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Original article

The decrease of cyclin B2 expression inhibits invasion and metastasis of bladder cancer

Cheng-yong Lei, M.D.[a,1], Wei Wang, M.D.[b,1], Yong-tong Zhu, Ph.D.[c], Wei-yi Fang, Ph.D.[d], Wan-long Tan, Ph.D.[a,*]

[a] *Department of Urology, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, China*
[b] *Department of Pathology, General Hospital of Guangzhou Military Command of PLA, Guangzhou, Guangdong Province, China*
[c] *Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong Province, China*
[d] *Cancer Center, Hospital of Integrated Chinese and Western Medicine, Southern Medical University, Guangzhou, Guangdong Province, China*

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Abstract

Objective: It has been shown that cyclin B2 is commonly overexpressed in many malignant tumors. This study aimed to investigate the potential role of cyclin B2 in bladder cancer.

Method and material: Fixed tissues for immunohistochemistry and fresh tissues for western blotting and quantitative real-time polymerase chain reaction assay were randomly selected from Nanfang hospital. Normal bladder urothelial cell and bladder cancer cell lines was stored in our laboratory, the bladder cancer cells were transfected to develop bladder cancer cell clones expressing decreased cyclin B2 levels, the clones were used for cell growth and metastasis experiments in vitro and in vivo.

Results: Western blot and immunohistochemical analysis both showed that the cyclin B2 protein expression was higher in bladder urothelial carcinoma than in normal bladder mucosa, especially in invasive cancer. Real-time polymerase chain reaction showed that the cyclin B2 messenger RNA expression exhibited the same trend. Results of cell lines experiments also showed higher cyclin B2 expression in cancer cells. In vitro tests the decrease of cyclin B2 expression that had little effect on cell growth and cell cycling according to the MTT assay and the Edu assay, whereas in the Boyden chamber transwell assay, the cyclin B2 low-expressing clones significantly inhibits the cells' invasion and metastatic abilities. This result was consistent with the scratch-wound assay result showing that the target clone needed more time for healing the wound. The in vivo experiment in nude mice produced similar results, the lung and liver target cell metastasis nodules were smaller and less than those of the negative control by the hepatic subcapsular injection assay, and the mice of the target clone group has longer survival time in no intervention observed test.

Conclusion: These results indicate that the cyclin B2 was overexpressed in bladder cancer, and the down-regulation of cyclin B2 expression in bladder cancer greatly inhibits the cell's invasion and metastatic abilities, and it prolonged the survival time of nude mice in vivo.

Keywords: Cyclin B2; Bladder cancer; Invasion; Metastasis; Survival time; Transfection

1. Introduction

Bladder cancer is the most common malignancy in urinary system, the incidence of which has been gradually increasing worldwide in recent years, especially in the developing countries [1]. Although the therapeutic efficacy has been considerably promoted, the recurrence rate of this incurable disease is still high and the prognosis is poor. Many of patients with bladder cancer developed eventually with metastatic complications. Therefore, novel biomarkers or molecular targets for the diagnosis and therapy of bladder cancer are urgently needed.

Generally, cell proliferation, differentiation as well as cell cycle are dysregulated in malignant cells [2,3]. It has been

[1] These authors contributed equally to this work.
* Corresponding author. Tel: +86-20-6164-1762.
E-mail address: twl@smu.edu.cn (W.-l. Tan).

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Table 1
The patients' basic clinical data of the specimen

Group	Age (average)	Male	Female	Biopsy normal	Cancer-adjacent normal tissue	Noninvasive cancer	Invasive cancer	Metastasis	TURBT	Radical cystectomy	Partial cystectomy
Fresh tissue	61.7	39	4	0	16	16	12	0	11	11	6
Fixed tissue	62.7	127	16	7	37	50	42	7	35	54	10

TURBT = transurethral resection of the bladder tumor.

indicated that the aberrant expression of cell cycle–related proteins is closely associated with tumorigenesis and cancer development. For example, the overexpression of cyclin D1, B1, E, and A were often observed in various types of cancers, and they were also well-correlated with the prognosis in some of these cancers [4–9]. Recently, some studies suggested that cyclin B2 expression was also up-regulated in a variety of cancer tissues, including colon, breast, lung, pancreas, and pituitary [10–14]. There were few researches mentioned about the up-regulation of cyclin B2 messenger RNA (mRNA) in patients with bladder cancer and its expression decreased after chemotherapy [15,16]. In our preliminary immunohistochemical assay, the cyclin B2 expression showed no obvious difference between bladder cancer and normal tissue, we further investigated the correlation between bladder cancer and cyclin B2 at the protein and mRNA level in this study.

2. Material and method

2.1. Tumor specimens

A total of 187 patients diagnosed with bladder cancer in the Nanfang Hospital between January 2010 and August 2014 were enrolled in this study. The bladder tumor was categorized according to the TNM staging system. The specimens were harvested after surgery, and immediately frozen in liquid nitrogen or fixed with formalin. The collection of the tissues was approved by the ethics committee of the Nanfang Medical University, and written informed consents were obtained from all patients. The clinical data about patients are summarized in Table 1.

2.2. Cell culture and transfection

Bladder cancer cells (UM-UC-3, EJ and 5637) and normal bladder cell (SV-HUC-1) were stored in our laboratory. The SV-HUC-1 cell was cultivated in F12k/DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), whereas the bladder cancer cells were grown in RPMI-1640 (Gibco, USA).

2.3. Short hairpin RNA transfection

The bladder cancer cells were transfected with a lentivirus containing the cyclin B2 short hairpin RNA (shRNA) labeled with green fluorescent protein (GFP) (Ruibo, China)

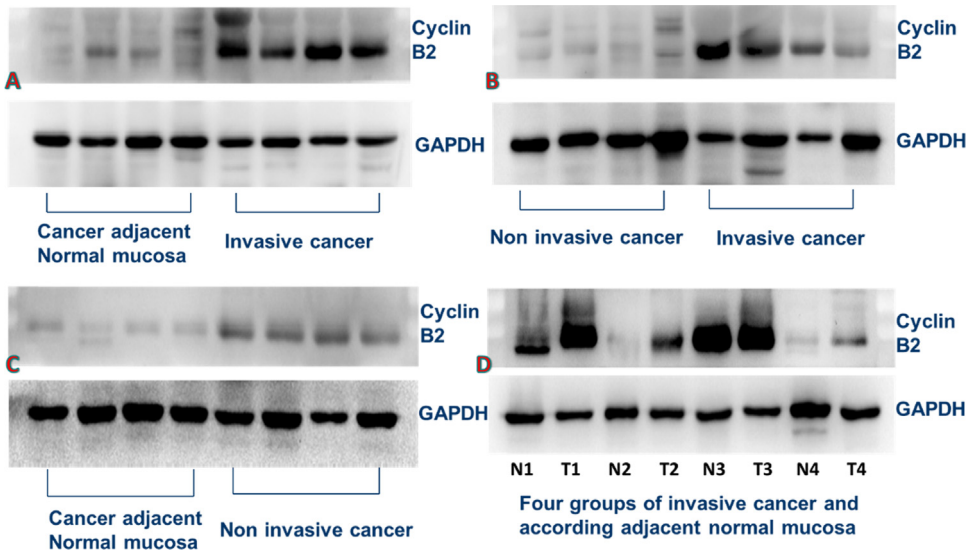

Fig. 1. The cyclin B2 expression in western blot assay. The cyclin B2 protein expression was higher in invasive cancer than in normal bladder mucosa (A); the cyclin B2 protein expression was higher in invasive cancer than in noninvasive cancer (B); the cyclin B2 protein expression was higher in noninvasive cancer than in normal bladder mucosa (C); the cyclin B2 protein expression in 4 cases of invasive cancer (T) and according cancer-adjacent normal mucosa (N). In 3 cases, we can see that the cyclin B2 protein expression was higher in cancer tissues; and only in 1 case, its expression difference was not obvious for the GAPDH was also little strong in cancer-adjacent normal mucosa (D). GAPDH = glyceraldehyde 3-phosphate dehydrogenase.