

Effect of Interventional Therapy on the Expression of Survivin mRNA in Cervical Cancer

SHAO-GUANG WANG¹, NAN MU¹ and HAI-YAN SUN²

¹Department of Gynecology, the Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, P.R. China;
²College of Medicine, Qingdao University, Qingdao, P.R. China

Abstract. *Aim: To examine the expression of survivin mRNA in cervical squamous cancer at different time points during interventional therapy in order to find the optimal time point for continual treatment. Patients and Methods: Fifty patients with stage IB2-IIIB cervical squamous cancer receiving transarterial infusion chemotherapy and chemoembolization were included in the present study. The expression of survivin mRNA in cancer samples before and after interventional therapy (on days 7, 14 and 21) were examined by reverse transcription-polymerase chain reaction. Results: The expression of survivin mRNA in cancer samples before the interventional therapy was significantly higher than that of any time point after the interventional therapy ($p < 0.05$). After treatment, the expression of survivin mRNA decreased until day 14. Conclusion: The expression of survivin mRNA was inhibited by the interventional therapy. It seems that day 14 after interventional treatment is the right time point for continuation of treatment.*

Cervical cancer is a common gynecological malignancy all over the world, especially in developing countries. Human papillomavirus is suggested to be responsible for the carcinogenesis of this malignancy. Surgery and radiation are principal therapeutics for this malignant disease. According to National Comprehensive Cancer Network guideline (1), patients with International Federation of Gynecology and Obstetrics (FIGO) Ia to IIa stage disease need surgical treatment. However, surgery for some patients is difficult due to large tumor body or parametrial metastasis. Recently, with the application of neoadjuvant chemotherapy (NAC) and interventional therapy in gynecology, the prognosis of

cervical cancer has been largely improved, such as increased survival time (2); improvement of sexual activity and bladder and bowel dysfunction (3) and even fertility persistence (4, 5). After NAC and interventional therapy, most of those patients were able to undergo further treatment. Nowadays, the evaluation of the effect of interventional therapy on cervical cancer is primarily through pathology and pharmacokinetics (6-8). Unfortunately, limited information about the right time point for further treatment after interventional therapy is available. Further treatment at an inappropriate time point would weaken its therapeutic effects. For these reasons, it is urgent to find a biomarker tightly associated with disease progress, thereby marking the most suitable time point for further treatment of patients with cervical cancer.

Survivin is a member of the inhibitor of apoptosis family of antiapoptotic proteins, which are expressed mainly in adult malignant tissues including cervical cancer (9). Overexpression of survivin leads to reduced apoptosis of cancer cells (10). In patients with cervical cancer, expression of this gene could reflect the progress of this malignant disease. After successful interventional therapy, the expression of survivin would be expected to decrease. We suggest that the time point when survivin expression is at a minimum is the optimal opportunity to conduct further treatment for patients with cervical cancer who have undergone interventional therapy. We had the opportunity to study the effect of interventional therapy on survivin mRNA expression (11), with the aim of finding the right time point for further treatment and report our findings here.

Patients and Methods

Selection of patients with cervical cancer. Fifty cases of cervical squamous cancer which received interventional treatment at Yantai Yuhuangding Hospital PRC between June 2012 and December 2013 were included in the current study. The patients ranged in age from 34 to 52 years with a mean age of 43.2 ± 6.4 years. According to FIGO 2009 (12), there were 18 cases of IB2 stage, 12 cases of IIA stage and 20 cases of IIB stage. Basic characteristics of study patients are presented in Table I.

Correspondence to: Shao-Guang Wang, MD, Ph.D., Department of Gynecology, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, No.20 Yuhuangding-East Road, Yantai 264000, P.R. China. Tel: +86 05356691999 ext. 83427, e-mail: 15653560356@163.com

Key Words: Cervical cancer, interventional therapy, survivin.

Cancer samples. All the participants gave their written consent for our investigation. All the patients received bilateral uterine artery embolization chemotherapy with cisplatin (70 mg/ml) and bleomycin (45 mg) (13). Gelfoam particles of 350-560 μ m (Alicon Pharm SCI &TEC Co., Ltd. HangZhou, Zhejiang Province, China) were selected as the embolization agent in our study. After intubation into the uterine artery, two-thirds of drugs were perfused into bilateral uterine arteries at the same dose. Then the remaining drugs combined with gelfoam particles and contrast agent were used to embolize uterine arteries. Before the interventional therapy and every 7 days after treatment until the day 21, a cancer tissue sample of 0.2x0.2x0.2 cm was drawn and stored for further investigation.

Primer design and synthesis. Primers used for quantification were as follows: survivin: forward primer, 5'-GGC ATG GGT GCC CCG ACG TT-3', reverse primer, 5'-AGA GGC CTC AAT CCA TGG CA-3', there were 320bp between forward and reverse primers; β -actin: forward primer, 5'-GTG GGG CGC CCC AGG CAC CA-3', reverse primer, 5'-CTC CTT AAT GTC ACCG CAC GAT TTC-3', these primers amplify a 539bp product. The primers were synthesized by Shanghai Boya Bio-engineering Company, PRC.

Reverse transcription-polymerase chain reaction (RT-PCR) methods. Total RNA was extracted from the cancer tissue samples using TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY, USA). RNA was synthesized into cDNA using MMLV reverse transcriptase. Real-time PCR was conducted using the following protocol: 94°C for 5 min, and then 30 cycles of 94°C for 1 min, 58°C for 1 minute, and 72°C for 1 minute, and 72°C for 10 min. The PCR product was separated in 2% agarose gel and analyzed using a gel image analysis system (Life Technologies Europe BV, he Netherlands). The expression of survivin mRNA was normalized by the absorbance ratio of survivin and β -actin.

Statistical analysis. Data were analyzed using SAS software package (version 9; SAS Institute, Cary, NC, USA). Significance of differences in continuous variables was assessed using the paired *t*-test. All *p*-values were two-sided and differences with *p*-values lower than 0.05 were considered statistically significant.

Results

Survivin expression in squamous cell cervical cancer after interventional therapy. The expression of survivin mRNA in cancer samples before the interventional therapy was higher than that after the interventional therapy ($p<0.05$). At day 7 after the treatment, the expression of survivin mRNA was significantly higher than that at days 14 and 21 after interventional treatment ($F=135.87$, $p<0.05$); However, the expression of survivin mRNA at day 14 was found to be lower than that on days 7 and 21 after treatment ($p<0.05$) (Table II).

Survivin expression in squamous cell cervical cancer after interventional therapy among different stages. The expression of survivin mRNA in samples of stage IB2, IIA and IIB cervical cancer before the interventional therapy was significantly higher than that after the interventional therapy ($p<0.05$). At day 7 post-therapy, the expression of survivin in all stages was

significantly higher than that of days 14 and 21 after treatment ($F=295.56$, $p<0.05$). At day 21 after treatment, the expression of survivin mRNA in all stages was significantly higher than that at day 14 and lower than that at day 7 ($p<0.05$) (Table III).

Discussion

Cervical cancer is a common malignant disease of the female genital tract. Conventionally, treatments are mainly surgery and radiation therapy. With the development of new anticancer drugs, interventional neoadjuvant chemotherapy of cervical cancer showed satisfactory effects (14). However, the disappearance of malignant tumor after interventional therapy was temporary. A study found that surgery after interventional therapy is difficult because of cellulose exudation and fiber cords formed around the cancer cells (15). At present, the evaluation of the effect of interventional NAC on cervical cancer is preliminary through clinical gynecological examination or imaging examination including ultrasonography computed tomography and magnetic resonance imaging (16, 17). Furthermore, the time point for further surgery was mainly determined by those examinations. Most researchers have suggested that the right time point for further treatment is 3 weeks after arterial infusion chemotherapy alone or 4 weeks after arterial chemoembolization (18). There are some other findings, including the following. (i) Genetic polymorphisms in the phosphatidylinositol 3 kinase (PI3K)/PKBPKB protein kinase B (PKB) pathway are associated with sensitivity to platinum-based chemotherapy in patients with cervical cancer. The heterozygous genotype of two loci in the *PIK3CA* gene was associated with an increased risk of chemoresistance. Non-responders to NAC had a higher frequency of the rs10416620 and rs62107593 G nucleotide in the alleles of the *AKT2* gene; rs2498786 and the GGCC haplotype of polymorphisms in *AKT1* gene showed a high risk for non-response to NAC (19); (ii) Protein Wee1 (pWEE1), r-histone family member X (r-H2AX), and protein checkpoint kinase 1 (pCHK1) are biomarkers of DNA damage and repair, predicting the efficacy of NAC in cervical cancer. Elevated levels of pWee1 and r-H2AX were significantly associated with a lower rate of pathological complete response and there was a significant association between pWEE1 and pCHK1 (20); (iii) ALDH1 expression pre-NAC was significantly associated with a low clinical chemotherapy response rate and clinical non-response; ALDH1 expression post-NAC was associated with poor disease-free and overall survival (21); (iv) glutathione metabolic pathway scores of cancerous tissue combined with uridine diphosphate-glucuronosyltransferase 1A1 (*UGT1A1*) genotyping of blood samples predicting the efficacy of NAC for cervical cancer. GMP was significantly up-regulated in NAC-resistant patients and *UGT1A1* genotyping revealed that patients with *UGT1A1* polymorphisms exhibited significantly higher response rates

Table I. Basic characteristics of patients with cervical cancer (n=50).

Characteristic	Value
Age (years)	43.2 (6.4)
Height (cm)	161.2 (11.5)
Weight (kg)	70.4 (16.1)
Birth weight >4 kg	16
Waist circumference (cm)	92.3 (10.7)
Abdominal circumference (cm)	97.4 (15.8)
Hip circumference (cm)	102.5 (17.4)
BMI (kg/m ²)	27.1(5.8)
WHR	0.91(0.05)
Age at menarche (years)	12.4(2.7)
Nulliparous	2 (4)
Diabetes	9 (18)
Hypertension	13 (26)
Family history of cancer	6 (12)

BMI, Body mass index; WHR, waist-to-hip ratio. Continuous variables are shown as means (SD), while categorical variables are shown as number (%).

Table II. Relative survivin expression in cervical squamous cancer before and after interventional therapy.

	Before (I)	Day 7 after (II)	Day 14 after (III)	Day 21 after (IV)
Survivin	1.17±0.26	0.97±0.15	0.53±0.32	0.72±0.42
<i>q</i> _{II-I} : 29.29*			<i>q</i> _{III-I} : 15.42*	
<i>q</i> _{IV-I} : 17.57*			<i>q</i> _{III-II} : 13.86*	
<i>q</i> _{IV-II} : 11.71*			<i>q</i> _{IV-III} : 2.06NS	

*Significant at $p < 0.05$, ^{NS} $p > 0.05$.

to NAC than those with the wild-type genotype (22). All the above findings may be prognostic markers, predicting the efficacy of NAC for cervical cancer, but none of them revealed the optimal time for further treatment of cervical cancer. However, in the current study, according to the expression of survivin mRNA, we found that day 14 after interventional treatment is the right time point for further treatment (23).

It is reported that survivin is responsible for the abnormal proliferation of cancer cells, moreover, it is also an inhibitor of cell apoptosis (24). This gene is located in chromosome 17, q25, with 14.7 kb length, including four exons and three introns. Deficiency and mutation of survivin will induce apoptosis of cancer cells (25). The expression of survivin gene is found in human endometrial tissue, ovary and thymus tissue (26). Three types of survivin have been found, with molecular weight of 431, 329 and 500 kDa, respectively. They all have the activity to inhibit apoptosis

Table III. Relative survivin expression in cervical squamous cancer before and after interventional therapy among International Federation of Gynecology and Obstetrics (FIGO) different stages.

Time point relative to therapy	FIGO stage		
	IB2	IIA	IIB
Before (I)	1.27±0.55	1.08±0.46	1.13±0.23
Day 7 after (II)	1.01±0.76	0.96±0.55	0.86±0.56
Day 14 after (III)	0.63±0.31	0.68±0.55	0.55±0.47
Day 21 after (IV)	0.73±0.47	0.81±0.54	0.75±0.45
<i>q</i> _{II-I}	25.20*	22.23*	27.24*
<i>q</i> _{III-I}	16.32*	15.35*	15.22*
<i>q</i> _{IV-I}	19.53*	18.47*	20.14*
<i>q</i> _{III-II}	14.16*	14.42*	14.85*
<i>q</i> _{IV-II}	12.21*	10.61*	13.12*
<i>q</i> _{IV-III}	4.65NS	5.25NS	5.12NS

*Significant at $p < 0.05$, ^{NS} $p > 0.05$.

(27). It is also suggested that the mechanism of survivin-mediated inhibition of apoptosis is closely related to caspase-3 (28), a key factor in the apoptosis signaling pathway.

In this study, every target of the serum samples was examined in the same batch and all these measurements were carried out in one laboratory. However, there are still some limitations to this study. Firstly, all the patients were accrued from one hospital. Multi-center studies including more patients would provide stronger evidence. Secondly, the patients were all Chinese women, and data from other ethnic groups would give us more information.

In the present study, the expression of survivin mRNA was found in cervical squamous cancer tissue of IB2-IIB stage before and after interventional therapy, which suggests that the apoptosis of cervical cancer cells was tightly associated with this protein. After therapy under the combined effect of chemotherapeutic drugs and embolization, the expression of survivin mRNA decreased. At day 14 after therapy, the expression of survivin mRNA was significantly lower than that at day 7. At this time, the effect of interventional therapy on cervical squamous cancer was ongoing. At day 21 after this therapy, the expression of survivin mRNA was higher than that at day 14 and significantly lower than that at day 7, which suggests that day 14 after the treatment is a milestone for future treatment. Accordingly, 2-3 weeks after arterial infusion chemoembolization for cervical squamous cancer is probably an optimum time for further treatment. The results provided by this study suggested that survivin is a useful biomarker for assessing the effect of interventional therapy on cervical squamous cancer. Furthermore, the period from day 14-21 after arterial infusion chemoembolization is the right time for further treatment.

References

- 1 Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Cho KR, Chu C, Cohn D, Crispens MA, Dorigo O, Eifel PJ, Fisher CM, Frederick P, Gaffney DK, Han E, Huh WK, Lurain JR 3rd, Mutch D, Fader AN, Remmenga SW, Reynolds RK, Teng N, Tillmanns T, Valea FA, Yashar CM, McMillian NR and Scavone JL: Cervical Cancer, Version 2.2015. *J Natl Compr Canc Netw* 13: 395-404, 2015.
- 2 Kumar L, Pramanik R, Kumar S Bhatla N and Malik S: Neoadjuvant chemotherapy in gynaecological cancers-Implications for staging. *Best Pract Res Clin Obstet Gynaecol* 29: 790-801, 2015.
- 3 Gargiulo P, Arenare L, Pisano C, Cecere SC, Falivene S, Gregg S, Tambaro R, Facchini G, De Palma G, Scaffa C, Della Pepa C, Pignata S and Di Napoli M: Long-term toxicity and quality of life in patients treated for locally advanced cervical cancer. *Oncology* 90: 29-35, 2016.
- 4 Yao YY, Wang Y, Wang JL, Zhao C and Wei LH. L: Outcomes of fertility and pregnancy in patients with early-stage cervical cancer after undergoing neoadjuvant chemotherapy. *Eur Gynaecol Oncol* 37: 109-121, 2016.
- 5 Salili R, Leunen K, Van Limbergen E, Moerman P, Neven P and Vergote I: Neoadjuvant chemotherapy followed by large cone resection as fertility-sparing therapy in stage IB cervical cancer. *Gynecol Oncol* 139: 447-498, 2015.
- 6 Ikeda O, Mizukami N, Murata Y, Arakawa A, Katabuchi H, Okamoto H, Yasunaga T, Tsunawaki A and Yamashita Y: Randomized comparison of intra-arterial chemotherapy versus intra-arterial chemotherapy and gelfoam embolization for treatment of advanced cervical carcinoma. *Cardiovasc Intervent Radio* 28: 736-743, 2005.
- 7 Chen Chunlin, Tan Daocai and Jang Lizhi: Comparison of drug concentration within cervical cancer tissue after transarterial infusion chemotherapy. *Chinese Gynecol Obstet J* 30: 298, 1995.
- 8 Liang Y, Lv B and Chen X: Prognostic value of pathological response to neoadjuvant chemotherapy in bulky stage IB2 and IIA cervical squamous cell cancer patients. *Virchows Arch* 468: 329-365, 2016.
- 9 Ambrosini G, Adida C and Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 3: 917-21, 1997.
- 10 Castedo M1, Perfettini JL, Roumier T, Andreau K, Medema R and Kroemer G: Cell death by mitotic catastrophe: a molecular definition. *Oncogene* 23: 2825-37, 2004.
- 11 Ip SW, Wei HC, Lin JP, Kuo HM, Liu KC, Hsu SC, Yang JS, Mei-Dueyang, Chiu TH, Han SM and Chung JG: Bee venom induced cell-cycle arrest and apoptosis in human cervical epidermoid carcinoma Ca Ski cells. *Anticancer Res* 28: 833-842, 2008.
- 12 Pecorelli S, Zigliani L and Odicino F: Revised FIGO staging for carcinoma of the cervix. *Int J Gynaecol Obstet* 105: 107-108, 2009.
- 13 Kokawa K, Mabuchi Y, Tanaka K, Yagi S, Utsunomiya T, Oba N, Yata C and Umesaki N: Apoptosis in cervical cancer after balloon-occluded arterial infusion of anticancer drugs. *Anticancer Res* 26: 1413-8, 2006.
- 14 Sardi JE: Comments on neoadjuvant chemotherapy in cervical cancer. *Gynecol Oncol* 108: 458-459, 2008.
- 15 Tian ZZ, Li S, Wang Y, Yue YJ, Zhu XH, Zhao R, Zhang CL and Wei SH: Investigation of uterine arterial chemoembolization and uterine arterial infusion chemotherapy for advanced cervical cancer before radical radiotherapy: a long-term follow-up study. *Arch Gynecol Obstet* 290: 155-162, 2014.
- 16 Lai CH, Yen TC and Chang TC: Positron-emission tomography imaging for gynecologic malignancy. *Curr Opin Obstet Gynecol* 19: 37-41, 2007.
- 17 Fu C, Feng X, Bian D, Zhao Y1, Fang X, Du W, Wang L and Wang X: Simultaneous changes of magnetic resonance diffusion-weighted imaging and pathological microstructure in locally advanced cervical cancer caused by neoadjuvant chemotherapy. *J Magn Reson Imaging* 42: 427-462, 2015.
- 18 Popovici LR, Ciulcu A, Dorobat B, Dumitraşcu M, Horhoianu VV and Cirstoiu M: Therapeutic approaches in pelvic bleeding of neoplastic origin. *J Med Life* 7: 391-395, 2014.
- 19 Guo L, Wu H, Zhu J, Zhang C, Ma J, Lan J and Xie X: Genetic variations in the PI3K/PKB pathway predict platinum-based neoadjuvant chemotherapeutic sensitivity in squamous cervical cancer. *Life Sci* 143: 217-241, 2015.
- 20 Vici P, Buqilioni S, Serqi D, Pizzuti L, Di Lauro L, Antoniani B, Sperati F, Terrenato I, Carosi M, Gamucci T, Dattilo R, Bartucci M, Vincenzoni C, Mariani L, Vizza E, Sanguineti G, Gadducci A, Vitale I, Barba M, De Maria R, Mottotese M and Maugeri-Saccà M: DNA damage and repair biomarkers in cervical cancer patients treated with neoadjuvant chemotherapy: an exploratory analysis. *PLoS One* 11: 1-11, 2016.
- 21 Xie Q, Liang J, Rao Q, Xie X, Li R, Liu Y, Zhou H, Han J, Yao T and Lin Z: Aldehyde dehydrogenase 1 expression predicts chemoresistance and poor clinical outcomes in patients with locally advanced cervical cancer treated with neoadjuvant chemotherapy prior to radical hysterectomy. *Ann Surg Oncol* 23: 163-70, 2016.
- 22 Horikawa N, Baba T, Matsumura N, Murakami R, Abiko K, Hamanishi J, Yamaguchi K, Koshiyama M, Yoshioka Y and Konishi I: Genomic profile predicts the efficacy of neoadjuvant chemotherapy for cervical cancer patients. *BMC Cancer* 15: 739, 2015.
- 23 Saito T, Takehara M, Tanaka R, Lee R, Horie M, Wataba K, Ito E and Kudo R: Correlation between responsiveness of neoadjuvant chemotherapy and apoptosis-associated proteins for cervical adenocarcinoma. *Gynecol Oncol* 92: 284-292, 2004.
- 24 Yue Z, Carvalho A, Xu Z, Yuan X, Cardinale S, Ribeiro S, Lai F, Ogawa H, Gudmundsdottir E, Gassmann R, Morrison CG, Ruchaud S and Earnshaw WC: Deconstructing Survivin: comprehensive genetic analysis of Survivin function by conditional knockout in a vertebrate cell line. *J Cell Biol* 183: 279-296, 2008.
- 25 Ai Z, Yin L, Zhou X, Zhu Y, Zhu Y, Zhu D, Yu Y and Feng Y: Inhibition of survivin reduces cell proliferation and induces apoptosis in human endometrial cancer. *Cancer* 107: 746-56, 2006.
- 26 Zhang H, Li M, Zheng X, Sun Y, Wen Z and Zhao X: Endometriotic stromal cells lose the ability to regulate cell-survival signaling in endometrial epithelial cells *in vitro*. *Mol Hum Reprod* 15: 653-63, 2009.
- 27 He XF, Wen DG, Hou JQ, He J and Chen JN: Expressions of survivin and the splice variants survivin-2B and survivin-Delta Ex3 in bladder cancer and their clinical significance. *Chin J Cancer* 28: 1209-1213, 2009.
- 28 White-Gilbertson SJ, Kasman L, Tirodkar T, Lu P and Voelkel-Johnson C: Oxidative stress sensitizes bladder cancer cells to TRAIL-mediated apoptosis by down-regulating anti-apoptotic proteins. *J Urol* 182: 1178-1185, 2009.

Received June 1, 2017

Revised June 20, 2017

Accepted June 22, 2017