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Multifaceted Role of Senescence Marker Protein-30 in Health and Diseases

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ABSTRACT

Senescence marker protein 30 (SMP30) is an age-linked marker protein, the expression of which declines with aging. SMP30 binds with calcium; however, the absence of a calcium-binding EF-hand motif makes it different from other calcium-binding proteins like calmodulin. Interestingly, previous studies have shown that it binds with other divalent metal co-factors also and catalyzes the cyclisation of L-gulonate required for the biosynthesis of ascorbate in non-primates. Remarkably, SMP30 is conserved among vertebrates indicating that it isa crucial protein performing certain physiological functions. Apparently, in primates, including humans, calcium homeostasis could be the primary function of SMP30 due to the absence of ascorbic acid biosynthesis in these species. In this review, we have discussed the expression pattern of SMP30 in various cells and tissues. SMP30 expression is modulated by different internal and external factors, which we have extensively discussed here. Subsequently, its role in calcium homeostasis, cell proliferation, apoptosis, and liver regeneration has also been explored. Further, the potentiality of SMP30 as a prophylactic agent against organophosphorus nerve agent poisoning has been elucidated due to its organophosphate hydrolysing activity as a promiscuous substrate.

Keywords: SMP30; Regucalcin; SMP30 expression; Modulators of SMP30; Calcium homeostasis

NOMENCLATURE

ARE	Antioxidant response element
BAX.BA	Bcl-2 family member
Bcl-2	B-cell lymphoma 2 (apoptosissuppressor gene)
cdk	Cyclin-dependent kinase
E2	17β-oestradiol
ERK	Extracellular signal-regulated kinase
FADD	Fas-associated death domain
FAS	Cell death receptor
IGF-I	Insulin-like growth factor
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MMECs	Mouse microvascular endothelial cells
NAME	N-nitro-L-arginine methyl ester
NFI-A1	Nuclear transcription factors
NO	Nitric oxide
NOS	Nitric oxide synthase
Nrf2	Nuclear erythroid 2-related factor
OP	Organophosphorus
PI3K	Insulin receptor
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphate
RGPR- p117	Regucalcin gene promoter region protein 117
ROS	Reactive oxygen species
SMP30	Senescence Marker Protein 30
TNFα	Tumor necrosis factor α
TRADD	Tumor necrosis factor receptor Type 1-associated DEATH domain protein
TRAF2	TNF receptor associated factor

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1. INTRODUCTION

Senescence Marker Protein 30 (SMP30) plays multiple roles in cell physiology.[1] In murine, the SMP30 gene contains seven exons and six introns, with an open reading frame of 897 nucleotides which encodes 299 amino acids.[1] The SMP30 gene was analysed in silico by using the biological process tool of Panther Go-slim, which resulted that SMP30 is co-expressed with several genes into many biological processes such as apoptosis, cell adhesion, and blood coagulation.[2] In hepatocellular carcinoma tissues, SMP30 expression was significantly reduced compared to non-tumor tissues, which was very noticeable in larger tumor sizes leading to worse survival of the patients. Further, it is hypothesised that DNA methylation might mediate the down-regulation of SMP30 in hepatocellular carcinoma.[2] It is also linked with several physiological disorders like liver fibrosis, osteoporosis, diabetes, and cancers.[1] Thus, it is considered a multifunctional protein involved in calcium homeostasis, cellular signaling, and ascorbic acid biosynthesis pathway in non-primate mammals.[1]

However, in primates, there is no ascorbic acid biosynthesis. Still, there is a high expression of SMP30, which indicates its importance in other physiological functions. SMP30 is characterised as a lactonase enzyme; however, it also possesses promiscuous enzyme activity, i.e., organophosphate hydrolase, which indicates that it might play a role in drug metabolism.

While investigating the exact physiological function and mechanism in humans, it is found to be involved in intracellular signaling pathways as it also shows protein kinases and phosphatase activities.[3] Owing to the multiple roles of SMP30 in cell physiology and possibly in drug metabolism, this review article aims to demonstrate the modulators of its expression and the mechanism by which SMP30 modulates the cell cycle, apoptosis, and calcium homeostasis.

2. STRUCTURE OF SMP30

Structural analyses of SMP30 show six β-sheet which are made up of 24 β-strands circular arrangements with a central active core and metal co-factor binding site. The crystal structure study of SMP30 shows the presence of a flexible α-helix from 268 to 275 residues, which connects with two different loops. These loops are situated at the top of the structure where the presumed active site is present. These loops are essential as it covers the active site and help in specific substrate recruitment. It has a 12 amino acid residues long tail at its C-terminal end (Fig. 1). The Calcium metal ion coordinates with Glutamate at position 18, Asparagine at position 154, and Aspartate at position 204.

At the place of Ca^{2+}, Zn^{2+} metal also binds with SMP30 in an almost similar way. A detailed crystal structure analysis of SMP30 revealed the single binding site for divalent cation per molecule of the protein[2,4]. The isoelectric point of the protein is 5.20. The protein has been reported as a metalloprotein[5], and the catalytic role has been established by mutagenesis of the residues. The kinetics study using substrate gluconolactone for the human SMP30 protein with different metals as a co-factor showed Kcat preferences for Zn^{2+} following Mn^{2+}, Ca^{2+}, and Mg^{2+}. The affinity constant, Kd value, compared with other metals shows that Ca^{2+} has a significantly high affinity among all other metals. The catalytic property of the protein was analysed in different studies through kinetics to study its lactonase, esterase, and organophosphate hydrolase activity, and the preferred divalent metal as co-factors for these activities. It has also been noted that with mouse SMP30 protein, Zn^{2+} has a role in lactonase activity wherein, for human SMP30, the preferential divalent metal is calcium. The protein also showed the preferential selection of divalent metal as a co-factor and organism-specific manner.

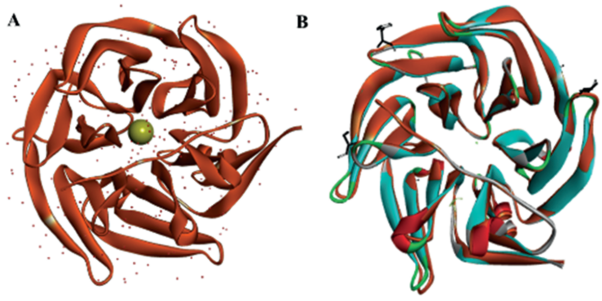

Figure 1. Crystal structure of human SMP30 (PDB: 3G4E). (A) The top view of the human SMP30 protein structure shows the β-propeller fold containing sixblades. The bound Ca2+is shown in a yellow ball, and the red dots are water molecules. (B) Superimposition of both human and mouse SMP30 (PDB: 4GN7) proteins crystal structure, the different amino acids residues are depicted in by stick structures. Superimposed Human SMP30 (red) and mouse SMP30(cyan) structures are showing high structural similarity.

3. SMP30 EXPRESSION

SMP30 expression at the mRNA level is not uniform in all the tissues. Data related to the expression of SMP30 in various tissue samples have been summarised in Table 1. The tissues isolated from mice have shown a noticeable level of mRNA expression in the kidney, liver, lung, testes, and cerebrum endogenously.[6] Similarly, the findings from immunoblotting and immunochemistry experiments have also stated its expression in rats. In addition, mammary glands and prostate in rats and human cell lines have also shown significant expression.[7-8] RNA*seq* data of 27 different tissues show varying levels of SMP30 expression in several organs, which can be summarised in the decreasing order like liver, adrenal gland, kidney, thyroid, ovary, small intestine, duodenum, prostate, heart, placenta, lungs, and other tissues.[9] However, there is a significant decrease in the expression level due to aging.

Gender-dependent alteration in SMP30 expression has been noticed in various tissues. Literature suggests that the SMP30 is linked with X-linked muscular dystrophy due to significantly low expression levels in mice diaphragm.[11]

Table 1. Expression of the SMP30 in tissues and body fluids of healthy animals

S. No.	Tissues	Animals	Biomolecule	Reference
1	Liver and Kidney	Human/Mouse	Protein/mRNA	10
3	Diaphragm muscle	Mouse	Protein	11
4	Cerebral cortex	Mouse	Protein	10
5	Locus coeruleus	Human	Protein	12
6	Stomach	Mouse	Protein/mRNA	13
7	Mammary gland	Human	Protein/mRNA	8
8	Submandibular gland	Mouse	Protein	14
9	Prostate, Testis	Human	Protein/mRNA	8,15
11	Plasma	Human	Protein	16-18
12	Pancreas	Human	Protein	2
13	Reproductive tract (Bulbourethral gland, Epididymis, prostate, Seminal vesicle, andTestis)	Male Buffalo	mRNA/Protein/ Immunolocalization	19
14	Spermatozoa	Male Buffalo	mRNA/Protein	20

Also, the presence of SMP30 in mice's heart and limb muscles was established in the same study.[11] In contrast, differential expression of SMP30 in the heart of rats may or may not be due to age differences in the animals used for the study.[21-22] The expression of SMP30 protein was found to have an androgen-independent decrease with aging when compared at age four weeks up to 30 months in Wistar/Slc male and female mice.[1] SMP30 was also seen to be up-regulated in sperms with aging in rats. The protein intensity was more significant at the acrosome of the sperm. This over expression of SMP30 in sperm cells suggests its association with reducing the age-related decline of sperm and the upkeeping of good spermatogenic yield.[23]

Moreover, a significant level of alterations in the expression was found to be associated with many pathological conditions, especially oxidative stress-related disorders such as Alzheimer's, and osteoporosis.[4] Most of the human cancer cell lines have shown downregulation of its expression at mRNA and protein levels. In addition, tumor cells isolated from the cancer patient samples also show decreased expression. These observations indicate that SMP30 plays an essential role in maintaining cellular homeostasis in normal cells.

4. MODULATORS OF SMP30 EXPRESSION

4.1 Internal Modulators

The expression of the SMP30 gene largely depends upon its nuclear transcription factors like NFI-A1, AP1, and RGPR-p117. These transcriptional factors could bind in the region at the TTGGC sequence after getting phosphorylated in the promoter sequence. RGPR-p117 protein gets a modification after post-translational modification, which is essential for its function as a transcription factor.[24] The expressionof RGPR-p117 is unaffected by the aging process.The overexpression study suggests its involvement in regulating expression by inhibiting protein synthesis.[25-26] It can also alter the expression of caspase protein at the mRNA level, thereby preventing apoptotic cell death. Moreover, studies also suggest that overexpression of RGPR-p117 alone cannot significantly alter SMP30 expression level but also involves hormonal control.[24]

Regulation of gene expression of SMP30 is associated with hormones like estrogen, testosterone, insulin, calcitonin, parathyroid hormone, TGF-β, and others, as depicted in (Fig. 2).[27] Enhanced expression in rat hepatoma cells (H4-II-E) inhibited the PI3K protein expression which is a receptor for insulin signaling, suggesting its role in insulin resistant.[28] The expression increases at the mRNA level when treated with parathyroid hormone (PTH) in mouse osteoblastic cells MC3T3-E129. The effect of the PTH on SMP30 expression in NRK52E cells might be due to the high expression of RGPR-p117 at mRNA level.[25] The studies on different rat tissues indicated that estrogen could either increase or decrease SMP30 mRNA expression in a tissue-specific manner.[7]

Endogenous testosterone administration during puberty of male mice shows an increase in SMP30 expression in the renal proximal tubular segment. Moreover, the study suggests that testosterone also regulates expression, particularly in the kidney, and contributes to calcium absorption in the urinary tract.[30] The 17β-oestradiol

(E2) administration in the rat lowered the expression of SMP30 in the mammary gland, which suggests the possible SMP30 connection in breast physiology through E2-dependent pathways.[31] Treatment with Thyroid hormone (T3,3,3'5 Triido L- thyronine) represses the expression in rat liver and human breast cancer cell line (MCF-7). The suppressed level leads to increased apoptosis as analysed by flow cytometry.[32] SMP30 knock-out mice show accelerated bone loss by inducing PPAR-γ expression; however, the control group of SMP30 knock-out mice supplemented with Vitamin C had healthy bone.[33,34] Therefore, it can be hypothesised that thyroid patients without any Vitamin C supplement could be more prone to cancer and bone-related diseases.

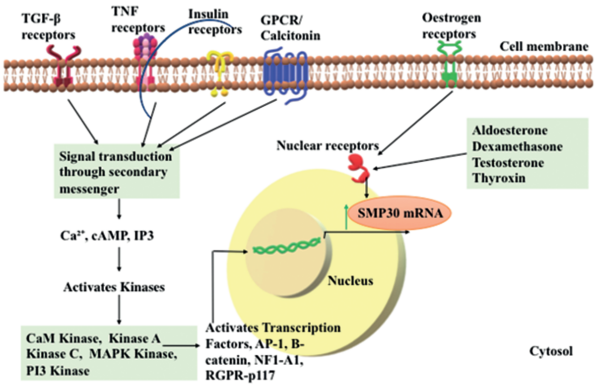

Figure 2. Regulation of SMP30 gene expression by estrogen, testosterone, insulin, calcitonin, parathyroid, and TGF-β. The steroid hormones bind to the receptors present in the cytoplasm of the cells, which activate/ modulate the secondary messengers such as cAMP, IP3, and the calcium level in the cytosol. These secondary messengers, in turn, activates kinases like CaM, Kinase A, KinaseC, MAPK, and PI3 kinases. These activated kinases further pass the signalthrough numerous cell-signaling factors such as AP-1, β-catenin, NF1-A1, and RGPR-p117, which induce the promoter for SMP30 gene expression in the nucleus. On the other hand, there is direct interaction of aldosterone, testosterone, thyroxin, and dexamethasone with nuclear receptors which also regulate gene expression.

4.2 External Modulators

In addition to internal modulators, many external agents could regulate the SMP30 expression. For example, Resveratrol found in red grapes and Quercetin present in red wine increase the SMP30 mRNA expression in HEK 293 cells.[32] However, high glucose exposure to mouse microvascular endothelial cells (MMECs) reduces expression. Whereas, counteracting the above scenario, Metformin increases the levels of SMP30 when given to high glucose-induced MMECs.[35] A decoction Yin Chen-Hao-Tang used in Taiwan and China also impacts the expression. This ancient herbal medicinal extract consists of Artemisia capillaries Thunb, Rheum officinale Baill, and Gardenia jasnimoides Ellis.[36] Another external agent, a novel metalloprotease obtained from Aranicola proteolyticus, also known as Arazyme, showed a protective post-hepatic injury by increasing cytosolic SMP30 expression and suppressing the TGF-β/Smad pathway.[37]

SMP30 expression could be manipulated by bile juice obtained from a semiaquatic rodent of South America, namely Nutria (Myocastor coypus). Nutria bile contains 37 % of ursodeoxycholic acid, and this is applied to mice treated with thioacetamide-induced liver injury, which reduces hepatic inflammation and increases the expression level.[38] Rats subjected to kainate induction showed up-regulation of SMP30 at the hippocampus region after four weeks of treatment. This upregulation correlates with increased fibrillary acidic protein in glial cells of the hippocampus and is associated with astrocytosis. Phosphorylation of ERK1/2 also significantly increased in CTX TNA2 cells along with SMP30 and glial fibrillary acidic protein after kainate induction.[39]

5. FUNCTIONS OF SMP30

5.1 Ascorbic Acid (Vitamin C) Biosynthesis

SMP30 is a crucial enzyme required for the reversible conversion of a linear L-gulonic acid and a cyclic L-gulono-γ-lactone at the penultimate step of Vitamin C biosynthesis. The SMP30, purified from rat liver, was characterised as an aldonolactonase enzyme catalyzing the interconversion of L-gulonate and L-gulono-γ-lactone. At the last step, ascorbic acid is formed from L-gulono-γ-lactone which is catalysed by Gulonolactone Oxidase (GULO) enzyme. However, primates including humans are not able to synthesize ascorbate as the last step of ascorbic acid biosynthesis is defective due to the absence of a functional Gulonolactone Oxidase gene. SMP30 also showed lactonase activity towards other aldonolactones due to its promiscuous nature.[1]

5.2 Calcium Homeostasis

As mentioned above, SMP30 is a cytosolic Ca^{2+} binding protein, which can also participate in Ca^{2+} signaling, regardless of its apparent structural scarcity of the EF-hand motif. Thus, it shows involvement in calcium homeostasis in various organs, most remarkably in the liver and kidney.[40] Calcium ion is vital in regulating many cell functions and cellular signaling as it functions as an essential intracellular secondary messenger. A (Ca^{2+}-Mg^{2+}) ATPase on the plasma membranes of hepatocytes pumps Ca^{2+} out of the liver cytoplasm. The activity of (Ca^{2+}-Mg^{2+}) ATPase increased in the plasma membrane when SMP30 was added in vitro, activating the Ca^{2+} pumps. SMP30 also stimulates the intake of Ca^{2+} by mitochondria in rat hepatocytes. The role of Ca^{2+} in the regulation of SMP30 expression has been observed in H4-II-E hepatoma cells.[13,28] In addition, calcitonin, estrogen, and insulin also stimulate hepatic SMP30 expression in rats[40]. A cell culture study on astrocytes isolated from the SMP30 knock-out mice suggests a modulation of the expression that can be a potential target for the treatment of Parkinson's disease.[41]

Under normal physiological conditions,the cytoplasmic Ca^{2+} concentration is ~0.1-1µM; however, under stressed conditions, its concentration in the cytoplasmic region increases significantly. The overexpression of SMP30 shows a cytoprotective role against the stress triggered due to the calcium-related molecules for instance Ca^{2+} ionophores (A23187) and thapsigargin.[22] Endogenous SMP30 participates in Ca^{2+} efflux in renal tubular cells by activating those Ca^{2+} pumps which are reliant on calmodulin for their functioning. It provides confrontation to the cells against the damagescaused due toenhanced intracellular Ca^{2+} levels, suggesting that it holds a physiological function in regulating the calcium homeostasis in kidney cells. Due to its role in calcium homeostasis, it also prevents apoptosis. Low expression of SMP30 in cancer cells also regulatesCa^{2+} concentration and calcium pumps, which regulate the metastasis-related matrix metalloproteinase expression, thereby increasing cell migration.[42]

5.3 Organophosphatase Activity

SMP30 shows similarity with mammalian Paraoxonaseand bacterial phosphotriesterase inhydrolyzingboth lactones and organophosphates43-44. Ithydrolyzes Di-isopropyl phosphorofluoridate (DFP) and other highly toxic organophosphorus compounds in the presence of metal cofactors such as Magnesium and Manganese1. These enzymes also show structural similarity to a typical β-propeller structure, however, there is minimal sequence identity.[4,43,45] Therefore, it could be hypothesised that the organophosphate hydrolase enzyme is evolved from the lactonase enzymes. Several other β-propeller structured proteins are reported to either have only lactonase or organophosphatase activity45.However, the exact mechanism of catalysis involved in organophosphate hydrolase and lactonase functions are yet to be exploredin SMP30. Recent research from our lab has evaluated that both human and mouse SMP30 proteins display organophosphatase activity in the presence of Zn^{2+} as well as Ca^{2+} ions, thus confirming its metal-dependent promiscuous activity.[44,46] In addition, for DFPase enzymatic activity, a recombinant human SMP30 has been expressed along with chaperones as a soluble protein.[44,47]

6. ROLE OF SMP30 IN DISEASES

6.1 Cancer Progression

SMP30 regulates cell proliferation and is linked to tumor formation.[7,8] The SMP30 expression in clinical hepatocellular carcinoma (HCC) tissues was noticed to be downregulated compared to normal tissues.[17,48] Improved expression of SMP30overturns the growth of renal carcinoma cells in vitro.[49] The research findings related to SMP30 expression have been summarised in Table 2.

Moreover, low SMP30 expression at mRNA levels is also correlated with the progressive stages of tumor formation.[8] These remarks back the theory that a reduction in SMP30 expression could encourage or play a significant role in the augmentation of a tumor. SMP30 expression patterns in several breast cancer cell lines like MDA-MB-231 and MCF-7, and human mammary cancer tissues revealed that it can be a potential therapeutic target and diagnostic marker.[54]

A study has shown that overexpression of SMP30 in rat liver cells downregulates the expression of cancer-linked genes like Ha-ras, c-myc, c-src, and similarly upregulates the expression of p53, and Rb (retinoblastoma protein) compared with contro.[11,8] SMP30 has also been revealed to inhibit metastasis and cell invasion in HCC cells by interacting with ROCK1 protein. These observations summarize SMP30 as a protective molecule against cancer. This metastasis-related protein, in turn, interacts with the cytoskeleton-related myosin light chain proteins and reduces its phosphorylation. SMP30 downregulation in cancer cells also results in the regulation of calcium ions concentration and calcium pumps, which regulate the matrix metalloproteinase protein expression leading to enhanced cell migration.[42]

6.2 Oxidative Stress-Related Disorders

Anti-oxidative and anti-aging properties of SMP30 establish it as a cardioprotective agent. Literature suggests its defensive role against adverse cardiac remodeling and repair induced by angiotensin II.[55] Shortage of SMP30 jointly with oxidative stress enhances hydrogen peroxide and angiotensin discharge from cardiomyocytes.

Table 2. The expression levels of SMP30 in various human and murine cancer cell lines at mRNA and protein level

S. No.	Cell line	Nature of cells	Biomolecule	Expression	References
1	HepG2	Human Hepatocarcinoma	Protein/mRNA	Downregulated	42, 50
2	MCF-7	Human breast	Protein/mRNA	Downregulated	8
3	LNCaP	Human prostate	Protein/mRNA	Downregulated	8
4	A549	Human lung	Protein/mRNA	Downregulated	51
5	RKO	Human colorectal	Protein/mRNA	Downregulated	52
6	MIA PaCa-2	Human pancreatic	Protein/mRNA	Downregulated	53
7	Huh7	Human hepatocarcinoma	Protein	Downregulated	3

Hence, SMP30 plays a vital role in regulating coronary vascular tenor by the myocardium.[56] Levels of protein carbonyls are more noticeable in SMP30 knock-out mice when compared to the control group of similar age. High oxidative stress levels are also detected in the brains of SMP30 knock-out mice[1]. At the same time, overexpression of SMP30 in embryonal carcinoma P19 cells shields the cells from *t*-butyl hydroperoxide and encourages cytotoxicity.[57]

Since ascorbate is a potent antioxidant, and the enzyme (SMP30) involved in its synthesis is recognised and credited, the SMP30 knockout mice have been the feasible animal model to study the role of ascorbate in various physiological conditions. *In vitro* experiments using recombinant human SMP30 protein have shown its ROS suppressive role in human hepatoma HepG^2 cells.[42] The reduced glutathione levels along with other ROS elements and superoxide dismutase activity were also observed in the same experiment which suggests the SMP30 as a potent antioxident.[50] It is also proposed that the recombinant human SMP30 alone may not directly scavenge the ROS. In this regard, its calcium-binding property might be playing a crucialrole insustaining the damage caused due to the enhanced ROS levels.[42]

In addition, SMP30 protects brain tissues from oxidative stress. It helps to maintain reduced levels of ROS and NADPH oxidase activity.[1] The overexpression of the SMP30 protein inhibits the Keap[1] and plays a vital role in neuronal protection by regulating cerebral reperfusion /ischemia by enhancing Nrf2/ARE (a protein called nuclear erythroid 2-related factor/ and antioxidant response element).[57] SMP30 is also able to influence superoxide dismutase activity in the rat heart.[58] Undeniably, overexpression of SMP30 in human liver carcinoma cell HepG^2 has accompanied the reduced superoxide dismutase protein levels. Four-week Kainate treatment induces oxidative stress which is leading to the overexpression of SMP30 in the hippocampus of rat brains. This could be also associated with ERK signaling as an off-target effect of it.[44]

6.3 Regulation of Cell Proliferation

Cell proliferation is an essential process in maintaining physiological homeostasis. SMP30 is an anti-proliferative molecule that might act on phosphatases and kinases involved in cell cycle regulation, as depicted in Fig. 3. Studies also revealed SMP30's anti-proliferative role in the liver and kidney cells of rat.[1] Similarly, proliferation was decreased in P19 cells when SMP30 was transfected and overexpressed in cells.[56] SMP30 could be a potential therapeutic target for cancer because it suppresses the mammary cell (MCF 10A) proliferation independent of p53 when treated with extracellular SMP30.[59] Studies in the cytoplasm of rat liver cells have shown that SMP30 may regulate protein phosphatase activity.[60] The same study also revealed that endogenous SMP30 may regulate protein kinase or tyrosine activity via Ca^{2+} / calmodulin. similarly, it can inhibit protein kinase C.[60]

Bay K8644, an L-type calcium channel blocker, treatment induces protein phosphatase activity in hepatoma cells which is suppressed when treated with trifluoperazine, this observation suggests Ca^{2+} /calmoduline-dependent protein phosphatase activity[60]. Whereas in the presence of SMP30, the protein phosphatase activity is suppressed, which strongly supports that endogenous SMP30 is enhanced through Ca^{2+} signaling and suppresses protein phosphatase activity via Ca^{2+}/calmoduline in proliferative cells. Endogenous SMP30 inhibits DNA synthesis in the H4-II-E proliferating cells[61]. Laminarin (extracted from seaweed) treatment enhances the expression of SMP30 which inhibits the proliferation of HepG2 cells.[62]

Anti-proliferative effect of SMP30 was observed in H4-II-E and can be related to its regulating property of MAP Kinase, and Ca^{2+} /calmoduline-based protein tyrosine kinase activity.[63] The same research also revealed that treatment of IGF-I does not suppress cell proliferation in SMP30 transfected H4-II-E cells compared with control cells.[63] On other hand, the p21 expression at the mRNA level was improved in SMP30 gene transfected cells.[64] Cyclin-dependent kinase (cdk) has a role in cell proliferation however p21 inhibits cdk. In contrast, SMP30 mayincrease p21 level and decreasethe progression of G1 phase through cdk; however, the direct inhibition of the cdk activity is not well established.

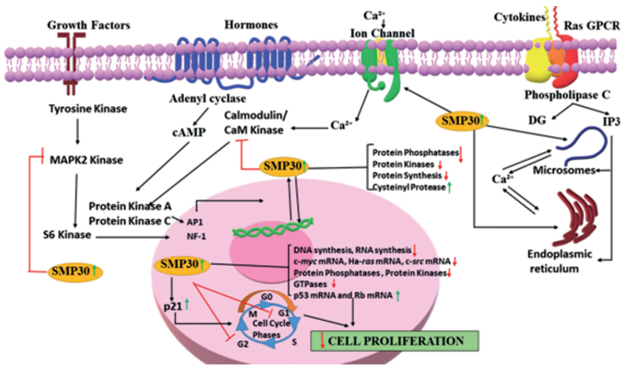

Figure 3. Regulation of the cell proliferation by modulating SMP30 expression. SMP30 expression at the mRNA level is mainly stimulated by Ca^{2+} /calmodulin (CaM)-dependent protein kinase. SMP30 obstructs the actions of multiple protein phosphatases and kinases and also restrains CaM-dependent enzyme activity and cyclic AMP level in the cytosol, which further activates the transcription factors AP1 and NF-1 in the nucleus. On the other hand, cytoplasmic SMP30 inhibits DNA, RNA, and protein synthesis in the nucleus. SMP30 inhibits the expression of cancer-stimulating genes (c-myc, Ha-ras, and c-src) at mRNAs levels. In contrast, it induces the expression of cancer-suppressing genes (p53 and Rb) at mRNA levels. Furthermore, SMP30 induces the G1 and G2/M phases.

6.4 Regulation of Apoptosis

Anti-apoptotic property of SMP30 is partly because of its cellular Ca^{2+} controlling property and interaction with calmodulin.[1,65] It inhibits apoptosis by inhibiting

the accumulation of reactive oxygen species induced by 4-hydroxynonenal in human lens epithelial cells. Overexpression of SMP30 increases the level of catalase and NrF2, decreasing the level of Keap[1] in the cells. Thus, regulating the oxidative functions of mitochondria and preventing the apoptosis of the lens epithelial cells.[66] Besides, oxidative stress induced by high glucose is also prevented by SMP30 in retinal ganglion cells by lowering apoptosis. It protects the cells by increasing NrF2 activation through the Akt/GSK-3β axis.[67] Apoptosis induced by IGF-I is significantly reduced by overexpressing the SMP30 in cloned H4-II-E cells.

Further study has revealed that the insulin effect is due to a decrease in the caspase3 inhibition, while in the case of IGF-I, the Nitric oxide synthase pathway was involved. Despite the involvement of different apoptotic pathways in both cases, the overexpressed SMP30 decreases apoptosis.[59] Lipopolysaccharide(LPS) initiates the expression of many genes inducing apoptosis in fibroblastic cells. The repressive effect of SMP30 on the apoptosis induced by LPS may, to some extent, be related to the suppressive effect on caspase3 in the H4-II-E cell line. Low expression of SMP30 is responsible for increased redox-related Protein Tyrosine Kinase/ Protein Tyrosine Phosphate(PTK/PTP) and PP1/PP2A gaps. It also elevates NF-kB-responsive inflammatory markers.[67] These studies suggest that SMP30 is a potent suppressive protein for oxidative stress and inflammation in mouse kidney cells.[2] A diagrammatic representation of SMP30's role as an anti-apoptotic factor is depicted in (Fig. 4.)

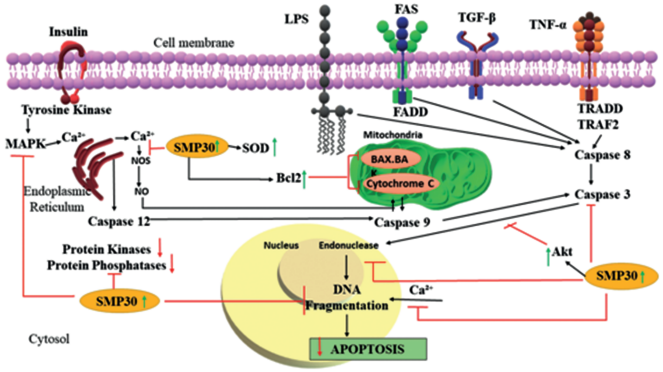

Figure 4. Role of SMP30 as an anti-apoptotic factor that rescuescell death via Ca^{2+} binding occurs at three key regulatory levels, which are (i) suppressive effects on activities of NO synthases (NOS) and hence suppressing Cytochrome C, (ii) suppressive effects on Protein Kinases and Protein Phosphatases and Akt-1, and at mRNA level of Bcl-2, and (iii) suppressive effects on Ca^{2+}dependent and independent endonucleases.

6.5 Role of SMP30 in Liver Regeneration

SMP30 has significance in regulating liver regeneration following hepatic injury. Its role in tissue development has been confirmed by increased expression in regenerating liver. Post 24h of partial hepatectomy surgery in rats, upsurge in SMP30 mRNA level advising that augmentation of SMP30 expression happens in the S-phase of the cell cycle.[68] Transgenic rats, treated to show a hyperlipidaemic disease condition, have demonstrated that SMP30 can decrease leptin mRNA expression in hepatocytes and adipocytes.[69] These studies designate that SMP30 might have a probable role in the differentiation of adipocytes as well.[70]

7. CURRENT STATUS AND FUTURE PERSPECTIVES

Proteins play crucial roles in different cellular functions according to their specific location in various organs. For instance, in acute liver failure, it was found by proteomics analysis that only the SMP30 level increases, and so it was taken as a marker for the disease.[29] Similarly, serum SMP30 level rises with liver damage, and urinary SMP30 increases with kidney injury, proposing SMP30 as a suitable tool for biomarker analysis. Additionally, the SMP30 expression level can also be utilised as an indicator for the initial detection of Alzheimer's disease and other neurodegenerative diseases.[71] The external modulators of SMP30 expression can also be further explored and utilised as a supplement for a healthy life. SMP30 knock-out mouse models can be used as a novel model for murine senile lung.[18] Even for hepatocellular carcinoma, this protein has displayed prognostic potential.[2] The SMP30 gene is proven to be associated with various pathophysiological states like cancer, osteoporosis, liver fibrosis, and diabetes.

Organophosphorus compounds are immensely used as pesticides worldwide, accounting for ailments like liver and kidney stones in the countries like Sri Lanka and Bangladesh. These pesticides are also misused as suicidal agents. A few subtypes of organophosphorus compounds can also be used as chemical warfare agents. SMP30, with its promiscuous nature as OP hydrolase, displays the high potential to be considered and used as a prophylactic agent against OP toxicity.

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