

# The Role of Skp2 and its Substrate CDKN1B (p27) in Colorectal Cancer

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## ABSTRACT

Colorectal cancer is one of the most frequent cancers worldwide, having the fourth mortality rate among cancers in both sexes. Numerous studies are investigating the signaling pathways and different factors involved in the development and progression of colorectal cancer. It has recently been shown that the S-phase kinase-associated protein 2 (Skp2) overexpression plays an important role in the pathogenesis of colorectal cancer. We review the role of Skp2 and its ubiquitin-proteasome pathway in colorectal cancer. The F-box protein Skp2, a component of the SCF (Skp1-Cullin 1-F-box) E3 ubiquitin-ligase complex, has been shown to regulate cellular proliferation, cancer progression and metastasis by targeting several cell cycle regulators for ubiquitination and subsequent 26S proteasome degradation. The best known protein substrate of the Skp2 is the cyclin-dependent kinase inhibitor 1B (CDKN1B), also known as p27<sup>Kip1</sup>. Overexpression of Skp2 and loss of CDKN1B (p27) was strongly associated with aggressive tumor behavior and poor clinical outcome in a variety of cancers, including colorectal cancer. An efficient interaction between Skp2 and CDKN1B (p27) requires the presence of an essential activator of the SCF-Skp2 complex, the cyclin-dependent kinase subunit 1 (Cks1) cofactor. Alterations in the Skp2, Cks1 and CDKN1B (p27) expression have major effects on colorectal carcinogenesis and may serve as an important and independent prognostic marker. Furthermore, we highlight that Skp2 may be a promising therapeutic target for colorectal cancer, and development of Skp2 inhibitors would have a great impact on colorectal cancer therapy.

**Key words:** cell cycle – CDKN1B – p27 – Skp2 – ubiquitin – prognosis – colorectal cancer.

**Abbreviations:** APC/C: anaphase-promoting complex/cyclosome; AR: androgen receptor; ATP: adenosine triphosphate; BCR-ABL: breakpoint cluster region-abelson leukemia gene; CDKN1B: cyclin-dependent kinase inhibitor 1B; Cdk: cyclin-dependent kinases; CEA: carcinoembryonic antigen; CIP/KIP: kinase inhibitor protein; Cks1: cyclin-dependent kinase subunit 1; CRC: colorectal cancer; EGFR: epidermal growth factor receptor; FKHR1: forkhead-related transcription factor 1; GSK3: glycogen synthase kinase 3; IGF: insulin-like growth factor; IKK: inhibitor of nuclear factor Kappa B Kinase; miRNAs: microRNAs; mRNA: messenger ribonucleic acid; NCCN: National Comprehensive Cancer Network; PI3K: phosphatidylinositol-3'-kinase; PPARγ: peroxisome proliferators activated receptor gamma; PTEN: phosphatase and tensin homolog; RECK: membrane-anchored matrix metalloproteinase-regulator; SCF: Skp1-Cullin 1-F-box; Skp2: S-phase kinase-associated protein 2; Thr: threonine; UPS: ubiquitin-proteasome system; VEGF: vascular endothelial growth factor.

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## INTRODUCTION

Colorectal cancer (CRC) is a major health problem, being one of the most frequently occurring types of cancer worldwide. It is the third most common cancer in men, after lung and prostate cancer, and the second in women, after breast cancer. Worldwide estimates rank CRC

as the fourth leading type of cancer with regard to mortality in both sexes, after lung, liver and gastric cancer [1].

The survival rate of CRC patients has risen in the past few years, owing to the improvement of therapeutic options and to the applied screening programs, which have led to the diagnosis of the disease in an early stage.

According to the NCCN Guidelines Version 2.2015, the therapeutic approach is strongly correlated to patients' prognosis. Until now, the TNM status is the most widespread and useful determinant factor in the clinical routine of the patients' prognosis. Also, it is known that the outcome

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correlates other clinico-pathological parameters including age, performance status, CEA level, vessel invasion and tumor differentiation grade [2-4].

In the last few years, the identification of tumor-related molecular and genetic factors in colorectal carcinogenesis was possible through the development of molecular genetics.

The biological markers that have a real impact on tumor progress and can become targets for molecular therapy are intensively studied in translational research.

The discovery of additional factors, closer to tumor cell biology can create the premise for personalized therapeutic approaches, which are much more effective. Numerous studies have been recently made regarding K-ras mutation, the intra-tumor lymphocyte infiltrate Treg FOXP3+, the mutation BRAFV600E, microsatellite instability, and microRNAs (miRNAs) [5].

These alterations in the various ways of the carcinogenesis appear in different stages of CRC, from the adenoma to the adenocarcinoma and metastasis. It is important to mention that the exact mechanism of carcinogenesis, in the case of CRC still remains poorly understood. Nevertheless, countless studies show that the Skp2 (S-phase kinase associated protein 2) protein expression has an important implication in CRC evolution and prognosis. Previous studies have reported an involvement of Skp2 in the regulation of cell cycle, cellular growth, proliferation, differentiation, apoptosis and metastasis [6-8].

Here, we systematically review the role of the Skp2-CDKN1B (p27) pathway in the CRC carcinogenesis and its therapeutic potential. To the best of our knowledge, the role of Skp2 has been only similarly reviewed in breast [9] and prostate cancer [10].

## THE ROLE OF CDKN1B (P27) PROTEIN IN CRC CARCINOGENESIS

In normal conditions, the cell cycle progression is based on the specific activation of the cyclin-dependent kinases (Cdks) [11]. These incorporate mitogenic and growth inhibitory signals. Two families of Cdk inhibitors regulate the deployment of this process: the Cdk4 (INK4) family members including p15<sup>INK4A</sup>, p16<sup>INK4B</sup>, p18<sup>INK4C</sup>, and p19<sup>INK4D</sup> and the CIP/KIP family (kinase inhibitor protein) enveloping p21<sup>CIP1</sup>, p27<sup>Kip1</sup> (CDKN1B) and p57<sup>Kip2</sup> [7]. The development of cancerous cells is strongly linked to the abnormal expression of the cell cycle regulators. Among these regulators, the tumor suppressor protein CDKN1B (p27) can be marked out as involved in cellular differentiation, proliferation, apoptosis, cellular adhesion and growth inhibition [7, 12, 13]. CDKN1B (p27) is a 198-aminoacid protein, and its gene can be found on the short arm of the 12th chromosome [7]. Previous studies have shown that the absence or reduction of CDKN1B (p27) expression can be associated with a poor prognosis in several types of cancer, including breast, prostate, lung and colon cancer [6, 12, 14-16]. The uncontrolled cellular growth and proliferation, which is an essential characteristic of cancer, is owed to the loss of control over the transition from G1 to S phase of the cell cycle. This is a period during which cells decide if they enter the cell cycle as a response to mitogenic stimuli or remain quiescent

in response to antimitogenic or senescence signals [12]. In a normal cell cycle, the level of CDKN1B (p27) is elevated in the G0/G1 phase. After the mitogenic stimulation, CDKN1B (p27) is rapidly degraded allowing the action of Cdk2/cyclinE and Cdk2/cyclinA and cell cycle progression [12]. CDKN1B (p27) degradation during the G1/S phase transition is triggered by the phosphorylation of CDKN1B (p27) at threonin (Thr)-187 by cyclinE/A-Cdk2 complexes [12,17,18]. This phosphorylation marks CDKN1B (p27) for recognition by the Skp2, which is the F-box protein component of a SCF ubiquitin ligase (E3) complex. In turn, this binding results in the polyubiquitination of CDKN1B (p27) [12,16,18]. Covalent ligation between the ubiquitin system molecules and the CDKN1B (p27) targets the 26S proteasome degradation [6, 12, 15]. The excessive nuclear loss of CDKN1B (p27) may contribute to the uncontrolled proliferation of malignant cells, due to the loss of cyclinE and cyclinA/Cdk2 inhibition, which drive cells in the S phase of the cell cycle [6,12,15,16]. One important difference between CDKN1B (p27) and other tumor suppressor proteins, such as p53 and p16, is that the decreased expression of CDKN1B (p27) in tumor cells is due to the increase in protein degradation and not to some genetic mutation which will interfere with the mRNA synthesis [6, 12, 15, 16]. The most known way of post-transcriptionally nuclear degradation of CDKN1B (p27) is due to its phosphorylation to Thr187 which allows the Skp2<sup>SCF</sup> binding and proteasome-dependent degradation [12].

Several inactivation mechanisms of CDKN1B (p27) have been described, such as CDKN1B phosphorylation at Ser10 and PI3K-Akt regulation. The latter can be at the transcriptional and post-transcriptional level, through the phosphorylation of CDKN1B at Thr157 and Thr198 by Akt. These phosphorylations determine the CDKN1B nuclear export to the cytoplasm, which leads to its inactivation [12]. The clinical and prognostical implication of nuclear and cytoplasmic CDKN1B (p27) expression in CRC were analyzed and afterwards published in 2009, using the database of the two independent prospective studies, the Nurses' Health Study and Health Professionals Follow-up Study [19, 20]. Moreover, mice model experiments have proved that CDKN1B (p27) is a haplo-insufficient tumor suppressor protein [16]. This idea is sustained by numerous studies in which patients have expressed low levels of CDKN1B (p27) rather than a total lack of CDKN1B (p27) expression in tumors [8].

## SKP2 IS A MEMBER OF THE F-BOX PROTEIN FAMILY AND A COMPONENT OF THE SCF E3 LIGASE COMPLEX

The F-box protein Skp2 is a recognition component for the substrate in SCF (Skp1-Cullin1-F-box) E3 ubiquitin-ligase complex. E3 ligase family belongs to the ubiquitin-proteasome system (UPS), which controls the stability of countless regulators in the cell cycle [8, 10]. There are three types of enzymes in the UPS component: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and ubiquitin-ligase (E3). These proteins determine a cascade of enzymatic reactions which result in the functioning of UPS [10]. The first phase is adenosine triphosphate (ATP)-dependent and assumes the binding of ubiquitin to E1. Ubiquitin, an evolutionarily well-

conserved 76-amino acid protein, is ATP-dependent activated by a thiol ester bond and subsequently transferred to the E2 enzyme [8, 10]. Further, ubiquitin linked to E2 interacts with a specific partner, E3, and transfers the ubiquitin molecules onto the proteic substrate, thus leading to the formation of a mono- or poly-ubiquitination (Fig.1). All this results in a proteic degradation on parts of 26S proteasome in an ATP-dependent manner [15]. Generally, target protein specificity depends on E3, this being the reason why the deregulation of E3 ligase often leads to cancer development [10, 21].

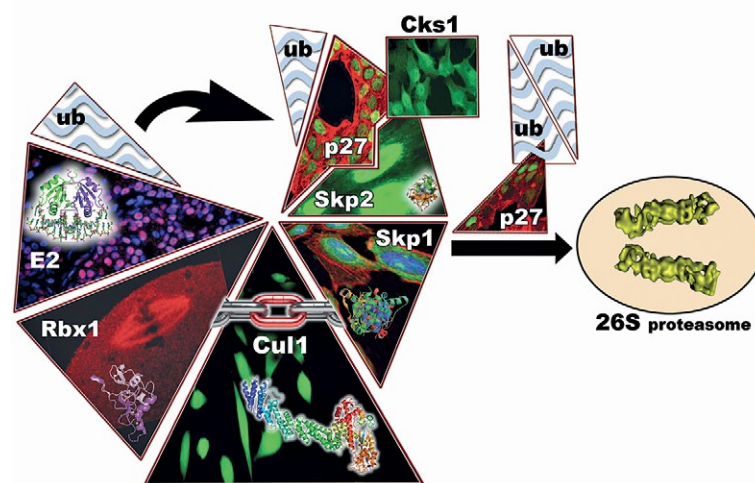
The SCF complex comprises four components: three invariable components Skp1, Rbx1 and Cullin1 (Cul1), and a variable component, the F-box protein [15, 22]. Cul1 is a rigid scaffold upon which the subunits Rbx1 and Skp1 are assembled. Rbx1 provides a docking site for conjugating enzymes (E2) and Skp1 binds the F-box protein subunit (Fig.1) [18, 23]. Each F-box protein binds a specific subset of protein substrates promoting their degradation [24]. To date, there are more than 70 F-box proteins encoded in the human genome [10, 25]. From these, only a few have been thoroughly studied, such as Skp2,  $\beta$ -TrCP1 and Fbw7 [25, 26]. Skp2 was discovered in 1995 by Beach and his collaborators as a protein associated with the S phase kinase Cdk2/cyclin A [27]. From then on, it was the research object for many studies which demonstrated its involvement in carcinogenesis through regulating the expression of various tumor suppressor proteins associated with cancer [8, 15].

### SKP2 FUNCTION AS A PROTO-ONCOPROTEIN

The Skp2 gene, located on the 5p13 chromosome, expresses an essential protein component of the SCF<sup>Skp2</sup> complex, which is capable of inducing proteic ubiquitination and subsequently the proteasome dependent degradation [14]. Upon analyzing the protein substrate of the Skp2 (i.e. p27, p16, p21, p57, E2F-1, TOB1, RBL2, cyclin D/E, BRCA2, FOXO1, RASSF1A) it can be inferred that this has an important role in regulating many cellular processes such as: cell cycle regulation, cell growth,

differentiation, cell proliferation, metastasis, apoptosis and survival, all linked to cancer development [10, 15, 22, 28, 29]. As the majority of the substrates are tumor suppressor proteins it can be asserted that Skp2 functions as an oncogene [9, 22, 30]. The most well-known substrate for Skp2 is the cell cycle inhibitor CDKN1B (p27). Skp2 overexpression induced the ubiquitination and the consecutive degradation of CDKN1B (p27), while a low level of Skp2 reduced its degradation. An efficient binding of Skp2 to CDKN1B (p27) requires the presence of an additional protein, the Cks1 (Cyclin-dependent kinase subunit 1) cofactor [6, 15, 28, 31, 32].

According to these data, Skp2 overexpression has been observed in various human cancers, including lymphomas [33], prostate cancer [10], melanoma [34], pancreatic cancer [35], gastric cancer [36], lung cancer [15,16], breast cancer [9], and colorectal cancer [6,14,15]. The oncogenic role of Skp2 was demonstrated in mouse models [8,16]. Xenografts of breast cancer cell lines overexpressing Skp2 grow faster than those showing a lower level of Skp2 [8]. Skp2<sup>-/-</sup> cellular phenotype included nuclear enlargement and polyploidy in cells of the liver, lung and testis, and an increased number of centrosomes in mouse embryonic fibroblasts. It is important that all these changes disappear in mice that also express CDKN1B<sup>-/-</sup>, indicating that CDKN1B (p27) is a key target of Skp2 [8]. According to the role in tumor progression, Skp2 knocks out mice that are resistant to tumor development induced by the loss of tumor suppressor proteins p53, p19<sup>ARF</sup> or PTEN (Phosphatase and Tensin Homolog) [30, 37]. The involvement of Skp2 overexpression in metastasis has been reported in many tumors including melanoma [34], nasopharyngeal carcinoma [38], pancreatic cancer [35], breast [9] and colorectal cancer [6,14]. By analyzing mice with CDKN1B (p27) gene mutations, which express a CDKN1B (p27) mutant that cannot be bound by Skp2 showed that the Skp2-dependent degradation of CDKN1B (p27) is crucial for the progression of adenomas to colon carcinomas [8, 16]. Skp2 overexpression has been associated with a poor prognosis and low expression of CDKN1B (p27) in different cancers, such as breast cancer



**Fig. 1.** CDKN1B (p27) ubiquitin-mediated degradation. Ubiquitin linked to E2 interacts with a specific partner, SCF<sup>Skp2</sup>, and transfers the ubiquitin molecules onto the proteic substrate. Covalent ligation between the ubiquitin system molecules and the CDKN1B (p27) targets the 26S proteasome degradation.

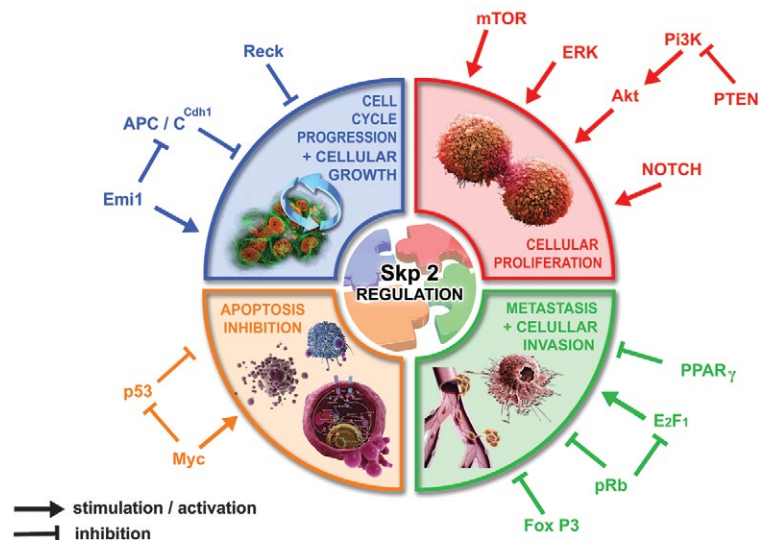


Fig. 2. Upstream regulators of Skp2 in human cancers.

[9], melanoma [34], nasopharyngeal carcinoma [38], prostate cancer [10] and colorectal cancer [6, 15]. Taken together, these data suggest that Skp2 serves as a proto-oncoprotein.

### CKS1, AN IMPORTANT COFACTOR OF SKP2

Cks1 is a member of the Cks/Suc1 protein family which interacts with Cdks. Cks proteins are essential for the functioning of Cdks and cell cycle control [39]. In mammals there are two types of Cks, Cks1 and Cks2, with different biological roles [39]. Cks1 bears a specific role, acting as an essential cofactor for the SCF-Skp2 complex in its interaction with Cdk inhibitors favoring their proteolytic degradation [40]. Certain studies have demonstrated that the overexpression of Cks favored tumor development allowing cells to continue their DNA replication even under replicative stress conditions, interacting with regulation mechanism from the S phase [39]. The role of Cks1 in targeting CDKN1B (p27) was indicated by demonstrating the lack of CDKN1B (p27) ubiquitylation in the absence of Cks1 *in vitro*, in cell-free systems [41]. *In vivo*, the accumulation of CDKN1B (p27) with a slow cell proliferation in Cks1 nullizygous mice was observed [41, 42]. mRNA and the protein expression of Cks1 strongly correlated to the protein expression of Skp2 and inversely to that of CDKN1B (p27) in CRC cancer [6, 24], breast cancer [43] and non-small cell lung cancer [15]. Cks1 deficiency decreases the Skp2-CDKN1B (p27) binding leading to CDKN1B (p27) accumulation. Studies completed in order to understand this cooperation have suggested that Cks1 binds to the Skp2 C-terminal leucine-rich repeats (LRRs) domain [23, 31, 44]. The results of this binding are underlined at the N-terminal domain through the stabilization of Skp2/Skp1 interaction [44]. Regarding the interaction with the substrate, Cks1 can serve as an adaptor between Skp2 and substrate, or can determine an allosteric change in Skp2, which increase its affinity for it (i.e. CDKN1B (p27), p21, RBL2) [18, 41, 42, 45]. The tumor suppressor p53 down-regulates Cks1 expression transcriptionally reducing mRNA and the proteic level [46].

The Myc family of transcription factors (c-Myc, n-Myc and l-Myc) which are overexpressed in approximately 70% of all cancers determine the CDKN1B (p27) increase degradation through the Cks1 overexpression, probably through down regulation of p53 [47]. An inverse correlation between the Cks1 expression and CDKN1B (p27) level has not been highlighted in all tumor biopsies. This aspect indicates the presence of multiple mechanisms where Cks1 could be involved in carcinogenesis [31]. Some studies demonstrated that retinoic acid [48], fluoxetine [49] and oncostatin M [50] can be potential anticancer agents through the regulation of Cks1 pathway either directly or indirectly.

### UPSTREAM REGULATORS OF SKP2 IN COLORECTAL CANCER

In the past years there have been many studies concentrating on Skp2 and its oncogenic role. However, upstream regulators of Skp2 in the progression of human cancer are not yet thoroughly known. Many genes and regulatory pathways of proteic expression have been discovered which could be involved in regulating the Skp2 expression (Fig. 2).

For example, it is said that in leukemic cells MYC directly regulates Skp2 expression [51], and BCR-ABL (breakpoint cluster region-abelson leukemia gene) controls gene transcription of Skp2 through PI3K/Akt/Sp1 [52]. In breast cancer it has been observed that overexpression of PPAR $\gamma$  (peroxisome proliferators activated receptor gamma) can decrease Skp2 expression [53]. Also in breast cancer cells an important role of the mTOR kinase in regulating the Skp2 expression [54] was observed. As for prostate cancer there were some more regulating mechanisms of Skp2 described such as PI3K/Akt, PTEN and AR (androgen receptor) [10].

Moreover, in the last few years, a growing number of miRNAs involved in Skp2 regulation have been described (Table I).

The following paragraphs will discuss several mechanisms that regulate Skp2 in colorectal cancer.

**Table I.** Skp2 miRNAs-regulation in different type of cells

miRNAs	Mechanism	Effects	Cells	Reference
miR-7	Skp2 downregulation	Cell cycle arrest	Chinese hamster ovary cells	[55]
miR-34a	cMyc-Skp2-Miz1 suppression	Inhibits invasion and migration	Prostate and renal cancer	[56,57]
miR-221 miR-222	CDKN1B (p27) translational inhibition	Compensates for loss of Skp2-mediated CDKN1B (p27) degradation	Prostate cancer and glioblastoma	[58-60]
miR-508-59	Suppression of Skp2 gene expression	Suppress Skp2 protein expression	HEK293T cells	[61]
miR-340	Skp2 downregulation	CDKN1B (p27) upregulation	Non-small cell lung cancer	[62]
miR-203	Skp2 suppression	Inhibits proliferation	Epidermal	[63]
miR-30s	Skp2 suppression	Inhibits metastasis	Lung fibroblast from tumor-bearing mice	[64]

miRNA- microRNA; Skp2- S-phase kinase associated protein 2; CDKN1B (p27) - cyclin-dependent kinase inhibitor 1B protein

### PI3K/Akt interacts with Skp2 pathway in colorectal cancer

The phosphatidylinositol-3'-kinase (PI3K) signaling cascade is involved in the regulation of several key cellular processes required for carcinogenesis. The PI3K activation as a response to certain growth factors such as EGFR, IGF1, FGF or IL-8 induces the phosphorylation and consequently the activation of Akt [65]. This, in turn, can promote cell survival and growth, proliferation and cell repair through the inhibition of apoptosis owing to the regulation of several signal pathways such as Bcl-xL/Bcl-2-Associated Death, IKK (Inhibitor of nuclear factor Kappa B Kinase), GSK3 (Glycogen synthase kinase 3), FKHR1 (Forkhead-related transcription factor 1), caspase-9 and mTOR [66, 67]. In human colon cancer cells the role of PI3K/Akt/GSK-3 to regulate proliferation and cellular differentiation has been proven [68]. GSK-3 is a substrate of Akt, which is inhibited after phosphorylation mediated by Akt. There are two types of isoforms, GSK-3 $\alpha$  and GSK-3 $\beta$ , which are involved in several biological processes through the phosphorylation of a great number of substrates, amongst which are also some transcription factors such as c-Myc, c-Jun and c-Myb [68, 69]. GSK-3 is involved in G0/G1 arrest, CDK2 inhibition, and Skp2 decreased expression [68]. High activation of Akt determines GSK-3 inhibition which causes increased cellular proliferation through the decrease of CDKN1B (p27) nuclear expression in a Skp2-dependent manner [68]. Moreover, activating mutations of K-ras in colorectal cancer, seen in approximately 40% of cases, determines the activation of some transduction pathways along with PI3K with results on the activation of Akt [69, 70]. Skp2 phosphorylation at Ser<sup>72</sup> by Akt has two consequences: promotes cytoplasmic translocation of Skp2 and protects Skp2 from the APC/C<sup>Cdh1</sup> mediated proteolysis through disrupting the interaction between Cdh1 and Skp2 [30,71]. There is also a positive feedback between Skp2 and Akt expression. SCF<sup>Skp2</sup> can trigger non-proteolytic K63-linked ubiquitination of Akt with the result on the increase of membrane localization and activation of Akt [72, 73].

### APC/C regulates Skp2 expression in colorectal cancer

APC/C (anaphase-promoting complex/cyclosome) is a multifunctional E3 ligase, which regulates certain highly important cellular events, including mitotic progression, DNA

replication, cellular differentiation, genomic integration and signal transduction [22, 74]. Pathological and epigenetic studies have demonstrated that the dysfunction of certain components of the APC/C complex, such as APC4, APC6/Cdc16, APC8/Cdc23 and Cdh1, correlates with different types of cancer, including colon cancer [75-77], B-cell lymphoma [74], gastric [75] and lung cancer [78]. APC/C activation is controlled by two WD40 family proteins, Cdh1 and Cdc20 [74, 79]. The activation of APC/C by Cdh1 targets Skp2 for proteolytic degradation and therefore prevents a premature entrance of the cell in the S phase. This is achieved by inhibiting the early degradation of CDKN1B (p27) mediated by SCF<sup>Skp2</sup> [74-76]. In the late mitosis and the G1 phase, Skp2 is maintained at a low level by the APC/C<sup>Cdh1</sup>-mediated ubiquitination [80]. Between the G1 and the S-phase, APC/C<sup>Cdh1</sup> function is blocked by the interaction of Cdh1 with the inhibitory-protein Emi1, as well as by the phosphorylation of Cdh1 mediated by Cdk [81]. The overexpression of Cdh1 in colon cancer cells was associated with a low histological tumor degree, with a reduced expression of Skp2 and a significant increase in CDKN1B (p27) levels [74]. Overexpression of Cdh1 in Skp2-depleted colon cells has not determined important changes regarding CDKN1B (p27) level, which suggests that CDKN1B- Cdh1-mediated regulation is achieved by controlling Skp2 expression. These data suggest that disorders in activating APC/C by Cdh1 can modify the regulation of Skp2-CDKN1B (p27) in CRC [74].

### PTEN down-regulates Skp2 in colorectal cancer

The PTEN tumor suppressor gene is located in the 10q23 chromosome [67, 82]. Through its capacity of negatively regulating the PI3K/Akt signaling pathway it has an important role in regulating cellular proliferation, apoptosis, signal arrest and angiogenesis [83]. Loss of PTEN function inhibits apoptosis and enhances cellular growth through upregulation of cyclin D and downregulation of CDKN1B (p27) [82]. The PTEN gene's inactivation is a frequent event in human malignancies. Loss of PTEN expression in CRC has been correlated to Akt activation and to aggressive tumor behavior [67, 82]. In CRC mutations of PTEN have been correlated with advanced TNM stage and metastasis, suggesting that PTEN involvement is more important in advanced than in early stage [84, 85]. Nuclear loss of PTEN expression has

been associated with liver metastasis, thus also being proven a predictive factor for poor prognosis and local recurrence [67, 84]. Moreover, loss of PTEN expression was correlated with tumor size and vascular invasion [69]. Furthermore, loss of PTEN expression has been associated with a weak response to cetuximab therapy. Cetuximab bears a superior efficacy when the transmission pathway of the growth signals continues to be regulated by wild-type PTEN expression [82, 86-88]. These data suggest that through the PTEN capacity of inhibiting the PI3K/Akt pathway it can downregulate Skp2-expression in CRC. Moreover, it can be considered that at least part of the consequences of loss of PTEN expression in CRC is due to the consecutive overexpression of Skp2.

### **RECK regulates Skp2 in colorectal cancer**

The membrane-anchored matrix metalloproteinase-regulator (RECK) is frequently found at low expression levels in cancer. In some studies, a correlation between the residual level of RECK in resected tumors and patients survival was observed [89, 90]. In xenografts models, restored RECK expression in cancer cells results in reduction of tumor angiogenesis, invasion and metastasis [91]. RECK's expression in colon carcinoma cells determines the cell cycle arrest through a decrease of Skp2 expression and upregulation of CDKN1B (p27) [89]. The induction of cell growth suppression by RECK is at least partially dependent on CDKN1B (p27) [89]. In patients with CRC, the RECK/Skp2 ratio is higher in normal tissue than in cancer tissue [89]. Molecular mechanisms through which RECK reduces the expression of Skp2 are not well-known. A hypothetical model can be that RECK protects pericellular extracellular matrix, enhancing integrin signaling which leads to a decrease of Skp2 and an increase of CDKN1B (p27) [89]. These data can sustain the idea that RECK reactivation may lead to a promising strategy for the manipulation of Skp2-CDKN1B (p27) pathway in cancer.

### **SKP2 INHIBITION IS A POTENTIAL STRATEGY FOR COLORECTAL CANCER TREATMENT**

Once the involvement of Skp2 as a common driving factor in carcinogenesis was proven, and its main mechanism of action was understood, Skp2 became an important pharmacological target for the development of new targeted therapies in cancer [92]. Moreover, understanding the role of the ubiquitin proteasome system in cell cycle regulation led to the discovery of molecular therapies, such as the inhibitors of 26S proteasome. Therefore, in 2004 the FDA approved the use of bortezomib (Velcade) in the treatment of multiple myeloma and in 2006 for mantle cell lymphomas [93]. Also, carfilzomib, another proteasome inhibitor with greater potency, with a better therapeutic index and tolerance was approved by FDA for patients with recurrences and refractory multiple myeloma [93]. Proteasome inhibitors induce cell cycle arrest, inhibit angiogenesis, cellular adhesion, cell migration and DNA repair [93]. These antitumor effects of proteasome inhibitors have been observed even on colon cancer-cell lines and animal models [93]. The treatment of CCR cell lines with bortezomib resulted in stabilizing and increasing CDKN1B

(p27) level via downregulation of Skp2. These cells showed a cell growth arrest and an apoptosis enhancement [94]. Furthermore, the apoptotic response was considerably higher when using bortezomib in combination with 5-fluorouracil, pointing to the synergism of the two components [94]. Hence, these data have demonstrated that on CRC cell line xenografts, one of bortezomib's multiple mechanisms of action is the downregulation of Skp2 paralleled by the upregulation of CDKN1B (p27) [94].

As a consequence of the pre-clinical studies, a phase II study using bortezomib was initiated on metastatic CRC patients, but unfortunately, no improvement of meaningful clinical endpoints was observed [95]. Data regarding the synergistic effect of bortezomib with other chemotherapeutic agents initiated some clinical trials using drug combinations. For example, in an early clinical trial in solid tumors bortezomib was used in combination with irinotecan [96], gemcitabin, docetaxel or a combination of carboplatin with paclitaxel [70]. Patients with CRC were also included, but there was no meaningful positive response. Regarding CRC, these studies have led to a new perspective concerning the association of bortezomib with COX-inhibitors (with important results in preclinical studies), anti-VEGF and anti-EGFR molecules [70]. Moreover, including only patients with Skp2 overexpression and using proteasome inhibitors with a better tolerance could lead to more promising results. But all these issues could be re-evaluated by developing a selective Skp2 inhibitor, which might have a greater impact on anti-cancer therapy.

These considerations have led to the discovery of an inhibitory molecule CPdA (Compound A) in multiple myeloma. This molecule induces cell cycle arrest, cell growth inhibition and apoptosis by blocking the binding of Skp2 to SCF ligase [97]. In addition, in prostate cancer cells, a component known as SMIP0004 was proven to stabilize CDKN1B (p27) by down-regulating Skp2 [98].

Researchers attention was as well directed towards natural components, with a better bioavailability and tolerance, that have the property of downregulating SKP2 [99]. Cellular studies have shown that certain components such as curcumin, quercetin, lycopene, silibinin and vitamin D, can induce cell cycle arrest in human cancer cells partly by downregulating Skp2 [99-101].

Unfortunately, specific molecules that block Skp2 in CRC are not yet available, but taking into account the great interest in developing such Skp2 inhibitors, pre-clinical and clinical testing of such compounds will proceed in the near future.

### **CONCLUSIONS AND PERSPECTIVES**

This review offers a brief summary regarding the role of Skp2 and its cofactor Cks1 in CRC, especially its interaction and influence on down-stream factors including CDKN1B (p27). Important to mention is the oncogenic role of Skp2, which is not yet fully understood, and that further studies will characterize other possible mechanisms of action.

Even so, we have succeeded in certifying and underlining the importance of Skp2 in CRC therapy. That is why the scientific community should focus on developing molecular target therapies, including Skp2 inhibitors.

We hope that through this review we support scientists to investigate further the mechanisms through which Skp2-Cks1-CDKN1B (p27) pathway could be blocked. These studies must continue, because the quality of life of patients with CRC cannot be improved without new molecular-target therapies.

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## REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: E359-E386. doi: [10.1002/ijc.29210](https://doi.org/10.1002/ijc.29210)
2. Ratto C, Sofo L, Ippoliti M, Merico M, Doglietto GB, Crucitti F. Prognostic factors in colorectal cancer. Literature review for clinical application. *Dis Colon Rectum* 1998; 41: 1033-1049. doi: [10.1007/BF02237397](https://doi.org/10.1007/BF02237397)
3. Derwinger K, Kodeda K, Bexe-Lindskog E, Taflin H. Tumour differentiation grade is associated with TNM staging and the risk of node metastasis in colorectal cancer. *Acta Oncol* 2010; 49: 57-62. doi: [10.3109/02841860903334411](https://doi.org/10.3109/02841860903334411)
4. Derwinger K, Kodeda K, Gerjy R. Age aspects of demography, pathology and survival assessment in colorectal cancer. *Anticancer Res* 2010; 30: 5227-5231.
5. Bonfrate L, Altomare DF, Di Lena M, et al. MicroRNA in colorectal cancer: new perspectives for diagnosis, prognosis and treatment. *J Gastrointest Liver Dis* 2013; 22: 311-320.
6. Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, Hershko DD. The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. *Cancer* 2005; 103: 1336-1346. doi: [10.1002/cncr.20917](https://doi.org/10.1002/cncr.20917)
7. Hershko DD, Shapira M. Prognostic role of p27(Kip1) deregulation in colorectal cancer. *Cancer* 2006; 107: 668-675. doi: [10.1002/cncr.22073](https://doi.org/10.1002/cncr.22073)
8. Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. *Nat Rev Cancer* 2008; 8: 438-449. doi: [10.1038/nrc2396](https://doi.org/10.1038/nrc2396)
9. Wang Z, Fukushima H, Inuzuka H, et al. Skp2 is a promising therapeutic target in breast cancer. *Front Oncol* 2012; 1. doi: [10.3389/fonc.2011.00057](https://doi.org/10.3389/fonc.2011.00057)
10. Wang Z, Gao D, Fukushima H, et al. Skp2: A novel potential therapeutic target for prostate cancer. *Biochim Biophys Acta* 2012; 1825: 11-17. doi: [10.1016/j.bbcan.2011.09.002](https://doi.org/10.1016/j.bbcan.2011.09.002)
11. Graña X, Reddy EP. Cell cycle control in mammalian cells: role of cyclins, cyclin dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). *Oncogene* 1995; 11: 211-219.
12. He W, Wang X, Chen L, Guan X. A crosstalk imbalance between p27(Kip1) and its interacting molecules enhances breast carcinogenesis. *Cancer Biother Radiopharm* 2012; 27: 399-402. doi: [10.1089/cbr.2010.0802](https://doi.org/10.1089/cbr.2010.0802)
13. Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation. *Semin Cancer Biol* 2003; 13: 41-47. doi: [10.1016/S1044-579X\(02\)00098-6](https://doi.org/10.1016/S1044-579X(02)00098-6)
14. Tian YF, Chen TJ, Lin CY, et al. SKP2 overexpression is associated with a poor prognosis of rectal cancer treated with chemoradiotherapy and represents a therapeutic target with high potential. *Tumor Biol* 2013; 34: 1107-1117. doi: [10.1007/s13277-013-0652-z](https://doi.org/10.1007/s13277-013-0652-z)
15. Hershko DD. Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer. *Cancer* 2008; 112: 1415-1424. doi: [10.1002/cncr.23317](https://doi.org/10.1002/cncr.23317)
16. Timmerbeul I, Garrett-Engle CM, Kossatz U, et al. Testing the importance of p27 degradation by the SCFskp2 pathway in murine models of lung and colon cancer. *Proc Natl Acad Sci U S A* 2006; 103: 14009-14014. doi: [10.1073/pnas.0606316103](https://doi.org/10.1073/pnas.0606316103)
17. Hoellein A, Graf S, Bassermann F, et al. Cks1 promotion of S phase entry and proliferation is independent of p27(Kip1) suppression. *Mol Cell Biol* 2012; 32: 2416-2427. doi: [10.1128/MCB.06771-11](https://doi.org/10.1128/MCB.06771-11)
18. Ungermannova D, Gao YF, Liu X. Ubiquitination of p27(Kip1) requires physical interaction with cyclin E and probable phosphate recognition by SKP2. *J Biol Chem* 2005; 280: 30301-30309. doi: [10.1074/jbc.M411103200](https://doi.org/10.1074/jbc.M411103200)
19. Ogino S, Nosho K, Irahara N, et al. A cohort study of cyclin D1 expression and prognosis in 602 colon cancer cases. *Clin Cancer Res* 2009; 15: 4431-4438. doi: [10.1158/1078-0432.CCR-08-3330](https://doi.org/10.1158/1078-0432.CCR-08-3330)
20. Ogino S, Shima K, Nosho K, et al. A cohort study of p27 localization in colon cancer, body mass index, and patient survival. *Cancer Epidemiol Biomarkers Prev* 2009; 18: 1849-1858. doi: [10.1158/1055-9965.EPI-09-0181](https://doi.org/10.1158/1055-9965.EPI-09-0181)
21. Kitagawa K, Kotake Y, Kitagawa M. Ubiquitin-mediated control of oncogene and tumor suppressor gene products. *Cancer Sci* 2009; 100: 1374-1381. doi: [10.1111/j.1349-7006.2009.01196.x](https://doi.org/10.1111/j.1349-7006.2009.01196.x)
22. Nakayama KI, Nakayama K. Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer* 2006; 6: 369-381. doi: [10.1038/nrc1881](https://doi.org/10.1038/nrc1881)
23. Hao B, Zheng N, Schulman BA, et al. Structural basis of the Cks1-dependent recognition of p27(Kip1) by the SCFskp2 ubiquitin ligase. *Mol Cell* 2005; 20: 9-19. doi: [10.1016/j.molcel.2005.09.003](https://doi.org/10.1016/j.molcel.2005.09.003)
24. Shapira M, Ben-Izhak O, Bishara B, et al. Alterations in the expression of the cell cycle regulatory protein cyclin kinase subunit 1 in colorectal carcinoma. *Cancer* 2004; 100: 1615-1621. doi: [10.1002/cncr.20172](https://doi.org/10.1002/cncr.20172)
25. Nakayama KI, Nakayama K. Regulation of the cell cycle by SCF-type ubiquitin ligases. *Semin Cell Dev Biol* 2005; 16: 323-333. doi: [10.1016/j.semcdb.2005.02.010](https://doi.org/10.1016/j.semcdb.2005.02.010)
26. Wang G, Chan CH, Gao Y, Lin HK. Novel roles of Skp2 E3 ligase in cellular senescence, cancer progression, and metastasis. *Chin J Cancer* 2012; 31: 169-177. doi: [10.5732/cjc.011.10319](https://doi.org/10.5732/cjc.011.10319)
27. Demetrick DJ, Zhang H, Beach DH. Chromosomal mapping of the genes for the human CDK2/cyclin A-associated proteins p19 (SKP1A and SKP1B) and p45 (SKP2). *Cytogenet Cell Genet* 1996; 73: 104-107.
28. Chan CH, Lee SW, Wang J, Lin HK. Regulation of Skp2 expression and activity and its role in cancer progression. *Scientific World Journal* 2010; 10: 1001-1015. doi: [10.1100/tsw.2010.89](https://doi.org/10.1100/tsw.2010.89)
29. Xu SY, Wang F, Wei G, et al. S-phase kinase-associated protein 2 knockdown blocks colorectal cancer growth via regulation of both p27

- and p16 expression. *Cancer Gene Ther* 2013; 20: 690-694. doi: [10.1038/cgt.2013.70](https://doi.org/10.1038/cgt.2013.70)
30. Wang Z, Inuzuka H, Zhong J, et al. Identification of acetylation-dependent regulatory mechanisms that govern the oncogenic functions of Skp2. *Oncotarget* 2012; 3: 1294-1300.
  31. Krishnan A, Nair SA, Pillai MR. Loss of cks1 homeostasis deregulates cell division cycle. *J Cell Mol Med* 2010; 14: 154-164. doi: [10.1111/j.1582-4934.2009.00698.x](https://doi.org/10.1111/j.1582-4934.2009.00698.x)
  32. Xu K, Belunis C, Chu W, et al. Protein-protein interactions involved in the recognition of p27 by E3 ubiquitin ligase. *Biochem J* 2003; 371: 957-964. doi: [10.1042/BJ20021722](https://doi.org/10.1042/BJ20021722)
  33. Lim MS, Adamson A, Lin Z, et al. Expression of Skp2, a p27(Kip1) ubiquitin ligase, in malignant lymphoma: correlation with p27(Kip1) and proliferation index. *Blood* 2002; 100: 2950-2956. doi: [10.1182/blood.V100.8.2950](https://doi.org/10.1182/blood.V100.8.2950)
  34. Rose AE, Wang G, Hanniford D, et al. Clinical relevance of SKP2 alterations in metastatic melanoma. *Pigment Cell Melanoma Res* 2011; 24: 197-206. doi: [10.1111/j.1755-148X.2010.00784.x](https://doi.org/10.1111/j.1755-148X.2010.00784.x)
  35. Schüler S, Diersch S, Hamacher R, Schmid RM, Saur D, Schneider G. SKP2 confers resistance of pancreatic cancer cells towards TRAIL-induced apoptosis. *Int J Oncol* 2011; 38: 219-225. doi: [10.3892/ijo\\_00000841](https://doi.org/10.3892/ijo_00000841)
  36. Mori M, Mimori K, Shiraishi T, et al. p27 expression and gastric carcinoma. *Nat Med* 1997; 3: 593.
  37. Lin HK, Chen Z, Wang G, et al. Skp2 targeting suppresses tumorigenesis by Arf-p53-independent cellular senescence. *Nature* 2010; 464: 374-379. doi: [10.1038/nature08815](https://doi.org/10.1038/nature08815)
  38. Xu HM, Liang Y, Chen Q, et al. Correlation of Skp2 overexpression to prognosis of patients with nasopharyngeal carcinoma from South China. *Chin J Cancer* 2011; 30: 204-212. doi: [10.5732/cjc.010.10403](https://doi.org/10.5732/cjc.010.10403)
  39. Liberal V, Martinsson-Ahlzen HS, Liberal J, et al. Cyclin-dependent kinase subunit (Cks) 1 or Cks2 overexpression overrides the DNA damage response barrier triggered by activated oncoproteins. *Proc Natl Acad Sci U S A* 2012; 109: 2754-2759. doi: [10.1073/pnas.1102434108](https://doi.org/10.1073/pnas.1102434108)
  40. Yao ZP, Zhou M, Kelly SE, Seeliger MA, Robinson CV, Itzhaki LS. Activation of ubiquitin ligase SCFSkp2 by Cks1: Insights from hydrogen exchange mass spectrometry. *J Mol Biol* 2006; 363: 673-686. doi: [10.1016/j.jmb.2006.08.032](https://doi.org/10.1016/j.jmb.2006.08.032)
  41. Spruck C, Strohmaier H, Watson M, et al. A CDK-independent function of mammalian Cks1: targeting of SCF(Skp2) to the CDK inhibitor p27Kip1. *Mol Cell* 2001; 7: 639-650. doi: [10.1016/S1097-2765\(01\)00210-6](https://doi.org/10.1016/S1097-2765(01)00210-6)
  42. Martinsson-Ahlzen HS, Liberal V, Grunenfelder B, Chaves SR, Spruck CH, Reed SI. Cyclin-dependent kinase-associated proteins Cks1 and Cks2 are essential during early embryogenesis and for cell cycle progression in somatic cells. *Mol Cell Biol* 2008; 28: 5698-5709. doi: [10.1128/MCB.01833-07](https://doi.org/10.1128/MCB.01833-07)
  43. Westbrook L, Ramanathan HN, Isayeva T, et al. High Cks1 expression in transgenic and carcinogen-initiated mammary tumors is not always accompanied by reduction in p27(Kip1). *Int J Oncol* 2009; 34: 1425-1431. doi: [10.3892/ijo\\_00000271](https://doi.org/10.3892/ijo_00000271)
  44. Yu V, Baskerville C, Grunenfelder B, Reed SI. A kinase-independent function of Cks1 and Cdk1 in regulation of transcription. *Mol Cell* 2005; 17: 145-151. doi: [10.1016/j.molcel.2004.11.020](https://doi.org/10.1016/j.molcel.2004.11.020)
  45. Sitry D, Seeliger MA, Ko TK, et al. Three different binding sites of Cks1 are required for p27-ubiquitin ligation. *J Biol Chem* 2002; 277: 42233-42240. doi: [10.1074/jbc.M205254200](https://doi.org/10.1074/jbc.M205254200)
  46. Rother K, Li YY, Tschöp K, et al. Expression of cyclin-dependent kinase subunit 1 (Cks1) is regulated during the cell cycle by a CDE/CHR tandem element and is downregulated by p53 but not by p63 or p73. *Cell Cycle* 2007; 6: 853-862. doi: [10.4161/cc.6.7.4017](https://doi.org/10.4161/cc.6.7.4017)
  47. Keller UB, Old JB, Dorsey FC, et al. Myc targets Cks1 to provoke the suppression of p27Kip1, proliferation and lymphomagenesis. *EMBO J* 2007; 26: 2562-2574. doi: [10.1038/sj.emboj.7601691](https://doi.org/10.1038/sj.emboj.7601691)
  48. Zancai P, Dal Col J, Piccinin S, et al. Retinoic acid stabilizes p27Kip1 in EBV-immortalized lymphoblastoid B cell lines through enhanced proteasome-dependent degradation of the p45Skp2 and Cks1 proteins. *Oncogene* 2005; 24: 2483-2494. doi: [10.1038/sj.onc.1208458](https://doi.org/10.1038/sj.onc.1208458)
  49. Krishnan A, Hariharan R, Nair SA, Pillai MR. Fluoxetine mediates G0/G1 arrest by inducing functional inhibition of cyclin dependent kinase subunit (CKS)1. *Biochem Pharmacol* 2008; 75: 1924-1934. doi: [10.1016/j.bcp.2008.02.013](https://doi.org/10.1016/j.bcp.2008.02.013)
  50. Halfter H, Friedrich M, Resch A, et al. Oncostatin M induces growth arrest by inhibition of Skp2, Cks1, and cyclin A expression and induced p21 expression. *Cancer Res* 2006; 66: 6530-6539. doi: [10.1158/0008-5472.CAN-04-3734](https://doi.org/10.1158/0008-5472.CAN-04-3734)
  51. Bretones G, Acosta JC, Caraballo JM, et al. SKP2 oncogene is a direct MYC target gene and MYC down-regulates p27(KIP1) through SKP2 in human leukemia cells. *J Biol Chem* 2011; 286: 9815-9825. doi: [10.1074/jbc.M110.165977](https://doi.org/10.1074/jbc.M110.165977)
  52. Chen JY, Wang MC, Hung WC. Transcriptional activation of Skp2 by BCR-ABL in K562 chronic myeloid leukemia cells. *Leuk Res* 2009; 33: 1520-1524. doi: [10.1016/j.leukres.2009.03.007](https://doi.org/10.1016/j.leukres.2009.03.007)
  53. Meng J, Ding Y, Shen A, et al. Overexpression of PPAR $\gamma$  can down-regulate Skp2 expression in MDA-MB-231 breast tumor cells. *Mol Cell Biochem* 2010; 345: 171-180. doi: [10.1007/s11010-010-0570-y](https://doi.org/10.1007/s11010-010-0570-y)
  54. Shapira M, Kakiashvili E, Rosenberg T, Hershko DD. The mTOR inhibitor rapamycin down-regulates the expression of the ubiquitin ligase subunit Skp2 in breast cancer cells. *Breast Cancer Res* 2006; 8: R46. doi: [10.1186/bcr1533](https://doi.org/10.1186/bcr1533)
  55. Sanchez N, Gallagher M, Lao N, et al. MiR-7 triggers cell cycle arrest at the G1/S transition by targeting multiple genes including Skp2 and Psme3. *PLoS One* 2013; 8: e65671. doi: [10.1371/journal.pone.0065671](https://doi.org/10.1371/journal.pone.0065671)
  56. Yamamura S, Saini S, Majid S, et al. MicroRNA-34a modulates c-Myc transcriptional complexes to suppress malignancy in human prostate cancer cells. *PLoS One* 2012; 7: e29722. doi: [10.1371/journal.pone.0029722](https://doi.org/10.1371/journal.pone.0029722)
  57. Yamamura S, Saini S, Majid S, et al. MicroRNA-34a suppresses malignant transformation by targeting c-Myc transcriptional complexes in human renal cell carcinoma. *Carcinogenesis* 2012; 33: 294-300. doi: [10.1093/carcin/bgr286](https://doi.org/10.1093/carcin/bgr286)
  58. le Sage C, Nagel R, Egan DA, et al. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J* 2007; 26: 3699-3708. doi: [10.1038/sj.emboj.7601790](https://doi.org/10.1038/sj.emboj.7601790)
  59. Galardi S, Mercatelli N, Giorda E, et al. miR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. *J Biol Chem* 2007; 282: 23716-23724. doi: [10.1074/jbc.M701805200](https://doi.org/10.1074/jbc.M701805200)
  60. Castagnino P, Kothapalli D, Hawthorne EA, et al. miR-221/222 compensates for Skp2-mediated p27 degradation and is a primary target of cell cycle regulation by prostacyclin and cAMP. *PLoS One* 2013; 8: e56140. doi: [10.1371/journal.pone.0056140](https://doi.org/10.1371/journal.pone.0056140)
  61. Bai J, Xing Y, Fan W, et al. Construction of miR-508-5P lentiviral vector and its targeted effect on S-phase kinase-associated protein-2 gene. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2014; 30: 28-32.
  62. Fernandez S, Risolino M, Mandia N, et al. miR-340 inhibits tumor cell proliferation and induces apoptosis by targeting multiple negative



- regulators of p27 in non-small cell lung cancer. *Oncogene* 2014. doi: [10.1038/onc.2014.267](https://doi.org/10.1038/onc.2014.267)
63. Jackson SJ, Zhang Z, Feng D, et al. Rapid and widespread suppression of self-renewal by microRNA-203 during epidermal differentiation. *Development* 2013; 140: 1882-1891. doi: [10.1242/dev.089649](https://doi.org/10.1242/dev.089649)
  64. Qi F, He T, Jia L, et al. The miR-30 family inhibits pulmonary vascular hyperpermeability in the premetastatic phase by direct targeting of Skp2 in mice. *Clin Cancer Res* 2015. doi: [10.1158/1078-0432.CCR-14-2785](https://doi.org/10.1158/1078-0432.CCR-14-2785)
  65. Saif MW, Chu E. Biology of colorectal cancer. *Cancer J* 2010; 16: 196-201. doi: [10.1097/PPO.0b013e3181e076af](https://doi.org/10.1097/PPO.0b013e3181e076af)
  66. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase-AKT pathway in human cancer. *Nat Rev Cancer* 2002; 2: 489-501. doi: [10.1038/nrc839](https://doi.org/10.1038/nrc839)
  67. Colakoglu T, Yildirim S, Kayaselcuk F, et al. Clinicopathological significance of PTEN loss and the phosphoinositide 3-kinase/Akt pathway in sporadic colorectal neoplasms: is PTEN loss predictor of local recurrence? *Am J Surg* 2008; 195: 719-725. doi: [10.1016/j.amjsurg.2007.05.061](https://doi.org/10.1016/j.amjsurg.2007.05.061)
  68. Wang Q, Zhou Y, Wang X, Evers BM. p27(Kip1) nuclear localization and cyclin-dependent kinase inhibitory activity are regulated by glycogen synthase kinase-3 in human colon cancer cells. *Cell Death Differ* 2008; 15: 908-919. doi: [10.1038/cdd.2008.2](https://doi.org/10.1038/cdd.2008.2)
  69. Park EJ, Chung HJ, Park HJ, Kim GD, Ahn YH, Lee SK. Suppression of Src/ERK and GSK-3/beta-catenin signaling by pinosylvin inhibits the growth of human colorectal cancer cells. *Food Chem Toxicol* 2013; 55: 424-433. doi: [10.1016/j.fct.2013.01.007](https://doi.org/10.1016/j.fct.2013.01.007)
  70. Voutsadakis IA. The ubiquitin-proteasome system in colorectal cancer. *Biochim Biophys Acta* 2008; 1782: 800-808. doi: [10.1016/j.bbadis.2008.06.007](https://doi.org/10.1016/j.bbadis.2008.06.007)
  71. Gao D, Inuzuka H, Tseng A, Chin RY, Toker A, Wei W. Phosphorylation by Akt1 promotes cytoplasmic localization of Skp2 and impairs APCdh1-mediated Skp2 destruction. *Nat Cell Biol* 2009; 11: 397-408. doi: [10.1038/ncb1847](https://doi.org/10.1038/ncb1847)
  72. Pascal LE, Wang Z. Virtual drug design: Skp1-Skp2 inhibition targets cancer stem cells. *Asian J Androl* 2013; 15: 717-718. doi: [10.1038/aja.2013.115](https://doi.org/10.1038/aja.2013.115)
  73. Chan CH, Li CF, Yang WL, et al. The Skp2-SCF E3 ligase regulates Akt ubiquitination, glycolysis, hereceptin sensitivity, and tumorigenesis. *Cell* 2012; 149: 1098-1111. doi: [10.1016/j.cell.2012.02.065](https://doi.org/10.1016/j.cell.2012.02.065)
  74. Fujita T, Liu W, Doihara H, Wan Y. Regulation of Skp2-p27 axis by the Cdh1/anaphase-promoting complex pathway in colorectal tumorigenesis. *Am J Pathol* 2008; 173: 217-228. doi: [10.2353/ajpath.2008.070957](https://doi.org/10.2353/ajpath.2008.070957)
  75. Kudo Y, Tsunematsu T, Takata T. Deregulation of Anaphase-promoting Complex/cyclosome-dependent Proteolysis in Cancer. *J Oral Biosci* 2010; 52: 388-401. doi: [10.2330/joralbiosci.52.388](https://doi.org/10.2330/joralbiosci.52.388)
  76. Bashir T, Pagano M. Don't skip the G1 phase: how APC/CCdh1 keeps SCFSkp2 in check. *Cell Cycle* 2004; 3: 850-852. doi: [10.4161/cc.3.7.977](https://doi.org/10.4161/cc.3.7.977)
  77. Wang Q, Moyret-Lalle C, Couzon F, et al. Alterations of anaphase-promoting complex genes in human colon cancer cells. *Oncogene* 2003; 22: 1486-1490. doi: [10.1038/sj.onc.1206224](https://doi.org/10.1038/sj.onc.1206224)
  78. Singhal S, Amin KM, Krukltis R, et al. Alterations in cell cycle genes in early stage lung adenocarcinoma identified by expression profiling. *Cancer Biol Ther* 2003; 2: 291-298. doi: [10.4161/cbt.2.3.399](https://doi.org/10.4161/cbt.2.3.399)
  79. Bassermann F, Eichner R, Pagano M. The ubiquitin proteasome system - Implications for cell cycle control and the targeted treatment of cancer. *Biochim Biophys Acta* 2014; 1843: 150-162. doi: [10.1016/j.bbamcr.2013.02.028](https://doi.org/10.1016/j.bbamcr.2013.02.028)
  80. Xu S, Abbasian M, Patel P, et al. Substrate recognition and ubiquitination of SCFSkp2/Cks1 ubiquitin-protein isopeptide ligase. *J Biol Chem* 2007; 282: 15462-15470. doi: [10.1074/jbc.M610758200](https://doi.org/10.1074/jbc.M610758200)
  81. van Leuken R, Clijsters L, Wolthuis R. To cell cycle, swing the APC/C. *Biochim Biophys Acta* 2008; 1786: 49-59. doi: [10.1016/j.bbcan.2008.05.002](https://doi.org/10.1016/j.bbcan.2008.05.002)
  82. Hsu CP, Kao TY, Chang WL, Nieh S, Wang HL, Chung YC. Clinical significance of tumor suppressor PTEN in colorectal carcinoma. *Eur J Surg Oncol* 2011; 37: 140-147. doi: [10.1016/j.ejso.2010.12.003](https://doi.org/10.1016/j.ejso.2010.12.003)
  83. Deevi R, Fatehullah A, Jagan I, Nagaraju M, Bingham V, Campbell FC. PTEN regulates colorectal epithelial apoptosis through Cdc42 signalling. *Br J Cancer* 2011; 105: 1313-1321. doi: [10.1038/bjc.2011.384](https://doi.org/10.1038/bjc.2011.384)
  84. Sawai H, Yasuda A, Ochi N, et al. Loss of PTEN expression is associated with colorectal cancer liver metastasis and poor patient survival. *BMC Gastroenterol* 2008; 8: 56. doi: [10.1186/1471-230X-8-56](https://doi.org/10.1186/1471-230X-8-56)
  85. Dicuonzo G, Angeletti S, Garcia-Foncillas J, et al. Colorectal carcinomas and PTEN/MMAC1 gene mutations. *Clin Cancer Res* 2001; 7: 4049-4053.
  86. Negri FV, Bozzetti C, Lagrasta CA, et al. PTEN status in advanced colorectal cancer treated with cetuximab. *Br J Cancer* 2010; 102: 162-164. doi: [10.1038/sj.bjc.6605471](https://doi.org/10.1038/sj.bjc.6605471)
  87. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 2007; 97: 1139-1145. doi: [10.1038/sj.bjc.6604009](https://doi.org/10.1038/sj.bjc.6604009)
  88. Sood A, McClain D, Maitra R, et al. PTEN gene expression and mutations in the PIK3CA gene as predictors of clinical benefit to anti-epidermal growth factor receptor antibody therapy in patients with KRAS wild-type metastatic colorectal cancer. *Clin Colorectal Cancer* 2012; 11: 143-150. doi: [10.1016/j.clcc.2011.12.001](https://doi.org/10.1016/j.clcc.2011.12.001)
  89. Yoshida Y, Ninomiya K, Hamada H, Noda M. Involvement of the SKP2-p27(KIP1) pathway in suppression of cancer cell proliferation by RECK. *Oncogene* 2012; 31: 4128-4138. doi: [10.1038/onc.2011.570](https://doi.org/10.1038/onc.2011.570)
  90. Noda M, Takahashi C. Recklessness as a hallmark of aggressive cancer. *Cancer Sci* 2007; 98: 1659-1665. doi: [10.1111/j.1349-7006.2007.00588.x](https://doi.org/10.1111/j.1349-7006.2007.00588.x)
  91. Oh J, Takahashi R, Kondo S, et al. The membrane-anchored MMP inhibitor RECK is a key regulator of extracellular matrix integrity and angiogenesis. *Cell* 2001; 107: 789-800. doi: [10.1016/S0092-8674\(01\)00597-9](https://doi.org/10.1016/S0092-8674(01)00597-9)
  92. Nalepa G, Wade Harper J. Therapeutic anti-cancer targets upstream of the proteasome. *Cancer Treat Rev* 2003; 29: 49-57.
  93. Frankland-Searby S, Bhaumik SR. The 26S proteasome complex: An attractive target for cancer therapy. *Biochim Biophys Acta* 2012; 1825: 64-76. doi: [10.1016/j.bbcan.2011.10.003](https://doi.org/10.1016/j.bbcan.2011.10.003)
  94. Uddin S, Ahmed M, Bavi P, et al. Bortezomib (velcade) induces p27Kip1 expression through S-phase kinase protein 2 degradation in colorectal cancer. *Cancer Res* 2008; 68: 3379-3388. doi: [10.1158/0008-5472.CAN-07-6109](https://doi.org/10.1158/0008-5472.CAN-07-6109)
  95. Mackay H, Hedley D, Major P, et al. A phase II trial with pharmacodynamic endpoints of the proteasome inhibitor bortezomib in patients with metastatic colorectal cancer. *Clinical Cancer Res* 2005; 11: 5526-5533. doi: [10.1158/1078-0432.CCR-05-0081](https://doi.org/10.1158/1078-0432.CCR-05-0081)
  96. Ryan DR, O'Neil BH, Supko JG, et al. A phase I study of bortezomib plus irinotecan in patients with advanced solid tumors. *Cancer* 2006; 107: 2688-2697. doi: [10.1002/cncr.22280](https://doi.org/10.1002/cncr.22280)
  97. Chen Q, Xie W, Kuhn DJ, et al. Targeting the p27 E3 ligase SCFSkp2 results in p27-and Skp2-mediated cell-cycle arrest and activation of autophagy. *Blood* 2008; 111: 4690-4699. doi: [10.1182/blood-2007-09-112904](https://doi.org/10.1182/blood-2007-09-112904)

98. Rico-Bautista E, Yang CC, Lu L, Roth GP, Wolf DA. Chemical genetics approach to restoring p27Kip1 reveals novel compounds with antiproliferative activity in prostate cancer cells. *BMC Biol* 2010; 8: 153. doi: [10.1186/1741-7007-8-153](https://doi.org/10.1186/1741-7007-8-153)
99. Huang HC, Lin CL, Lin JK. 1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucose, quercetin, curcumin and lycopene induce cell-cycle arrest in MDA-MB-231 and BT474 cells through downregulation of Skp2 protein. *J Agric Food Chem* 2011; 59: 6765-6775. doi: [10.1021/jf201096v](https://doi.org/10.1021/jf201096v)
100. Roy S, Kaur M, Agarwal C, Tecklenburg M, Sclafani RA, Agarwal R. p21 and p27 induction by silibinin is essential for its cell cycle arrest effect in prostate carcinoma cells. *Mol Cancer Ther* 2007; 6: 2696-2707. doi: [10.1158/1535-7163.MCT-07-0104](https://doi.org/10.1158/1535-7163.MCT-07-0104)
101. Yang ES, Burnstein KL. Vitamin D inhibits G1 to S progression in LNCaP prostate cancer cells through p27Kip1 stabilization and Cdk2 mislocalization to the cytoplasm. *J Biol Chem* 2003; 278: 46862-46868. doi: [10.1074/jbc.M306340200](https://doi.org/10.1074/jbc.M306340200)