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 p27Kip1. 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.

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...
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 CCR 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 the mitogenic and growth inhibitory signals. Two families of Cdk inhibitors regulate the deployment of this process: the Cdk4 (INK4) family members including p15INK4A, p16INK4B, p18INK4C, and p19INK4D and the CIP/KIP family (kinase inhibitor protein) enveloping p21CIP1, p27Kip1 (CDKN1B) and p57Kip2 [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 Skp2SCF 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, β-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 cromosome, expresses an essential protein component of the SCF<sub>Skp2</sub> 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 supressor 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 polyplody 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 theSkp2-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.
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**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 Bel-xL/Bcl-2-Associated Death, IKK (Inhibitor of nuclear factor Kappa B Kinase), GS3K (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-3α and GSK-3β, 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 by Akt has two consequences: promotes cytoplasmic translocation of Skp2 and Akt expression. SCFSkp2 can trigger non-proteolytic K63-linked ubiquitination of Akt with the result on the increase of membrane localization and activation of Akt [72, 73].

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

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Mechanism</th>
<th>Effects</th>
<th>Cells</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>miR-7</td>
<td>Skp2 downregulation</td>
<td>Cell cycle arrest</td>
<td>Chinese hamster ovary cells</td>
<td>[55]</td>
</tr>
<tr>
<td>miR-34a</td>
<td>cMyc-Skp2-Miz1 suppression</td>
<td>Inhibits invasion and migration</td>
<td>Prostate and renal cancer</td>
<td>[56, 57]</td>
</tr>
<tr>
<td>miR-221</td>
<td>CDKN1B (p27) translational inhibition</td>
<td>Compensates for loss of Skp2-mediated CDKN1B (p27) degradation</td>
<td>Prostate cancer and glioblastoma</td>
<td>[58-60]</td>
</tr>
<tr>
<td>miR-508-59</td>
<td>Suppression of Skp2 gene expression</td>
<td>Suppress Skp2 protein expression</td>
<td>HEK293T cells</td>
<td>[61]</td>
</tr>
<tr>
<td>miR-340</td>
<td>Skp2 downregulation</td>
<td>CDKN1B (p27) upregulation</td>
<td>Non-small cell lung cancer</td>
<td>[62]</td>
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<tr>
<td>miR-203</td>
<td>Skp2 suppression</td>
<td>Inhibits proliferation</td>
<td>Epidermal</td>
<td>[63]</td>
</tr>
<tr>
<td>miR-30s</td>
<td>Skp2 suppression</td>
<td>Inhibits metastasis</td>
<td>Lung fibroblast from tumor-bearing mice</td>
<td>[64]</td>
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**PI3K/Akt 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 pathological, 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 SCFSkp2 [74-76]. In the late mitosis and the G1 phase, Skp2 is maintained at a low level by the APC/C<sub>Cdh1</sub>-mediated ubiquitination [80]. Between the G1 and the S-phase, APC/C<sub>Cdh1</sub> 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].
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 decrease of Skp2 and an increase of CDKN1B (p27) [89].

**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], gemcitabine, 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 downregulating 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.

Conflicts of interest: No conflict to declare.

Authors’ contribution: O.V. B.: study concept and design, literature search and selection, data interpretation; conceived and drafted the manuscript. A.I.: obtained funding, supervised the study and revised it for important intellectual content. M.P.: critical comments and manuscript revision. I.B.-N.: literature search and manuscript revision for important intellectual content. All authors read and agreed with the final version of the manuscript.

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