RESEARCH ARTICLE

Expression of PGDH Correlates with Cell Growth in Both Esophageal Squamous Cell Carcinoma and Adenocarcinoma

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Abstract

Esophageal cancer represents the fourth most common gastrointestinal cancer and generally confers a poor prognosis. Prostaglandin-producing cyclo-oxygenase has been implicated in the pathogenesis of esophageal cancer growth. Here we report that prostaglandin dehydrogenase, the major enzyme responsible for prostaglandin degradation, is significantly reduced in expression in esophageal cancer in comparison to normal esophageal tissue. Reconstitution of PGDH expression in esophageal cancer cells suppresses cancer cell growth, at least in part through preventing cell proliferation and promoting cell apoptosis. The tumor suppressive role of PGDH applies equally to both squamous cell carcinoma and adenocarcinoma, which enriches our understanding of the pathogenesis of esophageal cancer and may provide an important therapeutic target.

Keywords: Prostaglandin hydrogenase - esophageal cancers - cell growth suppressor

Asian Pac J Cancer Prev, **16 (3)**, 997-1000

Introduction

Esophageal cancers represent the fourth most common gastrointestinal cancer and rank among the ten most common cancers worldwide (Siegel et al., 2011; Dabrowski et al., 2012; Silva et al., 2012). During the past two decades, the incidence of squamous cell cancers has decreased, although the incidence of adenocarcinoma of the esophagus is continuing to increase rapidly (Gonzalez et al., 2012).

Although many patients undergo neoadjuvant combined-modality therapy (chemotherapy and radiation) followed by surgery, the five-year mortality rate is still low compared to other cancers (Fedeli et al., 2012; Guo and Li, 2012; Song et al., 2012). Newer and more effective chemopreventive agents are thus needed to better prevent esophageal cancer (Garewal et al., 1992).

Cyclooxygenase-2 (COX-2), the enzyme leading to prostaglandin synthesis, is over-expressed in esophageal cancer and other gastrointestinal cancers and generally not expressed in normal epithelium (Shan et al., 2012; Wang et al., 2012; Zhang et al., 2012; Castro-Sanchez et al., 2013; Lee et al., 2013). Therefore, Cox-2 specific inhibitors have been developed to target on malignant tissue growth and have achieved clinical benefit, although the cardiovascular side-effects associated with their use have greatly hampered their popularity (Doll et al., 2012; Ho et al., 2012; Katkoori et al., 2012). However, the definitive chemopreventive benefit derived from use of Cox-2 specific inhibitors confirms that prostaglandin pathway can serve as a valid target for chemoprevention. In this way, the question remains how to better target prostaglandin pathway for chemoprevention.

Prostaglandin hydrogenase (PGDH) is the key enzyme that is responsible for prostaglandin degradation in human body (Thill et al., 2009; Thill et al., 2010). PGDH catalyzes the conversion of the 15-dydroxyl group on major prostagnadins such as PGE2 or PGF2alpha to 15-keto group, which greatly diminished their affinity to prostaglandin receptors (Walker and Eisen, 1979; Thill et al., 2010). Mutation of PGDH was associated with high level of prostaglandin E2 and congenital osteoarthricular hypertrophy (cite) (Thill et al., 2010; Young et al., 2013). Furthermore, high physiological level of PGDH was found in various normal human epithelial tissue, including lung (Ding et al., 2005; Tai et al., 2007; Li et al., 2014), colon (Chi et al., 2009; Roberts et al., 2011), stomach and breast (Brocklehurst et al., 1986). The expression of PGDH in normal human tissue helps control of the level of prostaglandins, which are produced by protumorigenic enzymes including cyclooxygenase-2. Recently, marked down-regulation of PGDH expression was found in various cancer, e.g. colon cancer (Chi et al., 2009; Roberts et al., 2011), lung cancer (Ding et al., 2005; Tai et al., 2007), gastric cancer and prostate cancer. Loss of PGDH expression contributes to cancer growth, which was supported by the fact that PGDH knockout mice develop colon cancer (Chi et al., 2009; Roberts et al., 2011). However, it is not known whether PGDH is expressed in normal esophageal epithelium and whether it plays a role in esophageal carcinogenesis.

Here we present data that demonstrates PGDH is lost

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in esophageal cancer in comparison to normal esophageal epithelium and when re-expressed, suppresses cancer cell growth.

Materials and Methods

Cell lines and culture conditions

Established esophageal squamous cell carcinoma cell line (OE21) and adenocarcinoma cell line (OE33) were initially obtained from Sigma-Aldrich and mainained in RPMI1640 medium, supplemented with 10% fetal calf serum (Gibco). Paired esophageal cancer tissue and adjacent normal tissue were obtained from pathology department of Qilu hospital Shandong University.

Realtime PCR

RNA from tissue and cell lines were purified using Rneasy mini kit (Qiagen, USA). Realtime PCR assay of PGDH expression is performed as described previously. Briefly, PCR products of PGDH transcripts with primers 5'-TGCTTCAA AGCATGGCATAG-3' and 5'-AACAAAGCCTGGACAAATGG-3' was recognized by a fluorogenic hybridization probe 5'FAM-CTCAGCAGCGTTGGCTGCTAATCTTA-BHQ-3' in an Icycler optical module (Bio-Rad). Thermal cycling was initiated at 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. β-2-Microglobulin (B2M) was amplified and detected by using human B2M TaqMan predeveloped assay reagents (Applied Biosystems), with PCR initiated at 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. The level of 15-PGDH RNA was determined as the ratio of 15-PGDH/B2M=2 exp (CTB2M-CTPGDH).

Western blot analysis

Cells were washed in cold PBS and harvested by trypsinization and resuspeneded in cold RIPA buffer with addition of protease inhibitor cocktail (Sigma-Aldrich). Equal concentrations of proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane (Perkin Elmer, MA) and blocked in 5% non-fat milk in Tris buffered saline and probed with rabbit polyclonal anti-human PGDH antibody (Cayman Chemical) and horseradish peroxidase-conjugated secondary antibody. After development, membranes were reprobed with anti-actin antibody (Sigma).

Tetracycline inducible PGDH expression

PGDH transcript was amplified from reverse transcribed human RNA and cloned into pcDNA4 series of vector from T-rex system (invitrogen). pcDNA6-TR and pcDNA4-PGDH was co-trasnfected to esophageal cancer cell lines at ratio of 6:1 and stable clones were selected with zeocin and blasticidin. Each single clone was screened for inducible PGDH expression following tetracycline treatment at 1ug/ml.

Colony formation Assay

Cells were seeded at 10,000 cells per well and 6 hours later were treated with tetracycline or control. Medium is changed every two days. Colonies were stained with 1%(w/v) methylene blue in methanol and counted.

Results

PGDH has been shown to be downregulated in multiple cancers versus their normal epithelial counterpart. We examined the PGDH expression at RNA and protein level in esophageal cancer and normal esophageal epithelium. Half of the cancer samples we examined are histologically adenocarcinoma and the other half are squamous carcinoma. In either case, adjacent normally appearing epithelium were peeled off, examined microscopically to confirm its histology and extracted for RNA and protein. Total RNA from cancer and normal tissue was reversed transcribed and PGDH level is determined with

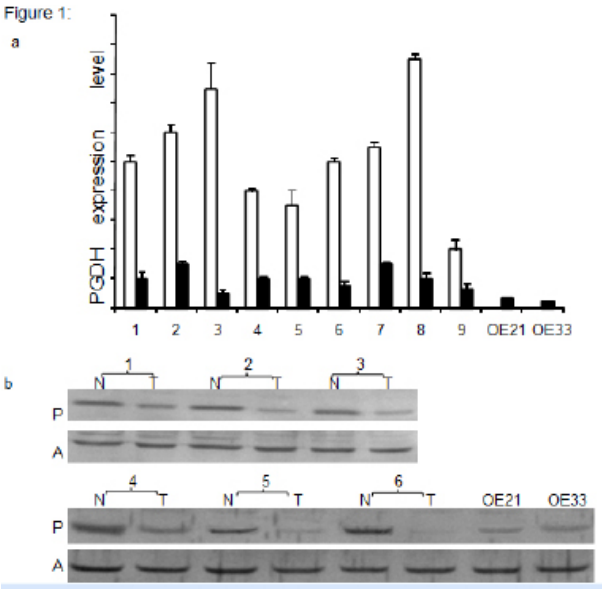

Figure 1. loss of PGDH expression in esophageal cancer a: realtime PCR analysis of PGDH expression in normal esophageal epithelium and paired esophageal cancer and two colon cancer cell lines. 1-9 represents ten patients. 1-5: squamous cell carcinoma; 6-9: esophageal adenocarcinoma. White bar: normal esophageal epithelium. Black bar: paired esophageal cancer. b: western blot analysis of PGDH expression in normal esophageal epithelium and paired esophageal cancer and two esophageal cancer cell lines. 1-6 represent samples from 6 patients. N: normal esophageal epithelium. T: paired esophageal cancer. P: PGDH A: Actin

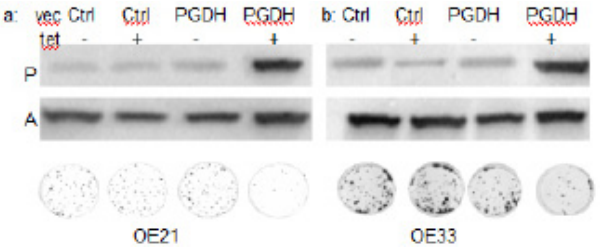

Figure 2. Reconstitutions of PGDH expression suppress cancer cell growth a: western blot analysis of PGDH expression in two esophageal cancer cell clones (OE21 and OE33) induced to express PGDH only in the presence of tetracycline. P: PGDH A: actin b: colony formation assay of esophageal cancer cell growth with or without PGDH induced for expression

quantitative realtime PCR. In all normal esophageal epithelium, there is a strong expression of PGDH, which is markedly reduced in paired esophageal carcinoma, as shown in Figure 1a. The loss of PGDH expression is seen in both squamous cancer and adenocarcinoma.

We further examined the expression of PGDH at protein level. Total protein was extracted from tumor and paired normal epithelium and analyzed with western blot. Similar to the RNA analysis, PGDH protein is expressed strongly in normal tissue, but lost in paired cancerous tissue. This loss is seen in both adenocarcinoma and squamous cancer, as shown in Figure 1b.

To determine whether the loss of PGDH expression functionally contributes to tumor growth, we examined how tumorigenicty of esophageal cancer cell lines may be affected by reconstitution of PGDH expression. OE21 and OE33 were derived from squamous esophageal cancer and esophageal adenocarcinoma respectively and lack PGDH expression (Figure 1b). The two cell lines were transfected with tet-on vector system into which PGDH expression is induced only when tetracycline is added to culture medium. Figure 2a showed stable cell clones expressing PGDH at level comparable to PGDH level in normal esophageal epithelium only when tetracycline is added.

The overall tumorigenicty of esophageal cancer cells expressing PGDH or control empty vector is determined by colony formation assay. As shown in Figure 2b, equal numbers of cells were seeded and grew into colonies. Cells induced to express PGDH by tetracycline treatment grow into considerably less and smaller colonies than cells not treated with tetracycline. The growth suppression is not caused by tetracycline toxicity as empty vector control cancer cells treated with tetracycline grows into colonies without difference from the same cells not treated with tetracycline. In conclusion, PGDH expression strongly suppresses the colony formation of esophageal cancer cells.

Discussion

We have shown that the major prostaglandin degrading enzyme, PGDH, is lost in esophageal cancer, both in squamous cell cancer and adenocarcinoma, and that when re-expressed, PGDH suppresses cancer cell growth. This is the first report that PGDH exhibits tumor suppressive properties in esophageal cancer. Our findings are consistent with previous reports demonstrating the tumor suppressor role of PGDH in other gastrointestinal cancers (Chi et al., 2009; Roberts et al., 2011; Li et al., 2014). This finding enriches our understanding of the tumorigenic pathway that leads to esophageal cancer growth. As mentioned earlier, prostaglandin pathway remains one of the well validated pathway for drug development for chemoprevention, but COX-2 specific inhibitors has formidable cardiovascular effects that has prohibited their widespread use (McKenna et al., 2001; Solomon et al., 2004; Wolfe et al., 2004; Zhao et al., 2004; Schaefer et al., 2005; O'Kane et al., 2010). On the other hand, it will be of enormous clinical benefit if PGDH expression can be induced in premalignant and malignant epithelial cells in esophageal cancer and subsequently suppress tumor growth. Obviously, more work remains to be done, especially to further prove the role of PGDH as tumor suppressor in vivo and to identify pathways that reduced PGDH expression in cancer and to find drugs that induced the expression of PGDH to suppress cancer growth.

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