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Increased expression of *GGN* promotes tumorigenesis in bladder cancer and is correlated with poor prognosis

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ABSTRACT

Bladder cancer has shown great challenge for people's life. Traditional therapeutics against bladder cancer including surgery could not bring much benefit for patients, particularly for the late stage patients. So it is necessary to keep in mind why and how bladder cancer cells survive in our body. In this study, we explored the function and the molecular mechanism of *GGN* gene in bladder cancer. *GGN* was shown to be expressed at a high level in bladder cancer tissues compared to the control and was associated with the unsatisfactory survival rate of patients. *GGN* was also expressed abundantly in bladder cancer cell lines such as T24, 5637 and BIU87. Then *GGN* was knocked down in 5637 cells and T24 cells at both RNA and protein level. In accordance, aberrant growth and proliferation were demonstrated in bladder cancer cells. The ability of migration and invasion of bladder cancer cells was also inhibited. The in vivo data further proved that the xenograft tumor growth was dramatically suppressed by *GGN* knockdown. Then we demonstrated that the level of IκB, bax and truncated caspase3 was upregulated after *GGN* was knocked down in 5637 cells. In contrast, expression level of NFκB, IKK, c-Myc, cyclin D1 and Bcl-2 was reduced. Further, the phosphorylation level of IκB was also downregulated. These data suggest that NFκB/caspase3-mediated apoptosis signaling was regulated by *GGN*. Conclusively, *GGN* played a tumor-promoting role in bladder cancer through regulation of NFκB/caspase3-mediated apoptosis signaling. This study provides a new clue for the treatment of patients with bladder cancer.

1. Introduction

Bladder cancer is one of the most common eight malignant cancers for men in the world, with the incidence rate ranking the fourth while the death rate ranking the eighth (Siegel et al., 2016; Siegel et al., 2017). Bladder cancer could be classified into muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC). NMIBC accounts for about 70% of all bladder cancer and 80% of NMIBC will progress into MIBC (Shimada et al., 2011; Ferreira-Teixeira et al., 2016). To date, the main therapies for bladder cancer are still radical cystectomy, radiotherapy and chemotherapy. But the prognosis of bladder cancer patients is not so satisfactory. About 70% bladder cancer patients would experience recurrence and the mean survival rate for MIBC patients was only 50% while the death rate for NMIBC arrived at 25% (Jin et al., 2014; Dalbagni et al., 2009). According to the newest data reported by the WHO, the estimated new cases will be 79,030 and the new deaths will be 16,870 in 2017 (Siegel et al., 2017). Therefore, it is in no time to try our best to develop new drugs for bladder cancer patients.

Almost all kinds of cancer could be attributed to multiple factors and elucidation of the major factor in particular cancer is an important way to develop new drugs against cancer. For example, antibody against PD-1 was approved by FDA in 2014 for clinic trials in metastatic and late stage bladder cancer patients (Kim, 2016). But how about the signaling network in bladder cancer and which is the dominant one? Human gametogenetin (*GGN*) is a sperm-specific gene and is located at chromosome 19q3.2. Human *GGN* was expressed in human testis and ovary (Jamsai et al., 2011). And the coding sequence of *GGN* showed some certain variance between the fertile and infertile Australian male, which suggested its potential role in spermatogenesis and male

Abbreviations: GGN, gametogenetin; QRT-PCR, quantitative real-time PCR; BrdU, bromodeoxyuridine; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; CRISP2, cysteine-rich secretory protein 2; FANCL, Fanconi anemia complementation group L; FA, Fanconi anemia; FACS, Flow cytometry analysis; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, inhibitor of NFκB; IKK, inhibitor of nuclear factor kappa-B kinase

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Table 1
Primers for qPCR of *GGN* and β-actin.

Gene	Primer	Sequence (5 to 3)	Product length
GGN	g-fp	CCATCTCTTACGCCGAGGTC	215 bp
	g-rp	CCAGTCGAATTTGGGCTTCG	
β-actin	a-fp	AGCTACGAGCTGCCTGACG	239 bp
	a-rp	GTGATCTCCTTCTGCATCCTGT	

Table 2
shRNA fragments designed for knockdown of *GGN*.

shRNA	Sequence (5 to 3)
shGGN-1	GGCAACTATCCGTGAAGGACA
shGGN-2	GCCCAAATTCGACTGGGTTAG
NC	TGCATTCTAAGCCATTCATGCA

infertility (Jamsai et al., 2011). In a yeast two-hybrid experiment, human *GGN* was shown to interact with cysteine-rich secretory protein 2 (CRISP2) (Jamsai et al., 2008a). CRISP2 is an ion channel regulator and is also enriched in human testis (Jamsai et al., 2008a). *GGN* is a conserved gene in human and mouse. Mouse *GGN*1 is also a sperm-specific gene and shares 69% amino acids homology with human *GGN*. Mouse *GGN*1 was reported to bind to CRISP2 and activate both MAPK signaling and NFκB signaling through MAT3K11 (Jamsai et al., 2008b; Zhang et al., 2005). In addition, mouse *GGN*1 could also interact with

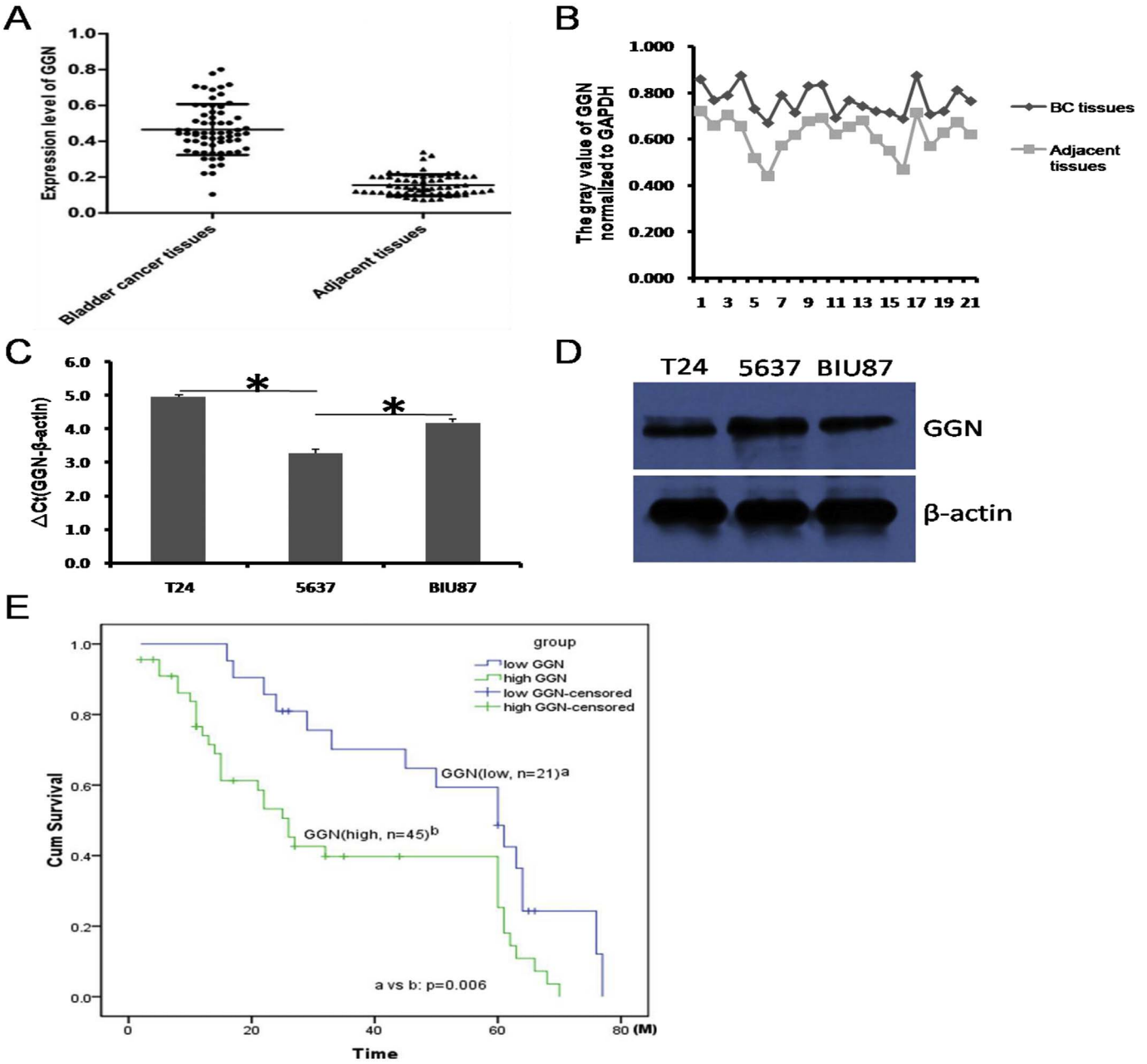

Fig. 1. *GGN* was clinically correlated with bladder cancer.
(A and B) The mRNA and protein level of *GGN* in bladder cancer tissues and adjacent normal tissues. The mRNA expression of 66 tumor tissues and adjacent tissues were determined by qRT-PCR. The protein level was detected with western blot method and the grey value of each band was quantified by Photoshop software. (C and D) The mRNA and protein level of *GGN* in bladder cancer cell lines including T24, 5637 and BIU87. *GGN* level in 5637 cells was the highest. (E) *GGN* expression level was associated with 5-year survival rate of bladder cancer patients. The fold change of *GGN* mRNA (tumor tissue/adjacent normal tissue) ≤2.5 was considered as low *GGN* expression while > 2.5 indicated high *GGN* expression. *P < 0.05.