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GPX2 promotes the development of bladder cancer with squamous cell differentiation through apoptosis control.

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Introduction

Nowadays, there is some evidence suggesting that oxidative stress induced by risk factors was concerning the carcinogenesis of bladder. And recently, there has been increased interest in research as to the role of oxidative stress and the status of antioxidant agents in carcinogenesis, and as to the mechanisms of antioxidant materials for the therapeutic targets for cancer prevention or prognostic biomarkers. Glutathione redox system is most important antioxidant agent that protects intracellular damage induced by oxidative stress in human body. And glutathione peroxidase 2 (GPX2), a selenoprotein and a member of glutathione peroxidase family, is a key enzyme of glutathione redox system, and mainly expressed in cytosol of mammary tissue and gastrointestinal tract, and in human, also expressed in liver. GPX2 reduces hydrogen peroxide and superoxide and is considered to play a major role in antioxidant defense system, moreover, has been suggested to protect against oxidative damage from food.

Therefore, in this study, we elucidated the proliferation role and prognostic significance of GPX2 in bladder cancer.

Material and methods

i. Six-week-old male F344 rats were given either 0.05% BBN [N-butyl-N(4-hydrobutyl) nitrosamine] in drinking water or 0.001 to 0.1% PEITC (Phenyl isothiocyanate) in their diet for 36 weeks. Each group consisted of 18 rats. Bladder tissue samples were collected from each animal for histopathological and protein expression.

ii. GPX2 small interfering RNA (siRNA) and negative control siRNA (NC) transfected BC31 cells (3×10^4) diluted in a buffered solution were subcutaneously implanted into nude mice. The tumor size was calculated weekly. Three weeks after implantation, the mice were sacrificed.

iii. Using radical cystectomy specimen, GPX2 expression was analyzed in immunohistochemical analysis.

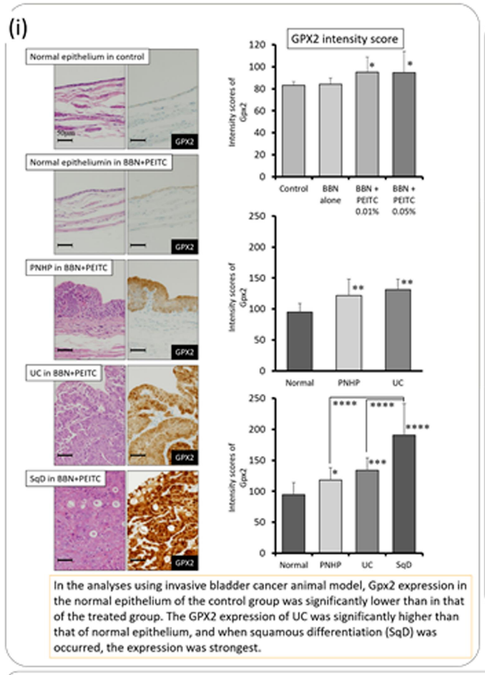
iv. GPX2 and antigen KI-67 (KI67) protein expression was analyzed by immunohistochemistry using BZ analyzer KEYENCE software in human transurethral resection specimens (Ta, T1 low grade UC: 25 cases Ta, T1 high grade UC: 144 cases). In addition, the correlation between the GPX2 expression and prognosis was also analyzed.

v. The rat bladder cancer cell line, BC31, and human cell lines, T24, RT4, TCC-SUP, and 5637, were used for mRNA and protein expression investigations by RT-PCR and western blotting, respectively. siRNA or NC were used to transfect 3×10^5 RT4 (v-a) and 3×10^4 BC31 cells (v-b). After transfection, we investigated the proliferation rates and ROS levels by employing cell counts, DCFH assay, western blotting, and flow cytometry.

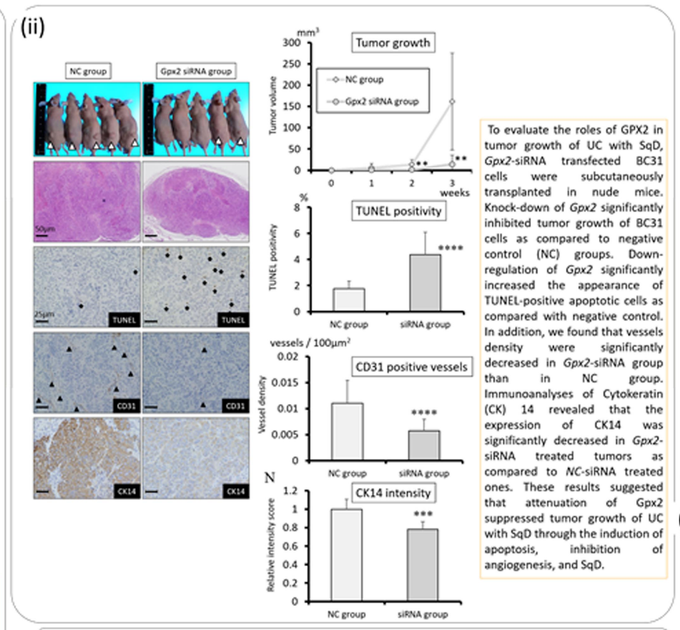
Discussion

Gpx2 overexpression was more marked in UC with squamous differentiation (SqD) than in pure UC. Clinical intraepithelial lesion of papillary UC, and invasive UC with SqD also had strong GPX2 expression in human radical cystectomy specimen. In addition, prognostic analyses using transurethral specimen revealed that low expression levels of GPX2 predicted poor prognosis in patient with pure UC. *In vitro*, UC cell lines, BC31 and RT4 also overexpressed GPX2. Knock-down of GPX2 induced significant inhibition of reactive oxygen species (ROS) production in BC31 cells. And GPX2 silencing induced significant growth inhibition, and increased apoptosis with activation of caspase 3 or 7 expressions both in BC31 and RT4 cells. Interestingly, tumor growth of BC31 cells subcutaneously transplanted in nude mice was significantly inhibited with induction of apoptosis, inhibition of angiogenesis, and SqD by Gpx2 down-regulation. Our findings demonstrated that GPX2 plays important roles in bladder carcinogenesis through the regulation of apoptosis against intracellular ROS, and may be considered as a novel marker or therapeutic target in bladder cancer.

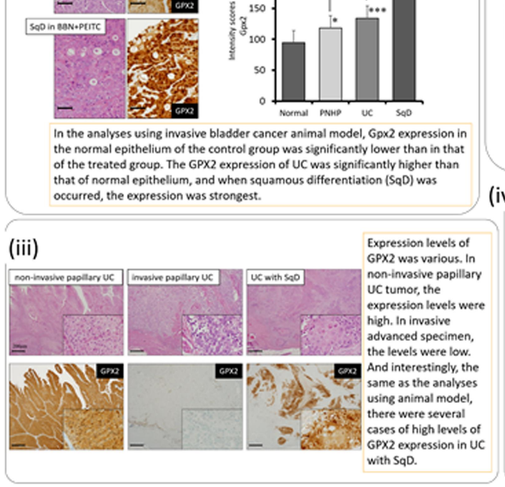
Results



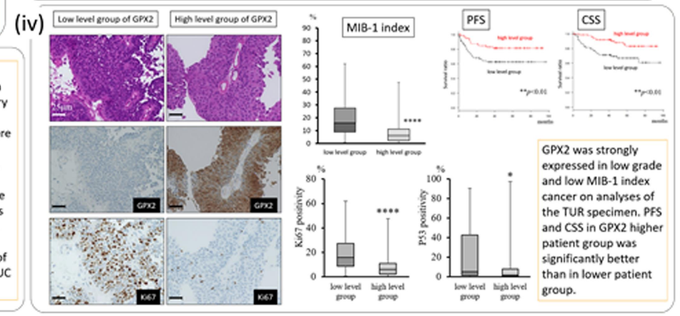
In the analyses using invasive bladder cancer animal model, Gpx2 expression in the normal epithelium of the control group was significantly lower than in that of the treated group. The GPX2 expression of UC was significantly higher than that of normal epithelium, and when squamous differentiation (SqD) was occurred, the expression was strongest.



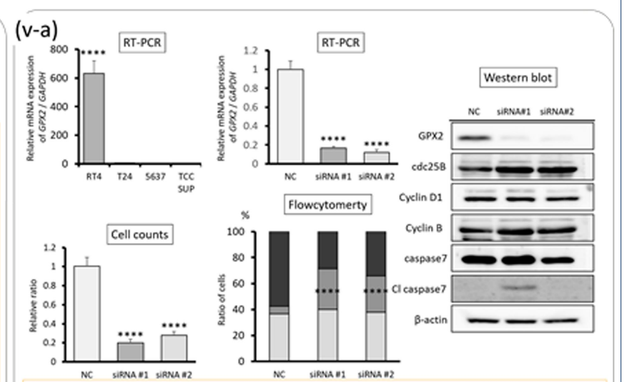
To evaluate the roles of GPX2 in tumor growth of UC with SqD, *Gpx2*-siRNA transfected BC31 cells were subcutaneously transplanted in nude mice. Knock-down of *Gpx2* significantly inhibited tumor growth of BC31 cells as compared to negative control (NC) groups. Down-regulation of *Gpx2* significantly increased the appearance of TUNEL-positive apoptotic cells as compared with negative control. In addition, we found that vessels density were significantly decreased in *Gpx2*-siRNA group than in NC group. Immunoanalyses of Cytokeratin (CK) 14 revealed that the expression of CK14 was significantly decreased in *Gpx2*-siRNA treated tumors as compared to *NC*-siRNA treated ones. These results suggested that attenuation of Gpx2 suppressed tumor growth of UC with SqD through the induction of apoptosis, inhibition of angiogenesis, and SqD.



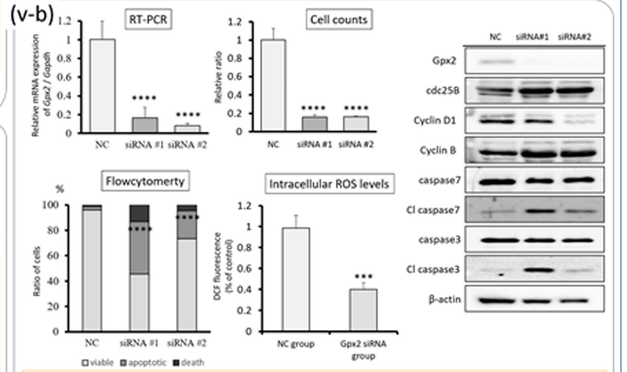
Expression levels of GPX2 was various. In non-invasive papillary UC tumor, the expression levels were high. In invasive advanced specimen, the levels were low. And interestingly, the same as the analyses using animal model, there were several cases of high levels of GPX2 expression in UC with SqD.



GPX2 was strongly expressed in low grade and low MIB-1 index cancer on analyses of the TUR specimen. PFS and CSS in GPX2 higher patient group was significantly better than in lower patient group.



Knock-down of GPX2 by two kinds of siRNA in RT4 was confirmed by quantitative RT-PCR (qRT-PCR). Cell proliferation of RT4 cells was significantly suppressed by *GPX2*-siRNA treatments, but not by negative control (NC) siRNA treatment. Treatment with *GPX2*-siRNA resulted in a marked induction in the expression of cleaved caspase 7, while no changes in the expression of cell cycle related proteins were observed. Flow cytometory analysis was performed, and we found that *GPX2*-siRNA treated RT4 cells appeared to significantly accumulate in the apoptotic phase. These results suggested that GPX2 is involved in maintenance of cell proliferation by protection against caspase-dependent apoptosis in RT4 cells.



Knock-down of Gpx2 by two kinds of siRNA in BC31 cells revealed the attenuation of cell growth via caspase dependent apoptosis. Furthermore, DCFH assay revealed that intracellular ROS levels were significantly decreased in the *Gpx2*-siRNA treated group as compared to *NC*-siRNA treated groups