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Detection of STAT2 in early stage of cervical premalignancy and in cervical cancer

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ABSTRACT

Objective: To measure the expression pattern of STAT2 in cervical cancer initiation and progression in tissue sections from patients with cervicitis, dysplasia, and cervical cancer. **Methods:** Antibody against human STAT2 was confirmed by plasmids transient transfection and Western blot. Immunohistochemistry was used to detect STAT2 expression in the cervical biopsies by using the confirmed antibody against STAT2 as the primary antibody. **Results:** It was found that the overall rate of positive STAT2 expression in the cervicitis, dysplasia and cervical cancer groups were 38.5%, 69.4% and 76.9%, respectively. The STAT2 levels are significantly increased in premalignant dysplasia and cervical cancer, as compared to cervicitis ($P< 0.05$). Noticeably, STAT2 signals were mainly found in the cytoplasm, implying that STAT2 was not biologically active. **Conclusions:** These findings reveal an association between cervical cancer progression and augmented STAT2 expression. In conclusion, STAT2 increase appears to be an early detectable cellular event in cervical cancer development.

1. Introduction

Uterine cervical cancer is the most common gynecological malignancy among women in China and the second most common cancer world-wide[1]. Diagnosis of in situ and early invasive cervical carcinoma is difficult due to lack of symptoms; as a result, 70% of patients diagnosed are already at advanced stages[1]. Standard therapeutic modalities for advanced cancer include surgical resection, chemotherapy and radiotherapy, but have limited value.

The biogenesis of cervical cancer is thought to be a continuous and complicated process starting from dysplasia to in situ cancer or to invasive cancer. Human papilloma virus (HPV) is a major risk factor for cervical cancer[1,2]. Despite this clear etiology, the molecular mechanism underlying cervical cancer development is still unclear.

Signal transducers and activators of transcription (STATs) proteins are transcription factors that regulate a variety of cellular events including differentiation, proliferation, cell survival and apoptosis[3,4]. Latent in the cytoplasm, STATs are activated and enter the nucleus upon cytokine, growth factor, and hormone stimulation[3,4]. Abnormal levels and pleiotropic effects of STATs contribute to tumor pathogenesis[5-9]. Constitutively activated STATs, especially STAT1, STAT3 and STAT5, have been found in a variety of human tumors and cancer cell lines, including blood malignancies and solid tumors[6-11]. STAT3 and STAT5 are considered as oncogenes, since they activate cyclin D1, c-Myc, and bcl-xl expression, which promotes cell-cycle progression, tumorigenic transformation, and concurrently inhibits apoptosis[6-11]. In contrast, STAT1 causes growth arrest, and promote apoptosis, and is therefore thought to be a potential tumor suppressor, even though its expression level may also increase in human neoplasias[12,13]. Therefore, the magnitude and specificity of STATs changes in various cancers underscores their potential values as molecular markers of tumor progression and their possible

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roles in carcinogenesis itself.

Compared to other STATs, STAT2 is an uncharacteristic member in the STAT family. It is unique to the biological response to interferon (IFN)[14,15]. Studies using STAT2-deficient cell lines and mice showed that STAT2 is necessary for the antiviral, apoptotic, and cell growth-inhibitory effects of type Ⅰ IFN[14,15]. Recently, studies with animal models have shown that STAT2-deficient mice have decreased tumor incidences to carcinogens, suggesting that STAT2 plays a positive role in tumorigenesis[16]. But the detailed mechanism is not clear. So far, no data are yet available on the expression status of STAT2 in cancer initiation and progression. In this study, we analyzed STAT2 expression by immunohistochemistry (IHC) staining in biopsies of human cervicitis, cervical intra-epithelial neoplasia (CIN) stages Ⅰ-Ⅲ, and cervical cancers in order to detect STAT2 expression pattern in cervical cancer development.

2. Materials and methods

2.1. Patients

Paraffin-embedded sections from a total of 176 cervical tissues, including 39 cervicitis, 72 CINs and 65 cervical invasive carcinomas from the Department of Pathology (Hunan Province Tumor Hospital, Hunan, China). The age ranged from 30 to 75 years old. Patients had received no cancer therapy before tissue collection. Informed consent was obtained from all patients. All diagnoses were made according to the published criteria of diagnostic histopathology of tumors. H&E-stained sections of cervical tissues were analyzed by a pathologist to confirm initial diagnosis, staging, and overall integrity of the tissue samples.

2.2. Cell culture, transfection, and Western blot

The plasmids, pCMV-Myc and pCMV-Myc-STAT2, were a gift from ZG Dong (Hormel Institute, University of Minnesota, MN, USA). Human cervical adenocarcinoma cell line Caski and human embryonic kidney 293 (HEK293) were grown in Dulbecco minimal essential medium, containing antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) supplemented with 10% heat-inactivated fetal calf serum (FCS), respectively. HEK293 cells were transfected with pCMV-Myc-mock or pCMV-Myc-STAT2 using Attractene transfection reagent (QIAGEN, Hilden, Germany) following the manufacturer's suggested protocol. The transfected cells were cultured for 36 hours and proteins were extracted by using Nonidet P-40 cell lysis buffer (50 mmol/L Tris-HCl, pH 8.0; 150 mmol/L NaCl; 0.5% Nonidet P-40; and protease-inhibitor cocktail). 30 μg of total protein was resolved on 10% SDS-PAGE and were transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk in TBS (50 mM Tris, 150mM NaCl) at room temperature for 1 hour, and were then incubated with rabbit anti-human STAT2 antibody (1:2 000, Santa Cruz Biotechnology, Santa Cruz, CA), or anti-Myc antibody (1:2 000, Invitrogen, Carlsbad, CA) at 4 ℃ overnight. Secondary horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology) was applied at a dilution of 1:2 000 for 2 hours at room temperature. Blots were developed by enhanced chemiluminescent detection (Amersham, Piscataway, NJ, USA) and exposed to X-ray film (Fuji).

2.3. Immunohistochemistry

Immunohistochemistry was done on paraffin-embedded sections of cervical tissues. The tissue was deparaffinized by placing slides into three changes of xylene and rehydrated in a graded ethanol series. The rehydrated tissue samples were rinsed in water and subjected to antigen retrieval in citrate buffer (pH 6.0). Slides were treated with 3% H_2O_2 for 5 minutes to reduce endogenous peroxidase activity and washed. STAT2 was detected using anti-STAT2 antibody (Santa Cruz Biotechnology) at a 1:100 dilution and visualized using a commercial streptavidin-biotin immuno-peroxidase complex kit (DAKO) following the manufacturer's instructions. Negative control slides were incubated with blocking serum (Santa Cruz Biotechnology) under similar conditions. Counter staining of the nucleus was archived with hematoxylin.

2.4. Evaluation of STAT2 expression

Each IHC-stained slide was examined by two independent pathologists without knowledge of the clinical diagnosis and staging of patients. The percentage of STAT2 positive cervical epithelial cells was obtained by counting 1 000 epithelial cells in 5-10 randomly selected fields. STAT2 expression was graded according to the frequency and the signal intensity of positively stained cells. Grading criteria for frequency were: 3, >75% of epithelial cells with positive staining; 2, 50%-75% of epithelial cells with positive staining; 1, 25%-50% of epithelial cells with positive staining; 0, <25% of cells with positive staining. Grading criteria for signal intensity were: 3, epithelial cells selectively stained brown; 2, epithelial cells stained yellow; 1, epithelial cells stained faintly yellow, 0, lack of staining. A sum of above two scores smaller than 1 was defined as negative; that of 2-3 assigned +; that of 4-5 assigned ++, and that of more than 6 assigned +++.

2.5. Statistics

Two-tailed Fisher's exact probability tests were used for comparison between categorical variables. The survival curve was plotted using the Kaplan-Meier estimator. Statistical difference in survival between groups of patients with positive or negative expression of STAT2 was compared by the log-rank. $P<0.05$ was considered statistically significant.

3. Results

In order to confirm the specificity of antibody against human STAT2, plasmid of pCMV-Myc-STAT2 or pCMV-Myc-mock was transfected into HEK293. 36 hours later, the HEK293 cell lysate as well as Caski lysate were analyzed by Western blot using antibody against STAT2 or Myc as the primary one, respectively. Figure 1 showed the positive signals at 113 kDa in Caski and HEK293 transfected with pCMV-Myc-STAT2, which demonstrated the specificity of antibody against human STAT2 (Figure 1).

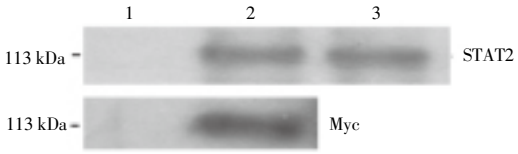

Figure 1. Specificity of primary antibody against human STAT2 was confirmed by Western blot.
pCMV-Myc-mock or pCMV-Myc-STAT2 was transfected into HER293 cells, respectively. 36 hours later, the cell lysates were collected and detected by Western blot with the anti-STAT2 antibody as the primary one. Caski cell lysate was also collected and detected by Immunoblot. Cell lysates were from 1.HEK293 transfected with pCMV-Myc-mock; 2. HEK293 transfected with pCMV-Myc-STAT2; 3.Caski. The primary antibody was STAT2 antibody against an epitope corresponding to amino acids 662-851 mapping at the C-terminus of human STAT2.

To determine whether STAT2 is relevant to human malignancy, we determined the expression levels of STAT2 by immunohistochemistry in 176 uterine cervices, which were obtained from patients in different cervical pathological stages. Representative images of different STAT2 expression levels are shown in Figure 2. Noticeably, STAT2 signals were mainly found in the cytoplasm although few signals were in the nuclear (Figure 2).

The distribution of scores that reflect both the frequency of STAT2 positive cells and the intensity of staining among the pathological groups is shown in Table 1. The overall rate of positive STAT2 expression in the cervicitis, dysplasia and cervical cancer groups were 38.5%, 69.4% and 76.9%, respectively, representing increasing trend of STAT2 rates from cervicitis to dysplasia till cervical cancer. Two-tailed Fisher's exact probability test showed that the increases in the overall rate of STAT2 detection in dysplasia biopsies and cervical cancer biopsies over the cervicitis biopsies were both highly statistically significant ($P<0.01$), whereas the small increase from the dysplasia tissues to the cancer group was not ($P>0.05$).

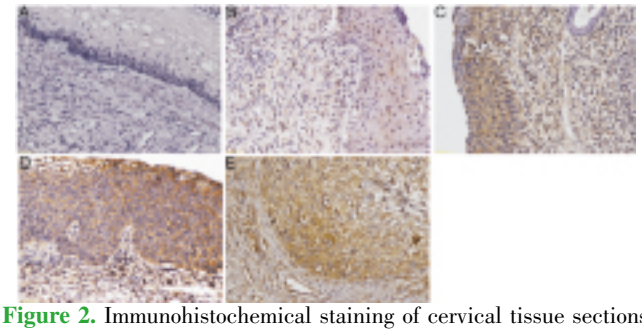

Figure 2. Immunohistochemical staining of cervical tissue sections showed different expression levels of STAT2.
Representative cervicitis (A), CIN Ⅰ (B), CIN Ⅱ (C), CIN Ⅲ (D) and cancer (E) specimens are shown (40× magnification).

We further performed statistical analysis for the expression of STAT2 in different stages of CIN (Table 2). It was found that CIN Ⅰ biopsies had a statistically higher STAT2 detection rate than cervicitis biopsies ($P=0.001$). However, there was no significant difference between the STAT2 detection rates of the CIN Ⅰ and CIN Ⅱ biopsies, or those of CIN Ⅱ and CIN Ⅲ biopsies ($P=0.090$, 0.081, respectively). There was no significant difference in the expression of STAT2 between dysplasia of different pathological types ($\chi^2=2.429$, $P=0.161>0.05$). Taken together, data presented in Table 1 and Table 2 suggested that, in most (about 70% patients), increase in STAT2 expression starts in the premalignant dysplasia and remains in cervical cancer.

To determine whether the STAT2 expression in the cervical cancer tissues correlates with patient 5-year post-diagnosis survival, a Fisher's exact probability with the positive or negative STAT2 immunoreactivity in the cervical cancer patients was performed (Table 3). STAT2 immunoreactivity appeared to be higher in the survival group (62%); however, the difference was not statistically significant ($P=0.150$). Finally, 5-year post-diagnosis overall survival analysis was performed in these cervical cancer patients (15

Table 1
Expression of STAT2 in cervicitis, displasia and carcinoma tissues ($n=176$).

Group	No of cases	Negative	Weak	Moderate	Strong	Total positive
Cervicitis	39	24(61.5%)	8(20.5%)	6(15.4%)	1(2.6%)	15(38.5%)
Dysplasia	72	22(30.6%)	34(47.2%)	15(20.8%)	1(1.4%)	50(69.4%)
Cancer	65	15(23.1%)	34(52.3%)	15(23.1%)	1(1.5%)	50(76.9%)

Table 2
Expression of STAT2 in displaysia stages, CIN Ⅰ, Ⅱ, Ⅲ.

Group	No of cases	Negative	Weak	Moderate	Strong	Total positive
Cervicitis	39	24(61.5%)	8(20.5%)	6(15.4%)	1(2.6%)	15(38.5%)
CIN Ⅰ	36	8(22.2%)	18(50.0%)	10(27.8%)	0(0.0%)	28(77.8%)
CIN Ⅱ	10	3(30.0%)	3(30.0%)	3(30.0%)	1(10.0%)	7(70.0%)
CIN Ⅲ	26	10(38.5%)	13(50.0%)	3(11.5%)	0(0.0%)	16(61.5%)

Table 3
Univariate analysis of clinicopathological variables for survival in 65 cervical cancer patients.

STAT2 immunoreactivity	5-year post-diagnosis overall survival	
	+	-
Positive ($n=50$)	31(62%)	19(38%)
Negative($n=15$)	6(40%)	9(60%)

Statistical analysis: Fisher's exact probability test. +, survival; -, death.

STAT2-negative, 50 STAT2-positive, comprising any positive staining) using the Kaplan-Meier method (Figure 3). The median survival time of STAT2-positive patients was 44.3 months, compared with 33.8 months for STAT2-negative patients. Nevertheless, this difference was still not statistically significant (P=0.10) (Figure 3), possibly due to relatively small number of patients in the STAT2 negative biopsies. However, it is not clear if STAT2 expression plays a role in patient survival.

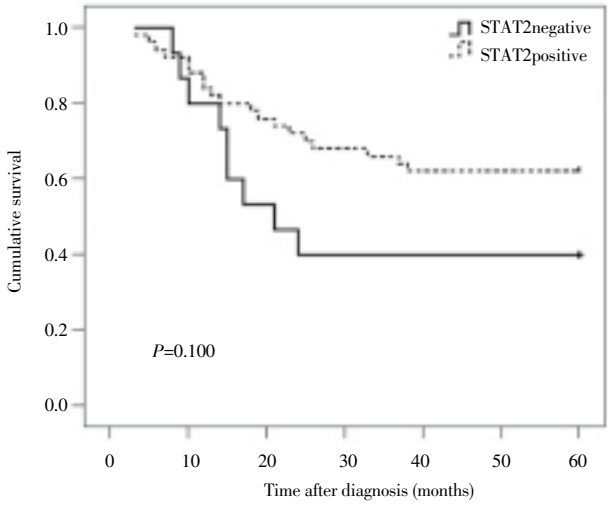

Figure 3. Kaplan-Meier survival estimates of the overall probability of post-diagnosis 5-year survival stratified by STAT2 expression determined from immunohistochemical staining, in patients with cervical cancer.
Group of STAT2 negative: black solid line; group of STAT2 positive, black dashed line.

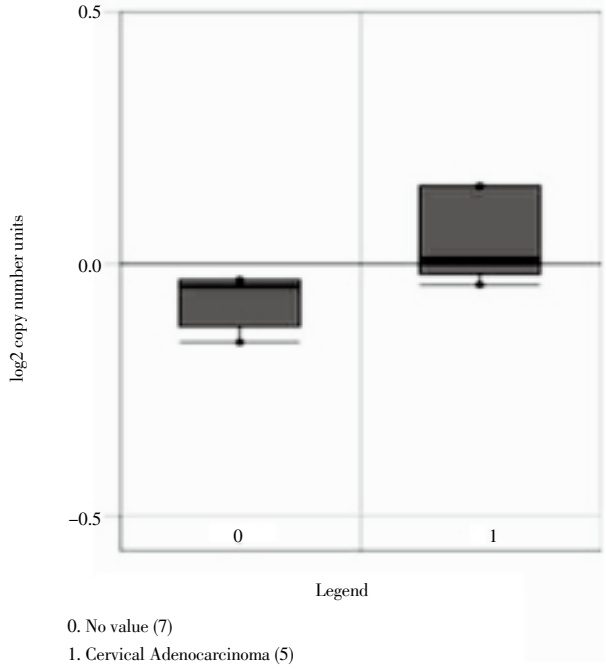

Figure 4. STAT2 expression was higher in cervical adenocarcinoma than normal cervical tissues by searching www.oncomine.com.
0: normal control group; 1: cervical adenocarcinoma; 7 and 5: number of samples.

4. Discussion

In the present study, we performed analysis of STAT2 expression for 176 cervical biopsies with different pathological stages using immunohistochemistry. The specificity of primary antibody against STAT2 was confirmed by Western blot in Caski cell line and HEK293 transiently transcfeted with pCMV-Myc-STAT2. We found significantly increased STAT2 detection rates in cervical displaysia biopsies and cervical cancer biopsies, as compared to cervicitis biopsies. However, the STAT2 detection rates were similar among biopsies of different displaysia stages, and between the displaysia biopsies and cancer biopsies. These results suggest that increased STAT2 expression frequently starts in a premalignant stage and remains in cancer. However, it is not clear if STAT2 expression plays a role in the development of cervical cancer. Further analyses with increased number of biopsies with increased statistical power will be helpful to address this question.

Although to our knowledge there is no published full-length literature directly evaluating STAT2 expression in the cervical tissue, at least several groups have indirectly corroborated our findings by including STAT2 on some custom chips in type I endometrial cancers as well as nasopharyngeal carcinoma[17,18]. From the Oncomine database (www.oncomine.com), we found that STAT2 was more highly expressed in cervical adenocarcinoma compared to normal tissues (Figure 4). The increased STAT2 expression in cervical cancer might be a result of long-term activation of the JAK-STAT pathway in response to HPV infection-stimulated production of IFNs.

STATs serve as a link between the cell surface and the nucleus, and this requires their physical movement from the cytoplasm to the nucleus[19,20]. Therefore, intra-nuclear staining of tumor cells was considered to indicate the presence of constitutively activated STATs[21]. However, the present data show that STAT2 was distributed in the cytoplasm of cervical dysplastic and cancerous epithelial cells, which implies that although STAT2 could be up-regulated in dysplastic and cancerous epithelial cells, but is not biologically active. Consistent with this hypothesis, functionally inactive STAT2 has been detected in prostate cancer[21].

The IFN system is a crucial component of the innate and adaptive immune response to virus infection. HPV infection is connected to 95 percent of all cervical cancer cases worldwide. Furthermore, HPV induces endogenous IFN expression resulting in STAT2 expression. In counterdefence, however, HPV has developed mechanisms to strand the STAT2 protein in the cytoplasm, suggesting that HPV has strategies to block STAT2 activity, resulting in avoiding innate immunity and adaptive immunity[22-24]. Therefore, in HPV infected cervical tissues, although STAT2 is highly expressed, it is retarded in cytoplasm, resulting in loss of its activity. Hence, the ability of the HPV to block IFN signaling may be important and necessary for the development of papilloma-virus-derived cancers[22-24]. In summary, whatever model accounts for the increased expression of STAT2, it is unlikely that the simple up-regulation of STAT2 expression results in their activation and nuclear accumulation.

In contrast with our findings, Clifford found a significant

decrease in expression of STAT2 in 76% of skin squamous cell carcinoma (SCC)[25]. In order to explore the role of STAT2 in skin carcinogenesis, Clifford et al. programmatically examined the expression of IFN-stimulated gene factor 3 (ISGF-3) proteins (including STAT 1 α / β , STAT2, and p48), which are important mediators of IFN- α signaling, in skin premalignancy and SCC. They observed a significant decrease in expression of one or more ISGF-3 proteins in SCC patients. Furthermore, they found that an IFN- α sensitive skin squamous cell line forced expression of a dominant negative-acting STAT2 (dnSTAT2) showed diminished antigrowth effects with IFN- α stimulation via suppressing the up-regulation of several IFN- α -inducible genes[26]. These data have led to the hypothesis that the suppressed expression of STAT2 resulting in consequent reduction in responsiveness to endogenous IFN likely are an early event in skin carcinogenesis. In all, combined with our findings, it was supposed that blocking STAT2 activity might be a molecular event in carcinogenesis.

In conclusion, we report an increased STAT2 detection rate in premalignant cervical displaysia biopsies as well as cervical cancer biopsies, as compared to cervicitis biopsies. It remains to be determined if STAT2 expression facilitates or inhibits cervical cancer development.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Davies P, Arbyn M, Dillner J, Kitchener HC, Meijer CJ, Ronco G, et al. A report on the current status of European research on the use of human papillomavirus testing for primary cervical cancer screening. *Int J Cancer* 2006; **118**: 791-796.

[2] Beglin M, Melar-New M, Laimins L. Human papillomaviruses and the interferon response. *J Interferon Cytokine Res* 2009; **29**: 629-635.

[3] Konjevic G. STAT proteins in cancerogenensis and therapy of malignancies. *Srp Arh Celok Lek* 2009; **137**: 98-105.

[4] Rawlings JS, Rosler KM, Harrison DA. The JAK/STAT signaling pathway. *J Cell Sci* 2004; 1281-1283.

[5] Lai SY, Johnson FM. Defining the role of the JAK-STAT pathway in head and neck and thoracic malignancies: implications for future therapeutic approaches. *Drug Resist Updat* 2010; **13**(3): 67-78.

[6] Koptyra M, Gupta S, Talati P, Nevalainen MT. Signal transducer and activator of transcription 5a/b: biomarker and therapeutic target in prostate and breast cancer. *Int J Biochem Cell Biol* 2011; **43**(10): 1417-1421.

[7] Ferrajoli A, Faderl S, Ravandi F, Estrov Z. The JAK-STAT pathway: a therapeutic target in hematological malignancies. *Curr Cancer Drug Targets* 2006; **6**(8): 671-679.

[8] Wang H, Lafdil F, Kong X, Gao B. Signal transducer and activator of transcription 3 in liver diseases: a novel therapeutic target. *Int J Biol Sci* 2011; **7**(5): 536-550.

[9] Benekli M, Baumann H, Wetzler M. Targeting signal transducer and activator of transcription signaling pathway in leukemias. *J Clin Oncol* 2009; **27**(26): 4422-4432.

[10] Gu L, Vogiatzi P, Puhr M, Dagvadorj A, Lutz J, Ryder A, et al. Stat5 promotes metastatic behavior of human prostate cancer cells *in vitro* and *in vivo*. *Endocr Relat Cancer* 2010; **17**:481-493.

[11] Lassmann S, Schuter I, Walch A, Gobel H, Jutting U, Makowiec F, et al. STAT3 mRNA and protein expression in colorectal cancer: effects on STAT3-inducible targets linked to cell survival and proliferation. *J Clin Pathol* 2007; **60**: 173-179.

[12] Wang H, Yang H, Sharma N, Tarasova NI, Timofeeva OA, Winkler-Pickett RT, et al. STAT1 activation regulates proliferation and differentiation of renal progenitors. *Cell Signal* 2010; **22**: 1717-1726.

[13] Widschwendter A, Tonko-Geymayer S, Welte T, Daxenbichler G, Marth C, Doppler W. Prognostic significance of signal transducer and activator of transcription 1 activation in breast cancer. *Clin Cancer Res* 2002; **8**: 3065-3074.

[14] Park C, Li S, Cha E, Schindler C. Immune response in Stat2 knockout mice. *Immunity* 2000; **13**: 795-804.

[15] Albert Zimmermann, Mirko Trilling, Markus Wagner, Manuel Wilborn, Ivan Bubic, Stipan Jonjic, et al. A cytomegaloviral protein reveals a dual role for STAT2 in IFN- γ signaling and antiviral response. *J Exp Med* 2005; **201** (10): 1543-1553.

[16] Gamero AM, Young MR, Mentor-Marcel R, Bobe G, Scarzello AJ, Wise J, et al. STAT2 contributes to promotion of colorectal and skin carcinogenesis. *Cancer Prev Res (Phila)* 2010; **3**: 495-504.

[17] Sugiyama Y, Dan S, Yoshida Y, Akiyama F, Sugiyama K, Hirai Y, et al. A large-scale gene expression comparison of microdissected, small-sized endometrial cancers with or without hyperplasia matched to same-patient normal tissue. *Clin Cancer Res* 2003; **9**: 5589-5600.

[18] Akervall J. Gene profiling in squamous cell carcinoma of the head and neck. *Cancer Metastasis Rev* 2005; **24**: 87-94.

[19] Reich NC. STAT dynamics. *Cytokine Growth Factor Rev* 2007; **18**: 511-518.

[20] Meyer T, Vinkemeier U. STAT nuclear translocation: potential for pharmacological intervention. *Expert Opin Ther Targets* 2007; **11**(10): 1355-1365.

[21] Ni Z, Lou W, Lee SO, Dhir R, DeMiguel F, Grandis JR, et al. Selective activation of members of the signal transducers and activators of transcription family in prostate carcinoma. *J Urol* 2002; **167**: 1859-1862.

[22] Antonsson A, Payne E, Hengst K, McMillan NA. The human papillomavirus type 16 E7 protein binds human interferon regulatory factor-9 via a novel PEST domain required for transformation. *J Interferon Cytokine Res* 2006; **26:** 455-461.

[23] Nees M, Geoghegan JM, Hyman T, Frank S, Miller L, Woodworth CD. Papillomavirus type 16 oncogenes downregulate expression of interferon- responsive genes and upregulate proliferation-associated and NF-kappaB- responsive genes in cervical keratinocytes. *J Virol* 2001; **75**: 4283-4296.

[24] Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J Gen Virol* 2008; **89**: 1-47.

[25] Clifford JL, Walch E, Yang X, Xu X, Alberts DS, Clayman GL, et al. Suppression of type I interferon signaling proteins is an early event in squamous skin carcinogenesis. *Clin Cancer Res* 2002; **8**: 2067-2072.

[26] Clifford JL, Yang X, Walch E, Wang M, Lippman SM. Dominant negative signal transducer and activator of transcription 2 (STAT2) protein: stable expression blocks interferon alpha action in skin squamous cell carcinoma cells. *Mol Cancer Ther* 2003; **2**: 453-459.