RESEARCH ARTICLE

Overexpression and Clinicopathological Contribution of DcR3 in Bladder Urothelial Carcinoma Tissues

Yi-Qiang Jiang[&], Teng-Fei Zhong[&], Yi-Wu Dang, Ling-Song Zou, Liu Yang, Xia Yang, Gang Chen*

Abstract

Background: To explore the expression of DcR3 protein and its clinicopathological significance in bladder urothelial carcinomas (BUC). Materials and Methods: Immunohistochemistry was performed to detect the expression of DcR3, caspase-3, Bcl-2, VEGF, Ki-67, PCNA and P53 in 166 BUC and 56 normal bladder tissues. Western blotting was used to detect the expression of DcR3 in the supernatants of cultured BUC cells. Results: Overexpression of DcR3 was found in BUC tissues and cell lines, with significant elevation as compared to normal bladder tissues ($p<0.0001$). Higher DcR3 expression was related to the status of invasion, lymph node metastasis and recurrence. Furthermore, DcR3 expression was negatively correlated with caspase-3 and positively associated with Bcl-2, VEGF, Ki-67 labeling index (LI), PCNA LI and P53 (all $p<0.0001$), respectively. Conclusions: DcR3 may play a crucial role as an oncogene in tumorigenesis, deterioration and progress of BUC via influencing related pathways of apoptosis, proliferation and angiogenesis. The detection of DcR3 protein in the formalin-fixed and paraffin-embedded samples could assist to predict in prognosis of BUC patients.

Keywords: DcR3 - BUC - tumorigenesis - invasion - metastasis - recurrence

Asian Pac J Cancer Prev, **15 (21)**, 9137-9142

Introduction

Bladder urothelial carcinoma (BUC) is the fifth most common cancer worldwide, with an estimated incidence of 73, 510 cases and 14, 880 deaths in the United States in 2012. In China, the morbidity of urothelial carcinoma is 6.61/100,000, ranked the ninth in all malignant tumors (O'Keeffe et al., 2008; Yang et al., 2011; Siegel et al., 2012; Bracha et al., 2014). The decoy receptor 3 (DcR3), locating on chromosome 20q13, is a member of the tumor necrosis factor receptor (TNFR) superfamily. Previously, we have reported the overexpression of DcR3 mRNA and protein in sera or tissues of several human malignancies, including hepatocellular carcinoma, gastric carcinoma and glioma (Chen and Luo, 2008a; 2008b; Chen et al., 2010; Huang et al., 2014). DcR3 has been considered as an oncogene for the aforementioned malignancies. So far, there has been only one report studying the relationship between DcR3 and urothelial carcinoma (UC). Yamana et al. (2005) investigated the amplification and protein expression of DcR3 in the tissues and cell lines of UC by using real-time quantitative PCR and immunohistochemistry in a small number of patients.

However, no study has been performed to explore the relationship between DcR3 and progression of UC patients. Furthermore, the role of DcR3 on BUC has not been fully clarified. Thus, the aim of the current study was to explore the expression and its clinicopathological significance in invasive stages and patients survival, in relation to tumor cell apoptosis, proliferation and angiogenesis in BUC formalin-fixed and paraffin-embedded (FFPE) tissues.

Materials and Methods

Patients population and histologic analysis

We studied 166 consecutive patients with BUC who had undergone partial or complete cystectomy and 56 cases of normal bladder from autopsies. The FFPE tissues were made into 2 tissue microarrays (TMAs). All cases were diagnosed at the First Affiliated Hospital, Guangxi Medical University, China, from January 2003 to October 2006. The age of BUC patients ranged from 27 to 96 years (mean age: 61.62 years) and the male/female ratio was 141/25. The age of normal bladder samples ranged from 44 to 66 years (mean age: 52.43) and the male/female ratio was 46/10. The histological sections were processed from tissues fixed in 10% formalin with standard methods and stained using hematoxylin and eosin (H&E). H&E-stained slides were used to evaluate histological grade as low or high grade (Jankovic Velickovic et al., 2009; 2011) for non-invasive BUCs, pathologic stage (pT) (Jankovic Velickovic et al., 2009; 2011), growth pattern of tumor (papillary/solid) for all BUCs. The invasive status of the tumors was divided into two stages: low-

*Department of Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning, China [&]Equal contributors *For correspondence: chen_gang_triones@163.com*

stage non-muscle invasive (pTa-pT1) and high-stage muscle invasive (pT2-pT4) tumors (Jankovic Velickovic et al., 2009; 2011). The status of lymph node metastasis, distant metastasis, tumor number and tumor size were also collected (Table 1). None of the BUC patients had received prior chemotherapy, intravesical instillation or radiation therapy. Of all 166 patients, 85 were followed up to 6~40 months. Till the end of follow-up, 19 cases had recurrence and 12 candidates were dead. The study protocol was approved by the Ethical Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was obtained from the patients and clinicians for the usage of the samples for research.

Immunohistochemistry

All tissues were analyzed using the monoclonal antibody DcR3 (37A565, Santa Cruz Biotechnology Inc., CA, USA, 1:300 dilution) and monoclonal antibodies: caspase-3, Bcl-2, vascular endothelial growth factor (VEGF), Ki-67, proliferating cell nuclear antigen (PCNA), P53 (Beijing Zhongshan Jinqiao Inc., Beijing, China), respectively. A standard avidin-biotin immunoperoxidase complexes detection system was performed as previously described (Chen and Luo, 2008a; Chen et al., 2010; Yang et al., 2010; Huang et al., 2014), according to the protocol of the manufacturer (Beijing Zhongshan Jinqiao Inc., Beijing, China). Before quantifying the immunohistochemical results, the technique quality was evaluated. Areas with greater positivity were chosen, while peripheral area measurement, necrosis, or artifacts were avoided. Slides were reviewed independently by three investigators (YJ, YD, GC). Intergroup inconsistencies were resolved via discussion by using an octal-headed microscope. Cytoplasmic and cytomembrane expression was recorded for DcR3. Cytoplasmic expression was recorded for caspase-3, Bcl-2 and VEGF, and nuclear expression was for Ki-67, PCNA and P53. For DcR3, caspase-3, Bcl-2 and VEGF, negative (-), weakly positive (+), moderately positive (++), and strongly positive (+++) were determined according to the immunohistochemistry of staining intensity and numbers of positive cells. All of (+), (++), and (+++) were considered as positive expression. The labeling indexes (LIs) of Ki-67, PCNA and the numbers of P53 were calculated with the formula (number of positive cells/total number of the cells×100%) by counting at least 10 random representative fields of high-power (40×40), distant from necrotic areas (Chen and Luo, 2008a; Chen et al., 2010; Yang et al., 2010; Huang et al., 2014).

Cell culture

Three human BUC cell lines (T24, HT1376 and RT4) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cell lines were cultured in Dulbecco's modified essential medium (DMEM, Invitrogen Corp., Grand Island, NY, USA) and supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen Corp., Grand Island, NY, USA), 2mM glutamine, and gentamicin at 37°C in a humidified incubator with 5% CO_2. Experiments were performed in triplicate.

Preparation of primary BUC cell cultures

Preparation of primary BUC cell cultures was performed as previously reported (Huang et al., 2014). Briefly, human BUC cells were achieved from 8 patients who underwent partial or complete cystectomy.

After surgery, the tissues were placed immediately in Petri dishes, minced mechanically, and digested enzymatically using collagenase (1h, 37°C). Next, the dissociated cells were filtered through 100μm cell strainers to remove cell debris. The BUC cells were centrifuged and washed with hypotonic water for the lysis of erythrocytes. Subsequently, the BUC cells were washed and re-suspended in full medium of DMEM. Conditioned medium was harvested after less than 5 passages. The diagnosis of BUC was confirmed by pathology.

Western blotting

BUC cells (5×10^6) were cultured in serum-free DMEM for 24h.The supernatants were collected and then concentrated by centrifugal filter devices.

The supernatants of freshly isolated ex vivo BUC cells were prepared as previously reported (Huang et al., 2014). The procedure of western blot was as reported (Chen et al., 2013a; 2013b; Rong et al., 2013; Huang et al., 2014). The protein concentration was detected by the Bio-Rad Bradford protein assay and 25μg of protein was subjected to SDS-PAGE (12 SDS-acrylamide gel) with a loading buffer containing 80mM Tris-HCl (ph 6.8), 5% SDS, 10% glycerol, 5mM EDTA (ph 8), 5% 2-Mercapto Ethanol, 0.2% Bromophenol blue, and 1mM phenylmethylsulfonyl fluoride. The separated proteins were transferred to PVDF membranes (Bio-Rad) for 2h at 100mA. The membrane was incubated with a DcR3 mouse monoclonal antibody (ab11930, Abcam, Cambridge, CB4 0FL, UK, 1: 1000 dilution,) or a β-actin antibody (A1978 AC-15 1: 2000 dilution, Sigma-Aldrich, St. Louis, NV, USA). Primary antibodies were detected with an HRP-conjugated secondary antibody (1: 4000 dilution, ECL Anti-mouse IgG Peroxidase linked Na 931, Sigma-Aldrich, St. Louis, NV, USA) and finally the membranes were subjected to chemiluminescence detection assay for assessment of expression levels.

Cell lysis sample from known DcR3 positive hepatocellular carcinoma cell line HepG2 was used as a positive control for western blot as previously reported (Huang et al., 2014).

Statistical analysis

SPSS20.0 (Munich, Germany) was used for statistical analysis. Mann-Whitney U test was employed to assess the difference of DcR3 expression between bladder tumor and non-cancer tissues, as well as in corresponding groups of each clinicopathological parameter. Values were presented as the mean ± standard deviation (SD) for Ki-67, PCNA LIs and P53 signals.

The Spearman Test was used to reveal the relationship between the expression of DcR3 and other markers (caspase-3, Bcl-2, VEGF, Ki-67, PCNA and P53). Kaplan-Meier and log-rank test were performed for the survival analysis. Statistical significance was determined at a $p<0.05$ level.

Results

Relationship between DcR3 expression and clinicopathological characteristics in BUC

DcR3 positive expression was found in 61 out of 166 BUC patients (36.7%), significantly higher than in normal bladder tissues (10.7%, 6/56, $p<0.0001$, Figure 1, Figure 2A, Table 1). The positive ratio of DcR3 expression was 43.7% (45/103) in the group of invasive stage T2-T4, significantly higher than that of stage Tα-T1 (25.4%, 16/63, $p=0.018$). Lymph node metastases were found only in 8 of current 166 cases. All of these 8 patients presented DcR3 positive. Fifty-three patients were DcR3 positive among 158 cases without lymph node metastases, with the positive ratio 33.5%, significantly lower than that with lymph node metastases (100%, 8/8, $p<0.0001$, Figure 2B, Figure 2C, Figure 2D). Of 85 followed-up patients, 19 patients had recurrence. The positive DcR3 expression of these 19 patients was 79.0% (15/19), significantly higher than that of those without recurrence (44.9%, 29/66, $p=0.007$). No difference of DcR3 expression was found in the corresponding groups of age, gender, distant metastasis, tumor number, diameter, or growth pattern ($p>0.05$).

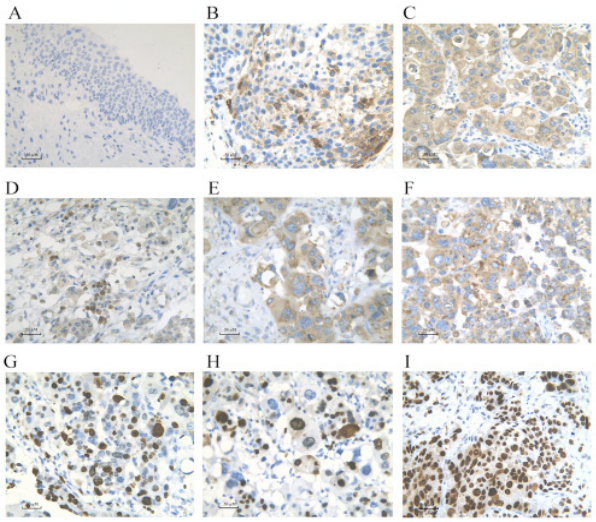

Figure 1. Expression of DcR3 and other Biomarkers in Bladder Urothelial Carcinomas (BUC). DcR3 in **(A)** normal bladder tissues, **(B)** low grade BUC , **(C)** high grade BUC; In high grade BUC **(D)** caspase-3, **(E)** Bcl-2, **(F)** VEGF, **(G)** Ki-67, **(H)** PCNA, **(I)** P53 (immunohistochemistry, ×400)

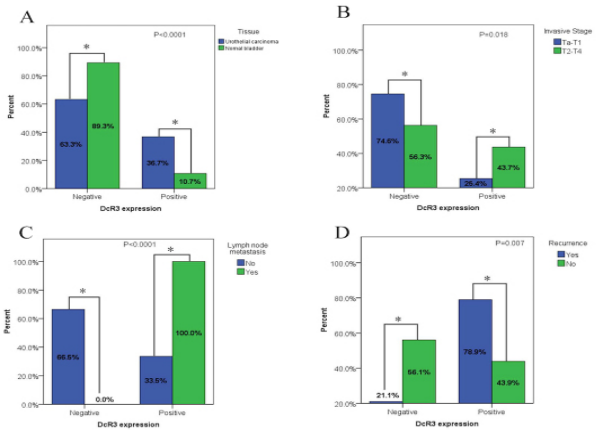

Figure 2. Relationship of DcR3 Expression and Clinicopathological Parameters in Bladder Urothelial Carcinoma. (A) DcR3 expression ratio in different bladder tissues. **(B)** Relationship of DcR3 expression and Invasive stage, **(C)** Lymph node metastasis, **(D)** Recurrence

Relationship between DcR3 expression and other markers

DcR3 expression was significantly higher in the caspase-3 negative group (50.5%, 55/109) than in the caspase-3 positive group (10.5%, 6/57, $p<0.0001$). A negative correlation was found between the expression of DcR3 and caspase-3 (r=-0.393, $p<0.0001$). Furthermore, DcR3 expression levels increased remarkably in the Bcl-2 and VEGF positive groups compared to their negative groups (both $p<0.0001$). There were positive correlations between the expressions of DcR3 and Bcl-2 (r=0.458, $p<0.0001$), also between the DcR3 and VEGF (r=0.408, $p<0.0001$, Table 2).

Table 1. Relationship between DcR3 Expression and Clinicopathological Features

Parameters		Total(n)	Expression of DcR3 n(%) Negative	Positive	*p*
Tissue	Normal bladder	56	50(89.3%)	6(10.7%)	<0.0001
	BUC	166	105(63.3%)	61(36.7%)	
Gender	Female	25	20(80%)	5(20%)	0.06
	Male	141	85(60.3%)	56(39.7%)	
Age	<65	90	54(60%)	36(40%)	0.346
	≥65	76	51(67.1%)	25(32.9%)	
Grade	low	26	21(80.8%)	5(19.2%)	0.35
	high	37	26(70.3%)	11(29.7%)	
Invasive Stage					
	Tα-T1	63	47(74.6%)	16(25.4%)	0.018
	T2-T4	103	58(56.3%)	45(43.7%)	
Lymph node metastasis					
	No	158	105(66.5%)	53(33.5%)	<0.0001
	Yes	8	0(0%)	8(100%)	
Distant metastasis					
	No	161	105(65.2%)	56(34.8%)	0.175
	Yes	1	0(0%)	1(100%)	
Tumor number					
	Single	157	101(64.3%)	56(35.7%)	0.23
	Multiple	9	4(44.4%)	5(55.6%)	
Diameter					
	<3cm	100	67(67.0%)	33(33.0%)	0.219
	≥3cm	66	38(57.6%)	28(52.4%)	
Growth pattern					
	Papillary	129	80(62.0%)	49(38.0%)	0.538
	Solid	37	25(67.6%)	12(32.4%)	
Recurrence					
	No	66	37(56.1%)	29(44.9%)	0.007
	Yes	19	4(21.0%)	15(79.0%)	

Table 2. Relationship between DcR3 and other Markers

Parameters		Total(n)	Expression of DcR3 n (%) Negative	Positive	*p*
caspase-3	Negative	109	54(49.5%)	55(50.5%)	<0.0001
	Positive	57	51(89.5%)	6(10.5%)	
Bcl-2	Negative	98	80(81.6%)	18(18.4%)	<0.0001
	Positive	68	25(36.8%)	43(63.2%)	
VEGF	Negative	103	81(78.6%)	22(21.4%)	<0.0001
	Positive	63	24(38.1%)	39(61.9%)	
Ki-67 LI	low	83	75(90.4%)	8(9.6%)	<0.0001
	high	83	30(36.1%)	53(63.9%)	
PCNA LI	low	91	86(94.5%)	5(5.5%)	<0.0001
	high	75	19(25.3%)	56(74.7%)	
P53	low	96	71(74.0%)	25(26.0%)	0.001
	high	70	34(48.6%)	36(51.4%)	

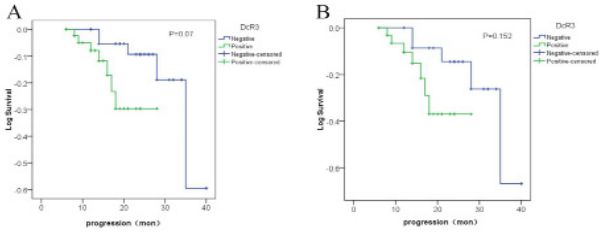

Figure 3. Correlation of DcR3 Expression with Survival. **(A)** Total BUC, **(B)** Invasive BUC

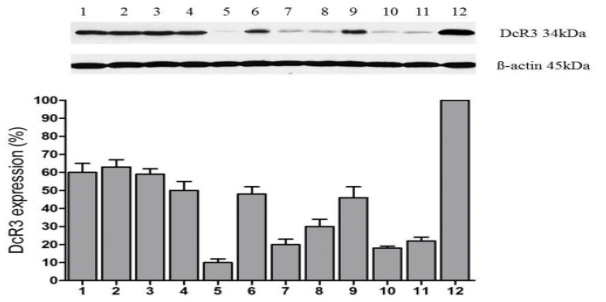

Figure 4. Expression of DcR3 in Supernatants of Cultured Bladder Urothelial Carcinomas (BUC) Cells. **(1)**: BUC cell line T24, **(2)**: HT1376, **(3)**: RT4. **(4-11)**: cells from eight freshly prepared BUC specimens. **(12)**: HepG2 HCC cells (western blot)

DcR3 expression enhanced markedly in the group of high Ki-67 LI compared to the low one (p<0.0001). The PCNA LI and P53 expression had the similar trends (Table 2). Spearman showed positive correlations between the expressions of DcR3 and Ki-67 LI (r=0.695, p<0.0001), PCNA LI (r=0.724, p<0.0001) and P53 (r=0.357, p<0.0001), respectively.

Relationship between DcR3 expression and survival

In the 85 patients with full follow-up information, the survival of the DcR3 negative group (n=41) was 35.57±1.72 months, longer than that of the DcR3 positive group (n=44, 24.52±1.163 months, Figure 3A). However, the difference showed no significance (p=0.07). In the subgroup of invasive BUC (n=59), the survival presented similar trend as above. The survival of the DcR3 negative group (n=25) was 34.43±2.05 months, longer than that of the DcR3 positive group (n=34, 23.76±1.369 months, Figure 3B). However, the difference showed either no significance (p=0.152).

Expression of DcR3 in the supernatants of BUC cells

DcR3 protein was expressed in the supernatants from all 3 cell lines (T24, HT1376 and RT4) tested, without significantly difference between each other (Figure 4). BUC cells were also freshly prepared from 8 BUC specimens and the supernatants were collected before the 5th passage. Soluble DcR3 could be detected in all samples tested by using western blot (Figure 4).

Discussion

In the present study, we established 2 TMAs of BUC and normal bladder epithelial tissues. Then we analyzed the expression of DcR3 and studied its correlation with clinicopathological parameters and various markers, which play vital roles in cell proliferation, apoptosis and angiogenesis.

DcR3 is a member of the tumor necrosis factor receptor superfamily, which locates on the chromosome 20q13.3. The expression of DcR3 protein was detected in various types of malignant tumors by our and other groups, especially in glioma, gastric, breast, liver and pancreatic cancer (Chen and Luo, 2008a; 2008b; Chen et al., 2010; Huang et al., 2014; Wu et al., 2014; Zhou et al., 2014). DcR3 is also closely related to the occurrence, development and prognosis of various malignant tumors (Yang et al., 2010; Toda et al., 2013; Zhou et al., 2013; Wu et al., 2014; Zhou et al., 2014). Moreover, it is absent or lowly expressed in normal tissues except cancerization. However, the role of DcR3 in bladder cancer has not yet been totally clarified. By far, only one research group has reported the relationship between DcR3 expression and urothelial cancers, including bladder, ureteral and renal pelvic cancer (Yamana et al., 2005). By using real-time quantitative PCR and immunohistochemistry, Yamana, et al. (2005) detected the amplification and expression of DcR3 in the tissues and cell lines of urothelial carcinoma (UC) and found a visible gene amplification in one UC cell line (T24) and five cases UC tissues (25%, 5/20). DcR3 expression was detected in UC (90.0%) and normal urothelium (80.7%) with immunohistochemical detection. This was inconsistent with the result from the current study. We found that the positive ratio of DcR3 expression was 36.7%, much lower than what was detected by Yamana et al. (2005). The difference of sample sources, sample sizes and detecting methods may partially lead to the discrepancy. However, even more noticeably low expression of DcR3 was found in the normal bladder epithelial tissues, indicating that the DcR3 protein expression increased in BUC compared to the normal tissues, which is similar to other malignancies. Furthermore, DcR3 protein could be detected in 3 BUC cell lines by using western blot (data not shown). Therefore, DcR3 might act as a proto-oncogene being involved in the process of BUC. DcR3 is a secretory protein, since the amino acid sequence of DcR3 protein lacks of membrane structure. The overexpression of serum DcR3 has also been detected in various types of body fluid of cancers and showed great potential to become a biomarker for the early non-invasive diagnosis for malignant tumors. In the current study, we found the expression of DcR3 in the supernatants of different BUC cell lines, as well as the freshly cultured BUC cells, which indicates the soluble characteristic of DcR3. However, the expression and clinical significance of serum DcR3 in BUC have never been investigated. Further work is warranted.

To explore the relationship between DcR3 expression and clinicopathological parameters in UC, Yamana et al. (2005) found no significant correlation between the expression of DcR3 protein and differentiation, stage or prognosis of UC. Distinct results were found in the current study. DcR3 expression was closely related to muscular invasion, lymph node metastasis and recurrence, which implies that BUC with higher DcR3 expression would progress faster and cause a poorer prognosis. Moreover, we, for the first time, have investigated the role of DcR3

on patient survival. The average survival time in the DcR3 positive group was shorter than in the DcR3 negative group, even it had no significant value, it still suggests that the DcR3 expression could possess the potential to become an indicator of prognosis for BUC. However, a larger cohort with more patients is required to support the current finding.

DcR3 can block LIGHT, FasL and TL1A mediated cell apoptosis and proliferation (Aiba et al., 2013; Fukuda et al., 2013; Toda et al., 2013; Zhou et al., 2013). To further investigate the potential mechanism of DcR3 to influence the biological function of BUC, we compared the DcR3 expression and other common biomarkers, including caspase-3, Bcl-2, VEGF, Ki-67 and PCNA labeling indexes (LIs) and P53. Among these biomarkers, sequential activation of caspase-3 plays a central role in the execution-phase of cell apoptosis (Cheng et al., 2013). The Bcl-2 gene is a proto-oncogene, and it can inhibit the tumor cells apoptosis (Jeon and Yoon, 2012; Kouri et al., 2012; Song et al., 2014). VEGF is a signal protein that stimulates vasculogenesis and angiogenesis (Chuangsuwanich et al., 2014; Nakamura et al., 2014). Ki-67 and PCNA are considered as the classical markers of cell proliferation and are in routine used by pathologists in diagnosis of some malignancies. P53 is the most prominent tumor suppressor, and many types of cancers result from functionally impaired P53 that loses its capability in DNA repairing and cell apoptosis induction (Shin and Kim, 2014; Song et al., 2014). In the current study, we found an inverse correlation between the expressions of DcR3 and caspase-3, confirming that higher DcR3 can block the pathway of apoptosis. This is also supported by the positive relationships between DcR3 and Bcl-2, P53. Additionally, DcR3 also leads to faster cell growth due to the positive correlation between DcR3 expression and Ki-67, PCNA LIs. More interestingly, we also found that DcR3 was related to the expression of VEGF, suggesting a role of DcR3 in prompting the angiogenesis of BUC. The relationship between DcR3 and these aforementioned biomarkers helps to explain its overexpression in the clinical BUC samples. DcR3 could influence the outcome of BUC patients via the related pathways of inhibiting apoptosis, promoting proliferation and inducing angiogenesis.

In conclusions, The current findings suggest the role of DcR3 as an oncogene in the process of carcinogenesis and deterioration of human BUC. The detection of DcR3 protein in the FFPE samples could assist to predict the progression of BUC patients. However, a larger cohort is needed to verify the evidence. DcR3 might influence the related pathways of apoptosis, proliferation and angiogenesis, thus to impact patients outcome. Further *in vitro* and *in vivo* functional experiments are warranted to investigate the mechanism of DcR3 in the malignant phenotype of BUC.

Acknowledgements

The study was supported by the fund of Guangxi Zhuang Autonomous Region University Student Innovative Plan (No. 201410598004), China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the paper.

References

Aiba Y, Harada K, Komori A, et al (2014). Systemic and local expression levels of TNF-like ligand 1A and its decoy receptor 3 are increased in primary biliary cirrhosis. *Liver Int*, **34**, 679-88.

Bracha S, McNamara M, Hilgart I, et al (2014). A multiplex biomarker approach for the diagnosis of transitional cell carcinoma from canine urine. *Anal Biochem*, **455**, 41-7.

Chen G, Luo D (2008a). Expression of decoy receptor 3 in liver tissue microarrays. *Natl Med J India*, **21**, 275-8.

Chen G, Luo D (2008b). Over-expression of decoy receptor 3 in gastric precancerous lesions and carcinoma. *Ups J Med Sci*, **113**, 297-304.

Chen G, Noor A, Kronenberger P, et al (2013a). Synergistic effect of afatinib with su11274 in non-small cell lung cancer cells resistant to gefitinib or erlotinib. *PLoS One*, **8**, 59708.

Chen G, Rong M, Luo D (2010). TNFRSF6B neutralization antibody inhibits proliferation and induces apoptosis in hepatocellular carcinoma cell. *Pathol Res Pract*, **206**, 631-41.

Chen G, Umelo IA, Lv S, et al (2013b). miR-146a inhibits cell growth, cell migration and induces apoptosis in non-small cell lung cancer cells. *PLoS One*, **8**, 60317.

Cheng Q, Blais MO, Harris G, et al (2013). PLGA-carbon nanotube conjugates for intercellular delivery of caspase-3 into osteosarcoma cells. *PLoS One*, **8**, 81947.

Chuangsuwanich T, Pongpruttipan T, P OC, et al (2014). Clinicopathologic features of breast carcinomas classified by biomarkers and correlation with microvessel density and VEGF expression: a study from Thailand. *Asian Pac J Cancer Prev*, **15**, 1187-92.

Fukuda K, Miura Y, Maeda T, et al (2013). Decoy receptor 3 regulates the expression of various genes in rheumatoid arthritis synovial fibroblasts. *Int J Mol Med*, **32**, 910-6.

Huang S, Chen G, Dang Y, et al (2014). Overexpression of DcR3 and its significance on tumor cell differentiation and proliferation in glioma. *ScientificWorld J*, **2014**, 605236.

Jankovic-Velickovic L, Stojnev S, Ristic-Petrovic A, et al (2011). Pro- and antiapoptotic markers in upper tract urothelial carcinoma associated with Balkan endemic nephropathy. *ScientificWorld J*, **11**, 1699-711.

Jankovic Velickovic L, Hattori T, Stefanovic V (2009). Molecular markers in upper urothelial carcinoma associated to Balkan endemic nephropathy. aristolochic acid as the major risk factor of the worldwide disease. *ScientificWorld J*, **9**, 1360-73.

Jeon BS, Yoon BI (2012). Altered expression of cellular Bcl-2 in the progression of hamster cholangiocarcinogenesis. *ScientificWorld J*, **2012**, 385840.

Kouri FM, Jensen SA, Stegh AH (2012). The role of Bcl-2 family proteins in therapy responses of malignant astrocytic gliomas: Bcl2L12 and beyond. *ScientificWorld J*, **2012**, 838916.

Nakamura I, Zakharia K, Banini BA, et al (2014). Brivanib attenuates hepatic fibrosis *in vivo* and stellate cell activation *in vitro* by inhibition of FGF, VEGF and PDGF signaling. *PLoS One*, **9**, 92273.

O'Keeffe MB, Devlin AH, Burns AJ, et al (2008). Investigation of pericytes, hypoxia, and vascularity in bladder tumors: association with clinical outcomes. *Oncol Res*, **17**, 93-101.

Rong M, Chen G, Dang Y (2013). Increased miR-221 expression in hepatocellular carcinoma tissues and its role in enhancing cell growth and inhibiting apoptosis *in vitro*. *BMC Cancer*,

13, 21.
Shin MK, Kim JW (2014). Clinicopathologic and diagnostic significance of p53 protein expression in papillary thyroid carcinoma. *Asian Pac J Cancer Prev*, **15**, 2341-4.
Siegel R, Naishadham D, Jemal A (2012). Cancer statistics, 2012. *CA Cancer J Clin*, **62**, 10-29.
Song HY, Deng XH, Yuan GY, et al (2014). Expression of bcl-2 and p53 in induction of esophageal cancer cell apoptosis by ECRG2 in combination with cisplatin. *Asian Pac J Cancer Prev*, **15**, 1397-401.
Toda M, Kawamoto T, Ueha T, et al (2013). 'Decoy' and 'non-decoy' functions of DcR3 promote malignant potential in human malignant fibrous histiocytoma cells. *Int J Oncol*, **43**, 703-12.
Wu Q, Zheng Y, Chen D, et al (2014). Aberrant expression of decoy receptor 3 in human breast cancer: relevance to lymphangiogenesis. *J Surg Res*, **188**, 459-65.
Yamana K, Bilim V, Hara N, et al (2005). Prognostic impact of FAS/CD95/APO-1 in urothelial cancers: decreased expression of Fas is associated with disease progression. *Br J Cancer*, **93**, 544-51.
Yang M, Chen G, Dang Y, et al (2010). Significance of decoy receptor 3 in sera of hepatocellular carcinoma patients. *Ups J Med Sci*, **115**, 232-7.
Yang MH, Chu PY, Chen SC, et al (2011). Characterization of ADAM28 as a biomarker of bladder transitional cell carcinomas by urinary proteome analysis. *Biochem Biophys Res Commun*, **411**, 714-20.
Zhou J, Song S, He S, et al (2013). Silencing of decoy receptor 3 (DcR3) expression by siRNA in pancreatic carcinoma cells induces Fas ligand-mediated apoptosis *in vitro* and *in vivo*. *Int J Mol Med*, **32**, 653-60.
Zhou J, Song S, Li D, et al (2014). Decoy receptor 3 (DcR3) overexpression predicts the prognosis and pN2 in pancreatic head carcinoma. *World J Surg Oncol*, **12**, 52.