

Peroxiredoxin 6 highly expressed in human cervical squamous cell carcinoma

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Abstract: The aim of this paper is to study the relativity between oxidative stress and cervical squamous cell carcinoma (CSCC) . Cervical cancer and healthy crowd cervical tissue were collected, and their content of Peroxiredoxin 6 (PRDX6) was measured and compared with Western Blot. PRDX6 was highly expressed in human CSCC tissue compared with healthy control, indicating that PRDX6 is a sensitive oxidative stress factor for the detection of cervical cancer.

Published by www.inter-use.com. Available online November 21 2014, Vol. 2 Issue 6, Page 32-35.

Keywords: Peroxiredoxin 6; oxidative stress; cervical squamous cell carcinoma

1. Introduction

Cervical cancer is the second most common cancer leading to the death of women worldwide. One of the main causes of this high prevalence of this cancer is the lack of awareness in women for its early detection and management. Cervical cancer is a multi-factorial disease process, and the infection with the human papilloma virus (HPV) has been suggested as the major risk factor. Other risk factors also have significant role in disease initiation, including early age at first intercourse, multiple sex partners and low socioeconomic status [1]. Several studies have reported that cervical cancer can also be associated with the impaired level of antioxidants due to the oxidative stress.

Oxidation is the most common biological and energy producing reaction. The body's own defense mechanism plays a crucial role to control the levels of the free radicals to prevent the harmful oxidative damage. The level of these antioxidants or function of the antioxidant enzyme systems in the body gets impaired due to the oxidative stress, which can damage DNA and proteins and induce various pathological conditions including cancer. A few articles have reported the alteration of antioxidant system in cervical cancer tissue [2]. The deficiency of antioxidants is due to the increased utilization as well as the sequestration of antioxidants by tumor cells, which indicates the impaired

level of serum antioxidants responsible for the pathogenesis of cervical cancer.

Peroxiredoxins (PRDXs) destroy peroxides, a reactive cysteine in the peroxiredoxin active site, and they are weakly oxidized by the destroyed peroxides. Six mammalian isoforms of PRDXs have been identified, and their roles in cellular redox regulation are individually different. Peroxiredoxin 6 (PRDX6) is a bifunctional 25 kDa protein with both Glutathione (GSH) peroxidase and phospholipase A2 activities. PRDX6 is expressed in many organs, and it is an important antioxidant enzyme and has a major role in lung phospholipid metabolism, whereas antisense treatment resulted in oxidant stress and apoptosis [3]. PRDX6 functions in antioxidant defense mainly by facilitated repair of damaged cell membranes through reduction of peroxidized phospholipids. Inhibition of phospholipase A2 activity results in alterations of lung surfactant phospholipid synthesis and turnover. However, the role of PRDX6 in cervical cancers remains unknown. In this study, the relativity between oxidative stress and cervical squamous cell carcinoma was analyzed and the potential application of PRDX6 as a biomarker was discussed for the detection of early stage of cervical cancer.

2. Material and Methods

2.1 Patients and the control groups

The cervical squamous cell carcinoma (CSCC) patients were histopathologically diagnosed and recruited from March to May in 2014 at the first affiliated hospital of Chongqing medical university (Chongqing, China), without the restrictions of age and sex. The exclusion criteria included previous cancer, metastasized cancer and family history of cancer. Cancer-free controls, having no history or family history of cancer and other genetic disease, were recruited from individuals who visited the same hospital for physical examination at the same time and were frequency matched to the cases on age, gender and residential area (urban or countryside). Totally, 40 incident cancer cases and 40 controls were genotyped in the current study. All the participants were genetically unrelated, ethnic Han Chinese.

2.2 Western blot test

CSCC tissues and paired adjacent esophageal tissue (at least 5 cm distal from primary tumor mass) were obtained during surgical resection. After excision, sample tissues were frozen immediately at -80° and stored until use. Total proteins, isolated from cancer and adjacent normal tissues using RIPA buVer, were separat-

ed on 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. After being blocked for 1 h with the Tris/NaCl containing 5% milk powder, the membranes were probed with speciWc antibodies against PRDX 1, 2, 6 (Abfrontier, Seoul, Korrea) and GAPDH (Kangcheng Corp., Shanghai, China). Following washing, the blots were incubated for 2 h with horseradish peroxidase labeled anti-goat/rabbit IgG (Zhongshan Corp., Beijing, China). The band of each protein was visualized by using the enhanced chemiluminescence system (Pierce, Rockford, IL, USA). The protein expression of each sample was normalized by that of GAPDH with Quantity One software.

2.3 Statistical analyses

Hardy-Weinberg equilibrium was tested by a chi-square test to compare the observed genotype frequencies to the statics.

3. Results

PRDX6 protein was expressed obviously in CSCC group by western blot test with the level of 5.27 ± 1.81 , while for the control group the protein was expressed few with the level of 3.58 ± 0.47 (Fig. 1). The expression of PRDX6 in CSCC group is significant higher than the control group ($P < 0.05$).

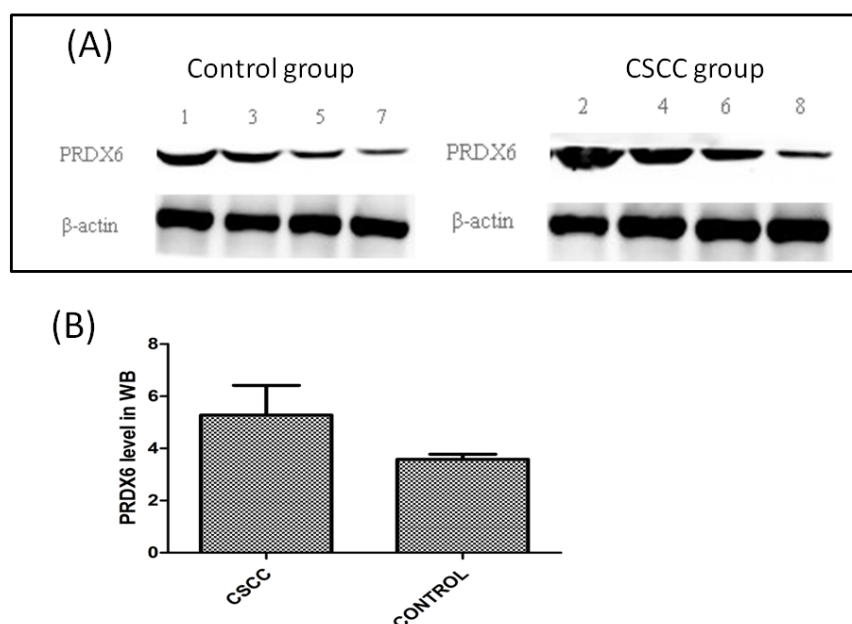


Figure 1. PRDX6 expression in human cervical squamous cell carcinoma (CSCC) and control groups. For (A) the western blot bands, 1,3,5,7 and 2,4,6,8 indicated randomly number in each group; (B) Western blot data was quantified from (A).

4. Discussion

PRDXs represent a superfamily of Se (selenium) independent peroxidases and some studies have showed a significant relationship between the expression of PRDXs and cervical cancer. Pak JH and Chang XZ [4, 5] reported that PRDX6 were over-expressed in cisplatin-induced apoptosis in human ovarian cancer and breast cancer cells, suggesting that PRDXs had a proliferative effect and might be related to cancer development or progression. Huang WS [6] demonstrated that enhanced PRDX6 expression was strongly associated with colorectal cancer development. Furthermore, over-expression of PRDX6 was also found [7] in malignant mesothelioma of the lung.

Increased PRDX levels in tumors would be that they were the results of responding to impending oxidative stress and that antiapoptotic feature of

PRDXs provided a growth advantage to tumor cells [8]. It was probable that PRDXs were able to inhibit reactive oxygen species (ROS)-mediated physiological apoptosis, causing abnormal proliferation and thereby leading to tumorigenesis. However, for the deregulation of PRDXs in some malignant cells, a lack of PRDXs lead to an accumulation of ROS, which promoted carcinogenesis in all its stages, for example initiation, promotion and progression [9].

Some groups have reported about the alteration of antioxidant system in cervical cancer tissue. Maldonado PA [10] reported decreased activities of antioxidant enzymes in the erythrocytes of cervical cancer patients, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Naidu MS [11] demonstrated the lowered concentrations of GSH and vitamin E, and decreased activity of CAT in erythrocytes of cervical cancer patients. Grace Nirmala J [12] observed low levels of GSH, GPx, vitamin E, and vitamin C and decreased activity of GST and SOD in the circulation of cervical cancer patients. Beevi SS [13] indicated impaired level of serum antioxidants responsible for the pathogenesis of cervical cancer. The deficiency of antioxidants may be due to the increased utilization as well as the sequestration of antioxidants by tumor cells.

Some molecules can damage the cellular components by exerting oxidative stress [14], including ROS like hypochlorous acid, free radicals like the superoxide

anion, hydrogen peroxide, and lipid eroxides. Oxidation is the most common biological and energy producing reaction, and the level of these antioxidants or function of the antioxidant enzyme systems in the body gets impaired due to the oxidative stress, resulting in the damage of DNA and proteins and causing various pathological conditions, such as cancer [15]. Our experiment showed PRDX6 was highly expressed in cervical cancer tissue, demonstrating the recent advances in research on the association of cervical cancer pathogenesis with the defects in antioxidant systems.

5. Conclusion

We identified upregulation of PRDX6 in human CSCC compared with their parental tissues. These results confirmed our previous hypothesis that PRDX6 expression contributes to cellular invasive and metastatic potential. PRDX6, which reduced intracellular peroxides as a novel kind of antioxidant protein, was extensively expressed in various types of cancers and was thought as a CSCC biomarker.

Acknowledgements

This project was funded by Chongqing City Health Bureau key item: (2013-1-002); Chongqing City Health Bureau (2011-2-079); Chongqing CityYuzhong District Commission: (20120221).

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