a cardiac hypertrophy inducer with the help of its intronic miRNAs, and has shown a key role in cell cycle regulation. This short review offers an explanation of the mechanism of action of small CTD phosphatases, in different biological activities and metabolic processes. [BMB Reports 2014; 47(4): 192-196]

INTRODUCTION

The RNA polymerase II (RNAPII) has specifically the C-terminal domain (CTD) of its largest subunit that can be dephosphorylated by CTD phosphatases. Among CTD phosphatases, Small CTD phosphatase (SCP1), demonstrates emerging roles, like gene silencing activities in neuronal genes, preferential Ser5 dephosphorylation, and phase regulation in the cell cycle. The repressor element 1 (RE-1) silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) forms complexes, and represses the transcription of neuronal genes in non-neuronal cells. Small CTD phosphatases (SCPs) are the potential transcriptional regulators; the novel biological activity helps to act in neuronal gene silencing in a global manner. Studies in the P19 stem cells of mouse were used to illustrate the mechanism behind the neuronal gene silencing in non-neuronal cells by silencing factors complex (REST/NRSF) (1), with the mediation of SCP1. The mechanism showed how inactive forms of SCP1 and neuronal repressor complex (REST/NRSF) interact with each other and repress the neuronal gene expression in non-neuronal cells.

The C-terminal domain is the largest subunit of RNA polymerase (RNAP) II, and regulates the processing and transcription of precursor-mRNA in eukaryotes by its dephosphorylating activity (2). The study has shown that small C-terminal domain phosphatases (SCPs) preferentially reverse the phosphorylation of Ser5 residues of consensus heptad repeat, and its attraction towards the specific substrate (Table 1). The RNA polymerase II-associated protein 74 (RAP74) is a subunit of transcription factor IIF (TFIIF), which enhances the SCP1 activity. Expressions of the active and inactive forms of the SCP1 demonstrate the inverse effect. The active form of SCP1 suppresses the transcription of various promoters, in contrast to this inactive form of SCP1, which intensifies the transcription. In similar manner, SCP1 regulates the gene expression, by limiting the switch between initiation and elongation in translation activity (3).

Extensive studies of SCP1 have revealed its vital role in osteoblastic differentiation; SCPs naturally possess phosphatase activity. The novel phosphatase activity is the key player in the suppression of osteoblastic differentiation. By suppressing the bone morphogenetic protein (BMP) signaling, the downstream region genes (Runt-related transcription factor 2, RUNX2) will be suppressed by dephosphorylating the effectors of the RUNX2, and regulates its activity in BMP signaling. The integrated profound effect of SCP1 dephosphorylation observed with Smads also acts together in osteoblastic differentiation, by suppressing BMP-Smad complex.

Table 1. Role of SCP1 in various biological activities

Biological activity	Involved genes	Regulation	Reference
Neuronal gene silencing	REST/NRSF	Negative	(17)
Ser5 dephosphorylation	RAP74 and Ser residues	Positive	(37)
BMP-Smad axis-induced osteoblastic differentiation	Runx2	Negative	(24)
Cardiac hypertrophy	miRNA-26a/b, TRPC3, GATA4	Negative	(31)
G1/S phase transition	miRNA-26a/b, ppRb	Negative	(33)

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Apart from the progressive research on SCP1, a novel activity has been identified in cardiac hypertrophy and cell cycle progression, where the entire mechanism has been concomitantly led by its intronic miRNA-26a and miRNA-26b (4). The expression of these miR-26a/b and dephosphorylation activities of SCPs imposed great influence on both biological activities. The brief review that is presented here takes into consideration all the above novel properties of SCPs.

PREFERENTIAL DEPHOSPHORYLATION OF Ser5 IN RNAPII CTD BY SCP1

The dephosphorylation of phospho-CTD by SCP1/FCP1 plays an important role in transcription initiation. SCP1/FCP1 are members of the Mg^{2+}-dependent phospho-serine/threonine phosphatase family. These phosphatases share 20% identity in their catalytic region, although they show high specificity in terms of its substrate and preferences, in the dephosphorylation of serine/threonine residues (5). The structural and functional analysis of SCP1 denotes the residues responsible for its CTD binding capability, and the preferential dephosphorylation of phospho-Ser5 in CTD heptad repeat. Structural determination studies were used to identify the binding properties of SCP1 with phosphopeptides of CTD. SCP1 showed strong attraction towards the phospho-Ser5 residue through crystallization study (6). From the results, it is clear that phospho-Ser5 residue is key molecules in forming the binding groove. SCP1 has conserved the catalytic domain with FCP1, which possess the residues coordinate with Mg^{2+}, and are essential for dephosphorylation. The steady-state kinetic studies determined that Ser2-Pro3-Thr4-Phospho-Ser5 is the key residues for structural stability and interaction, and also confirmed that Phe106 in SCP1 is the determinant of preferential selection of Phospho-Ser5 for SCP1 mediated dephosphorylation (7).

The CTD of RNAPII is built with repeats of consensus sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7. The specific site in the consensus repeat region, and the state of CTD phosphorylation greatly influence the RNAII activation (8). A class C phosphatase, FCP1, shows specificity in the dephosphorylation of Ser2 and Ser5, and FCP1 may possess the specificity in dephosphorylation of Ser residues at its specific sites (8). Human SCP1 shows 20% homology in the active region with FCP1; in contrast, the other three SCPs show 90% homology (9). They are playing in the initial stages of the transcription cycle, by involving in crucial steps the promoter initiation/capping and elongation. The phosphorylation of consensus heptad repeat residues Ser2 is detected at its coding region, and Ser5 at the promoter region.

In order to determine the phosphatase activity in SCP1, studies were carried out, and observed that the DXDX conserved domain mediated the phosphatase activity. They also identified that the consensus repeat site specific dephosphorylation is dependent on CTD kinase activity (5). The results of a study conducted using RNAPII isozymes, RNAPIIo and RNAPIIa with CTD kinases with known specificity, showed that SCP1 demonstrated more substrate specificity, compared to FCP1; and also observed the interesting results that an increased amount of SCP1 preferentially dephosphorylated Ser5, rather than Ser2. On the other hand, another study observed the RAP74 largest subunit of transcription factor IIF regulates the SCPs activity (10). Hence it is suggested that the RAP74 of TFIIF stimulations plays a vital role in SCP1 phosphate activity, and regulates the gene expression *in vivo* (11).

From the results of gene assays illustrating the influence on expression profiles of wild type SCP1 and mutant SCP1 in gene expression regulation, the over expression of mutant SCP1 results in enhanced transcription; whereas, wild type SCP1 inhibited the phosphorylation in a selective manner (12). The preferential Ser5 phosphorylation activity of SCP1 denotes the vital role of CTDs in stage specific phosphorylation of the transcription cycle, and the importance of CTD mediated phosphorylation in gene expression regulation.

ROLE OF SCP1 IN SILENCING NEURONAL GENE EXPRESSION

The ventricular zones of neuroepithelial precursor cells tend to produce the central nervous system, and neuronal cells are derived based on different signaling patterns. Transcription factors of activator and repressor systems of transcription activity regulate the expression patterns of neuronal genes. Earlier studies identified that repressor element 1 (RE-1)-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) is the best known repressor protein transcription factor, which globally regulates the neuronal gene expression, specificity, and maturity (13). REST/NRSF binds to RE-1 DNA predominantly found in numerous neuronal gens, and forms a multi protein complex, which represses gene transcription by covalent modifications like methylation of DNA and deacetylating histones, and which results in neuronal gene silencing (14-16). The CTD phosphatase FCP1 negatively regulates the RNAPII activity, and combined structural studies of SCP1 and FCP1 elicited similarities of these phosphatases in the core fold, and the conserved active site signatures, like all other SCPs, belong to the DXDX(T/V) family. The phosphate activity of SCP1 plays a vital role in gene silencing (Fig. 1).

The expression studies of SCPs observed that wide expression of SCP1 in cervical tissues, mesenchymal tissues, ectodermal tissues, and undifferentiated neuroepithelial cells at embryonic stages and higher concentrations of SCP1, were detected in skeletal muscles, compared to brain. The expression pattern studies by co-immunoprecipitation denoted that parallel exclusion of SCP1 and REST/NRSF in differentiated neural tissues is expected to have a function in neuronal gene silencing (17), and the chromatin immunoprecepitation (ChIP) study confers the role of REST/NRSF role in the binding of SCP1 with RE-1 elements.

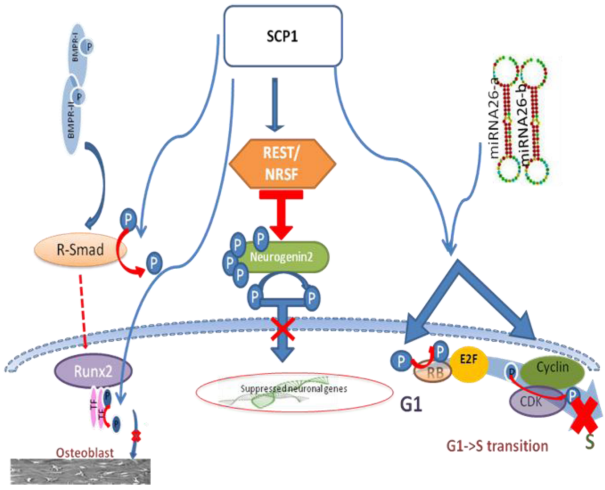

Fig. 1. SCP1 related signaling pathways.

An experiment has been carried out to illustrate the role of SCP1 in neuronal differentiation, by using P19 mouse embryonic stem cells. Differentiating neuronal cells induced with REST/NSRF results in cell death, while with expression of SCP1, no cell death was observed. In contrast, down regulation of SCP1 observed cell differentiation at a doubled rate. The results conferred the importance of REST/NSRF mediatory role in SCP1 neuronal gene silencing. The study also observed how co-expression profiles of SCP1 and REST/NSRF influence the neuronal differentiation pathway, by inhibiting the basic helix-loop-helix (bHLH) transcription factor neurogenin 2 (Ngn2). Down regulation of SCP1 and REST/NSRF induced the neuronal differentiation (18). Conversely, the study revealed insights into how SCP1 plays its vital role in neuronal gene silencing when co-expressed with REST/NSRF, and its mechanism of action.

SUPPRESSION OF BMP-SMAD SIGNALING BY SCP1 AND ROLE OF SCP1 IN AXIS-INDUCED OSTEOBLASTIC DIFFERENTIATION

BMP is a key molecule in bone and cartilage formation, and also influences myogenic and osteoblast differentiation. BMP type I receptors play a vital role in the phosphorylation of downstream signaling molecules R-Smads, and BMPs phosphorylate the Ser residues at the C-terminal domain of R-Smads (19, 20). SCP1 regulates the BMP activity by dephosphorylating the Ser-X-Ser motif in the C-terminal region, and MAPK-phosphatase sites at its linker regions in Smads (Fig. 1). The phosphate activity of SCP1 plays an important role in the suppression of BMP activity, and higher concentrations of SCP1 regulate the activity of alkaline phosphatase (ALP) as a BMP4-induced osteoblastic differentiation marker (21). From the results of co-expression studies on wild type SCP1 and mutant SCP1, wild type SCP1 reduced the BMP4-dependent phosphorylation at the C-terminus end in Smad1 (22); in contrast, the linker region phosphorylated at Ser206 in both BMP4-dependent and independent manner, but mutant SCP1 did not affect the phosphorylation of the linker region. Similarly, protein phosphatase 1A (PPM1A) possesses the dephosphorylation activity that will inhibit the BMP-Signaling, by reducing Smad protein levels (23, 24).

SCP1 specifically regulates transcription factor RUNX2 through the BMP signaling, and it greatly enhances the differentiation of osteoblastic cells by BMP-Smad pathway (25). SCP1 has shown better suppression of osteoblastic differentiation, in contrast to regulation of RUNX2 (26). PPM1A suppressed the osteoblast differentiation, by disruption of Smad; whereas SCP1 suppressed the RUNX2 by inhibiting the effectors of its downstream region (24). The localization of SCP1 in the tissues of myogenic and skeletal muscle physiologically affects the BMP-induced osteoblast differentiation in myogenic cells. The study indicated that SCP1 suppresses the BMP-Smad induced signaling via RUNX2 by inhibiting downstream effectors (27).

ROLE OF SCP1 AS A KEY CARDIAC HYPERTROPHY INDUCER

SCPs are highly expressed in the heart, and contain miRNAs (miR)-26b, 26a-2 and 26a-1 in their introns (28); these introns play a specific function in the heart. A comparative study has been carried out to determine the roles of SCP1 and its intronic miR-26b in cardiomyocytes, and the results indicated that SCP1 itself has the potential to induce cardiac hypertrophy (Fig. 1).

Mouse cardiomyocytes were used to find out the role of the SCP1 gene in the heart, and then overexpressed it in cardiomyocytes. The overexpression of SCP1 results in significant increasing of cell surface area of cardiomyocytes, and the expression levels of responsible genes (29). That siRNA against SCP1 significantly reduced the mRNA expression levels in cardiomyocytes (30).

From a comparative knockdown study related to its effect of cardiac hypertrophy in mouse heart upon expression of SCP1, a cDNA was constructed with the 5' end of exon1, and the 3' end of exon 7, and tested against the SCP1. The expression of SCP1 induces a cardiac hypertrophic phenotype, and the expression of its intronic miR-26b has the opposite effect compared to the effect of the overexpression of SCP1 *in vitro*. To overexpress SCP1 with its introns, they cloned a DNA fragment from mouse genome DNA (gDNA), and compared its function to that of SCP1 cDNA. miR-26b levels were significantly elevated in cardiomyocytes with gDNA, compared with vector- or cDNA-transduced cardiomyocytes. cDNA significantly increased related gene expression, and the cell surface area in cardiomyocytes, compared with cells transduced with gDNA. Moreover, miR-26b target genes transient receptor potential cation channel, subfamily C, member 3 (TRPC3) and

transcription factor GATA4 are down regulated by gDNA, compared to cDNA. Hence, the results show that SCP1 potentially induces cardiac hypertrophy, which is negatively regulated by its intronic miR-26b (31).

ROLE OF SCP1 IN G1/S TRANSITION

Many miRNAs are produced from its intron regions of host genes, and the functional relationship between these molecules is an enigma. SCPs produce the miRNAs, like miR-26a and miR-26b. It is revealed from the serum starvation stimulation assays in primary fibroblasts and two-thirds partial-hepatectomies in mice, that miR-26a/b and SCPs were co-expressed during the cell cycle process (32).

The retinoblastoma protein (pRb) pathway is the checkpoint for cell cycle progression, as iterative phosphorylation and dephosphorylation processes of the pRb protein control the entry of cells into S-phase. A synergistic effect of miR-26a/b and SCPs is found in decreasing the phosphorylation level of pRb (ppRb), and blocking the G1/S progression (33). The intronic miRNAs of small CTD family genes, miR-26a and miR-26b indirectly suppressed the amount of ppRb, by repressing the CDK and cyclins that mediate the phosphorylation of pRb (34). The host genes SCPs also performed a similar function directly with its natural dephosphorylation activity; thus, both small CTD family genes SCPs and its intronic miRNAs miR-26a/b concomitantly suppress the ppRb level, and obstruct the cell cycle progression in G1/S transition (35). C-Myc has the capability to down-regulate the SCPs and the miR-26a/b for progression of the cell cycle, and this mechanism has been clearly observed in mouse hepatocytes (36). The results indicated that CTD family members were down regulated in hepatocytes tissues, and dysfunction of these genes may result in hepatocarcinogenesis (Fig. 1).

CONCLUSION

The extensive study of protein phosphatases has taken a new pace in the last decade, with special interest being shown to newly emerging RNAPII small C-terminal domain phosphatases, due to its unique roles in various biological process, and conserved catalytic properties among the different taxa of organisms.

The novel substrate properties and dephosphorylation activity of SCPs, and the distinctive roles in various biological activities like neuronal gene silencing have shown how it acts when expressed in non-neuronal cells and neuronal cells. Further, it has been shown how it associates with REST/NRSF complex, and silences the neuronal gene expression. Another notable role in preferential dephosphorylation of Ser5 in CTD of RNAPII shows its specificity towards the substrate. The novel mechanisms have been identified concomitantly with its intronic miRNA-26a/b in cardiac hypertrophy, and hepatocellular carcinoma due to the regulation of cell cycle at G1/S transition. Conversely, the C-Myc was able to suppress the SCP1, and its intronic miRNA-26a/b for progression of G1/S transition; hence there is a need to focus on the enigmatic mechanism behind the C-Myc and SCPs in ppRb pathway.

Perhaps further intensive research on small CTD phosphatases would focus in a new dimension, due to the many notable and mysterious roles that are emerging from recent findings, which could contribute considerable milestones to the biomedical field in developing novel drugs.

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