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 reductase system is the 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 reduct system, and mainly expressed in cytosol of mammary tissue and gastrointestinal tract, and in human to express 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

1. Six-week-old male F344 rats were given either 0.05% BBN (N,N-dimethyl-N-(4-hydroxybutyl) nitrosamine), in drinking water or 0.001 to 0.01% PEITC (Phenyl isothiocyanate) in their diet for 36 weeks. Each group consisted of 30 rats. Bladder tissue samples were collected from each animal for histopathological and protein expression.

2. GPX2 small interfering RNA (siRNA) and negative control (NC) transfect BC31 cells (3×10⁶) 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.

3. Using retrovirus system, GPX2 expression was analysed in immunohistochemical analysis.

4. GPX2 and antigen Ki-67 (KI-67) protein expression was analysed by immunohistochemistry using B2 antibody. KI-67 immunohistochemical analyses were performed using a rabbit polyclonal antibody against KI-67 (1:200). A 5-μm-thick section was prepared from the formalin-fixed, paraffin-embedded tissue of each specimen. The slides were stained using standard avidin-biotin peroxidase methods. Slides were counterstained with hematoxylin.

Discussion

GPX2 overexpression was more marked in UC with squamous differentiation (SqD) than in pure UC. Clinical intravesical lesion of papillary UC, and invasive UC with SqD also had strong GPX2 expression in human UC histocytological 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 RTA 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 RTA 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.