Expression of survivin in bladder cancer cell lines using quantitative real-time polymerase chain reaction

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Abstract

Objective: Previous studies have reported that survivin expression is significantly associated with various malignancies including bladder cancer. However, the relationship between the expression of survivin and the tumor stage and grade of bladder cancer still require further study.

Methods: To determine whether survivin plays a role in the differentiation of bladder cancer cells, we conducted a preliminary study to examine the expression of survivin in bladder cancer cell lines.

Results: In this study, we observed that the gene expression fold changes of survivin ranged from 3.2 to 16.7 in various tumor grades (G1–G4) of bladder cancer cell lines, which were higher than that in normal human urothelial cell line. With the worse differentiation of bladder cancer cell lines, the gene expression fold changes of survivin increased significantly (3.2-fold in RT4, 5.8-fold in 5637, 6.6-fold in T24, and 16.7-fold in HT1197). In addition, we observed different genotypes among various cell lines (C/C in HUC4449, C/G in RT4, C/G in 5637, G/G in T24, and C/C in HT1197). The relationship between survivin/C031 C/G polymorphism and various bladder cancer cell lines was non-significant. However, the overexpression of survivin may be associated with aggressive features of bladder cancer.

Conclusion: Our findings suggest that survivin could be a potential therapeutic target through the inhibition of cell proliferation in bladder cancer.

Keywords: bladder cancer; cell lines; gene expression; polymorphism; survivin

1. Introduction

Survivin is an inhibitor of apoptosis protein involved in anti-apoptosis pathways.1 Survivin is generally expressed in embryonic tissues and in various cancers, but it is almost undetectable in well-differentiated tissues.2 Therefore, survivin not only plays a critical role in carcinogenesis but is also associated with the poor prognosis of various cancers.3−5

Previous studies have reported that survivin expression is significantly associated with various malignancies, including bladder cancer.6,7 Shariat et al.8 observed that a high level of survivin in urine is associated with the high tumor grades of bladder cancer but not with the advanced tumor stages. Other studies have reported that survivin is overexpressed in tumor cells but not in normal urothelial cells.9,10 However, the relationship between the expression of survivin and the tumor stage and grade of bladder cancer require further investigation.

To determine whether survivin plays a role in the differentiation of bladder cancer cells, we conducted a preliminary study to examine the expression level of survivin in bladder cancer cell lines.

2. Methods

The survivin expression in five cell lines, HUC4449 (normal human urothelial cell line), RT4 [human urinary bladder papilloma cell line; tumor grade 1 (G1)], 5637 [human bladder carcinoma cell line; tumor grade 1 (G1)], T24 [human bladder carcinoma cell line; tumor...
polymorphism, the promoter region of the survivin gene, was determined using PCR and real-time PCR. Total RNA was isolated from $1 \times 10^6$ normal human urothelial cells: HUC4449 (ScienCell Research Lab, Carlsbad, CA, USA) and $1 \times 10^6$ cells from four bladder cancer cell lines [Bioresource Collection and Research Center (BCRC), Hsinchu City, Taiwan]: RT4 (BCRC60011), 5637 (BCRC60061), T24 (BCRC60062), and HT1197 (BCRC60059), by using a Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the total RNA (1.5 ug) was then transcribed to cDNA by using Oligo(dT)$_{15}$ primers and an ImPromII reverse transcription system (Promega Corporation, Madison, WI, USA) according to the manufacturers’ instructions.

The expression level of the survivin transcript was determined using real-time PCR and an ABI instrument (ABI7500; ABI, Foster City, CA, USA). The survivin primers were used from position A07 (Unigene Hs.514527) in an RT2 Profiler PCR array system (SABiosciences Corporation, Frederick, MD, USA). The survivin expression level was normalized using a housekeeping gene (GAPDH) as the internal reference to control the loading difference. The following formula was used to calculate the relative gene expression level: 2$^{-\Delta\Delta Ct}$ is $\Delta Ct = Ct$ (GOI) - avg. [Ct (HKG)], where GOI is each gene of interest, and HKG is the housekeeping gene. The fold change (2$^{-\Delta\Delta Ct}$) is the ratio of the bladder cancer cell line to the normal urothelial cell line.

In addition, the –31 C/G polymorphism (rs9904341) located in the promoter region of the survivin gene, was determined using PCR-restricted fragment length polymorphism. For the survivin –31 C/G polymorphism, the specific primers used were 5’-GGTCTCTTAAAGGAGTCTGACG-3’ (sense) and 5’-GGGACTTTTTAAGGAAGTG-3’ (antisense). The PCR conditions were one cycle at 95°C for 5 min; 35 cycles of 95°C for 30 sec, 58°C for 60 sec and 72°C for 30 sec and a final extension at 72°C for 5 min. The genotypes (C/C: 341 bp; C/G: 341 bp, 236 bp, and 105 bp; and G/G: 236 bp and 105 bp) were determined after digestion with EcoO109I (New England Biolabs, Ipswich, MA, USA) at 37°C overnight.

3. Results

Based on our preliminary results, the gene expression fold changes of survivin ranged from 3.2 to 16.7 in various tumor grades of bladder cancer cell lines (RT4, G1; 5637, G2; T24, G3, and HT1197, G4), which were higher than that in normal human urothelial cell line (HUC4449; Fig. 1). With the worse differentiation of bladder cancer cell lines, the gene expression fold changes of survivin ranged from 3.2 to 16.7 in various tumor grades of bladder cancer cell lines, which were higher than that in normal human urothelial cell line.

4. Discussion

Bladder cancer is one of the most common male malignancies in Taiwan. Survivin, an inhibitor of apoptosis protein, has been reported to play a role in carcinogenesis, and is associated with poor clinical outcomes in various cancers. A previous study evaluated the association between the survivin –31 C/G polymorphism and urothelial carcinoma (UC). The major findings were: (1) compared with individuals carrying the C/G genotype of the survivin gene, significantly increased UC risks of 2.8 and 4.0 were observed for those who carried the C/G genotype and those who carried the C/C genotype, respectively; (2) those who have ever smoked and carried the C/G and C/C genotypes of the survivin gene have a significantly higher UC risk of 5.6; and (3) UC cases carrying the C/C genotype of the survivin gene have a significantly higher prevalence of having muscle-invasive or higher-grade tumors, compared with those carrying the C/G genotype. However, the clinical application of survivin in bladder cancer requires further study.

In the present study, we used an in vitro approach to evaluate the expression of survivin in various bladder cancer cell lines. The gene expression fold changes of survivin from 3.2 to 16.7 in various tumor grades of bladder cancer cell lines, which were higher than that in normal human urothelial cell line (Fig. 1). These findings imply that the increasing expression of survivin is
significantly associated with the poor differentiation of bladder cancer cells.

A previous study indicated that the lack of a promoter region in the survivin gene can lead to a deficiency of cell cycle-dependent expression in HeLa cells. Therefore, we also examined the survivin –31 C/G polymorphism in bladder cancer cell lines. Our results did not reveal a significant relationship between survivin –31 C/G polymorphism and various tumor grades of bladder cancer cell lines, possibly because the gene expression level or enzyme activity of survivin is modulated by single nucleotide polymorphisms or epigenetic changes in other susceptible genes.

According to a review of the literature, this is the first in vitro study to investigate the expression of survivin in various bladder cancer cell lines. With the worse differentiation of bladder cancer cell lines, the gene expression fold changes of survivin significantly increased in this study. Although the relationship between the survivin –31 C/G polymorphism and various tumor grades of bladder cancer cell lines was not significant, additional studies with larger sample size are required to clarify the relationship between genotypes and phenotypes in various ethnic groups.

The present study has some limitations. First, we only examined the expression of survivin in several bladder cancer cell lines. Our findings should be further validated by using bladder cancer tumor tissues. Second, we investigated only one polymorphism, which incompletely represents the entire function of survivin gene. More functional polymorphisms of the survivin gene should be included to examine the combined effects on bladder cancer risk. Therefore, the findings of our analyses should be interpreted with caution.

In conclusion, the overexpression of survivin was significantly associated with an aggressive clinical behavior of bladder cancer. These findings suggest that survivin could be a potential therapeutic target through inhibition of cell proliferation in bladder cancer.13–15

Conflicts of interest statement

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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References