The oncoprotein hepatitis B X-interacting protein promotes the migration of ovarian cancer cells through the upregulation of S-phase kinase-associated protein 2 by Sp1

FUQIANG XU¹,²,³*, XIAOMING ZHU¹,⁴*, TAO HAN⁵*, XIAONA YOU³, FABAO LIU⁶, LIHONG YE⁶, XIAODONG ZHANG³, XIAOHONG WANG⁴**, and YUANQING YAO¹,⁴**

¹Department of Gynecology and Obstetrics, General Hospital Chinese PLA, Beijing 100853; ²Department of Gynecology and Obstetrics, Beijing Shijingshan Hospital, Shijingshan Teaching Hospital of Capital Medical University, Beijing 100043; ³Department of Cancer Research, Key Laboratory of Molecular Microbiology and Technology of Ministry of Education, Institute for Molecular Biology, College of Life Sciences, Nankai University, Tianjin 300071; ⁴Department of Obstetrics and Gynecology, Tangdu Hospital, Fourth Military Medical University, Xi’an, Shaanxi 710038; ⁵Department of Orthopedics, Hainan Branch of PLA General Hospital, Sanya, Hainan 572013; ⁶Department of Biochemistry, State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, Tianjin 300071, P.R. China

Received September 21, 2013; Accepted November 18, 2013

DOI: 10.3892/ijo.2014.2411

Correspondence to: Dr Yuanqing Yao, Department of Gynecology and Obstetrics, The General Hospital of the Chinese People’s Liberation Army, No. 28 Fuxing Road, Beijing 100853, P.R. China
E-mail: yqyao@126.com

Dr Xiaohong Wang, Department of Obstetrics and Gynecology, Tangdu Hospital, Fourth Military Medical University, Xi’an, Shaanxi 710038, P.R. China
E-mail: wangxh-99919@163.com

*, **Contributed equally

Key words: hepatitis B X-interacting protein, S-phase kinase-associated protein 2, Sp1, cell migration, ovarian cancer

Abstract. Hepatitis B X-interacting protein (HBXIP) is a novel oncoprotein. We have previously reported that HBXIP promotes the proliferation and migration of breast cancer cells. S-phase kinase-associated protein 2 (Skp2) is another oncoprotein which is important for migration. In this study, we investigated whether Skp2 is involved in the migration enhanced by HBXIP in ovarian cancer. The expression of HBXIP and Skp2 in ovarian cancer tissues was examined by immunohistochemistry using tissue microarrays. The role of HBXIP and Skp2 in the migration of ovarian cancer cells was investigated by wound-healing assay and Transwell migration assay. The effect of HBXIP on Skp2 was assessed by reverse transcription polymerase chain reaction (RT-PCR), western blot analysis, luciferase reporter gene assays and chromatin immunoprecipitation in ovarian cancer cells (SKOV3 and CAOV3). We found that both HBXIP and Skp2 were highly expressed in ovarian cancer tissues. We observed that the overexpression of HBXIP enhanced the migration of ovarian cancer cells, while Skp2 siRNAs decreased the cell migration enhanced by HBXIP. The HBXIP siRNAs inhibited ovarian cancer cell migration and Skp2 rescued the migration inhibition induced by HBXIP siRNA. HBXIP could upregulate Skp2 at the levels of mRNA and protein in ovarian cancer cells. Moreover, HBXIP increased the activity of Skp2 promoter via binding to the transcription factor Sp1. HBXIP is highly expressed in ovarian cancer tissues. HBXIP enhances the migration of ovarian cancer cells. HBXIP was able to stimulate the activity of Skp2 promoter via transcription factor Sp1 thus promoting the migration of ovarian cancer cells.

Introduction

Mammalian hepatitis B X-interacting protein (HBXIP) is a conserved 18 kDa protein, which was originally identified because of its interaction with the C-terminus of hepatitis B virus X protein (HBx) (1,2). HBXIP sequences are well conserved among mammalian species, with close orthologues found in all vertebrate species where sequence data exist. HBXIP formed a complex with survivin, an anti-apoptotic protein that is overexpressed in most human cancers, resulting in the suppression of cell apoptosis through the mitochondrial/cytochrome pathway. HBXIP also regulates centrosome duplication, causing excessive centrosome production and multipolar mitotic spindles in HeLa cells (3,4). HBXIP is involved in mTORC1 pathway regulating cell growth (5). Our previous studies reported that HBXIP was able to promote cell proliferation and migration through S100A4 and IL-8 in breast cancer cells (6,7). However, the mechanism by which HBXIP enhances migration of ovarian cancer cells is poorly understood.

S-phase kinase-associated protein 2 (Skp2) belongs to the family of the F-box proteins. Skp2, which was originally
discovered by Zhang et al in 1995, because of its ability to interact with the cell cycle protein cyclin A, is necessary for DNA replication (8). The Skp2 protein levels changes during the cell cycle, which is low in early G1 phase, while it is high during G1/S transition (9). This alteration in the Skp2 protein level during cell cycle progression is partly due to a change in its gene expression and protein stability (10). A previous report showed that Skp2 overexpression in prostate cancer cells markedly promoted cancer cell growth and tumorigenesis in a xenograft tumor model (11). And other groups showed that Skp2 deficiency displayed a defect in cell migration and metastasis, while Skp2 overexpression promoted cell migration and invasion (11,12). Inuzuka et al have shown that acetylation of Skp2 enhanced cellular migration through ubiquitination and destruction of E-cadherin (13). Subsequent experiments revealed that Skp2 was involved in cell cycle progression. Cardozo and Pagano have shown that Skp2 plays an important role in governing cell cycle progression and cell survival by promoting the destruction of numerous tumor suppressor proteins, including p27, p21, p57, p130 and FOXO1 (14). Aberrant Skp2 signaling has been implicated as a driving event in tumorigenesis. Overexpression of Skp2 was frequently observed in numerous human cancers, such as ovarian, colorectal, gastric, prostate, lung, sarcoma, breast and other cancers (15-26). These observations suggest that Skp2 may contribute to the development of human cancers. Accumulated evidence suggests that Skp2 displays a proto-oncogenic role in vitro and in vivo. Previous report showed that rapamycin, an mTOR inhibitor, could downregulate the expression of Skp2 in breast cancer (27). Thus, we speculate Skp2 may play an important role in the function mediated by HBXIP.

In the present study, we investigated the role of HBXIP and Skp2 in migration of ovarian cancer cells, with the hope that such associations might provide insight into the causal mechanisms by which HBXIP enhances the migration of ovarian cancer cells.

Materials and methods

Immunohistochemistry. The ovarian carcinoma tissue microarrays were obtained from the Xi’an Aomei Biotechnology Co., Ltd. (Xi’an, China). These microarrays (catalog no. C1026) were composed of 80 ovarian carcinoma tissue samples (average age 39), which included duplicate core biopsies (1 mm in diameter) from fixed, paraffin-embedded tumors. Immunohistochemical staining of samples was performed as previously reported (28) and the primary antibody of rabbit anti-HBXIP (1:100, Proteintech Group, Chicago, IL, USA) or the primary antibody of rabbit anti-Skp2 (1:30, Boster Group, Wuhan, China) was used. Immunostained slides were evaluated under a microscope. Categorization of immunostaining intensity was performed by three independent observers. The staining levels of HBXIP and Skp2 were classified into three groups using a modified scoring method based on the intensity of staining (0, negative; 1, low; and 2, high) and the percentage of stained cells (0, 0% stained; 1, 1-49% stained; and 2, 50%-100% stained). A multiplied score (intensity score x percentage score) lower than 1 was considered to be a negative staining (-), 1 and 2 were considered to be moderate staining (+), and 4 was considered to be intense staining (+++).

Plasmid construction and small interference RNA (siRNA). pCMV-tag2B, pGL3-Basic vectors (Promega, Madison, WI, USA), pCMV-HBXIP was maintained in our laboratory (6). The 5′-flanking region (from -1309 to +235 nt) of Skp2 gene was inserted into the KpnI/Xhol site upstream of the luciferase gene in the pGL3-basic vector, termed pGL3-Skp2 promoter. Mutant construction of Skp2 promoter, termed as pGL3-Skp2 promoter mut, carried a series substitution of nucleotides within Sp1 binding site. The complete human Skp2 (GenBank accession no. NC 000005.9) gene was subcloned into pcDNA-tag2B vector to generate the pCMV-Skp2 construct. siRNA duplexes targeting human HBXIP (or Skp2) gene and siRNA duplexes with non-specific sequences using as negative control (NC) were synthesized by Ribobio (Guangzhou, China) (3,30). All primers and siRNA sequences are listed in Table I.

Transfection. One day before transfection, cells were harvested and seeded into 6- or 24-well plates. Cells were transfected with plasmid or siRNAs using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Wound-healing assay and Transwell migration assay. Cells transfected with plasmid of HBXIP, plasmid of HBXIP and siRNAs of Skp2, siRNAs of HBXIP, siRNAs of HBXIP and pCMV-Skp2 were seeded in a 6-well plate and cultured for 24 h to form confluent monolayers. A wound was created by dragging a pipette tip through the monolayer, and plates were washed using pre-warmed PBS to remove cellular debris. Wound images were photographed at 0, 24, 48 and 72 h after wounding. The wound gaps were measured at each time point. For Transwell migration assay using SKOV3 and CAOV3 cells with indicated treatment, 5x10⁵ cells were plated on 8 μm Transwell filters (Corning Incorporated, Corning, NY, USA). The cells were induced to migrate towards medium containing 10% FBS for 20 h. Non-migrating cells were removed with a cotton swab. The remaining cells were stained, stained with hematoxylin and eosin, and analysed by a bright-field microscope.

RNA extraction and reverse-transcription polymerase chain reaction (RT-PCR). Total RNA of cells was extracted using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized by PrimeScript reverse transcriptase (Takara Bio, Dalian, China) and oligo (dT) following the manufacturer's instructions. The primers are listed in Table I.

Western blot analysis. Western blot analysis was carried out with standard protocols. Primary antibodies used were rabbit anti-Skp2 (1:300, Boster Group), rabbit anti-HBXIP (1:1,000, Proteintech Group), and mouse anti-β-actin (1:800, Sigma-Aldrich, St. Louis, MO, USA). All experiments were repeated 3 times.
Luciferase reporter gene assays. For luciferase reporter gene assays, the ovarian cancer cells were transfected with plasmids encoding HBXIP by Lipofectamine 2000. The luciferase activities were determined 48 h after transfection, and the results are the average of 3 independent repeats. The luciferase activities in the cell lysates were measured by a dual luciferase reporter assay kit (Promega), and the luciferase activity was normalized with renilla luciferase activity.

Chromatin immunoprecipitation (ChIP) and ReChIP assay. The ChIP assay was performed using the EpiQuik™ chromatin immunoprecipitation kit from Epigentek Group Inc (Farmingdale, NY, USA) according to the published methods (6,31). Protein-DNA complexes were immunoprecipitated with HBXIP antibodies, whereas rabbit preimmune serum served as a control. DNA from input or immunoprecipitated samples was assayed using SYBR-Green-based quantitative PCR with specific primers designed to amplify the Skp2 promoter around the SREs. ChIP/ReChIP: ChIP was performed as above, binding complexes from the first immunoprecipitation were eluted from the sepharose beads using Re-ChIP buffer. The eluted protein-DNA complexes were diluted in radioimmunoprecipitation buffer and resubjected to ChIP using a different antibody.

Statistical analysis. Each experiment was repeated at least three times. Statistical significance was assessed by comparing mean values (± SD) using a Student's t-test for independent groups or pairing $\chi^2$ for dependent groups and was assumed for *P<0.05, **P<0.01 and ***P<0.001.

Results

The expression of HBXIP is positively associated with that of Skp2 in clinical ovarian cancer tissues. Our previous reports showed that HBXIP was overexpressed in breast cancer and other cancer cells. However, there is no report concerning the expression of HBXIP in ovarian cancer. In this study, we examined the expression of HBXIP in ovarian cancer. The data revealed that HBXIP was overexpressed in ovarian cancer tissue (Fig. 1). It has been reported that Skp2 is also overexpressed in ovarian cancer tissues (23). Thus, we supposed that overexpression of Skp2 might be correlated with enhanced HBXIP in ovarian cancer. Then, we investigated the expression correlation between HBXIP and Skp2 by IHC using tissue microarrays from the same tissue paraffin block. Our data showed that the positive rate of HBXIP was 75% (60/80) in clinical ovarian cancer tissue samples, and the positive rate of Skp2 was 81.67% (49/60) in the HBXIP-positive specimens (Fig. 1). Pairing $\chi^2$ analysis showed that there was no significant difference between the positive rate of HBXIP and that of Skp2 in the tissues (P>0.05, Table II), suggesting that the expression of Skp2 is relevant to that of HBXIP in ovarian cancer tissues. Additionally, in this study, IHC staining showed that the expression of HBXIP could be observed in both cytoplasm and nucleus in ovarian cancer tissues (Fig. 1B). We also observed that Skp2 was expressed in both cytoplasm and nucleus in ovarian cancer tissues (Fig. 1C), which is consistent with a previous study (32). Thus, we speculated that HBXIP might be involved in the transcriptional regulation of Skp2.

Table I. The primers and sequences used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers for Skp2 promoter</td>
<td>Forward</td>
<td>CGGGGTACCCCGTCCCTTTCATAACAAATCTC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CCGCTCGAGCGCCGTTTACCTGTGCATAGCG</td>
</tr>
<tr>
<td>Primers for qRT-PCR</td>
<td>Forward</td>
<td>CTTTCTGGGTGTCTTGATTTCCTCT</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGGGAATTCTGTATTTGGAGGG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>CATCACACTTCCAGAGAGCC</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGACCTTTGCCCCACAGCTTGG</td>
</tr>
<tr>
<td>Primers for ChIP</td>
<td>Forward</td>
<td>GCGGGACGGAAAATCAA</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGCAATTAAGCTGAGAGCTGC</td>
</tr>
<tr>
<td>HBXIP siRNA</td>
<td>Sense</td>
<td>CGGAACGCGAUGAUGUUUUTdT</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>AAAACUACUCCCCGGUUCCGTdT</td>
</tr>
<tr>
<td>Skp2 siRNA</td>
<td>Sense</td>
<td>GCCAGGGAGTGACAAAAdT</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>TTTTGCACTCCTTGTGCdT</td>
</tr>
<tr>
<td>Control siRNA</td>
<td>Sense</td>
<td>UUCUCCGAGACUGUCGUACGAdT</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>ACGUGACAGCUUGCGAGAAAdT</td>
</tr>
</tbody>
</table>

Luciferase reporter gene assays. For luciferase reporter gene assays, the ovarian cancer cells were transfected with plasmids encoding HBXIP by Lipofectamine 2000. The luciferase activities were determined 48 h after transfection, and the results are the average of 3 independent repeats. The luciferase activities in the cell lysates were measured by a dual luciferase reporter assay kit (Promega), and the luciferase activity was normalized with renilla luciferase activity.

Chromatin immunoprecipitation (ChIP) and ReChIP assay. The ChIP assay was performed using the EpiQuik™ chromatin immunoprecipitation kit from Epigentek Group Inc (Farmingdale, NY, USA) according to the published methods (6,31). Protein-DNA complexes were immunoprecipitated with HBXIP antibodies, whereas rabbit preimmune serum served as a control. DNA from input or immunoprecipitated samples was assayed using SYBR-Green-based quantitative PCR with specific primers designed to amplify the Skp2 promoter around the SREs. ChIP/ReChIP: ChIP was performed as above, binding complexes from the first immunoprecipitation were eluted from the sepharose beads using Re-ChIP buffer. The eluted protein-DNA complexes were diluted in radioimmunoprecipitation buffer and resubjected to ChIP using a different antibody.

Statistical analysis. Each experiment was repeated at least three times. Statistical significance was assessed by comparing mean values (± SD) using a Student's t-test for independent groups or pairing $\chi^2$ for dependent groups and was assumed for *P<0.05, **P<0.01 and ***P<0.001.
Figure 1. The expression levels of HBXIP are positively associated with those of Skp2 in clinical ovarian cancer tissues. The expression of HBXIP and Skp2 was examined by immunohistochemical staining in ovarian cancer tissues using tissue microarrays. (A) negative control; (B) ovarian cancer tissues with HBXIP-positive staining; (C) ovarian cancer tissues with Skp2-positive staining.

Figure 2. Skp2 is responsible for HBXIP-enhanced migration of ovarian cancer cells in vitro. (A-D) SKOV3 (or CAOV3) cells were transfected with either pCMV, pCMV-HBXIP, or pCMV-HBXIP and si-Skp2. The cell migration was determined by wound healing and transwell assays. The images are representative of at least three independent experiments. Statistically significant differences are indicated: *P<0.05, **P<0.01, ***P<0.001, Student’s t-test.
Skp2 is responsible for HBXIP-enhanced migration of ovarian cancer cells in vitro. Cell migration is an essential process in cancer metastasis and HBXIP can promote cell migration in breast cancer cells (6,7). Other reports showed that Skp2 can modulate cell migration of breast cancer, prostate cancer and myxofibrosarcoma (18,21,22,24). Thus, we supposed that Skp2 might be involved in the migration enhanced by HBXIP. Wound-healing and Transwell assays showed that treatment with plasmid encoding HBXIP enhanced the migration of SKOV3 cells, while additionally treated with Skp2 siRNAs abolished the effect (Fig. 2A and B).

Furthermore, we performed the same assay using another ovarian cancer cell line CAOV3, and obtained similar results (Fig. 2C and D). We found that the inhibited migration of SKOV3 ovarian cancer cells induced by HBXIP siRNAs, was rescued by the overexpression of Skp2 (Fig. 3A and B). Similar results occurred in CAOV3 ovarian cancer cell line (Fig. 3C and D). Furthermore, as shown in Fig. 4, the RNA interference of HBXIP (or Skp2) decreased the expression of protein levels in SKOV3 and CAOV3 cells. Thus, our data suggest that HBXIP promotes the migration of ovarian cancer cells through Skp2 in vitro.
HBXIP upregulates the expression of Skp2 in ovarian cancer cells. Next, we evaluated whether HBXIP was able to upregulate Skp2 in ovarian cancer cell lines. After transfection with plasmid encoding HBXIP, we observed that the levels of mRNA and protein of Skp2 were upregulated by HBXIP in SKOV3 and CAOV3 cell lines in a dose-dependent manner (Fig. 5). Thus, we verified that HBXIP was able to upregulate Skp2 in ovarian cancer cells.

**Discussion**

Our studies have showed that HBXIP is a novel oncoprotein. HBXIP was highly expressed in breast cancer tissues and metastatic lymph node tissues and significantly associated with the growth and metastasis of breast cancer cells (6,7,33). However, the expression and role of HBXIP in ovarian cancer cells is poorly understood. Many studies have shown that overexpression of Skp2 is observed in a variety of human cancers, including ovarian cancer, gastric cancer, colorectal cancer, prostate cancer, sarcoma, breast cancer, lung cancer, pancreatic cancer and other cancers (15,26). In addition, Skp2 has an estab-
Established role in the migration of cancer cells. Therefore, we are interested in the effect of HBXIP on cell migration in ovarian cancer and the role Skp2 plays in the signaling pathway.

Latest study showed that HBXIP was a regulator component that is required for mTORC1 activation by amino acids (5). Cross-talk between mTOR pathway and Skp2 pathway has been reported recently. Shapira et al showed that repamin, an mTOR inhibitor, could downregulate the expression of Skp2 in breast cancer (27). Shigemasa et al proved that Skp2 was expressed in nearly half of the 91 ovarian adenocarcinomas (23). In this study, we first observed that HBXIP is overexpressed in ovarian cancer tissues. We noted that the expression of HBXIP is significantly correlated with Skp2 expression in ovarian cancer tissues. Skp2 overexpression has been correlated with tumor progression such as stage and survival in ovarian cancer and other human cancers (16), indicating that Skp2 may be important in

**Figure 6.** HBXIP activates Skp2 promoter via transcription factor Sp1. (A and B) SKOV3 (or CAOV3) cells were co-transfected with renilla luciferase plasmid containing Skp2 promoter and either pCMV or pCMV-HBXIP. Luciferase activity was determined 48 h after transfection. Statistically significant differences are indicated: *P<0.05, **P<0.01, ***P<0.001, Student's t-test. (C) The interaction between HBXIP and promoter region of Skp2 was examined by ChIP assay. (D) The binding site region in Skp2 promoter. (E) ChIP/ReChIP analysis of HBXIP and Sp1 binding to the Skp2 promoter. (F and G) The promoter activities of Skp2 mediated by HBXIP were measured by luciferase reporter gene assay when the Sp1 binding site was mutated in SKOV3 (or CAOV3) cells. Statistically significant differences are indicated: *P<0.05, Student's t-test.
cancer cell migration, invasion and metastasis. Previous reports showed that Skp2 deficiency displayed a defect in cell migration and metastasis, while Skp2 overexpression promoted cell migration and invasion (11,12). It has also been shown that acetylation of Skp2 enhanced cellular migration (13). Consistent with this notion, we found that Skp2 is responsible for the enhanced migration of ovarian cancer cells mediated by HBXIP.

We observed that HBXIP was able to upregulate the mRNA and protein levels of Skp2 in ovarian cancer cells. Next, we sought to elucidate the underlying mechanism by which HBXIP upregulates Skp2. We previously observed the nuclear localization of HBXIP in MCF-7 cells (6), we found a similar phenomenon in ovarian cancer tissues, implying that HBXIP may be involved in the transcriptional regulation of Skp2. Then, we predicted the putative transcription factor binding sites in the -640/-443 promoter region of Skp2. Strikingly, we found a Sp1 binding site in the region. Sp1 is a transcription factor that either enhance or repress the activity of promoters of genes involved in differentiation, cell cycle progression and oncogenesis (34). In comparison to normal tissues or cells, Sp1 level is greater in breast carcinomas, thyroid cancer, hepatocellular carcinomas, pancreatic cancer, colorectal cancer, gastric cancer and lung cancer (34-37). Sp1 is also overexpressed in ovarian cancer (38) and plays an important role in the process of cancer. We found that HBXIP was able to bind to the Skp2 promoter region through interacting with Sp1 by ChIP/ReChIP assays. We further demonstrated that HBXIP activated Skp2 promoter through the transcription factor Sp1. Thus, we report that the transcription factor Sp1 plays a role in regulating Skp2 mediated by HBXIP in ovarian cancer cells.

In summary, our finding indicates that HBXIP promotes the migration of ovarian cancer cells through upregulating Skp2, in which HBXIP activates the transcription of Skp2 through interaction with transcription factor Sp1. HBXIP may act as a co-activator of transcription factors to upregulate many genes in the development of cancer. Therefore, our finding provides new insight into the mechanism of HBXIP in promotion of migration of ovarian cancer cells.

Acknowledgements

This study was supported by grants from the National Basic Research Program of China (973 Program, nos. 2011CB512113 and 2009CB521702) and National Natural Science Foundation of China (nos. 8017623, 8017624 and 81272217).

References

1. Melegrari M, Scaglioni PP and Wands JR: Cloning and chara-
acterization of a novel hepatitis B virus x binding protein that 
2. Lok AS: Hepatitis B infection: pathogenesis and management.
Zhang W, Zhang X and Ye L: miR-520b regulates 
and p45Skp2 are essential elements of the cyclin A-CDK2 S 
7. Kurland JF and Tansey WP: Crashing waves of destruction: 
the cell cycle and APc(Cdh1) regulation of SCF(Skp2). Cancer 

XU et al: HBXIP PROMOTES OVARIAN CANCER CELL MIGRATION THROUGH UPREGRULATING SKP2 BY SPI

262

9. Kurland JF and Tansey WP: Crashing waves of destruction: 
the cell cycle and APc(Cdh1) regulation of SCF(Skp2). Cancer 
10. Bretones G, Acosta JC, Caraballo JM, Ferrándiz N, 
Gómez-Casares MT, Albajar M, Blanco R, Ruiz P, Hung WC, 
Albero MP, Perez-Roger I and León J: SKP2 oncogene is a 
direct MYC target gene and MYC down-regulates p27(Kip1) 
through SKP2 in human leukemia cells. J Biol Chem 286: 
9815-9825, 2011.
Yang WL, Erdjument-Bromage H, Nakayama KI, Nimer S, 
Tempst P and Pandolfi PP: Phosphorylation-dependent regulation 
of cytosolic localization and oncogenic function of Skp2 by 
Nakayama KI, Kang HY, Huang HY, Hung MC, Pandolfi PP 
and Lin HK: Deciphering the transcriptional complex critical for 
13. Inuzuka H, Gao D, Finley LW, Yang W, Wang L, Fukushima H, 
Nakayama K, Teruya-Feldstein J, Toker A, Haigis MC, 
Pandolfi PP and Wei W: Acetylation-dependent regulation of 
15. Bretones G, Acosta JC, Caraballo JM, Ferrández N, 
Gómez-Casares MT, Albajar M, Blanco R, Ruiz P, Hung WC, 
Albero MP, Perez-Roger I and León J: SKP2 oncogene is a 
direct MYC target gene and MYC down-regulates p27(Kip1) 
through SKP2 in human leukemia cells. J Biol Chem 286: 
9815-9825, 2011.
16. Einama T, Katagata Y, Tsuda H, Morita D, Ogata S, Ueda S, 
Takigawa T, Kawarabayashi N, Fukusuki T, Sugiyura I, 
Matsubara O and Hatusue K: High-level Skp2 expression in 
pancreatic ductal adenocarcinoma: correlation with the extent of 
lymph node metastasis, higher histological grade, and poorer 
17. Hung WC, Tseng WL, Shiea J and Chang HC: Skp2 overex-
pression increases the expression of MMP-2 and MMP-9 
Wang YH, Wu WR, Li SH, Yu SC, Li CT, Liu J, Shie YL, 
Wu LC and Huang HY: Characterization of gene amplification-
driven SKP2 overexpression in myxofibrosarcoma: potential 
implications in tumor progression and therapeutics. Clin 
19. Lim MS, Adamson A, Lin Z, Perez-Ordonez B, Jordan RC, 
Tripp S, Perkins SL and Elenitoba-Johnson KS: Expression of 
Skp2, a p27(Kip1) ubiquitin ligase, in malignant lymphoma: 
decline of Skp2 expression in primary breast cancer. Mol Cancer 


