JBUON 2017; 22(5): 1122-1130
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

The prognostic role of Skp2 and the tumor suppressor protein p27 in colorectal cancer

Ovidiu Vasile Bochis[1], Patriciu Achimas-Cadariu[2], Catalin Vlad[2], Bogdan Fetica[3], Daniel Corneliu Leucuta[4], Constantin Ioan Busuioc[5], Alexandru Irimie[2]

[1]Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca; Department of Medical Oncology, "Prof. Dr. Ion Chiricuta" Institute of Oncology, Cluj-Napoca; [2]Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca; Department of Surgery, "Prof. Dr. Ion Chiricuta" Institute of Oncology, Cluj-Napoca; [3]Department of Pathology, "Prof. Dr. Ion Chiricuta" Institute of Oncology, Cluj-Napoca; [4]Iuliu Hatieganu University of Medicine and Pharmacy, Department of Medical Informatics and Biostatistics, Cluj-Napoca; [5]Iuliu Hatieganu University of Medicine and Pharmacy, Department of Pathology, Cluj-Napoca, Romania

Summary

Purpose: *This study aimed at exploring the role of Skp2, p27 and Cks1 expression as prognostic factors for colorectal cancer (CRC) patients, and to identify a correlation between their expression and the cell proliferation marker Ki67.*

Methods: *We conducted a retrospective study on 130 patients with CRC treated with surgery and adjuvant treatment at the Oncology Institute Cluj-Napoca between 2006-2010. The Skp2, p27, Cks1 and Ki67 immunoexpression was grouped from 1 to 4, according to percents of tumor cells with nuclear reactivity. Their correlation with overall survival (OS), recurrence free survival (RFS) and with the classical histopathological prognostic factors were analyzed. All patients had 5-year follow-up.*

Results: *The majority of patients had locally advanced TNM stages II and III. More than a half of the tumors had low immunoexpression of Skp2 and Cks1, and high and moderate p27 and Ki67 expression. Skp2 overexpression negatively influenced the p27 (p=0.002). Both OS and RFS were significantly higher in patients with moderate and high expression of p27 (p=0.005). Skp2 and Cks1 overexpression negatively influenced OS and RFS. Skp2 overexpression positively correlated with TNM stage (p<0.001), node capsular invasion (p=0.002) and lymphovascular invasion (p=0.042). Ki67 expression did not correlate with Skp2 (p=0.88), Cks1 (p=0.67) and p27 (p=0.40), neither with OS (p=0.841) and RFS (p=0.84).*

Conclusions: *The most important prognostic factor was the Skp2 overexpression. It was the only protein we studied that correlated with the other well-known prognostic factors in CRC. The expression of Ki67 did not bring any novel prognostic information regarding CRC.*

Key words: *colorectal cancer, Ki67, p27, prognosis, Skp2, survival*

Introduction

CRC is one of the most frequent cancers worldwide, being the third most common cancer in men and the second in women. It is more frequent in economically more developed countries, but the mortality is higher in the less developed ones [1].

According to international guidelines (NCCN and ESMO) the therapeutic management for advanced-stage CRC is based primarily on chemotherapy. For early-stage the multimodality approach, including surgery, chemotherapy and radiotherapy (for rectal cancer), is the gold standard. However, the efficacy of current treatment modalities is limited, and the development of new molecular targeted treatments is necessary.

The therapeutic approach in CRC depends on the patients' prognosis, based on TNM status, performance status, microsatellite instability (MSI), age, tumor aggressiveness, all-RAS and BRAF

Correspondence to: Ovidiu Vasile Bochis, MD. Ion Chiricuta Institute of Oncology, 34-36 Republicii street, 400015, Cluj-Napoca, Cluj, Romania. Tel: +40 264 598362, Fax: +40 264 598365, E-mail: bochis.ovidiu@umfcluj.ro
Received: 05/06/2017; Accepted: 19/06/2017

mutations and tumor location (right vs left) [2-5].

In the last few years, after the development of molecular biology techniques, many translational researches were focused on the identification of molecular and genetic factors involved in CRC oncogenesis [6]. The discovery of the molecular mechanisms for activation of oncogenes and inactivation of tumor suppressor genes can create the premise for targeted therapeutic approaches, which are much more effective and less toxic.

The molecular and genetic disorders that appear in the development of invasive CRC, from the epithelial normal mucosa, through adenoma and *in situ* carcinoma, are still poorly understood. However, numerous studies are focused in identified cell cycle regulators, including cyclin, cyclin-dependent kinases (CDKs) and CDK inhibitory proteins. Abnormal expression of the cell cycle's regulators is mandatory for carcinogenesis. The tumor suppressor protein p27, a negative regulator of cyclinE and cyclinA/Cdk2, which drive cells in the S phase of the cell cycle [6-9] is involved in cellular differentiation, proliferation, apoptosis, cellular adhesion and growth inhibition [7,10,11]. The F-box protein Skp2 (S-phase kinase associated protein 2), a recognition component for the substrate in SCF (Skp1-Cullin1-F-box) E3 ubiquitin-ligase complex, is an important component of the ubiquitin-proteasome system (UPS) which controls the stability of the cell cycle regulators [12,13]. The most well-known substrate for Skp2 is the tumor suppressor protein p27. The Skp2 overexpression induces ubiquitination and the consecutive degradation of p27. The proteolytic degradation demands an efficient binding between Skp2 and p27, which requires the presence of the cyclin-dependent kinase subunit 1 (Cks1) cofactor, that acts like an adaptor between Skp2 and p27 [9,14-17]. The excessive degradation of p27 may contribute to uncontrolled proliferation of cancer cells. Among p27, Skp2 is involved in degradation of other tumor suppressor proteins, as p21, p57, FOXO1 and RBL2 [6,13], having an important role in cell cycle regulation, cell growth, differentiation, cell proliferation, metastasis, and apoptosis, all linked to the cancer development [6,9,10,12,13].

This study aimed at exploring the role of Skp2, p27 and Cks1 expression as prognostic factors for CRC patients, and to identify a correlation between their expression and the Ki67 expression, a cell proliferation marker.

Methods

Patient selection

After the approval of the institution's research ethics board, we conducted a retrospective study of 130 patients with CRC treated with surgery and adjuvant chemotherapy, or radiochemotherapy for rectal cancer cases, at the Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, from January 2006 to December 2010. All patients were newly diagnosed with colorectal adenocarcinoma based on pathological examination and underwent surgical treatment in our institute. Patients considered eligible had to be above the age of 18 years, with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 to 1 and without previous treatments with cytotoxic drugs for other tumors. The tumor stage was classified by using the 7th edition of the American Joint Committee on Cancer (AJCC) staging system. After completion of treatment all patients were regularly monitored until death or their last visit at the hospital.

Immunohistochemistry

After the procurement of formalin-fixed and paraffin-embedded tissues, immunoexpression of Skp2, p27, Cks1 and Ki67 was scored by two pathologists, without knowledge of the clinical data. Four groups of various expression levels from 1 to 4 were defined according to percents of tumor cells with nuclear reactivity, respectively 0-24, 25-49, 50-74 and 75-100% (Figure 1). Rabbit monoclonal antibodies against Skp2, p27, Cks1 and Ki67 were used.

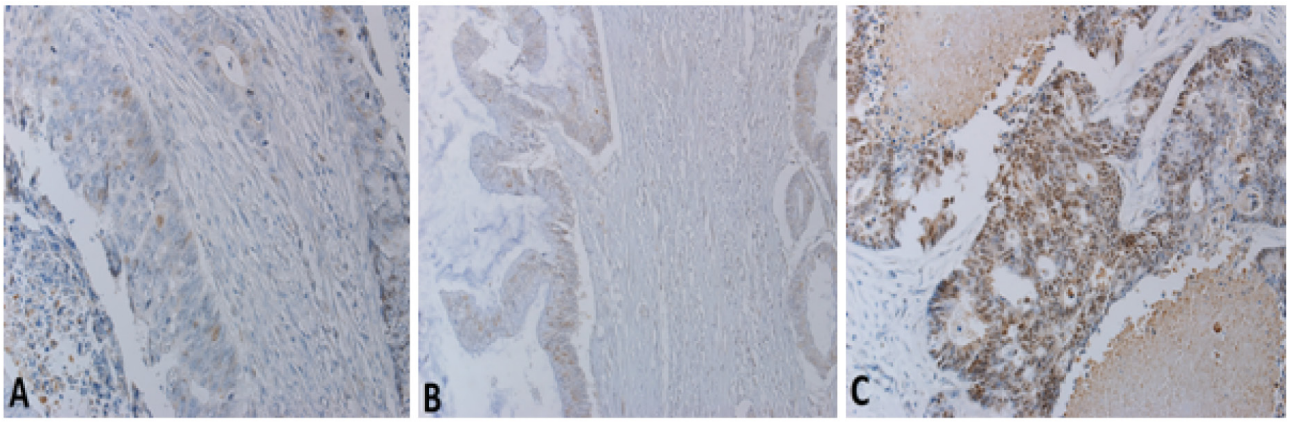

Figure 1. Different types of immunohistochemical staining of Skp2: low **(A)**, moderate **(B)** and high **(C)**.

Statistics

The statistical program R version 3.2.1 was used to perform all the analyses. Cases with immunoreactivity of 0 and 1 were defined as low expression, those with 2 were defined as moderate expression, and those with 3 or 4 as high expression. The Kaplan-Meier method was used to estimate OS and RFS, calculated from the date of surgery to the date of event. Categorical variables were analyzed by means of the x^2 test or Fisher's exact test, and the t-test or Mann–Whitney U test were used for continuous data. Log-rank test was used in univariate analysis for evaluate prognostic differences between groups. The Cox proportional hazards model was used to develop multivariate models. For all analyses, hazard ratios and confidence intervals were obtained at 95% significance, and a p value <0.05 was considered statistically significant.

Results

A total of 130 patients with CRC were evaluable for this study. Female to male ratio was 1:1.16. More than two thirds of the patients had left-sided tumors (82.3%). The majority of patients had locally advanced TNM stages II and III, and 19.23% of the cases had metastatic stage IV. Regarding the histology, the majority of patients had adenocarcinoma (85.38%) and more than a half (60.77%) were moderately differentiated. Lymphatic invasion was seen in 55.38%, followed by perineural and vascular invasion. Node capsular invasion was encountered in 16.92%. More than half of the tumors had low immunoexpression of Skp2 and Cks1, while for the p27 and Ki67 expressions more than half had high or moderate expression. All patients were followed for at least 5 years. Recurrence occurred in 1/3 of the patients (41/130). At the end 41.54% patients were deceased with a median OS of 63 months (range 2-117). Patient and tumor characteristics are shown in Tables 1 and 2.

From the data displayed in Table 1 it can be observed the correlation between OS and the prognostic factors currently well-known and accepted by the international guidelines for several years, being validated by multiple clinical studies. Thus, poorer performance status, higher TNM stage, lymphovascular and perineural invasion, positive resection margin and node capsular invasion were negatively correlated with OS. In the studied population, no significant statistical correlation was found between the histological grade and the OS, nor between the tumor localizations (left vs. right colon) and the OS.

Analyzing the correlation between the expression of p27 and Skp2, it was demonstrated that the Skp2 overexpression negatively influenced significantly the p27 expression, (p=0.002; Figure 2).

Table 1. Patient characteristics

Characteristics	*n=130 n (%)*	*OS p value*
ECOG (1 vs. 0)	73/130 (56.15)	0.001
Anatomic location	left side: 107/130 (82.3)	0.849
	right side: 23/130 (17.7)	
Location (rectum vs. colon)	76/130 (58.46)	0.98
TNM stage	I: 7/130 (5.38)	<0.001
	II: 43/130 (33.08)	
	III: 55/130 (42.32)	
	IV: 25/130 (19.23)	
Resection margin (R0 vs. R1)	104/130 (80)	0.002
Vascular invasion (yes vs. no)	36/130 (27.69)	0.018
Lymphatic invasion (yes vs. no)	72/130 (55.38)	<0.001
Perineural invasion (yes vs. no)	45/130 (34.62)	<0.001
Node capsular invasion (yes vs. no)	22/130 (16.92)	<0.001
Histologic grade	1: 23/130 (17.69)	0.979
	2: 79/130 (60.77)	
	3: 28/130 (21.54)	
Adjuvant therapy	chemotherapy: 90/130 (69.23)	N/A
	chemotherapy+RT 45Gy: 31/130 (23.85)	N/A
	chemotherapy+RT 50Gy: 9/130 (6.92)	N/A
Targeted therapy (yes vs. no)	24/130 (18.46)	N/A
Recurrence (yes vs. no)	41/130 (31.54)	<0.001
Distant metastases (yes vs. no)	35/130 (26.92)	<0.001
Local recurrence (yes vs. no)	14/130 (10.77)	0.109
Death (yes vs. no)	54/130 (41.54)	N/A

N/A: not available

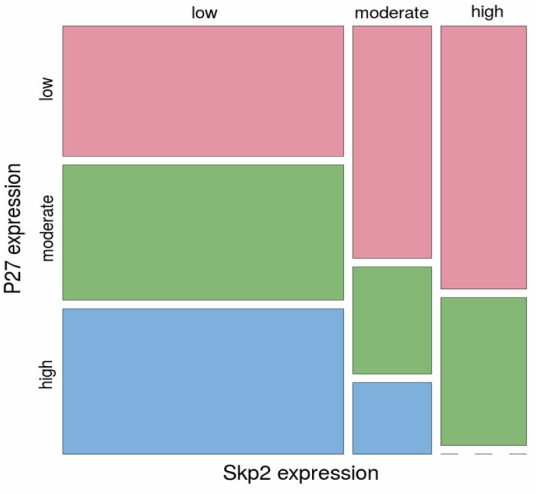

Figure 2. Correlation between p27 expression according to Skp2 expression (p=0.002). This Figure presents an inversely proportional correlation between the expression of Skp2 and p27. Of notice, no case was identified with both proteins' overexpression. In each of the cases, if one of the proteins is overexpressed, the other one shows a low or moderate expression.

Both OS and RFS were statistically significantly higher in patients with moderate and high expression of p27 (Figures 3 and 4), in contrast to Skp2 (Figure 5 and 6) and Cks1 (Figure 7) expression which were statistically significantly higher in cases with low immunoexpression. Regarding Ki67 expression, OS or RFS were not influenced by its immunoexpression (Table 2).

Regarding the classical histopathological prognostic factors, like TNM stage, node capsular invasion and lymphovascular invasion, they were all significantly correlated with the expression of Skp2. A significant statistical correlation as well in relation to the expression of Cks1 and these factors, excepting the lymphatic and vascular invasion (p=0.234). No statistically significant correlation between these prognostic factors and

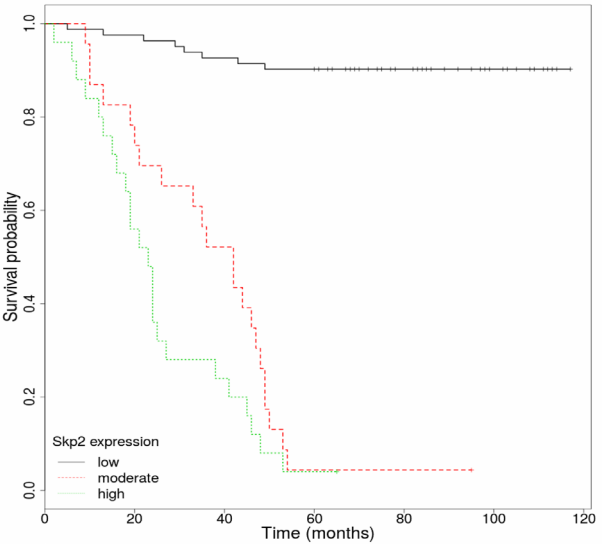

Figure 5. Overall survival according to Skp2 expression (p<0.001).

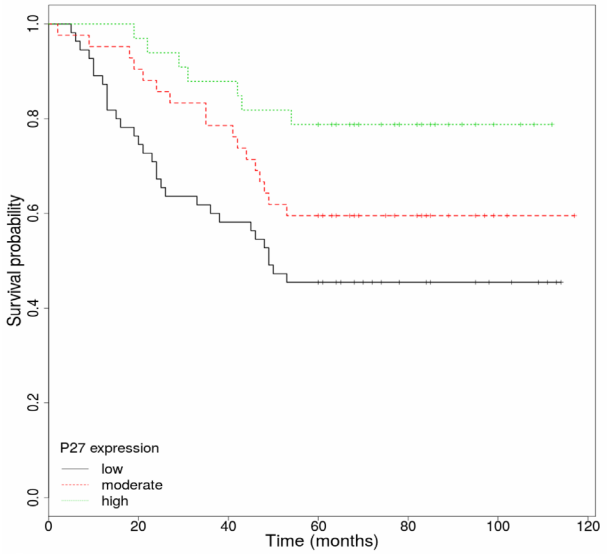

Figure 3. Overall survival according to p27 expression (p=0.005).

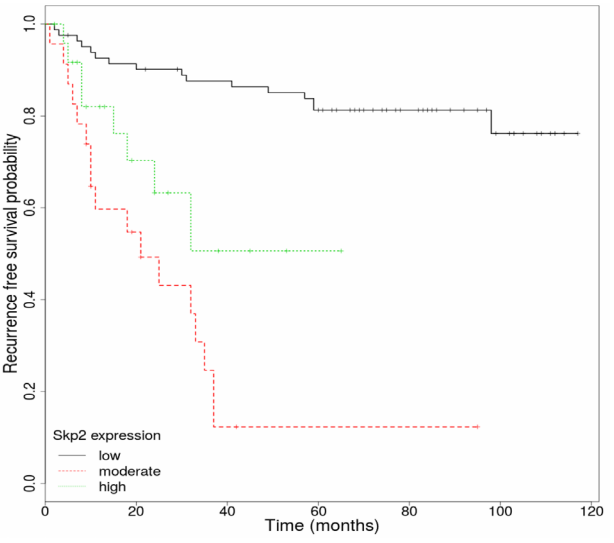

Figure 6. Recurrence free survival according to Skp2 expression (p<0.001).

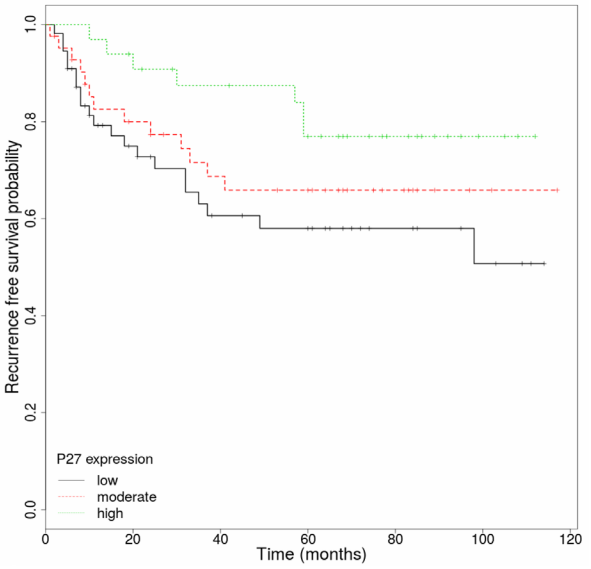

Figure 4. Recurrence free survival according to p27 expression (p=0.04).

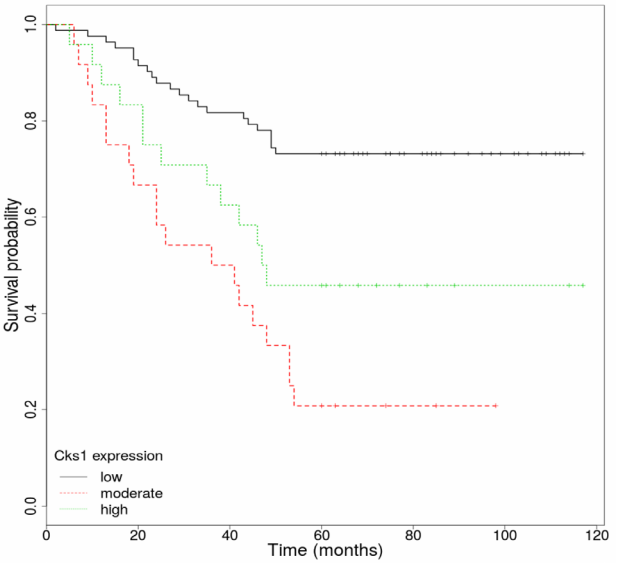

Figure 7. Overall survival according to Cks1 expression (p<0.001).

the expression of p27 and Ki67 was identified. Moreover, the histological grade was not significantly correlated with the expression of the immunohistochemical markers studied. Recurrence, local or distant, was significantly correlated only with the expression of Skp2. No significant difference between the expression of these regulatory proteins of the cycle cell in relation to the tumor localization, on the right or on the left colon, was noted (Table 3). Also, no statistically significant correlation was identified between the immunohistochemical expression of Ki67 and the expression of the other markers studied [Skp2 (p=0.88), Cks1 (p=0.67) and p27 (p=0.40)].

The impact on OS and RFS of the expression of the regulatory proteins of the cycle cell, grouped in high expression vs other (moderate+low) was assessed (Table 4). A statistically significant difference regarding the OS in relation to the expression of Skp2 (Figure 8) and p27 was registered (Figure 9). When the cases were grouped into high+moderate vs low expression, a statistically significant difference arose regarding the OS, as well as on the expression of Skp2, p27 on the expression of Cks1. The expression of high+moderate Skp2 and Cks1 negatively influenced OS, while the expression of high+moderate of p27 positively influenced OS. RFS was negatively influenced in a statistically significant manner only by the expression high+moderate of Skp2 (Table 4).

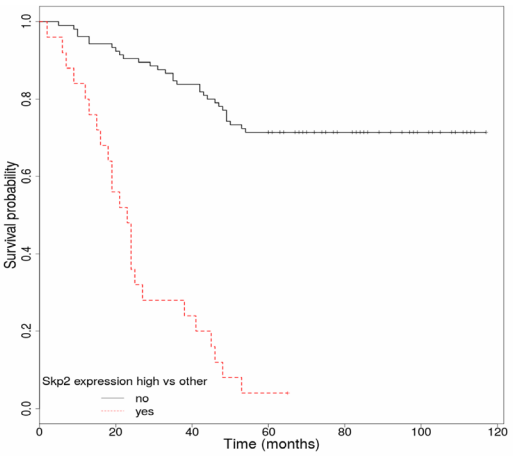

Figure 8. Overall survival according to Skp2 expression (high vs other; p<0.001).

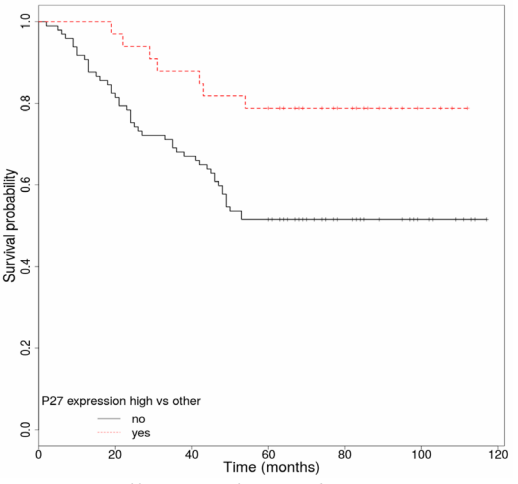

Figure 9. Overall survival according to p27 expression (high vs other; p=0.007).

Table 2. Disease immunoexpression

Immunoexpression	*n=130* *n (%)*	*OS* *p value*	*RFS* *p value*
p27 expression	high: 33/130 (25.38) moderate: 42/130 (32.31) low: 55/130 (42.31)	0.005	0.04
Skp2 expression	high: 25/130 (19.23) moderate: 23/130 (17.69) low: 82/130 (63.08)	<0.001	<0.001
Cks1 expression	high: 24/130 (18.46) moderate: 24/130 (18.46) low: 82/130 (63.08)	<0.001	0.023
Ki67 expression	high: 33/130 (25.38) moderate: 39/130 (30) low: 58/130 (44.62)	0.841	0.84

Table 3. Correlation between classical prognostic factors and cell-cycle regulators (Skp2, Cks1, p27) plus Ki67

Correlations	*Skp2*	*Cks1*	*p27*	*Ki67*
Recurrence	p<0.001	p=0.23	p=0.251	p=0.828
TNM stage	p<0.001	p=0.029	p=0.579	p=0.605
Node capsular invasion	p=0.002	p=0.015	p=0.271	p=0.774
N stage at diagnosis (N0, N1, N2)	p=0.002	p=0.044	p=0.996	p=0.622
M stage at diagnosis (M0, M1)	p<0.001	p=0.003	p=0.546	p=0.178
Histologic grade	p=0.694	p=0.52	p=0.702	p=0.881
Lymphovascular invasion	p=0.042	p=0.234	p=0.982	p=0.86
Anatomic location, right vs left	p=1	p=0.566	p=0.134	p=0.85

Table 4. Overall survival and recurrence free survival according to different percentages of immunoexpression

Immunoexpression	*n=130* *n (%)*	*OS* *p value*	*RFS* *p value*
p27 expression	high: 33/130 (25.38) moderate+low: 97/130 (74.62)	0.007	0.058
p27 expression	high+moderate: 75/130 (57.70) low: 55/130 (42.30)	0.004	0.072
Skp2 expression	high: 25/130 (19.23) moderate+low: 105/130 (80.77)	<0.001	0.103
Skp2 expression	high+moderate: 48/130 (36.93) low: 82/130 (63.07)	<0.001	<0.001
Cks1 expression	high: 24/130 (18.46) moderate+low: 106/130 (81.54)	0.136	0.767
Cks1 expression	high+moderate: 48/130 (36.93) low: 82/130 (63.07)	<0.001	0.119

Table 5. Multivariate analysis for overall survival according to Skp2, Cks1 and p27 expression after setting controls for age, gender, tumor grade and disease stage

Immunohistochemical expression	*HR adjusted*	*(95% CI)*	*p value*
Skp2 expression high vs other	5.08	(2.52 - 10.25)	<0.001
Skp2 expression high+moderate vs low	19.79	(8.7 - 45.01)	<0.001
Skp2 expression high vs low	21.46	(8.50 – 54.21)	<0.001
Skp2 expression moderate vs low	20.73	(8.81 - 48.79)	<0.001
p27 expression high vs other	0.35	(0.15 - 0.78)	0.010
p27 expression high+moderate vs low	0.41	(0.24 - 0.72)	0.002
p27 expression high vs low	0.24	(0.11 – 0.58)	0.001
p27 expression moderate vs low	0.59	(0.32 - 1.08)	0.089
Cks1 expression high vs other	1.25	(0.66 - 2.36)	0.489
Cks1 expression high+moderate vs low	2.73	(1.52 - 4.91)	<0.001
Cks1 expression high vs low	1.93	(0.96 – 3.92)	0.066
Cks1 expression moderate vs low	3.13	(1.61 - 6.08)	0.0007

Furthermore, when Skp2, Cks1 and p27 expressions were separately introduced to Cox regression multivariate analysis, after setting controls for gender, age, histologic tumor grade and TNM stage (Table 5), high and also moderate Skp2 expression was evidently strongly and independently associated with OS. Regarding the expression of p27 this strongly correlated with OS only in the cases with high expression or high+moderate expression. The expression of Cks1 significantly correlated with OS in the cases with moderate or high+moderate expression. Due to the small number of cases with Cks1 with high expression we did not identify a statistically significant difference for these cases taken separately.

Discussion

A malignant cell has the ability to promote an uncontrolled cellular growth with a higher proliferation rate, most frequently due to the loss of apoptosis, as well as of the control over the transition from G1 to S phase of the cell cycle [10,21]. In a normal cell cycle, the level of p27 is elevated in the G0/G1 phase. After the mitogenic stimulation allowing the cells to enter into the S phase, p27 is rapidly degraded after its phosphorylation at threonin-187 that marks p27 for recognition by the Skp2 [10,22,23]. To date, p27 is the most well-known substrate for Skp2. An Skp2 overexpression induced ubiquitination and the consecutive degradation of p27 through the 26S proteasome degradation, promoting the cell cycle division [8,10,15,16,23]. The role of Cks1 in targeting p27 was indicated by demonstrating the lack of p27 ubiquitination in the absence of Cks1 *in vitro* in cell-free systems [24,25].

Previous studies have shown that the absence or reduction of p27 expression can be associated with a poor prognosis in several types of cancer, including breast, prostate, lung and colon cancer [8,11,12,16]. In our study more than half of the patients had moderate or high immunoexpression of p27 (57.69%). Both OS (p=0.005) and RFS (p=0.04) were significantly higher in patients with moderate and high expression of p27. From the obtained data after the analysis of the impact on OS of the expression of p27 and after grouping

the cases in low+moderate vs high expression and low vs moderate+high expression, it can be concluded that the positive expression of p27 in the tumor cells significantly improved the survival. As shown in Table 3 we did not identify a statistically significant correlation between the classical prognostic factors used (TNM stage, node capsular invasion, lymphovascular invasion, histological grade or anatomic location) and the expression of p27. This can support the hypothesis that the p27 expression is an independent prognostic factor [8,10,12].

The oncogenic role of Skp2 was demonstrated in mouse models and cancer cell lines . These studies show that xenografts of breast and colon cancer overexpressing Skp2 grow faster than those showing a lower level of Skp2 [11,16]. By analyzing mice with p27 gene mutations which generate a mutant p27 protein that cannot be bound by Skp2 showed that the Skp2-dependent degradation of p27 is crucial for the progression of adenomas to colon carcinomas [11,16]. The involvement of Skp2 overexpression in metastasis has been reported in many solid tumors including CRC [8,12,26]. Skp2 overexpression has been associated with poor prognosis and low expression of p27 in different cancers, such as breast cancer [27], lung cancer [11], prostate cancer [15] and CRC [8,12,26]. In this study we managed to demonstrate once again that Skp2 overexpression negatively influenced the p27 expression (p=0.002) (Figure 2). In our cases, more than half of the tumors had low immunoexpression of Skp2 and Cks1. Both OS (p<0.001) and RFS (p<0.001) were negatively influenced by the Skp2 overexpression. In the analysis after grouping Skp2 expression in low+moderate vs high + low vs moderate+high expression, we demonstrated that OS was negatively influenced as well in the moderate+high cases as in those with high expression of Skp2. RFS was negatively influenced only in the high Skp2 expression. It was also demonstrated a proportionally direct correlation, statistically significant, between the overexpression of Skp2 and the presence of known negative prognostic factors, such as advanced TNM stage, presence of node capsular invasion and lymphovascular invasion. Moreover, recurrence was more frequent in the cases with Skp2 overexpression (p<0.001). These data demonstrate once again that Skp2 overexpression is a negative prognostic factor, with a great impact both on OS and RFS.

In some kinds of cancer, including breast, CRC and gastric cancer, Cks1 expression has been discovered to be an independent prognostic marker as it offers additional information to that given by Skp2 and p27 alone [8,28]. In our cases, as well, the expression of Cks1 was negatively correlated with OS (p<0.001) and RFS (p=0.023).

Even in multivariate analysis, after setting controls for gender, age, histologic tumor grade and TNM stage (Table 5) it was demonstrated that immunoexpression of Skp2, p27 and Cks1 was strongly and independently associated with OS.

No statistically significant difference between the expression of regulatory proteins of the cell cycle and the tumor localization (right or left colon) was identified (Table 3). Also, no statistically significant correlation was noted between the immunohistochemical expression of Ki67 and the expression of the other studied markers: Skp2 (p=0.88), Cks1 (p=0.67) and p27 (p=0.40). The expression of Ki67 did not significantly correlate with either OS or RFS. Moreover, no significant correlation between Ki67 and already known prognostic factors was registered. Thus, we consider that the expression of Ki67 does not bring any novel prognostic information regarding CRC.

Finally, we can assert that in this study, the most important prognostic factor was the overexpression of Skp2. Skp2 had influenced OS and RFS. It was the only protein we studied that was also correlated with the other well-known prognostic factors in CRC. Thus, the present study could be added to other already published studies that have demonstrated the prognostic importance of Skp2 overexpression in CRC, as well by p27 downregulation as by the correlation to other negative prognostic factors for OS and RFS. Moreover, we consider that routine testing of Skp2 expression would bring new information regarding the prognosis in CRC, especially in locally advanced cases, and, no doubt, that developing molecular-targeted anti-Skp2 therapies would be of great necessity.

The first steps in developing anti-Skp2 therapies have started to appear after understanding the role of the ubiquitin proteasome system in cell cycle regulation and the importance of enzymatic reactions that promote the 26S proteasome degradation. Hence, different molecules from ubiquitin proteasome system become an important pharmacological target for the development of new target therapies in cancer [29]. In preclinical studies the treatment of CRC cell lines with Bortezomib, a proteasome inhibitor, has resulted in stabilizing and increasing the p27 level via downregulation of Skp2. These cells showed cell growth arrest and enhancement of apoptosis [30]. Adding 5-fluorouracil to Bortezomib determined an apoptotic response considerably higher, pointing to a potential synergism between classical chemotherapy and proteasome inhibitors [30].

As a consequence of the preclinical studies, some phase II studies using Bortezomib in solid tumors were conducted, including CRC patients. Bortezomib was used in association with other chemotherapy agents, like Irinotecan, Gemcitabin, Taxanes and Platinum agents, but there was no meaningful positive response [31-33]. Maybe, if these studies had included only patients with Skp2 overexpression we could have expected promising results. Moreover, using a selective Skp2 inhibitor and not a proteasome inhibitor, results could be more resonant. Multiple studies have demonstrated that Skp2 downregulation induces p27 stabilization and inhibits the development of colon cancer cells line [34,35]. Consequently, the development of targeted Skp2 inhibitors would have a great impact on anticancer therapy, including CRC.

Conclusions

Skp2 overexpression is a very important prognostic factor in CRC. It was the only protein studied that correlated with the other well-known prognostic factors in CRC. Its routine testing can bring important prognostic information, especially in locally advanced stages. Moreover, the discovery of targeted anti-Skp2 therapy can bring promising results in CRC patients overexpressing Skp2. Also, the expression of Ki67 does not bring any novel prognostic information regarding CRC.

Conflict of interests

The authors declare no confict of interests.

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-E86.
2. Vlad C, Kubelac P, Vlad D, Irimie A, Achimas Cadariu P. Evaluation of clinical, morphopathological and therapeutic prognostic factors in rectal cancer. Experience of a tertiary oncology center. JBUON 2015;20:92-9.
3. Arnold D, Lueza B, Douillard JY et al. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomised trials. Ann Oncol 2017. Epub ahead of print, doi: 10.1093/annonc/mdx175.
4. 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;41:1033-49.
5. 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.
6. Derwinger K, Kodeda K, Gerjy R. Age aspects of demography, pathology and survival assessment in colorectal cancer. Anticancer Res 2010;30:5227-31.
7. Muresan M, Zaharie F, Bojan A et al. MicroRNAs in liver malignancies. Basic science applied in surgery. JBUON 2015;20:361-75.
8. Hershko DD. Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer. Cancer 2008;112:1415-24.
9. Vlad C, Kubelac P, Fetica B, Vlad D, Irimie A, Achimas-Cadariu P. The prognostic value of FOXP3+ T regulatory cells in colorectal cancer. JBUON 2015;20:114-9.
10. He W, Wang X, Chen L, Guan X. A Crosstalk Imbalance Between p27(Kip1) and Its Interacting Molecules Enhances Breast Carcinogenesis. Cancer Biother Radiopharmaceut 2012;27:399-402.
11. Timmerbeul I, Garrett-Engele CM, Kossatz U et al. Testing the importance of p27 degradation by the SCFskp2 oathwav in murine models of lung and colon cancer. Proc Natl Acad Sci USA 2006;103:14009-14.
12. 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;103:1336-46.
13. Hershko DD, Shapira M. Prognostic role of p27(Kip1) deregulation in colorectal cancer. Cancer 2006;107:668-75.
14. Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation. Semin Cancer Biol 2003;13:41-7.
15. Wang Z, Gao D, Fukushima H et al. Skp2: A novel potential therapeutic target for prostate cancer. Biochim Biophys Acta-Rev Cancer 2012;1825:11-7.
16. Frescas D, Pagano M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. Nat Rev Cancer 2008;8:438-49.
17. Yao Z-p, 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;363:673-86.
18. Chan C-H, Lee S-W, Wang J, Lin H-K. Regulation of Skp2 Expression and Activity and Its Role in Cancer Progression. Thescientificworldjournal 2010;10:1001-15.
19. Krishnan A, Nair SA, Pillai MR. Loss of cks1 homeostasis deregulates cell division cycle. J Cell Mol Med 2010;14:154-64.
20. Xu K, Belunis C, Chu W et al. Protein-protein interactions involved in the recognition of p27 by E3 ubiqui-

tin ligase. Biochem J 2003;371:957-64.

21. Braicu C, Pileczki V, Pop L et al. Dual targeted therapy with p53 siRNA and Epigallocatechingallate in a triple negative breast cancer cell model. PLoS One 2015;10(4):e0120936.
22. 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;32:2416-27.
23. Ungermannova D, Gao YF, Liu XD. Ubiquitination of p27(Kip1) requires physical interaction with cyclin E and probable phosphate recognition by SKP2. J Biol Chem 2005;280:30301-9.
24. Spruck C, Strohmaier H, Watson M et al. A CDK-independent function of mammalian Cks1: Targeting of SCFSkp2 to the CDK inhibitor p27(Kip1). Mol Cell 2001;7:639-50.
25. Ooi LC, Watanabe N, Futamura Y, Sulaiman SF, Darah I, Osada H. Identification of small molecule inhibitors of p27(Kip1) ubiquitination by high-throughput screening. Cancer Sci 2013;104:1461-7.
26. 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;3:1107-17.
27. Wang Z, Fukushima H, Inuzuka H et al. Skp2 is a promising therapeutic target in breast cancer. Front Oncol 2012;1:57.
28. 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;34:1425-31.
29. Nalepa G, Harper JW. Therapeutic anti-cancer targets upstream of the proteasome. Cancer Treat Rev 2003;29:49-57.
30. Uddin S, Ahmed M, Bavi P et al. Bortezornib (velcade) induces p27Kip1 expression through S-phase kinase protein 2 degradation in colorectal cancer. Cancer Res 2008;68:3379-88.
31. 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. Clin Cancer Res 2005;11:5526-33.
32. 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;107:2688-97.
33. Voutsadakis IA. The ubiquitin-proteasome system in colorectal cancer. Biochim Biophys Acta (BBA) - Molecular Basis of Disease 2008;1782:800-8.
34. Chung YK, Chi-Hung Or R, Lu CH, Ouyang WT, Yang SY, Chang CC. Sulforaphane down-regulates SKP2 to stabilize p27(KIP1) for inducing antiproliferation in human colon adenocarcinoma cells. J Biosci Bioeng 2015;119:35-42.
35. Chen H, Mo X, Yu J, Huang S, Huang Z, Gao L. Interference of Skp2 effectively inhibits the development and metastasis of colon carcinoma. Mol Med Rep 2014;10:1129-35.