RESEARCH ARTICLE

SKP2/P27Kip1 pathway is associated with Advanced Ovarian Cancer in Saudi Patients

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Abstract

Background: Ovarian cancer is the most common gynecological malignancy and constitutes the fifth leading cause of female cancer death. Some biological parameters have prognostic roles in patients with advanced ovarian cancer and their expression may contribute to tumor progression. The aim of this study was to investigate the potential prognostic value of SKP2, genes P27Kip1, K-ras, c-Myc, COX2 and HER2 genes expression in ovarian cancer. Materials and Methods: This study was performed on two hundred formalin fixed paraffin embedded ovarian cancer and normal adjacent tissues (NAT). Gene expression levels were assessed using real time PCR and Western blotting. Results: Elevated expression levels of SKP2, K-ras, c-Myc, HER2 and COX2 genes were observed in 61.5% (123/200), 92.5% (185/200), 74% (148/200), 96 % (192/200), 90% (180/200) and 78.5% (157/200) of cancer tissues, respectively. High expression of SKP2 and down-regulation of P27 was associated with advanced stages of cancer. Conclusions: The association between high expression of c-Myc and SKP2 with low expression of P27 suggested that the Skp2-P27 pathway may play an important role in ovarian carcinogenesis. Reduced expression of P27 is associated with advanced stage of cancer and can be used as a biological marker in clinical routine assessment and management of women with advanced ovarian cancer.

Keywords: Ovarian cancer - gene expression - protein expression - Skp2-P27 pathway

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Introduction

Ovarian cancer is the fifth leading cause of cancer death worldwide. It is one of the worst prognoses among gynecologic malignancies with ~90% of cancers arise are carcinomas (Ozols, 1992; Auersperg et al., 2001; Poorolajal et al., 2014). Most patients with ovarian cancer are diagnosed at advanced stage with standard therapy limitation (Auersperg et al., 2001). In Saudi Arabia, the ovarian cancer ranks the seventh among females and accounts for 3.1% of newly diagnosed cases with median age of 50 years (Haya S. Al-Eid, 2007). Multiple alterations in genes product involved in cell cycle control-related are supposed to be concerned in ovarian cancer pathogenesis. Therefore it is important to clarify the cell cycle control mechanisms involved in the development and progression of ovarian cancer (Shigemasa et al., 2003). Cyclin dependent kinase inhibitors (CDK) regulate cellular proliferation through the regulation of cyclin/cyclin dependent kinase (Cdk) complexes (Yang and Sun, 2015). These inhibitors are classified into two families: p21 ($p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$) and p16INK4a (p15, p16, p18, and p19). $p27^{Kip1}$ inhibits most cyclin/Cdk complexes, in particular cyclin E/Cdk2 and its expression causes cell cycle arrest in G1 phase, while its loss increase rate of cell proliferation (Hulit et al., 2006). The mechanisms by which p27Kip1 regulates tumorigenesis are less well understood. In the majority of studies, reduced p27Kip1 levels in tumors, correlates with poor prognosis, tumor aggressiveness and dedifferentiation although elevated levels are reported in a subset of tumors (Catzavelos et al., 1997; Fredersdorf et al., 1997). The p27Kip1degradation caused by SCF-type ubiquitin ligase complex that contains S-phase kinase protein 2 (Skp2) as the specific substrate-recognizing subunit (Carrano et al., 1999; Sutterluty et al., 1999). Skp2 is a member of the F-box protein family that helps in DNA replication (Hung et al., 2010). Its expression associated with cell cycle regulation and is accumulated during S-phase of the cell cycle. The aberrant expression of Skp2 during cell cycle can promote S-phase entry associated with loss of p27 (Frontini et al., 2012). Thus, the overexpression of Skp2 may play an important role in the pathogenesis of ovarian cancer as well, and assessed the significance of its expression with $p27^{Kip1}$ as a predictive marker for the prognosis of primary cases of this disease. Patients with tumors having low or undetectable levels of $p27^{Kip1}$ protein have a very poor outcome (Slingerland and Pagano, 2000).

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c-Myc is an oncogenic transcription factor that regulate cell growth, metabolism and division, as well as differentiation, cell migration, and apoptosis (Henriksson and Luscher, 1996). MYC genes are overexpressed in ~70% of all rapidly dividing tumors, at levels sufficient to drive primary quiescent cells into S phase (Cavalieri and Goldfarb, 1987). MYC genes expression accelerates rates of cell proliferation through the ability to down-regulate the expression of Cdk inhibitor $P27^{Kip1}$via increasing the expression levels of the Skp2 F-box component, CUL1 and CKS1 both components of the SCFSKP2 complex (Coppola and Cole, 1986; Roussel et al., 1991), which inactivates cyclin E-Cdk2 and cyclin A-Cdk2 complexes that are coordinate entry and progression through S phase. Therefore SKP2 is a direct MYC target gene and that MYC-mediated SKP2 up-regulation contributes to p27 degradation (Zhao et al., 2013).

The Ras gene family consists of Kirsten- (K), Harvey- (H), and N-ras, and these genes play a central part in the control of cellular growth, production, and differentiation by the transduction of extracellular growth signals (Makrodouli et al., 2011; Pan, 2012). Mutations in the K-ras gene, particularly in codons 12, 13, and 61 produce active forms of K-ras protein, which continuously transmit growth signals even in the absence of extracellular growth stimuli(Mannan and Hahn-Stromberg, 2012). Prolonged activation of RAS can also occur through mechanisms other than mutations in RAS. For example, reduced expression of let-7 microRNAs, which suppresses RAS by targeting the 3'untranslated region of RAS mRNAs, is often associated with a higher RAS protein level in tumors (Johnson et al., 2005). Expression in k-ras gene can reduce p27 by three pathways: MAPK in G1, PI3K in G1 and S, and via increased SKP2 in S and G2 phases(Wang et al., 2013).

The human epidermal growth factor receptor (HER) family consists of EGFR, HER2 (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4). It is responsible for cell proliferation and survival via the activation of the RAS/RAF/ERK and PI3K/PTEN/AKT pathways(Steelman et al., 2011). Several studies suggested that the elevated expression levels of EGFR gene is related to the response to anti-EGFR agents, whereas the resistance to anti-EGFR MoAbs associated with mutations in the *KRAS, NRAS, BRAF* or *PIK3CA* genes or loss of PTEN protein expression (Moroni et al., 2005; Frattini et al., 2007).

Cyclooxygenase 2 (COX2) is a key enzyme in prostaglandin biosynthesis. It is expressed in a limited number of cell types but is inducible by tumor promoters, pro-inflammatory cytokines, and growth factors. Overexpression COX2 gene has been reported in many malignancies, and deregulated in epithelial tumors (Ali-Fehmi et al., 2005). There are a link between HER2 and COX2 showing a HER2/COX2 regulated axis. Previous study reported that constitutive up-regulation of the COX2 pathway in different type of cancer by paracrine/autocrine activation of HER2/HER3 heterodimers (Vadlamudi et al., 1999). In ovarian cancer, there is an association between HER2 amplification and elevated COX2 expression in serous ovarian carcinomas was observed by Erkinheimo et al. (2004), on the other hand Ferrandina et al could not find an association between HER2 and COX2 immunohistochemical expression (Ferrandina et al., 2002). In epithelial ovarian cancer, COX2 expression has been associated with worse prognosis(Khalifeh et al., 2004; Ali-Fehmi et al., 2005)and less response to platin based chemotherapy(Brosens et al., 2005), but the mechanisms by which COX2 may mediate ovarian cancer progression are not understood.

The aim of the present study was to determine the potential role of Skp2/P27 pathway in ovarian cancer via investigating p27, Skp2, COX2 and HER2 differential expression and their association with clinico-pathologic parameters in ovarian cancer, also investigating the potential prognostic significance of these genes in ovarian cancer.

Materials and Methods

This study was conducted in compliance with Helsinki Declaration 2013 and was approved by the ethical committee of the college of medicine, King Saud University. Two hundred formalin-fixed paraffin-embedded (FFPE) ovarian cancer and their normal adjacent tissues (NAT) were included in this study and were collected from the pathology department, college of medicine, King Saud University and the Pathology Department, Riyadh Regional Laboratory and blood bank, King Saud Medical City, Ministry of Health.

RNA extraction and cDNA Synthesis

Total RNA were extracted from 200 FFPE blocks using Recover All total Nucleic Acid Isolation Kit (Ambion cat #: AM1975). The extraction procedure was performed in triplicate according to the manufacturer instructions. The quantity and integrity of the extracted RNA was characterized using a UV spectrophotometer (NanoDrop 8000, thermo, CA, USA). RNA was electrophoresed on ethidium bromide stained agarose gel. The isolated RNA has 260/280 ratio of 1.9-2.1.First-strand cDNA was synthesized from 1μg total RNA by reverse transcription with a SuperScript™ first-strand synthesis system kit (Invitrogen, CA, USA), according to the manufacturer's instructions.

Gene expression of K-ras, HER2, SKP2, P27, c-Myc and COX2 using real time PCR

The genes expression levels were measured using qPCR Master with ROX (Applied biosystem, life science, CA, USA) using $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene has been used as the internal control. The primers and probes used in this study have been purchased from applied biosystemHs00364284_g1, Hs01076078_ml, Hs01021864_m1, Hs01597588_m1, Hs00153408_m1, Hs00377726_m1and Hs99999905_m1 (applied biosystem, life technology,CA, USA). Target genes were amplified in ABI 96-well optical reaction plate (Applied biosystem, life science, USA). No template control (NTC) was used as a negative control. Experiments were performed in triplets for all data points. The cycling conditions are 95 °C for 10 min followed by 40 cycles of 94 °C for 30 s, annealing/extension at 60 °C for 1 min.

Western Blot Analysis of K-ras, HER2, SKP2, P27, c-Myc and COX2 Protein

For western blot analysis, protein extracts from ovarian and NAT tissues paraffin sections were prepared using ice cold cell lysis buffer supplemented with protease inhibitor cocktail (IBI SCIENTIFIC, Peosta, USA). Protein concentrations were measured using the Bradford assay (Bio-Rad, CA, USA) according to manufacturer's protocol. Proteins were separated on 7.5-12% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels and transferred to PVDF membrane. The membrane was blocked with 5% skimmed milk in TBS-T (10mM Tris-HCl, 150mM NaCl, 0.25% Tween 20, pH 7.5) at room temperature for 2 h followed by incubation with primary antibody for *K-ras (anti-mouse IgG-sc-30)*, P27 (anti-mouse IgG-sc-393380), HER2 (anti-mouse IgG-sc-373746), SKP2 (Rabbit polyclonal IgG-sc-7164), c-Myc (anti-mouse IgG-sc-373712), GAPDH (anti-mouse IgG-sc-365062) (Santa cruze, USA) and COX2 (anti-mouse polyclonal IgG-160106 Cayman) (Cayman Chemical Company, USA)) in TBS and 5% skimmed milk overnight at 4°C. After washing, the membrane was incubated with horseradish peroxidase (HRP) labeled secondary antibody (Santa cruze, USA) in TBS-T buffer for 2 h at room temperature, followed by three washes with TBS-T. The detection was performed using chemiluminescence assay (Amersham, GE Healthcare, life science, UK). Membranes were exposed to X-ray film to observe the bands (Kodak, Rochester, NY, USA). Protein bands were quantified using the Kodak Scientific ID software.

Statistical analysis

For statistical analysis, one way ANOVA test were used to analyze. Significance was defined as $P<0.05$. The SPSS version 22.0 program (SPSS, Chicago, Il, USA) was used for statistical analysis.

Results

The patients' mean age was 48 ± 11.9 years (range 23-85 years). The histological stages were determined according to the International Federation of Gynecology and Obstetrics system (FIGO). The initially diagnosed disease was FIGO stage I for 36 patients (18%), FIGO stage II for 67 (33.5%), FIGO stage III for 63(31.5 %) and FIGO stage IV for 34 patients (17 %). The pathological grade for the ovarian carcinoma was as follows: FIGO grade I: 42 cases (21%), FIGO grade II: 55 cases (27.5%) and FIGO grade III: 105 cases (52.5%).

There was no association between the expression levels of the studied genes and the age in group less or more than 40 years (Table 1).

The expression level of Skp2 in ovarian cancer

High expression levels of SKP2 were observed in ovarian cancer tissues compared to NAT. The Skp2 gene expression was significantly correlated with advanced histological stage. Approximately, seventy six and eighty eight percent of stage 3 and 4 showed high Skp2 expression levels compared to 16.7% and 58.2% of stage 1 and 2 (Table 1). Statistically significant mean expression levels was 3.0 and 3.9 in stage 3 and 4 respectively compared to 1.2 and 1.9 in stage 1 and 2 ($P=0.04$) (Table 2). As for the histological grade, Skp2 expression was higher in grade-1 and -2 with mean 3.0 and 2.5 fold expression respectively, than in grade IIIwith mean 2.3 fold expression ($P=0.05$) (Table 2). Skp2 protein expression was significantly correlated with advanced clinical stage (Figure 1). On the other hand, no significant correlation was found between

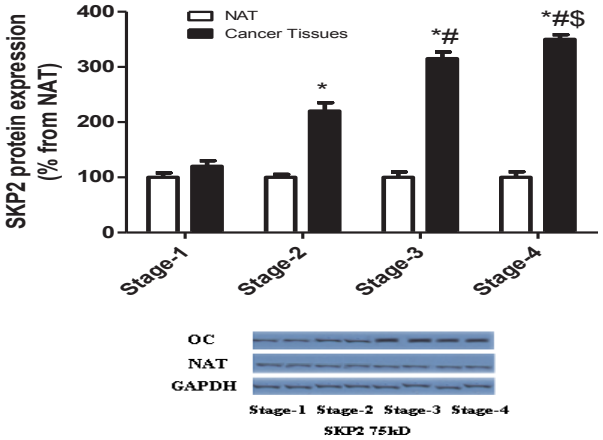

Figure 1. SKP2 Protein Expression Levels in Relation to Tumour Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT. *, # and $ indicate significant change from stage-2, -3 and -4, respectively, at $p<0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test

Table 1. Genes Expression Levels in Ovarian Carcinoma Tissue in Relation to Clinco-Pathological Data

Parameter	N=200	SKP2 (%)	P27 (%)	c-Myc (%)	K-ras (%)	HER2 (%)	COX2 (%)
Age (years)							
<40	55	31 (56.4%)	37 (67.3%)	53 (94.5%)	38 (69%)	49 (89.1%)	44 (81.5%)
>40	145	92 (63.4%)	93 (64.1%)	139 (95.9%)	110 (75.9%)	131 (90.3%)	113 (77.9%)
Histologic grade							
Grade-1	42	26 (61.9%)	25 (59.5%)	41 (97.6%)	28 (66.7%)	37 (88.1%)	30 (71.4%)
Grade-2	53	38 (71.7%)	33 (62.3%)	48 (90.6%)	41 (77.4%)	49 (92.5%)	45 (84.9%)
Grade-3	105	59 (56.2%)	72 (68.6%)	102 (97.1)	79 (75.2%)	94 (89.5%)	82 (78%)
Histologic Stage							
Stage-1	36	6 (16.7%)	15 (41.7%)	31 (86.1%)	27 (75%)	27 (75%)	23 (63.9%)
Stage-2	67	39 (58.2%)	28 (41.8%)	63 (94%)	51 (76%)	58 (86.6%)	44 (66.7%)
Stage-3	63	48 (76.2%)	56 (88.9%)	63 (100%)	47 (74.6%)	61 (96.8%)	57 (90.5%)
Stage-4	34	30 (88.2%)	31 (91.2%)	34 (100%)	23 (67.6%)	34 (100%)	33 (97.1%)

Skp2 protein expression levels and histological grade.

The expression level of P27 in ovarian cancer

P27 was down-regulated in 130/200 (65%) of ovarian cancer tissues in relation to NAT. The down-regulation of P27was significantly correlated with tumor stages (table 1). The down-regulation was observed in 41.7%with stage-1 and 41.8% with stage-2 compared to 88.9% in stage-3 and 91.2% in stage-4 of cancer patients. The P27 mean fold expression levels were 0.48 in stage-1 and 0.52 in stage-2 and were statistically significant compared to 0.67 and 0.68 in stage-3 and -4 respectively (P=0.04) (Table 2). In relation to the histological grade, P27 expression was more frequently observed in grade 3 (68.6%, with mean 0.62 fold expression) than in grade-2 (62.3%, with mean 0.7 fold expression) and -3 (59.5%with mean 0.56 fold expression), (P=0.08) (Table 2). In ovarian cancer tissues, there was no significant difference observed in the protein expression in relation to tumor stage or grade (Figure2).

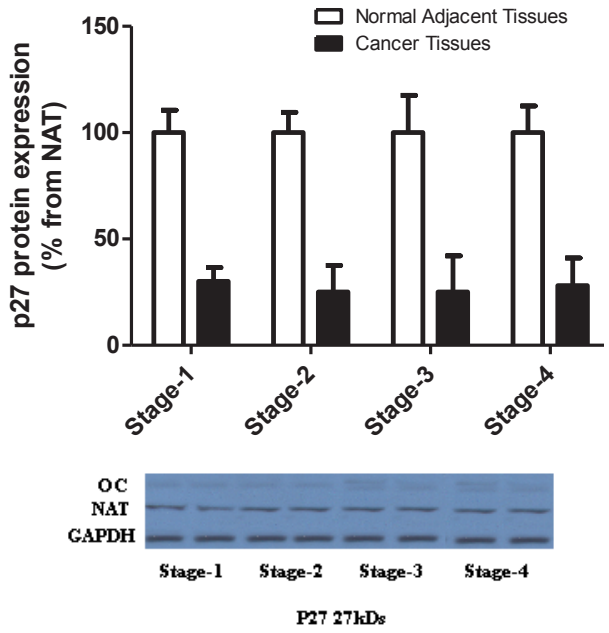

Figure 2. The P27 Protein Expression Levels in Relation to Tumor Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT

The expression level of c-Myc in ovarian cancer

The c-Myc expression levels were observed in 96% of cancer tissues compared to NAT. The c-Myc expression levels were increased with tumor stage increasing. The mean fold expression levels were 9.4 and 6.4 in advanced stage (stage-4 and -3) compared to 2.5 and 2.8 in stage-1 and -2, respectively (Table 2). Also a statistically significant difference in the c-Myc expression levels were observed in grade-2 and -3 compared to grade-1 (P=0.01). c-Myc protein expression levels increased with tumor stage increasing. In advanced stage high percentage of protein expression was observed in stage-3 (480%) and in stage-4 (450%) compared to stage-1 (380%)and stage-2 (320%) (Figure3). There is no significant difference observed in c-Myc protein expression in relation to tumor grade.

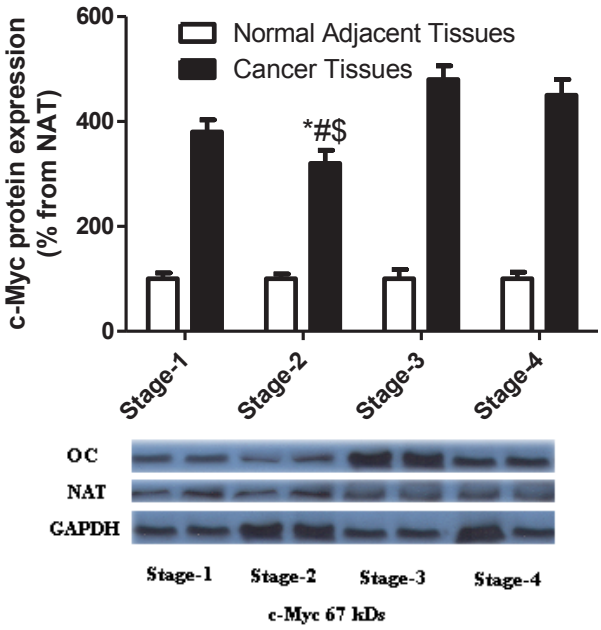

Figure 3. c-Myc Protein Expression Levels in Relation to Tumor Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT. *, # and $ indicate significant change from stage-1, -3 and -4, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test

Table 2. Genes Expression Levels in Ovarian Carcinoma Tissue in Relation to Clinco-Pathological Data

parameter	SKP2 Mean ±SE	P27 Mean ±SE	c-Myc Mean ±SE	K-ras Mean ±SE	HER2 Mean ±SE	COX2 Mean ±SE
Age (years)						
<40	2.3±0.19	0.54±0.06	5.0±0.42	3.1±0.35	3.9±0.38	3.9±0.38
>40	2.6±0.24	0.62±0.04	5.0±0.27	3.5±0.23	3.7±0.22	3.9±0.22
Histologic grade						
Grade-1	3.0±0.74	0.56±0.06	4.2±0.43	3.3±0.47	2.9±0.3	3.2±0.47
Grade-2	2.5±0.19	0.57±0.06	5.3±0.47	3.4±0.37	4.4±0.45+	4.4±0.45
Grade-3	2.3±0.16	0.62±0.05	5.3±0.31	3.3±0.26	3.7±0.25	3.9±0.26
Histologic Stage						
Stage-1	1.2±0.09	0.48±0.05	2.5±0.18	3.5±0.46	1.7±0.1	2.5±0.29
Stage-2	1.9±0.14	0.52±0.05	2.8±0.15	3.7±0.36	2.4±0.12	2.5±0.19
Stage-3	3.0±0.5*#	0.67±0.06	6.4±0.31*#	3.2±0.33	4.7±0.32*#	5.1±0.35*#
Stage-4	3.9±0.3*#	0.68±0.09	9.4±0.4*#$	2.8±0.44	6.6±0.54*#$	6.0±0.52*#

*, # and $ indicate significant changes from stage- 1, -2 and stage-3; + indicate significant difference from grade-1

The expression level of K-ras in ovarian cancer

High expression levels of K-ras gene was observed in tumor tissues compared to normal adjacent tissues. K-ras expression was more frequently observed in stage-1,-2 and -3 by 75%, 76% and 74.6% respectively compared to stage-4 by 67.6% (Table1). The mean fold expression levels were 3.2 and 2.8 in advanced stage (stage-3 and -4) compared to 3.5 and 3.7 in stage-1 and -2, respectively (Table 2). There was no significant correlation between expression levels of K-ras and histological grade (table2). The K-ras protein expression levels were correlated with the tumor stages, in which high expression levels was in stage-1 compared to advanced stages (stage-3 and -4). A high percentage (330%) in stage-1 compared to 260% and 240% in stage-3 and -4 respectively) and also compared to NAT was observed (Figure4).

The expression level of HER2 in ovarian cancer

There was high expression levels of HER2 compared to NAT. The expression levels were increased with tumor stage increasing. In advanced stage, the HER2 mean fold expression levels were 4.7 and 6.6 in stage-3 and -4, compared to 1.7 and 2.4 in stage-1 and -2 respectively (table2). There was an insignificant increase in the expression levels of HER2 in relation to tumor grade (table 2). There is no association between protein expression levels of HER2 and tumor grade. A significant protein expression level of HER2 was observed in stage-4 compared to stage-1 and -2 (Figure 5).

The expression level of COX2 in ovarian cancer

The COX2 gene expression was positively correlated with advanced histological stage. The mean fold expression level was 5.1 and 6.0 in stage-3 and -4, compared to 2.5 in both stage-1 and -2 respectively (table 2). As for the histological grade, COX2 fold expression levels were more frequently observed in grade-2 and -3 (4.4, 3.9 folds) than in grade-1 (3.2 fold) (P=0.05). High protein expression level of COX2 was observed in stage-3 (350%)and in stage-4 (380%) compared to stage-1 (220%)

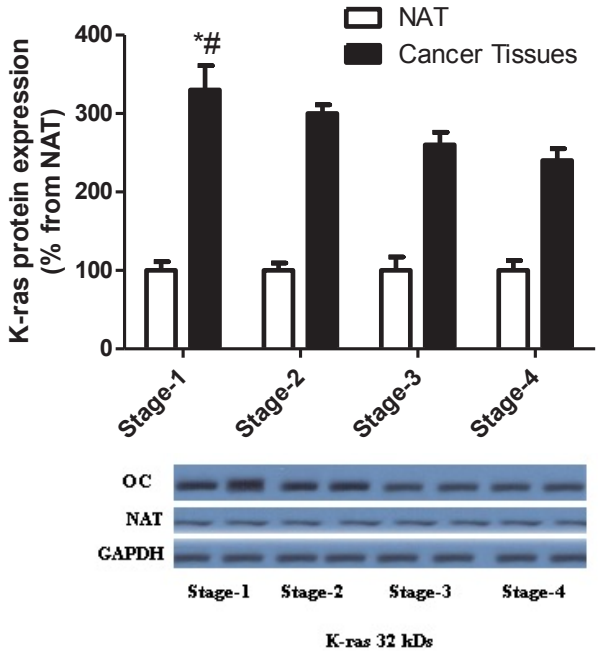

Figure 4. K-ras Protein Expression Levels in Relation to Tumor Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT. * and # indicate significant change from stage-3, and -4, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test

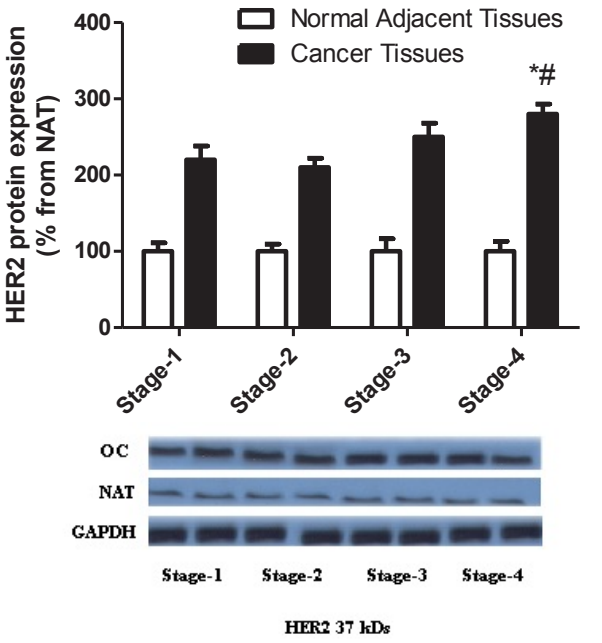

Figure 5. HER2 Protein Expression Levels in Relation to Tumor Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT. * and # indicate significant change from stage-1, and -2, respectively, at $p<0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test

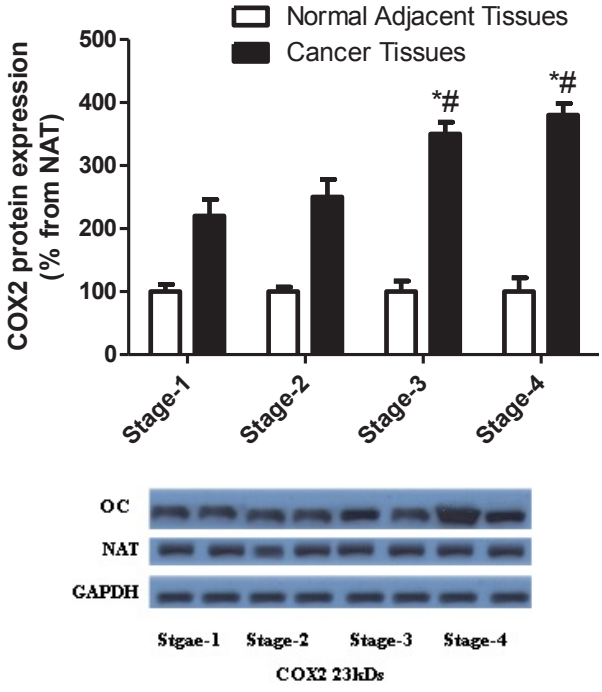

Figure 6. COX2 Protein Expression Levels in Relation to Tumor Stage in Cancer Tissues Compared to NAT. Data are presented as percentage from the NAT. * and # indicate significant change from stage-1, and -2, respectively, at $p < 0.05$ using ANOVA followed by Tukey-Kramer as a post ANOVA test

and in stage -2 (250%) (Figure 6). There was no significant difference observed in protein expression levels in relation to tumor grade.

Discussion

Ovarian cancer is one of the worst prognoses cancer among gynecologic malignancies worldwide, more than 90% of this cancers are carcinomas (Ozols, 1992; Auersperg et al., 2001). Molecular techniques are used to study molecular profiles of cancer which lead to better understand the biological background of the disease. Also, to find new molecular markers, therapeutic targets, and/or new classification approaches that will enable better treatment of patients.The expression of Skp2 is associated with cell cycle regulation and is accumulated during S-phase of the cell cycle. The aberrant expression of Skp2 during cell cycle can promote S-phase entry associated with loss of $p27^{Kip1}$.Previous studies suggest that Skp2 expression may play a significant role in the etiology and pathogenesis of a number of systemic malignancies and associated with decreased survival probability rates and a worse clinical outcome (Ben-Izhak et al., 2009; Wang et al., 2012). $p27^{Kip1}$, a key cell cycle inhibitor, is a cdk3 inhibitor that regulates progression from G1 into S-phase by inhibiting a variety of cyclin/cdk complexes, including cyclin D/cdk4, cyclin E/cdk2, and cyclin A/cdk2 (MacLachlan et al., 1995).

Overexpression of Skp2 has been reported in several malignant tumors, including hepatocellular carcinoma (Zhang et al., 2002), colorectal carcinoma (Li et al., 2004),oral squamous cell carcinoma (Shintani et al., 2003), and ovarian cancer (Shigemasa et al., 2003). In the present study high expression levels of SKP2 are associated with tumor stages. Also found a reversal relationship between elevated levels of c-Myc and Skp2 and low p27 expression levels. Similarly Lu et al, found significant association between Skp2 expression in ovarian carcinomas with tumor stage and lymph node metastasis (Lu et al., 2012). Other study suggested that Skp2 is a receptor for dihydrotestosterone that regulates p27 degradation in ovarian carcinomas (Shi et al., 2011). The high expression levels of SKP2 may be associated with high risk HPV infection that reported in our previous study. Similarly, previous study suggested that Skp2 expression may contribute to the development and progression of different human cancers. Other study reported that high expression of Skp2 with H-Ras had oncogenic potential in primary rodent fibroblasts cells (Gstaiger et al., 2001). Also, the activated Skp2 associated with activated N-Ras are responsible for the tumorigenesis of T-cell lymphoma in a transgenic mice model (Latres et al., 2001). Therefore, assessment of Skp2 overexpression could be used as clinical management of ovarian cancer patients, especially those with advanced clinical stage, that allow identifying of patients with poor prognoses.

Previous studies showed an inverse relationship between Skp2 and p27 expression levels in cancers (Gstaiger et al., 2001; Kudo et al., 2001). However the down-regulation of $p27^{Kip1}$ induced by Myc promotes cell cycleentry and accelerates the rates of cell proliferation (Baudino et al., 2003; Keller et al., 2007; He et al., 2014). Skp2 is a component of SCFSkp2 ubiquitin ligase complex that is regulated by Myc (O'Hagan et al., 2000). Myc directs the ubiquitin-mediated degradation of $p27^{Kip1}$ by increasing the expression of Skp2 (O'Hagan et al., 2000; Spruck et al., 2001).

There were several reports concerning the prognostic role of p27 expression in cancer. The suppression of p27 expression was associated with the risk of cancer recurrence or death (Catzavelos et al., 1997; Cote et al., 1998). Moreover, the association between loss of p27 expression and poor survival is significant in different cancer therapy and survival (Peter and Heintz, 1996). In the present study a significant suppression was observed in P27 levels in ovarian cancer tissue and this suppression was associated with tumor stages. Some study on ovarian cancer, found a prognostic significance of p27 in patients with ovarian cancer. An association was reported between p27 positivity and a better clinical outcome (Baekelandt et al., 1999). Newcomb et al. found neither relation between the negative prognostic roles of p27suppression with clinic-pathological features nor biological markers of cancer aggressiveness (Martino et al., 2014; Yang et al., 2014). The current data are in agreement with other studies (Valeria Masciullo, 2000; Akman et al., 2008), and in contrast with other (Baekelandt et al., 1999), which found no prognostic significance of p27 in patients with stage III ovarian cancer. The use of different detection methods, the cut-off value, and the selected stage of patients (stage III only) in that study may account for the different results from the present study. There was evidence of a second proteolytic pathway for controlling p27, one that is activated by mitogens and degrades p27 exclusively during G1 (Malek et al., 2001). More *in vitro* and *in vivo* studies are necessary to clarify whether alterations in p27 expression are responsible for the reduced response to chemotherapy in ovarian cancer cells.

HER2 gene over-expression is reported in a subset of ovarian cancer and is associated with poor clinical outcome (Felip et al., 1995; Hogdall et al., 2003; Verri et al., 2005). In the present study, high expression levels of HER2 mRNA and protein are found in tumor tissues and these expressions are associated with advanced stages. Previous study found that HER2 mRNA and protein were expressed in different ovarian cancer cell lines and in fresh tumor tissues (Lanitis et al., 2012; Missaoui et al., 2014). Farley and his colleague found that HER2, using fluorescence in situ hybridization (FISH) technique, has no predictive or prognostic value in advanced stage epithelial ovarian cancer treated (Farley et al., 2009). Also, other study on Asian metastatic ovarian cancer showed similar clinic-pathological features between HER2+ and HER2- patients (Chay et al., 2013). Similarly, Anglesio and his colleagues found high prevalence of HER2 in mucinous ovarian cancer cohort (Anglesio et al., 2013) . Some of these differences in the expression levels of HER2 among different studies may likely to be attributable to the diagnostic technique used to measure this expression. HER2 protein expression is commonly measured using western blotting or immunohistochemistry (IHC), whereas HER2 gene amplification is typically measured using

hybridization techniques, such as fluorescence in situ hybridization (FISH) (Wolff et al., 2007). HER2 mRNA expression levels can be detected using the quantitative PCR in archival samples (Muller et al., 2011). Therefore, the proportion of ovarian cancers overexpressing HER2 is a matter of debate.

COX2 is an important enzyme in prostaglandin biosynthesis. COX2 overexpression is associated with carcinogenesis and is linked to proliferation, neoangiogenesis, and inhibition of apoptosis (Ali-Fehmi et al., 2005). In the present study the high expression levels of COX2 was observed in 78.5% of tumor specimens. Similarly, previous studies found that high COX2 expression in 42-83% ovarian carcinoma (Seo et al., 2004; Steffensen et al., 2007). The overexpression of COX2 is associated with advanced stages. Also the expression level of COX-2 is correlated with HER2expression. Several data have established a link between HER2 and COX2 showing a HER2/COX2 regulated axis. The constitutive up-regulation of the COX2 pathway induced by activation of HER2/HER3 heterodimers in different types of cancer (Vadlamudi et al., 1999; Demir et al., 2014). In ovarian cancer, Erkinheimo et al. investigate the co-expression of HER2/COX2 and found an association between HER2 amplification and elevated COX2 expression in serous ovarian carcinomas (Erkinheimo et al., 2004), other not found an association between HER2 and COX2 expression (Ferrandina et al., 2002). The stimulation of COX2 transcription from HER2 complex forming at the COX2 promoter has proposed a link between the COX2 and the EGFR system (Wang et al., 2004; Demir et al., 2014).

The Skp2/P27 pathway has an important role and Skp2 overexpression may be a prognostic factor in patients with ovarian adenocarcinoma. High Skp2 may confirm the role of Skp2 in the development of ovarian carcinoma. The Skp2-p27 pathway may represent a molecular target for ovarian cancer prevention or treatment via antisense oligonucleotides or antibodies. Also the overexpression of HER2 suggests the potential for HER2 targeted treatment

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