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Review Article

SPOP and cancer: a systematic review

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Abstract: The initiation and progression of cancer is dependent on the acquisition of mutations in oncogenes or tumor suppressor genes that ultimately leads to the dysregulation of key regulatory pathways. Though these mutations often occur in direct regulators of such pathways, some may confer tumorigenic potential by indirectly targeting several pathways congruently thereby exerting pleiotropic effects. In recent years, the tumor suppressor gene Speckle Type POZ Protein (SPOP) has gained a lot of attention as it has been found to be altered in a variety of different cancers. SPOP appears to exert pleiotropic tumorigenic effects as multiple different regulatory pathways become dysregulated upon SPOP alterations. SPOP has been identified as an E3 ubiquitin ligase substrate binding subunit of the proteasome complex. Since protein degradation is critical in regulating proper cellular function it is not surprising that the proteasome pathway is often found to be disrupted in cancer. Many studies have now indicated that mutations or changes in the expression of SPOP are one of several underlying reasons of proteasome pathway disruption in different cancers. Ultimately, either SPOP downregulation or mutation promotes stabilization of direct SPOP targets which subsequently promotes cancer through the dysregulation of key regulatory pathways. In this review, we will discuss the current literature on cancer-specific SPOP alterations as well the SPOP targets that are stabilized, and the pathways that are dysregulated, as a result.

Keywords: SPOP, cancer, proteasome, ubiquitin ligase, review

Introduction

Neoplastic transformation of cells is a multistep process that involves acquisition of certain characteristics that confers survival advantage in their microenvironment. These traits, coined as the hallmarks of cancer, are acquired as neoplastic cells evolve and enable them to be tumorigenic and malignant [1]. The multistep process of cancer initiation and progression often entails alterations in the genome of neoplastic cells. Accordingly, these alterations result in constitutive activation of oncogenic signaling circuits, or dysregulation of key pathways that regulate tumor suppressor activities.

Protein degradation pathways are important in order to regulate proper cellular function. There are two major proteolytic pathways: the lysosomal-mediated proteolysis and the ubiquitin-proteasome pathway [2, 3]. Whereas the lysosomal pathway degrades extracellular proteins, the latter targets proteins marked for destruction by ubiquitin [2]. The proteasome pathway is responsible for the majority of protein degradation in the cell and is essential for cellular homeostasis [4]. It helps maintain intracellular levels of proteins, including those involved in cell cycle progression, apoptosis, DNA damage and repair, and drug resistance [4, 5]. Therefore, it is not surprising that disruption of proteasome activity is present in many types of cancers. In fact, targeting aberrant proteasome activity has become the focus of many anti-cancer therapies.

Proteasome-mediated degradation is composed of multiple steps, starting with the ubiquitination of target proteins and culminating in the degradation of the ubiquitinated substrates by the 26S proteasome complex. Consecutive enzymatic reactions ensure the proper ubiquitination of target proteins, which are catalyzed by ubiquitin activating enzyme E1, ubiquitin-conjugating enzyme E2, and a ubiquitin protein E3 ligase [5]. Alterations in one or more of these components has been reported in cancers. More recently, the speckle-type POZ protein

(SPOP), an E3 ubiquitin ligase adaptor protein, has garnered attention in cancer research. Many studies have demonstrated SPOP to be frequently mutated in many types of malignancies, including cancers of the prostate, breast, endometrium, liver, and colon.

SPOP was first identified in 1997 through an immunoscreening approach using serum from a scleroderma patient [6]. A rough speckled nuclei enriched staining pattern was observed in COS7 cells, which was later identified, through immunoscreening of a human HeLa cell cDNA library to be a novel POZ/BTB domain-containing protein [6]. Later studies revealed the presence of a meprin and TRAF homology (MATH) domain thereby first presenting a role of SPOP in substrate recognition through a Cul3 based mechanism [7, 8]. SPOP is evolutionarily well conserved with homologs that have high sequence similarity and conserved functions in *Caenorhabditis elegans* and *Drosophila melanogaster* [7, 9]. Furthermore, a human paralog of SPOP, termed SPOPL (containing 81% sequence similarity) has been identified through GenBank database interrogation, which also contains high sequence similarity to SPOPL homologs in other species [10]. SPOP and SPOPL both act as the substrate adaptor of a cullin-3-RING ubiquitin ligase (CRL3) and serve to recruit substrates to the CRL3. Albeit the high sequence similarity, SPOP and SPOPL appear to target different substrates and carry out unique functions. Functions that are unique specifically to SPOPL include inhibition of E3 ligase activity (discussed later) and degradation of EPS15 at endosomes to aid in endocytic trafficking [11]. The multifaceted functions of SPOP will be discussed in the remainder of this review article.

It is evident that SPOP plays a big role in tumorigenesis of a variety of different cancer subtypes. In most cancers, with a few minor exceptions, SPOP acts as a tumor suppressor gene and promotes tumorigenesis when either mutated or downregulated. As a result, the mutational landscape and gene expression status of SPOP has been extensively studied which has shown that SPOP is altered in a large number of different cancers (**Table 1**). In this review, the overall structure of SPOP will be discussed, followed by the mechanistic roles of SPOP in non-cancer and cancer related pathways.

Structure and activation of SPOP

SPOP is comprised of a N-terminal MATH domain, an internal BTB/POZ domain, an internal BACK domain (with smaller 3-box domain), and a C-terminal nuclear localization sequence (**Figure 1A**). SPOP-Cul3 recognition occurs primarily through the BTB domain at the α3-β4 loop and the α5/α6 helical hairpins with additional Cul3 recognition occurring through the BACK domain (**Figure 1B**) [12]. In fact, one study has suggested that the N-terminal and C-terminal regions adjacent to the BTB domain contribute to the interaction of SPOP with Cul3 as deletions mutants containing only the BTB domain were unable to associate with Cul3 [13]. Substrate recognition through the MATH domain is mediated by several critical residues, including Y87, F102, Y123, W131, and F133 that aid in recognizing a 5-residue SPOP-binding consensus (SBC) motif [14]. Earlier studies have shown that optimal ubiquitination activity occurs when SPOP forms a homodimer through a BTB dimerization interface thereby recruiting two Cul3 proteins and exposing two asymmetrically arranged MATH domains (**Figure 1B**) [14, 15]. The dimeric configuration is believed to increase binding efficiency to substrates containing multiple suboptimal SBC motifs in a substrate-specific manner as the binding affinity of one MATH domain in the dimer is independent of the association state of the other MATH domain [16]. Furthermore, the presence of multiple SBC motifs within substrates is critical for polyubiquitination and subsequent degradation as substrates with only one SBC motif only undergo monoubiquitination [16]. Substrate SBC motifs appear to adopt an extended conformation to bind MATH domains in a 2:1 ratio of one dimeric SPOP-containing complex to one substrate (**Figure 1B**) [12, 14]. More recent studies have suggested that SPOP also undergoes high order oligomerization through a dimerization interface in the BACK domain thereby increasing E3 ubiquitin ligase activity (**Figure 1C**) [12, 16-18]. BTB domain-mediated dimerization has a very low dissociation constant, whereas BACK domain-mediated oligomerization is highly dynamic and dissociable. Interestingly, one study has indicated that oligomerization, but not dimerization, significantly increases binding efficiency to substrates [16]. It is thus proposed that high order oligomerization provides flexibility in E3

Table 1. SPOP alterations

TUMOR TYPE	ALTERATION	%	REFERENCE
Prostate cancer	Mutation in 2/7	28.6	[53]
	Mutation in 8/78 (Caucasian men)	10.3	[54]
	Mutation in 4/88 (African American men)	4.5	[54]
	Mutation in 2/45	4.4	[55]
	Mutation in 9/90	10	[56]
	Downregulation in 248/265	93.5	[56]
	Upregulation in 17/265	6.5	[56]
	Mutation in 4/84 (African American men)	4.7	[57]
	Mutation in 6/81 (Korean men)	6.9	[57]
	Mutation in 6/93 (Swedish men)	6.1	[57]
	Mutation in 19/222 (Zurich men)	7.9	[57]
	Mutation in 11/83	13.3	[58]
	Mutation in 5/49	10.2	[59]
	Mutation in 11/66	16.7	[60]
	Downregulation in 28/111	25.2	[67]
	Mutation in 2/29	6.9	[61]
	Mutation in 3/34	8.8	[62]
	Mutation in 6/62	9.7	[63]
	Mutation in 22/89	24.7	[64]
Breast cancer	Loss of heterozygosity in 26/45	57.8	[70]
	Copy number loss	60-70	[70]
Colorectal cancer	Mutation in 1/45	2.2	[55]
	Downregulation (RNA level) in 78/126	61.9	[94]
	Downregulation (protein level) in 18/24	75	[46]
	Downregulation in 12/60	20	[55]
	Downregulation in 31/118	26.2	[41]
Endometrial cancer	Mutation in 9/63	14.3	[97]
	Mutation in 5/28	17.9	[98]
	Mutation in 4/52 (serous endometrial tumors)	7.7	[99]
	Mutation in 2/23 (clear-cell endometrial tumors)	8.7	[99]
Gastric cancer	Downregulation in 18/60	30	[55]
Glioma	Downregulation in 61/98	62.2	[120]
Carcinosarcoma	Mutated in 3/22	13.6	[121]
Liver cancer	Upregulation in 231/300	77	[112]
	Upregulation in 33/70	47.1	[113]
Kidney cancer	Mutation in 1/6	16.7	[102]
	Downregulation in 10/33	30.3	[102]
	Downregulation in 25/54	46.3	[103]
	Downregulation in 26/44	59.1	[104]
Lung cancer	Downregulation in 132/157	84.1	[106]
Ovarian cancer	Deletion in 46/88	52.3	[111]
	Mutation in 1/10	10	[110]

ubiquitin ligase activity with optimal activity occurring in higher order complexes [12, 17, 18]. Consistent with this is the finding that self-association deficient SPOP is defective in proper protein localization. Wildtype SPOP localizes to nuclear membrane-less organelles whereas oligomerization-defective mutations lead to diffused nuclear localization [6, 18]. Another pro-

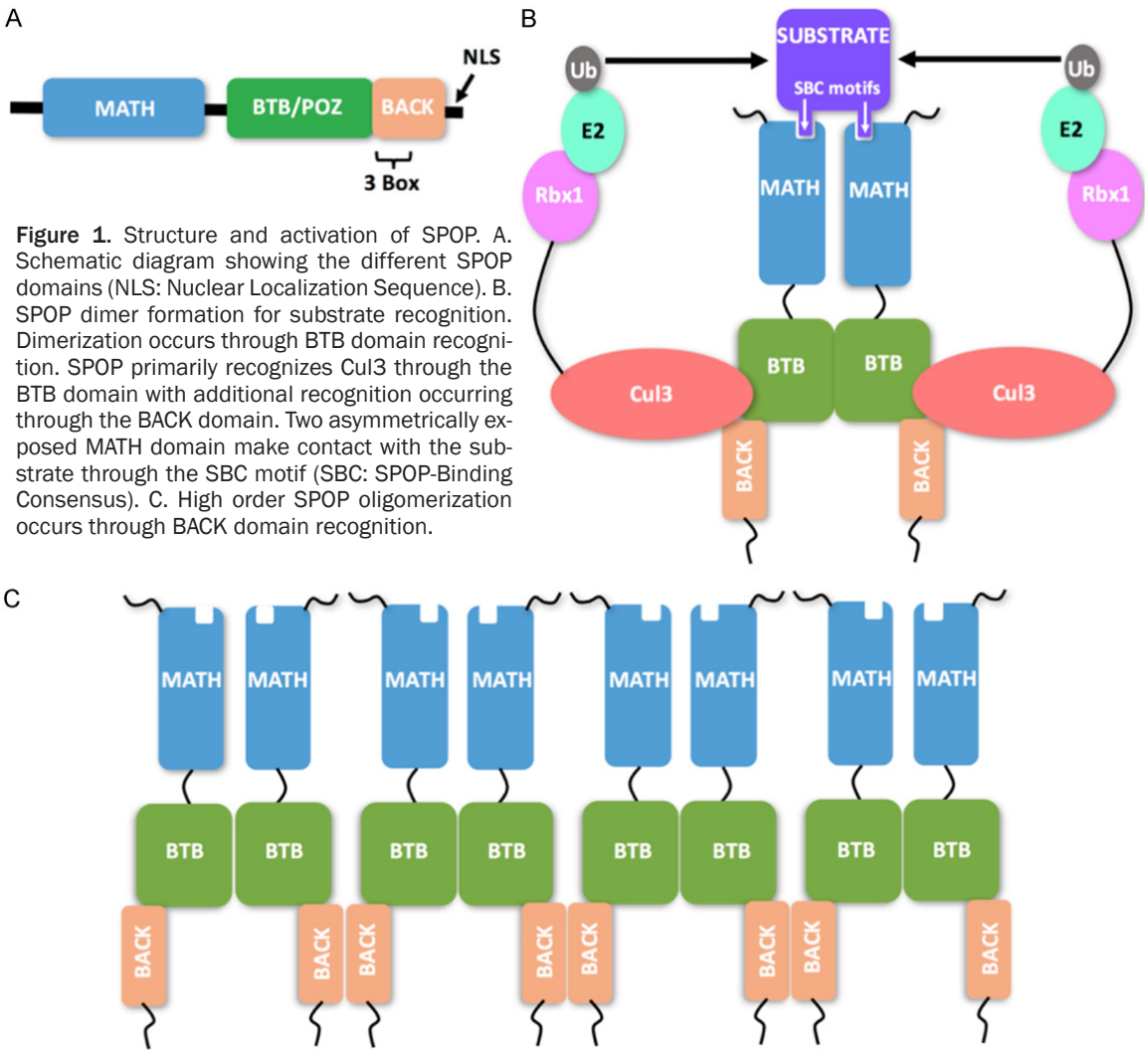

Figure 1. Structure and activation of SPOP. A. Schematic diagram showing the different SPOP domains (NLS: Nuclear Localization Sequence). B. SPOP dimer formation for substrate recognition. Dimerization occurs through BTB domain recognition. SPOP primarily recognizes Cul3 through the BTB domain with additional recognition occurring through the BACK domain. Two asymmetrically exposed MATH domain make contact with the substrate through the SBC motif (SBC: SPOP-Binding Consensus). C. High order SPOP oligomerization occurs through BACK domain recognition.

posed mechanism is that high order complexes allows for recruitment of multiple E2 conjugating enzyme molecules close to the substrate on the same face of the assembly [12]. Fine-tuning of E3 ubiquitin ligase activity is also regulated through hetero-dimerization of SPOP with SPOPL. At least one study has shown that SPOP/SPOPL complexes are correlated with a decrease in E3 ubiquitin ligase activity thereby indicating that SPOPL acts as a negative regulator of SPOP ubiquitination activity [12].

Although not much is known about the activation of Cul3-SPOP complexes, at least one study has indicated involvement of p38 MAPK signaling [19]. This study has shown that increased phosphatidylinositol 5-phosphate levels promote ubiquitination of the SPOP targets type II phosphatidylinositol phosphate kinase β (PIPKIIβ), pancreatic and duodenal homeobox 1 (PDX1), and death domain associated protein (DAXX) and that this stimulation is transduced by p38 MAPK signaling (**Figure 2**). Furthermore, a negative feedback mechanism has been proposed where SPOP itself is ubiquitinated along with its substrates in a similar manner (**Figure 2**) [19]. Importantly, total ubiquitination was unchanged in these studies thereby indicating that the role of MAPK signaling is specific to the ubiquitination of Cul3-SPOP substrates [19]. As mentioned previously, full activation of Cul3-SPOP complexes is also dependent on the ability of SPOP to properly dimerize and/or oligomerize [12, 17, 18]. Mutations within the BACK domain that prevent proper oligomerization, or mutations within the BTB domain that prevent dimerization significantly impede ubiquitination activity [12, 14, 17, 20]. Substrate binding-defective mutations within the MATH domain greatly affect E3 ligase

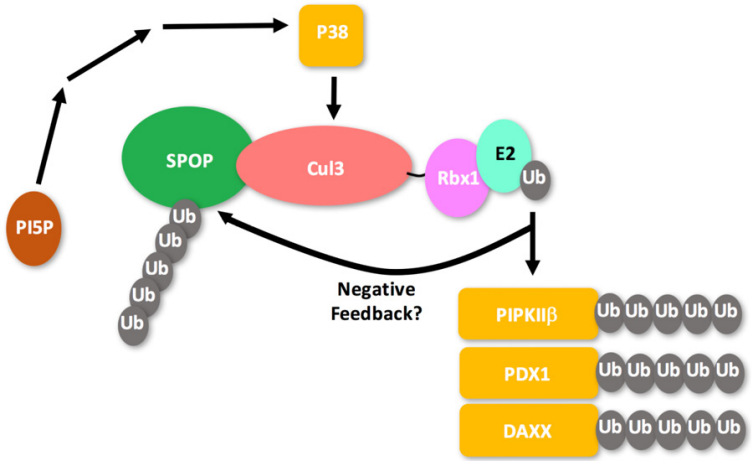

Figure 2. Activation of SPOP through p38 MAPK signaling. Phosphatidylinositol 5-phosphate (PI5P) activates p38 MAPK signaling through unknown intermediate effector proteins. Activated p38 MAPK signaling promotes the ubiquitination of several known SPOP targets as well as SPOP itself in a negative feedback loop.

activity and in most cases fully abolish SPOP-mediated ubiquitination and degradation of substrates (as will be discussed in detail later). Furthermore, SPOP mutations within the MATH domain are commonly found in a variety of cancers thereby indicating that abolishment of SPOP-mediated substrate degradation can play a major role in cell regulatory pathways (as will be discussed in detail later). SPOP activity can also be impeded by mutation-independent mechanisms. As mentioned previously, SPOPL acts as a negative regulator of SPOP activity through dimerization to form SPOP/SPOPL complexes [12]. Furthermore, SPOP protein levels have been shown to be regulated by miRNA-145 and Daz interacting protein 1 (Dzip1) thereby providing yet another level of SPOP regulation [21, 22]. Specifically, these studies have demonstrated that SPOP is downregulated at the post-transcriptional level by miRNA-145 and Dzip1 protects SPOP from proteasome-dependent degradation [21, 22].

Non-cancer related roles of SPOP

The major function of SPOP has been designated to its role in the ubiquitin-mediated proteasome pathway, as attributed to its E3 ubiquitin ligase activity, though some studies have highlighted a role of SPOP in a particular pathway without identifying a specific substrate. In recent years, many SPOP substrates have been uncovered and implications of SPOP-mediated substrate degradation has been studied in a variety of systems (**Table 2**). The majority of SPOP-related studies focus on the role of SPOP in tumorigenesis although some studies have focused on non-cancer related roles as well. Summarized below are non-cancer related pathways in which SPOP plays a critical role.

Development

The first reported SPOP substrates are gli family zinc finger 2 and 3 (GLI2 and GLI3), downstream effectors in Sonic Hedgehog (SHH) signaling [22-27]. One study has uncovered three SBC motifs within the N-terminus of GLI3 that display high binding affinity for high-order SPOP oligomers by binding to the canonical SPOP substrate-binding groove [16]. In these early studies, it was indicated that SPOP only targets the full-length activator form of GLI3, but not the truncated repressor form [23, 25]. GLI2 and GLI3, but not GLI1, are both ubiquitinated and targeted for degradation by SPOP in a proteasome-dependent manner in mouse embryos and mouse embryonic fibroblasts thereby highlighting a critical role of SPOP during mouse development [23-25]. In another line of study, SPOP was shown to play a critical role in regulating the patterning of the mouse ventral spinal cord. Specifically, it was indicated that loss of SPOP suppresses the loss of floor plate and V3 interneuron phenotypes displayed by GLI2 mutants [28]. Furthermore, they showed that loss of SPOP correlates with an increase in the level of GLI3 and that SHH signaling is restored in these mutants thereby highlighting the negative role of SPOP in the level and activity of SHH signaling and ventral spinal cord patterning [28]. Similar results were found in *Drosophila melanogaster* (*D. melanogaster*) where SPOP was shown to target both GLI2 and GLI3 for degradation, a process that is critical for proper eye development [9, 29]. Another study also reported the importance of SPOP in *D. melanogaster* wing disc development where it reportedly downregulates Suppressor of Fused (SUFU), a negative SHH regulator [30]. In accordance with these previous studies, a recent

Table 2. SPOP targets

SPOP TARGET PROTEIN	PROCESS/PATHWAY AFFECTED	CANCER	REFERENCE
GLI2	SHH signaling, IHH signaling, mouse and *D. melanogaster* development, apoptosis	Colorectal cancer, gastric cancer, lung cancer	[9, 23-25, 27-30, 32, 41, 117]
GLI3	SHH signaling, IHH signaling, mouse and *D. melanogaster* development	Lung cancer	[9, 23-25, 28-30, 32, 117]
MacroH2A	X chromosome inactivation		[35, 36]
MBI1	X chromosome inactivation		[36]
PDX1	Apoptosis, glucose homeostasis, maintenance of β cell mass		[38, 39]
DAXX	Apoptosis, extracellular matrix degradation, angiogenesis		[13, 15, 48]
BCL2	Apoptosis	Colorectal cancer	[41]
FADD	Pancreatic stellate cell activation, NF-κβ signaling	Lung cancer	[51, 52]
SRC-3	Androgen receptor signaling, PI3K/mTOR signaling, estrogen receptor signaling	Prostate cancer, breast cancer	[70-72]
AR	Androgen receptor signaling	Prostate cancer	[73, 74]
ERG		Prostate cancer	[40, 75]
DDIT3	ER stress-induced apoptosis	Prostate cancer	[76]
DEK		Prostate cancer	[20]
TRIM24	Androgen receptor signaling, progression to CRPC	Prostate cancer	[78]
NANOG	AMPK/BRAF signaling	Prostate cancer	[80, 81]
CDC20	Cell cycle	Prostate cancer	[82]
CYCLIN E1	Cell cycle	Prostate cancer	[83]
c-MYC	Epithelial to mesenchymal transition	Prostate cancer, breast cancer	[84, 92]
Eg1N2		Prostate cancer	[85]
HDAC6		Prostate cancer, colorectal cancer	[86]
ATF2		Prostate cancer	[69]
FASN	Lipid homeostasis	Prostate cancer	[87]
ER	Estrogen receptor signaling	Endometrial cancer	[37, 100]
PR	Progesterone receptor signaling	Breast cancer	[91]
BRMS1	Breast cancer metastasis	Breast cancer	[93]
PTEN		Kidney cancer	[114]
ERK		Kidney cancer	[114]
SENP7		Liver cancer	[104]
SIRT2		Lung cancer	[108]
BET proteins	Akt/mTOR signaling	Prostate cancer, endometrial cancer	[85, 89]

finding has indicated that double knockout mice for SUFU and SPOP show a significant upregulation of GLI2 which subsequently results in abnormal gut mesenchymal development [31]. SPOP has also been shown to play a role in mouse development by directly regulating Indian Hedgehog signaling (IHH). Specifically, SPOP was shown to target GLI2 and GLI3 for degradation which in turn leads to a downregulation of Patched 1 (PTCH1) and parathyroid hormone-like peptide (PTHLH) which subsequently causes major skeletal defects, including brachydactyly and osteopenia [32]. Interestingly, the results from this study showed that SPOP targets the full length as well as the repressor forms of GLI3, an outcome that directly contradicts previous findings [32].

SPOP has also been shown to play a development role in mechanisms that do not involve the GLI proteins and SHH signaling. One study has shown that SPOP expression is induced by E2 and P4 hormones in the mouse uterus in the primary decidual and second decidual zone during embryo implantation [33]. In congruence with this finding, it was shown that high SPOP expression is critical for embryo implantation and endometrial decidualization [33]. Furthermore, another study showed that conditional knockout mice (in which SPOP is abolished in uterine cells) display development of large glandular cysts with foci of epithelial proliferation thereby further supporting a role of SPOP in maintaining a healthy uterus [34]. Although a direct mechanistic role was not discussed, SPOP conditional knockout mice show a decrease in progesterone receptor (PGR) expression and an increase in estrogen receptor 1 (ESR1), GATA binding protein 2 (GATA2), and steroid receptor co-activator 2 (SRC2) thereby indicating that SPOP likely plays a role in uterine health through modulation of these target proteins [34].

X-chromosome inactivation

At least two studies have highlighted the role of SPOP during the process of X inactivation. Through yeast two-hybrid screening, SPOP was found to bind directly to MacroH2A to target it for degradation, and furthermore, that this was critical for promoting stable X chromosome inactivation [35, 36]. Another SPOP target that is critical for X-inactivation is BMI1, a polycomb group protein that acts as an oncogenic collaborator together with MYC [36]. MacroH2A and BMI1 are both recruited to the inactive X chromosome in a highly dynamic manner and, importantly, knockdown of SPOP promotes loss of MacroH2A from the inactivated X chromosome thereby subsequently affecting X-inactivation [36].

Apoptosis and cellular senescence

A pro-apoptotic role of SPOP has been demonstrated in several studies as indicated by enhanced apoptosis upon SPOP overexpression and reduced apoptosis upon SPOP knockdown [27, 37, 38]. More specifically, studies in mice have found that PDX1, a major mediator of insulin transcription, is a SPOP target in adult β cells and that proper PDX1 targeting is critical for glucose homeostasis and maintenance of β cell mass through apoptotic mechanisms [38, 39]. Additionally, SPOP-mediated degradation of DAXX, a multifunctional protein regulating a variety of cellular processes, has been linked to apoptosis and extracellular matrix degradation [13, 40]. Specifically, it was demonstrated that SPOP-mediated degradation of DAXX reverses repression of ETS1 and p53 dependent transcription thereby inducing apoptosis [13]. Finally, SPOP has also been shown to repress BCL2, an apoptosis inhibitor, in colorectal cancer, thereby inhibiting the function of resisting apoptosis in these cells [41]. In a direct contradiction to these studies, SPOP has also been shown to negatively regulate apoptosis in renal cell cancer cells as indicated by enhanced apoptosis upon SPOP knockdown [42]. At least one study has also demonstrated a role of SPOP in cellular senescence through SPOP-mediated degradation of the desumoylase SENP7 [43].

DNA damage response

The role of SPOP in the DNA damage response (DDR) has been demonstrated in several studies. In one study, the authors indicate that SPOP forms nuclear foci in response to DNA damage in a manner that is dependent on ATM, a protein that has been shown to play a big role in facilitating optimal DNA damage response [44]. Furthermore, SPOP depletion results in reduced DNA damage repair and hypersensitivity to irradiation [44]. In congruence with these findings, another study has shown that SPOP knockdown leads to spontaneous replication stress and impaired recovery from replication fork stalling through a mechanism that involves

repression of breast cancer type 2 susceptibility protein (BRCA2), ATR serine/threonine kinase, checkpoint kinase 1 (CHK1), and RAD51 recombinase transcription [45]. Furthermore, SPOP knockdown also impairs RAD51 foci formation and activation of CHK1 in the DNA damage response pathway [45]. The role of SPOP in the DDR pathway has also been implicated in different cancers, including prostate cancer and lung cancer [46, 47]. In prostate cancer, SPOP modulates DNA double strand break (DSB) repair and SPOP mutations show a high correlation with genomic instability [46]. Specifically, homology-directed repair of DSBs is impaired in SPOP mutant prostate cancer, a phenotype that resembles breast cancer type 1 susceptibility protein (BRCA1) inactivation in breast cancer [46]. Furthermore, SPOP mutant prostate cancer appears to be sensitized to DNA damaging therapeutic agents thereby indicating that SPOP mutant prostate cancer patients could respond better to these therapeutic agents [46]. SPOP also appears to play a critical role in regulating the DDR pathway in lung adenocarcinoma in response to radiation [47]. SPOP protein levels are sharply upregulated when lung adenocarcinoma cells experience DNA damage and, incongruence with this, SPOP knockdown affects DDR repair kinetics upon exposure to ionizing radiation [47]. This process appears to involve the DDR factors RAD51 and KU80 as both of these are downregulated upon SPOP knockdown [47].

Angiogenesis, inflammation, hemoglobin regulation, and chronic pancreatitis

As mentioned above, DAXX has been identified as a SPOP target protein and SPOP-mediated DAXX regulation has been shown to play a role in apoptosis. Another line of study found that knockdown of SPOP led to the upregulation of DAXX protein, which in turn promotes downregulation of vascular endothelial growth factor receptor 2 (VEGFR2) levels in vascular endothelial cells [48]. Since VEGFR2 plays a critical role in angiogenesis, this finding attributes a function of SPOP to the process of angiogenesis.

SPOP also plays a role in modulating systemic inflammation as SPOP-negative cells are able to induce inflammatory activation of hematopoietic stem cells (HSCs) in an unresolved manner which often results in a lethal phenotype caused by hyper-inflammatory syndrome or sepsis [49]. SPOP-mediated systemic inflammation regulation appears to involve the innate signal transducer myeloid differentiation primary response protein 88 (MYD88) as SPOP has been shown to directly target it for degradation [49].

Another line of study has implicated the role of SPOP in the regulation of fetal hemoglobin levels, a finding that could provide new treatment strategies for patients with sickle cell disease and β-thalassemia. Specifically, it was shown that depletion of SPOP raises fetal globin messenger RNA and protein levels in human erythroid cells [50].

Recently, SPOP has been shown to play an important role in pancreatic fibrosis, a critical feature of chronic pancreatitis. Specifically, SPOP targets Fas-associated death domain (FADD) for degradation, which subsequently has an effect on pancreatic stellate cell activation [51, 52]. It was demonstrated that SPOP is downregulated in chronic pancreatitis-induced mice and, furthermore, that SPOP knockdown leads to significant pancreatic stellate cell activation through modulation of FADD [52]. These findings thus highlight that SPOP plays an important role in preventing the onset of chronic pancreatitis.

SPOP and cancer

Through recent studies it is evident that mutant or downregulated SPOP plays a key role in driving tumorigenesis in a variety of cancers. A common theme among all of these studies is that wildtype SPOP acts as a tumor suppressor by targeting critical oncogenic proteins for degradation. Downregulation of SPOP due to mutations within the SPOP MATH domain or a decrease in SPOP expression promote stabilization of its targets thereby driving tumor progression. Highlighted below are all the different cancer subtypes in which SPOP has been shown to play a role.

Prostate cancer

The majority of SPOP-related studies in the context of cancer have been carried out in prostate cancer. Not only have many studies shown that SPOP is downregulated or mutated in a high proportion of prostate cancers, many underlying mechanisms have also been identified. Overall, SPOP has been found to be mutated in prostate cancer in anywhere from 4.4% to 28.6% of cases studied (**Table 1**) [53-64]. SPOP

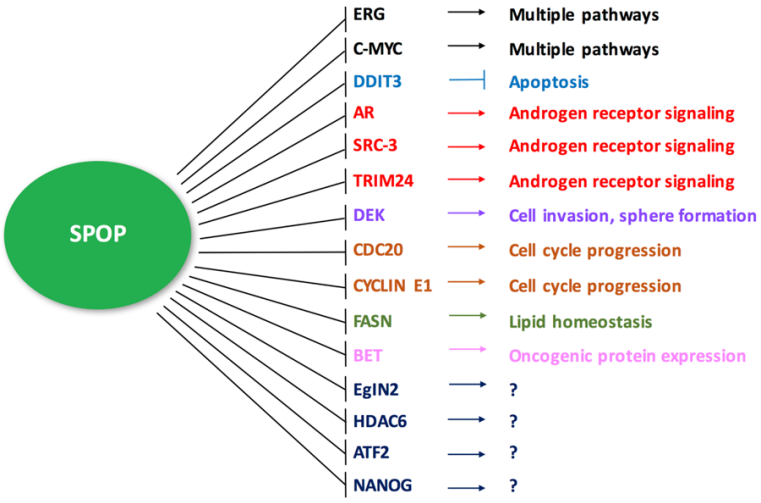

Figure 3. SPOP targets in prostate cancer. Diagram illustrating all the known SPOP targets in prostate cancer and the regulatory pathways that are affected as a result of SPOP mutation or downregulation.

mutations seem to primarily play a role in disruption of substrate recognition as the majority of mutations lie within the substrate binding MATH domain [57-60]. One study has also identified a mutation within the BTB domain although no further studies have been done to determine the implication of this mutation in prostate cancer tumorigenesis [65]. Interestingly, SPOP mutations appear to define a unique molecular subtype of prostate cancer as they show mutual exclusivity with ETS family gene rearrangements, a chromosomal arrangement that is highly prevalent in prostate cancer [56-58, 66]. The BTB domain SPOP mutation identified by Zuhlke et al. is thus far the only exception to the mutual exclusivity rule [65]. In addition to the high mutational rate, SPOP has also been shown to be downregulated in 25.2-93.5% of prostate cancers thereby further solidifying the tumor suppressive role of SPOP in prostate cancer (**Table 1**) [56, 67]. Several studies have suggested that SPOP mutations are associated specifically with prostate cancer progression and that SPOP alterations show a high correlation with worse disease prognosis [56, 59, 68]. When comparing the mutational status between high grade prostatic intraepithelial neoplasia (HGPIN), a precursor for prostate cancer development, with prostate cancer samples, it was found that SPOP mutations were specific to prostate cancer thereby suggesting a role of mutated SPOP in progression from HGPIN to prostate cancer [68]. Consistent with this finding, one study has shown that SPOP mutations were associated with aggressive prostate cancer, though it should be noted that this was based on a study with a low number of samples and therefore lacks statistical significance [59].

It is now clear that dysregulation of effector substrates plays a major role in the tumorigenic properties of mutant SPOP in prostate cancer (**Figure 3**; **Table 2**). Importantly, several studies have indicated that SPOP acts in a dominant negative fashion thereby enhancing its tumorigenic role when mutated [20, 58, 69]. The first identified SPOP target in prostate cancer was steroid receptor coactivator 3 (SRC-3) [70, 71]. Geng et al. was the first to demonstrate that prostate cancer-associated SPOP mutations disrupt ubiquitination and subsequent degradation of its targets, in this case SRC-3 [71]. Since SRC-3 is an important coactivator of the androgen receptor (AR), SRC-3 stabilization in SPOP-mutant prostate cancer was further shown to promote the transcriptional activity of AR [71]. This has important implications in prostate cancer tumorigenesis since prostate cancer onset and progression is critically dependent on AR signaling to promote cell growth and survival. Stabilized SRC-3 in SPOP mutant prostate cancer also promotes prostate cancer progression through a mechanism involving PI3K/mTOR signaling [72]. AR signaling and PI3K/mTOR signaling normally exert a negative feedback on each other, thus SPOP mutations not only lead to an increase in the oncogenic signaling by activating AR and PI3K/mTOR signaling, but it also effectively uncouples the normal negative feedback between these two pathways [71, 72].

Interestingly, a more direct link between SPOP and AR was found in two later independent studies that uncovered AR as a direct SPOP target [73, 74]. Both of these studies found that SPOP binds to, ubiquitinates, and degrades AR in a manner that is disrupted by prostate cancer-associated SPOP mutations, and that this subsequently promotes an increase in AR signaling [73, 74]. Furthermore, the presence of mutant SPOP promotes larger prostate adeno-

carcinomas in immunocompromised mice, which correlates with higher AR protein expression levels [74]. The interaction between SPOP and AR is mediated by a perfectly matched SPOP binding motif in the hinge domain region of AR [73, 74]. This finding is important as commonly found AR splice variants lack the hinge domain region, thereby indicating that only certain AR variants are subject to SPOP-mediated degradation.

Approximately 50% of prostate cancer have a translocation that fuses the ETS transcription factor ERG to TMPRSS2 thereby resulting in ERG overexpression and indicating that ERG plays an important role in prostate cancer tumorigenesis. Interestingly, one study has reported that loss of SPOP expression is strongly correlated to ERG overexpression [67]. Furthermore, several studies have now demonstrated that SPOP binds to ERG through an N-terminal degron motif thereby ubiquitinating and targeting it for degradation in a manner that is disrupted by prostate cancer-associated SPOP mutations [40, 75]. The interaction between SPOP and ERG is specific to full length ERG as truncated ERG (D ERG) evades SPOP-mediated destruction [75]. The SPOP/ERG interaction appears to be mediated by CKI-dependent phosphorylation as DNA damaging drugs that trigger CKI activation can effectively restore the SPOP/D ERG interaction [75]. These findings are of importance since SPOP mutations are normally found to be mutually exclusive with ERG-TMPRSS2 gene rearrangements, with the exception of one study that found 1.7% of ERG-rearranged prostate cancers to also harbor a SPOP mutation [56-58, 66, 67]. Thus, prostate cancer cells can utilize two separate mechanisms to promote ERG overexpression: through ERG-TMPRSS2 gene rearrangement or mutating/downregulating SPOP.

DNA damage inducible transcript 3 (DDIT3) has also been designated as a SPOP target since wildtype SPOP, but not prostate cancer-associated mutant SPOP, can bind to DDIT3 through a SPOP binding motif in its transactivation domain to target it for ubiquitination and degradation [76]. Stabilization of DDIT3 has important implications since DDIT3 plays a role in ER stress-mediated apoptosis, a process that has been exploited for the treatment of advanced prostate cancer [77]. As expected prostate cancer-associated SPOP mutants were defective in suppressing ER stress-induced apoptosis thereby providing a plausible mechanism of stabilized DDIT3-mediated prostate cancer tumorigenesis [76].

DEK proto-oncogene (DEK) was identified as a SPOP target in a genome-wide ubiquitylome study using glycine-glycine remnant identification through mass spectrometry [20]. Again, SPOP was found to bind directly to DEK to target it for ubiquitination and subsequent degradation [20]. DEK degradation is compromised specifically by prostate cancer-associated SPOP mutations as two endometrial cancer-associated SPOP mutations had no effect on DEK levels [20]. Furthermore, stabilized DEK levels due to SPOP mutations have been linked to increased cell invasion and sphere formation to promote prostate cancer tumorigenesis [20].

Since prostate cancer is critically dependent on circulating androgens, androgen deprivation therapy (ADT) is commonly used as a frontline choice of treatment. Though ADT has proven to be highly successful, most patients succumb to recurrence of the disease in a lethal form of castration resistant prostate cancer (CRPC). Interestingly, SPOP has been shown to target tripartate motif containing 24 (TRIM24) for degradation and, furthermore, stabilization of TRIM24 in a SPOP mutant setting promotes prostate cancer cell proliferation under low androgen conditions [78]. In congruence with this finding, TRIM24 protein levels significantly increase during the progression of primary prostate cancer to CRPC [78]. Furthermore, stabilized TRIM24 enhances AR signaling thereby leading to upregulation of AR and TRIM24 co-activated genes [78]. It is therefore postulated that the AR/TRIM24 gene signature can be utilized as a prediction of disease recurrence to CRPC. A recent study has now provided insight into the regulation of TRIM24 in SPOP wildtype prostate cancer. It was shown that tripartate motif containing 28 (TRIM28) acts as an upstream regulator of TRIM24 by forming a direct interaction to prevent SPOP-mediated degradation of TRIM24 [79]. Furthermore, TRIM28 aids in TRIM24 occupancy on chromatin and enhances AR signaling in a manner that is similar to TRIM24-mediated AR signaling [79]. In congruence with these findings, TRIM28 is upregulated in aggressive prostate cancer where it directly promotes cell proliferation and, furthermore, the AR/TRIM24 signature previously found is similar in tumors with high TRIM28 expression [79].

NANOG, an essential transcription factor required for embryonic stem cell and cancer stem cell maintenance, has been shown to be targeted for degradation by SPOP in two independent studies [80, 81]. Both studies have indicated that SPOP-mediated degradation of NANOG is abrogated in either SPOP mutant or S68Y NANOG mutant prostate cancer cells [80, 81]. Interestingly, PIN1 oncoprotein acts as an upstream NANOG regulator by impairing SPOP recognition thereby subsequently stabilizing NANOG and promoting prostate cancer progression [31]. This finding is of importance as it provides insight into the rationale behind using PIN inhibitors for patients with wildtype SPOP prostate cancer. An additional NANOG regulatory axis involves AMPK-BRAF signaling. Specifically, NANOG phosphorylation as mediated through AMPK-BRAF signaling blocks the SPOP-NANOG interaction thereby providing another possible therapeutic strategy for SPOP wildtype prostate cancer patients [81].

As highlighted in the studies above, disruption of SPOP-mediated protein degradation in prostate cancer promotes tumorigenesis in a variety of mechanisms, but none have indicated SPOP-mediated regulation of the cell cycle directly. Two studies have now provided a direct link between SPOP mutant prostate cancer and disruption of the cell cycle. In one study SPOP was shown to target cell division cycle 20 (CDC20) for degradation in a manner that was specifically disrupted by prostate cancer associated SPOP mutations [82]. Consequently, SPOP mutant prostate cancer cells were shown to be highly resistant to pharmacological CDC20 inhibitors [82]. In another independent study, wildtype SPOP was shown to target cyclin E1 for degradation in prostate cancer cells [83]. Importantly, CDK2 competes with the SPOP-cyclin E1 interaction thereby suggesting that SPOP specifically regulates CDK2-free cyclin E1 [83].

c-MYC, a critical regulator of cell proliferation, is one of the most commonly mutated oncogenes in a variety of cancers. Interestingly, one study has now found that wildtype c-MYC can drive prostate cancer tumorigenesis through a mechanism involving SPOP since wildtype, but not mutant or downregulated, SPOP can target c-MYC for degradation in prostate cancer cells [84]. Moreover, gene set enrichment analysis has identified a strong overlap between the gene signatures in SPOP mutant and c-MYC overexpressing cells which is correlated with inferior clinical outcomes [84]. To further support these findings, a clear inverse correlation was found between c-MYC activity and SPOP mRNA levels in prostate cancer patient cohorts [84].

Additional SPOP targets that have been found to play important roles in prostate cancer include the prolyl hydroxylase protein Eg1N2, histone deacetylase 6 (HDAC6), activating transcription factor 2 (ATF2), fatty acid synthase (FASN), and bromodomain and extraterminal domain (BET) proteins all of which were shown to be targeted for degradation in a manner that is disrupted by prostate cancer-associated SPOP mutations [69, 85-89]. Minimal mechanistic insight is provided in these studies, however, stabilization of FASN by mutant SPOP provides the first link to the role of SPOP in maintaining lipid homeostasis in prostate cancer [87]. Furthermore, stabilization of BET proteins was shown to likely promote enhanced tumorigenic properties through a mechanism that involves the hyper-activation of AKT-mTOR signaling [85].

Finally, inverted formin 2 (INF2) has been shown to be regulated by SPOP in a degradation-independent manner thereby highlighting the first role of mutant SPOP in prostate cancer that is not attributed to its proteasome-mediated ubiquitin ligase activity [90]. In the absence of SPOP regulation, INF2 localizes to the ER where it mediates actin polymerization and facilitates dynamin related protein 1 (DRP1) recruitment to induce mitochondrial fission. Interestingly, SPOP targets INF2 for polyubiquitination, however, this subsequently leads to reduced localization of INF2 to the ER rather than INF2 degradation [90]. In congruence with this function, DRP1 puncta formation and mitochondrial fission are enhanced in SPOP mutant prostate cancer cells which in turn promotes cell migration and invasion [90].

Breast cancer

Though there are several studies that have found SPOP-regulated targets specifically in breast cancer, there currently are no peer reviewed studies that have investigated the mutational status of SPOP in breast cancer. One study, however, has indicated that SPOP

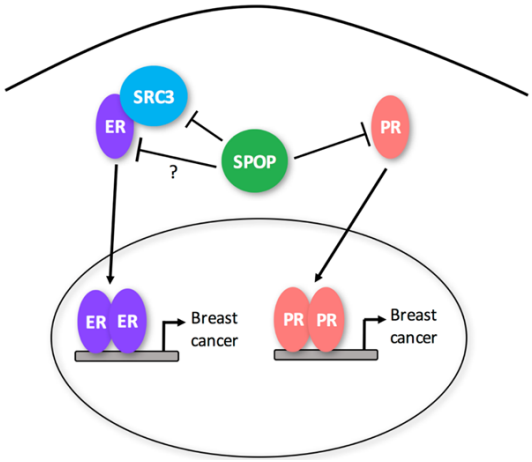

Figure 4. Dysregulated estrogen and progesterone signaling in SPOP altered breast cancer. Wildtype SPOP targets the estrogen receptor (ER) co-activator SRC3 for degradation to constrict ER signaling. SPOP downregulation or mutation promotes SRC3 stabilization and subsequent dysregulated ER signaling. ER itself is also a likely SPOP target although this has not been shown in a breast cancer setting. Wildtype SPOP also targets the progesterone receptor (PR) for degradation to constrict PR signaling. SPOP downregulation or mutation promotes PR stabilization and subsequent dysregulated PR signaling.

frequently undergoes copy number loss and is one of the highest loci to undergo loss of heterozygosity [70]. Through single-nucleotide polymorphisms arrays, the copy numbers at the SPOP locus were found to undergo copy number loss in 60-70% of 42 observed breast cancer cell lines (**Table 1**) [70]. Furthermore, in 45 breast cancer samples, the region around the SPOP locus was determined to undergo loss of heterozygosity in 57.8% of the samples thereby indicating that somatic mutations in this region are likely (**Table 1**) [70]. Moreover, SPOP depletion in breast cancer cell lines was shown to dramatically affect cell invasion and anchorage-independent growth thereby further highlighting the tumor suppressive role of SPOP in breast cancer [70].

The role of SPOP in breast cancer has been attributed to SPOP-mediated degradation of SRC-3, the progesterone and estrogen receptors, c-MYC, and breast cancer metastasis suppressor 1 (BRMS1) (**Table 2**) [37, 70, 91-93]. In breast cancer cells, casein kinase Iε phosphorylates SRC-3 thereby priming it for SPOP-dependent turnover [70]. Since SRC-3 is a co-activator for the estrogen receptor (ER) and ER is critical for breast cancer tumorigenesis, it is believed that stabilized SRC-3 in SPOP mutant or downregulated breast cancer promotes ER signaling and thereby tumor initiation and/or progression (**Figure 4**) [70]. SPOP also appears to directly regulate ER signaling by targeting ER itself, a finding that was demonstrated in human embryonic 293 cells (**Figure 4**) [37]. Another critical signaling pathway in breast cancer tumorigenesis involves progesterone receptor (PR) signaling, a pathway that is also directly regulated by SPOP. Specifically, one study has shown that SPOP targets PR for degradation in breast cancer cells and that progesterone induced PR activation is directly suppressed by wildtype SPOP (**Figure 4**) [91]. c-MYC overexpression is known to play a critical role in the progression of triple negative breast cancer through a mechanism that involves promoting the epithelial to mesenchymal transition. As highlighted above, c-MYC is a known target of SPOP in prostate cancer, a finding that is also true for breast cancer [84, 92]. Therefore, it is postulated that SPOP mutations or SPOP downregulation in triple negative breast cancer aids to promote tumorigenesis through an upregulation of c-MYC and subsequent promotion of the epithelial to mesenchymal transition. BRMS1 is a gene that specifically suppresses breast cancer metastasis without affecting primary tumorigenesis. SPOP has been shown to ubiquitinate and target BRMS1 for degradation although it has not been tested whether this occurs in a cancer mutation-dependent manner [93]. However, knockdown of SPOP does show an increase in BRSM1 expression and a subsequent decrease in BRMS1 repressive genes thereby indicating that SPOP likely plays a role in regulating breast cancer metastasis [93].

Colorectal cancer

The mutational landscape of SPOP in colorectal cancers has not been extensively studied, although one study found 1 SPOP mutation in a cohort of 45 colorectal cancer patients (**Table 1**) [55]. Although SPOP mutations appear to be rare in colorectal cancer, SPOP downregulation at either the RNA or protein level has been observed in 20-61.9% of colorectal cancer patients (**Table 1**) [41, 55, 94, 95]. One of these studies found that SPOP downregulation is negatively correlated to promoter hypermethylation status by altering transcription factor retinoid X receptor alpha (RXRA) binding [41]. In congruence with these findings, SPOP knock-

down promotes cell proliferation, migration and colony formation whereas SPOP overexpression leads to suppressed cell proliferation, migration, and colony formation [94]. Furthermore, downregulation of SPOP is significantly correlated to poor differentiation, distant metastasis, and poor overall prognosis [94].

Tumorigenesis in SPOP mutant or SPOP downregulated colorectal cancer patients likely involves regulation of matrix metallopeptidase 2 and 7 (MMP2 and MMP7), E-cadherin, vimentin, HDAC6, and GLI2 (**Table 2**) [41, 86, 94, 96]. SPOP has been shown to significantly downregulate MMP2, MMP7, and vimentin whereas it upregulates E-cadherin in colorectal cancer [94, 96]. It is currently unclear that this regulation can be attributed to the ubiquitin ligase activity of SPOP, although at least MMP2 regulation does appear to involve PI3K/Akt signaling [96]. As highlighted above, Tan et al. has demonstrated that SPOP can target HDAC6 for degradation in prostate cancer, a finding that was also shown to be consistent in colorectal cancer [86]. Importantly, cell proliferation and migration in SPOP downregulated colorectal cancer cells can be effectively reverses by depletion of HDAC6 thereby indicating that SPOP-mediated regulation of HDAC6 plays a critical role in colorectal cancer tumorigenesis [86]. SPOP has also been found to promote GLI2 degradation in colorectal cancer cells, which subsequently alters regulation of the apoptotic BCL-2 protein thereby suggesting that SPOP could promote tumorigenesis through regulation of apoptosis [41].

Endometrial cancer

Several studies have investigated the mutational status of SPOP in endometrial cancers, the results for which mimic the high mutation rate that is observed in prostate cancer as 8-17.9% of endometrial cancers harbor SPOP mutations (**Table 1**) [97-99]. The endometrial cancer-associated SPOP mutations all appear to lie within the MATH domain, a finding that is also consistent to the mutational status of SPOP in prostate cancer [99]. SPOP mutations are specific to subtypes of endometrial cancer since one study has reported that serous endometrial tumors and clear-cell endometrial tumors harbor SPOP mutations in 8 and 9% of cases respectively, whereas endometrioid and mixed-histology tumors did not contain SPOP mutations [99].

One reported SPOP target in endometrial cancer is the estrogen receptor α (ERα), a finding that is of high importance since ER signaling is critical to endometrial growth and development (**Table 2**) [100]. It was reported that SPOP binds to the AF2 domain within ERα thereby targeting it for degradation in endometrial cancer cells [100]. Importantly, ERα degradation is abrogated by endometrial-associated SPOP mutations, which subsequently drives endometrial cancer cell growth [100].

Gastric cancer

To date, there are no peer reviewed studies that have reported SPOP mutations in gastric cancer, although one study has reported significant SPOP downregulation in 18 of 60 gastric cancer tissues (**Table 1**) [55]. One mechanism that could underlie SPOP downregulation in gastric cancer involves microRNA-mediated regulation since miRNA-543 has been shown to target SPOP in gastric cancer cells [101]. Furthermore, SPOP downregulation is correlated with increased cell migration and invasion thereby highlighting the role of SPOP in gastric cancer progression [101].

The only potential SPOP target that has been identified in gastric cancer is GLI2 thus indicating that SPOP potentially regulates SHH signaling to promote gastric tumorigenesis (**Table 2**) [27]. Specifically, this study showed an inverse relationship between GLI2 and SPOP expression which was abrogated in the presence of proteasome inhibitors, thereby suggesting that SPOP targets GLI2 for degradation through the proteasome pathway [27]. One downstream effect of stabilized GLI2 in downregulated SPOP gastric cancer appears to include inhibition of apoptosis since pro-apoptotic genes are significantly upregulated in SPOP overexpressing gastric cancer cells [27].

Liver cancer

The SPOP mutational and expression status in different types of liver cancer has been explored in three independent studies. It was found that SPOP is mutated in 16.7% and downregulated in 30.3% of hepatoblastoma cases and downregulated in 46.3-59.1% of hepatocellular carcinoma (HCC) cases (**Table 1**) [49, 102-104].

Importantly, SPOP downregulation has a negative correlation to tumor grade, differentiation, and metastasis of HCC patients and SPOP knockdown promotes cell proliferation of HB cells [102-104]. Furthermore, the cos regression model indicates that low SPOP expression is a risk factor related to the prognosis of HCC patients [104].

Several possible SPOP targets have been identified in liver cancer, including cyclin dependent kinase inhibitor 2B (CKN2B), zinc finger E-box binding homeobox 2 (ZEB2), and SUMO specific peptidase 7 (SENP7) (**Table 2**). Wildtype SPOP significantly increases CDKN2B, an important cell growth regulator, expression in a manner that is disrupted by an HB-associated SPOP mutation [102]. ZEB2 is an important regulator of the epithelial to mesenchymal transition and has been shown to be negatively regulated by SPOP [103]. It should be noted that CDKN2B and ZEB2 expression are both modulated by SPOP, however, it is unclear whether this occurs through proteasome-mediated degradation. On the contrary, SENP7 has been shown to be a direct target of SPOP in HCC where it gets targeted for degradation via ubiquitin-dependent proteolysis [104]. As a result of increased SENP7 expression in SPOP downregulated HCC cells, vimentin expression increases which in turn promotes HCC cell metastasis [104].

Lung cancer

Though several studies have investigated the role of SPOP in lung cancer tumorigenesis, there is only one study to date that has looked at the overall expression levels or mutational status of SPOP in lung cancer. In this study it was found that SPOP is significantly downregulated in 84.1% of non-small cell lung cancer (NSCLC), which appears to be directly correlated to CpG island hypermethylation in the SPOP promoter region (**Table 1**) [105, 106]. MicroRNA regulation could play a role in SPOP downregulation since miR520b is shown to be upregulated in NSCLC where it decreases SPOP expression [107]. Importantly, low SPOP expression is positively correlated with tumor size, differentiation status, lymph node metastasis, clinical stages, and overall patient survival [106].

Several direct SPOP targets have been discovered in NSCLC, including GLI2, GLI3, the NAD-dependent deacetylate SIRT2, and FADD, all of which are degraded via ubiquitin-mediated proteolysis (**Table 2**) [47, 51, 107, 108]. As mentioned previously, stabilization of GLI2/GLI3 in SPOP downregulated NSCLC promotes SHH signaling which in turn plays a pivotal role in promoting cell proliferation [107]. SIRT2 is widely known to have broad anti-cancer activity in a variety of cancers, thus indicating that stabilization of SIRT2 as a result of low SPOP expression can play a central role to NSCLC tumorigenesis [108]. Finally, high FADD expression is correlated to poor prognosis in NSCLC, which appears to be a direct result of increased NF-κβ signaling [51].

SPOP has also been shown to play a role in lung adenocarcinoma as previously discussed. Upon DNA damage, SPOP expression is upregulated in lung adenocarcinoma cells where it is required to activate the DDR pathway [47]. Therefore, low SPOP expression prevents activation of DDR and subsequently creates genomic instability in lung adenocarcinomas. RAD-51, and KU80 play a direct role in this mechanism since both are shown to be downregulated upon SPOP knockdown [47].

Oral squamous carcinoma

Only one study has provided insight into the role of SPOP in oral squamous carcinoma (OSCC). In this study it was demonstrated that miR-373 is significantly upregulated in OSCC where it promotes tumorigenesis by decreasing SPOP expression [109]. Overexpression of miR-373 in OSCC cells leads to higher cell proliferation, cell migration, and cell invasion, which can be effectively rescued by overexpressing SPOP, thus indicating that miR-373 regulated tumorigenesis is mediated by a mechanism involving SPOP [109].

Ovarian cancer

Two independent studies have reported SPOP mutations in 10-52.3% of ovarian cancer patients (**Table 1**) [110, 111]. Interestingly, Hu et al. has reported that the specific SPOP mutation found was a full deletion as caused by monosomy of chromosome 17, a finding that has not been duplicated in other cancer types [111]. Importantly, chromosome 17 monosomy has a significant positive correlation to more aggressive histological subtypes and grades

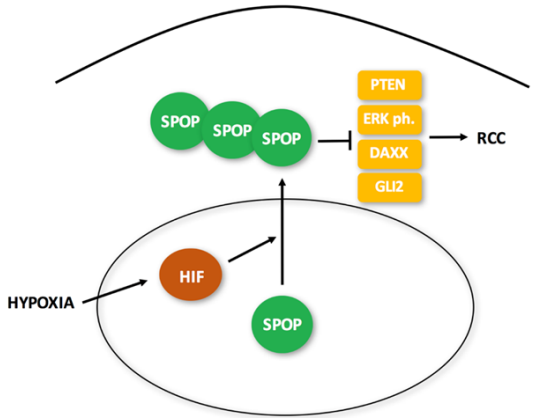

Figure 5. Elevated SPOP expression promotes renal cell carcinoma (RCC) tumorigenesis. In hypoxic conditions, upregulation of hypoxia-inducible factor (HIF) promotes cytoplasmic accumulation of SPOP. Cytoplasmic SPOP targets PTEN, ERK phosphatases, DAXX, and GLI2 for degradation which subsequently promotes RCC through unknown mechanisms.

[111]. Presently, the mechanistic basis of SPOP mutations in ovarian cancer have not been studied.

Kidney cancer

Interestingly, two independent studies have indicated that SPOP expression is significantly elevated in 47.1-77% renal cell carcinomas (RCC) tested, a finding that directly contradicts the mutational status and expression levels of SPOP in other cancers (**Table 1**) [112, 113]. Moreover, high SPOP expression correlates significantly with higher grade tumors and presence of distant metastases [113]. In longitudinal studies, it was found that 25 of 29 (86.2%) RCC cases with high SPOP expression had progression of the disease while 18 of 21 (85.7%) RCC cases with negative SPOP expression did not show progression [113]. Furthermore, SPOP knockdown promotes apoptosis, and decreases cell viability, colony formation, and cell migration [42]. All these findings thus indicate that wildtype SPOP plays a positive in role in promoting RCC tumorigenesis.

High SPOP expression in RCC could be partially explained by the finding that SPOP is a direct transcriptional target of hypoxia-inducible factor (HIF) in RCC [114]. When RCC tumors experience hypoxic conditions, HIF gets activated which subsequently promotes cytoplasmic accumulation of SPOP [114]. Upon cytoplasmic accumulation, SPOP targets several cell proliferation and apoptotic regulators for degradation, including phosphatase and tensin homolog (PTEN), ERK phosphatases, DAXX, and GLI2 which then subsequently leads to the SPOP-mediated tumorigenic effects (**Figure 5**) [114]. Another possible mechanism involves modulation of E-cadherin, vascular endothelial growth factor receptor (VEGFR), matrix metallopeptidase 9 (MMP9), and vascular cell adhesion molecule 1 (VCAM1) (**Table 2**) [42]. Specifically, SPOP knockdown promotes expression of E-cadherin, whereas it decreases expression of VEGFR, MMP9, VCAM1 [42]. Since these factors play a central role in integrin-mediated cell surface interactions and extracellular matrix organization, it can be postulated that these pathways play an important role in promoting RCC tumorigenesis when SPOP expression is elevated.

Discussion

Since a significant number of cancers harbor SPOP alterations, it is of great interest to translate these findings to personalized treatment strategies for patients with SPOP-altered cancers. Thus far, one study has set out to discover novel therapeutics for SPOP altered cancer through the means of a targeted molecular screen for SPOP-overexpressed RCC [115]. As mentioned above, a significant number of RCCs have overexpression of SPOP, which is a direct contradiction to the SPOP mutations or SPOP downregulation that is commonly found in all other cancers discussed. Since SPOP overexpression appears to drive RCC, it can be assumed that downregulation of SPOP target proteins drives RCC tumorigenesis and that targeting the SPOP-substrate interface could be an attractive therapeutic target. Guo et al. therefore used a structure-based design followed by hit optimization to yield small molecules that specifically inhibit the SPOP-substrate interaction [115]. From the screening process, 109 molecules were identified, from which three molecule showed promising results in blocking RCC tumorigenesis as shown by a reduction in proliferation of RCC primary cells and RCC xenografts [115]. It was further shown that these molecules blocked tumorigenesis by disrupting the interaction of SPOP with two of its targets, PTEN and DUSP7 [115]. Though this study has shown promising results in the use of

small molecules as a therapeutic strategy for cancers that overexpress SPOP, they are useless for the majority of cancer subtypes as most harbor SPOP mutations or SPOP downregulation.

In addition to finding novel therapeutic strategies, several studies have also reported that SPOP-altered cancers could respond differently to already existing treatments and that specific combination treatments may prove more beneficial for these patients. For instance, one study has specifically reported results of differential response to treatment in SPOP-mutant CRPC. The results of this study indicated that out of a cohort of 89 patients, the 22 patients who harbored SPOP mutations responded significantly better to the hormone therapy abiraterone as compared to the SPOP wildtype patients [64]. One extreme example of differential response to treatment is shown by a case study where a SPOP-mutant prostate cancer patient showed complete response to a combination of ADT and docetaxel [116]. This is a highly impressive finding as pathologic complete response is very rare in advanced prostate cancer. Finally, one study also found that prostate cancers belonging in the SPOP mutant subclass were less likely to have adverse pathological features and, furthermore, that this subclass had the highest biochemical-free, metastasis free, and lowest prostate cancer-specific mortality after radical prostatectomy compared with other subtypes [117]. Collectively, these studies demonstrate that the presence of SPOP mutations can predict response to treatment which can therefore be beneficial in regards to positive clinical outcomes.

Despite the promise of SPOP as a therapeutic marker, there is a need to approach this with caution. On the contrary to the studies listed above that show improved response to treatment in SPOP-mutant cancer, several studies have also demonstrated drug resistance in these cancers. The common theme amongst these studies is that SPOP-mutant cancers are highly resistant to BET inhibitors as a direct consequence of the finding that BET proteins are targeted for SPOP-mediated proteolysis [85, 88]. BET proteins comprise four different members, BRD2, BRD3, BRD4, and BRDT, all of which drive tumorigenesis by increasing the expression of several different oncogenic proteins thereby prompting the development of various different BET inhibitors as a therapeutic agent. Thus far, several BET inhibitors have been developed that show promising results in the treatment of a variety of different cancers [118, 119]. Problematically, BET resistance is commonly observed in SPOP mutant prostate cancer as a result of BET stabilization [85, 88, 89]. Thus, therapeutic strategies that can overcome BET resistance in SPOP mutant cancers are currently being investigated. At least one study has reported that the AKT-mTORC1 pathway becomes hyper-activated as a result of BRD4 stabilization in SPOP mutant prostate cancer [85]. Consequently, it was reported that BET resistance in SPOP-mutant prostate cancer can be overcome by a combination treatment with AKT inhibitors [85]. Though most SPOP mutant cancers thus far have been shown to provoke resistance to BET inhibitors, one study has provided contradictory results. Janouskova et al. reported that BET protein levels are reduced as a result of SPOP mutations in endometrial cancer and, furthermore, that SPOP-mutant endometrial cancers are sensitized to BET inhibitors [89]. These findings thus demonstrate that differential drug sensitivities can be provoked in different cancers even if mutations are present within the same gene.

The aforementioned studies indicate that SPOP mutations are promising biomarkers as a prediction to drug response. As more knowledge about SPOP emerges, we learn more about what targets become stabilized and subsequently which pathways altered upon SPOP alterations. More importantly, with this new knowledge, comes the prospect in utilizing those targets/pathways to uncover novel therapeutic strategies. For instance, many studies have demonstrated alterations in the DDR pathway when SPOP mutations are present, suggesting that the use of DNA damaging agents in the clinic could prove to be beneficial [46]. Other attractive signaling pathways that could prove to be beneficial in treating SPOP mutant cancers include the SHH, AR, ER, PR, PI3K/mTOR, and NF-κβ pathways, among others. It is clear that although many strides have been made in providing mechanistic insight behind SPOP mediated tumorigenesis, there is still a critical need in how this knowledge can ultimately be translated into providing superior treatment strategies for SPOP altered cancers.

Disclosure of conflict of interest

None.

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