Differential Expression of Desmocollin2, Desmoglein2, and Plakophilin2 in the Progression of Esophagitis to Esophageal Adenocarcinoma

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Research article

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Abstract

Background

Desmosomes play a key role in intercellular adhesive, but also contribute to tumorigenesis. This study aimed to examine the differential expression of desmocollin2 (DSC2), desmoglein2 (DSG2), and plakophilin2 (PKP2) in the progression of reflux esophagitis to esophageal adenocarcinoma (EAC) in the rat model of reflux disease established by esophagogastroduodenal anastomosis (EGDA).

Methods

EGDA was performed on rats to induce gastroesophageal reflux leading to the development of EAC. All rats were randomly divided into four groups: group A rats received EGDA only (n=27); group B rats received EGDA and iron supplementation (n=28); group C received pseudo surgery only (n=20); group D received pseudo surgery and iron supplementation (n=20). Animals were randomly selected from each group and euthanized at 8 weeks and 32 weeks following EGDA. Esophageal tissues were harvested and divided into 4 types (normal esophagus, esophagitis, dysplasia, and EAC). On these tissue types, immunohistochemistry was performed to characterize the localization and distribution of DSC2, DSG2, and PKP2, while qRT-PCR and western blot were performed to detect the expression of DSC2, DSG2, and PKP2 at the gene and protein levels.

Results

At 8 weeks after surgery, 80% of rats in group A and 100% in group B had esophagitis. At 32 weeks, 29.41% and 17.65% of rats in group A developed dysplasia and EAC, respectively, while in group B, dysplasia and EAC accounted for 44.44% and 38.89%, respectively. The expression of DSC2, DSG2, and PKP2 at both the gene and protein levels increased progressively from esophagitis to dysplasia, and EAC. Of note, all of the three genes were significantly upregulated in EAC tissues compared with tissues of esophagitis.

Conclusion

DSC2, DSG2, and PKP2 may play an important role in the progression of esophagitis to EAC. Their expression levels may therefore be utilized as molecular biomarkers for early diagnosis and targeted therapy for EAC.

Background

Esophageal cancer is the eighth most common cancer worldwide and is associated with high morbidity and mortality. Since the 1970s[4], the incidence of esophageal adenocarcinoma (EAC) has increased in many Western and several Asian countries[1–3] by approximately 600%, with the incidence of adenocarcinoma type exceeding those of esophageal squamous cell carcinoma (ESCC)[5]. China has approximately 18% of all cases worldwide given its large population[5]. The overall mortality of EAC is high, with a 5-year survival rate of less than 15%[4], primarily because of patients presenting and diagnosed at an incurable late-stage[6, 7]. Although several risk factors have been associated with EAC, Gastroesophageal reflux disease (GERD)

represents the strongest risk factor[8] that has been demonstrated in several population-based studies[6, 9]. GERD causes reflux esophagitis, leading to Barrett's esophagus and the dysplasia of the esophagus, which predisposes to esophageal adenocarcinoma[10, 11]. The molecular mechanisms underlying such progression of a chronic benign condition to esophageal carcinogenesis remain to be elucidated.

Previously, we presented a functional module-based approach to explore link between inflammation and esophagus cancer based on GEO database[12]. We observed a functional module containing genes Desmocollin2(DSC2), Desmoglein2(DSG2), and Plakophilin2(PKP2), was served as both inflammation and cancer module. DSG2, PKP2 and DSC2 supposed to play a vital role in progression from esophagitis to esophageal cancer. However, the mechanism underlying the effect of the three genes on tumor progression remains unknown, with only a limited number of studies reporting on the role of them in esophageal cancer. As previously reported, DSG2, PKP2 and DSC2 are major components of cell desmosomes[13, 14] in mediating desmosomal cell-cell adhesion in epithelial tissues and cardiac muscle and maintaining epithelial homeostasis. In addition, they play a key role in regulating epithelial cell proliferation and tumorigenesis[15, 16]. Desmosomal cadherins have been shown to be downregulated in several cancers to promote tumor progression[17] but upregulated in other cancer types. For instance, in patients with non-small cell lung cancer (NSCLC), the DSG2 expression has been associated with a poorer prognosis, suggesting that overexpression of DSG2 promotes the development and progression of NSCLC[18]. While in another study, downregulation of DSG2 and DSG3 expression has been found in lung cancer cell lines, which might be contributed by DNA metheylation[19]. The composition of desmosomes may be dependent on the tissue-specific or differentiation-specific expression of particular isoforms of the constituent proteins[20]. Therefore, the role of desmosomes-adhesion complexes in carcinogenesis remains elusive[14]. However, to date, the changes in the expression of DSC2, DSG2, and PKP2 with their roles in the progression of esophagitis to esophageal adenocarcinoma have not been fully elucidated. Therefore, this study aimed to examine the expression levels of these proteins in esophageal tissues (of esophagitis to esophageal adenocarcinoma) harvested from reflux rat models simulating human gastroesophageal reflux disease using the esophagogastroduodenal anastomosis (EGDA) method.

Methods

Animal experiment

This study was approved by the Medical Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University. Eight-week-old male Sprague-Dawley rats (Beijing Vital River Laboratory Animal Technology Co., Beijing, China) were housed five per cage. They were given water and food ad libitum, maintained on a twelve-hour light/dark cycle, and allowed to acclimate for two weeks. Eight-hour prior to surgery, the rats were kept nil per os (NPO). Anesthetics were administered in an acrylic anesthetizing chamber (2% isoflurane in 1 ml/L oxygen)[8]. Based on previously described, EGDA was performed in an end-to-side esophagoduodenostomy manner with gastric preservation through a left upper abdominal incision. Briefly, two 1.0 cm incisions were made each on the gastroesophageal junction and the duodenum on the anti-mesenteric border and then were anastomosed together with precise mucosal-to-mucosal opposition. All

surgically treated rats (n = 55) were randomly allocated into two groups: group A rats treated with EGDA only (n = 27); group B treated with EGDA and iron dextran (4 mg Fe/Kg/week, i.p.), starting from 2 weeks after surgery and continuing for the duration of the experiment (n = 28). On the other hand, 40 pseudo-surgery rats were randomly assigned into two groups: Group C received pseudo-surgery only (n = 20); group D received pseudo-surgery and iron dextran (4 mg Fe/Kg/week, i.p.) starting 2 weeks after surgery. The animals were weighed weekly and euthanized at 8 weeks (n = 10 in every group), and the rest were kept for 32 weeks following surgery. The animals were euthanized by using isoflurane overdose.

Histopathology

To examine the morphological changes, a longitudinal incision was made in the middle and lower esophagus of rats. The esophagus was fixed with 40 g/L paraformaldehyde for 12–24 hours and then embedded in paraffin. Paraffin tissue sections were stained with hematoxylin and eosin (H&E) for histopathological analysis.

Based on the established classification criteria, the histopathological changes of the rat esophagus were defined as the following: Type 1. Normal esophagus: esophageal mucosa was a laminated squamous epithelium, where the basal cells were confirmed in size, low in the nucleus/cytoplasm ratio, cells were aligned and good polarity; Type 2. Esophagitis: the presence of inflammatory cell infiltration in the esophageal mucosa and submucosa, which were categorized into mild, moderate, and severe according to the number of inflammatory cells; Type 3. Esophageal dysplasia: basal cells were enlarged, the nucleo-plasmic ratio was increased, the cell arrangement was disordered, and the polar orientation was poor; Type 4. Esophageal adenocarcinoma (EAC): the presence of “mucosa lake” in the mucosal and submucosal layer, in which cancer cells appeared floating. Changes in cancer cells included nuclei enlargement, chromatin condensation, and higher nucleo-cytoplasmic ratio.

Immunohistochemistry

Tissue sections were stained for the expression of Desmocollin2 (DSC2), Desmoglein2 (DSG2), and Plakophilin2 (PKP2) proteins in the esophagus. Paraffin sections were dewaxed in xylene, then rehydrated in gradient alcohols to distilled water. Tissue sections were repaired by the EDTA (EDTA/distilled water: 1:500; MXB, China; MVS-0098) in 100 ^{0}C. Antibodies of DSC2 (biorbyt, United Kingdom; orb100766), DSG2 (Abcam, United States; ab150372), and PKP2 (Abcam, United States; ab189323) were used and incubated for 1 h at room temperature. The Elivision™ super HRP (Mouse/Rabbit) IHC kit (MXB, China; KIT-9921) and Peroxidase-Conjugated Rabbit anti-Goat IgG (H + L) (ZSGB-BIO, Peking, China; ZB-2306) were applied and incubated for 30 min, followed by staining with diaminobenzidine (DAB). Then, sections were counterstained with hematoxylin, dehydrated by the gradient alcohol, cleared in xylene, and covered by the neutral balsam.

The IHC slides were analyzed by the pathologist. The cellular localization and the staining degree of DSC2, DSG2, PKP2 were assessed, with the expression of these proteins graded according to the intensity of the staining: no staining (-), mild staining (yellow) (+), medium staining (light brown) (++), and strong staining (dark brown) (+++).

RNA isolation and quantitative real-time PCR

The total liver RNA was isolated from the esophageal tissue by using the E.Z.N.A.®Total RNA Kit I (OMEGA, United States; R6834-1) according to the manufacturer's protocols. The isolated RNA samples were then converted to cDNA using the ReverTra Ace qPCR RT Kit (TOYOBO CO., Osaka, Japan; Code No.FSQ-101). All PCR reactions were performed on the Applied Biosystems QuantStudio™ 3 Real-Time PCR System (ThermoFisher, MA, United States) using the FastStart Universal SYBR® Green Master (ROX) (Roche Diagnostics, Indianapolis, IN, United States) in standard 20μL reaction volumes as follows: 1μL (600 ng/uL) cDNA, 1μL of 10 pmol/L sense primer, 1μL of 10 pmol/L antisense primer, 7 ul DEPC water, and 10μL FastStart Universal SYBR® Green Master. The following RT^2 Primer Assays were used: DSC2 (Invitrogen, Carlsbad, CA, United States), DSG2 (Invitrogen, Carlsbad, CA, United States), PKP2 (Invitrogen, Carlsbad, CA, United States), with GAPDH (Invitrogen, Carlsbad, CA, United States) selected as an endogenous control. The primer sequences were as follows: DSC2-Forward primer: 5'-TGAGAAGGTGCAGTTTTGCC-3'; DSC2-Reversed primer: 5'-TCCAAAAACTCAAGCCCGTC-3'; DSG2-Forward primer: 5'-AGACCCTAGCCGAAGTTTGC-3'; DSG2-Reversed primer: 5'-TCTGAGCTGGCTGTCACTTG-3'; PKP2-Forward primer: 5'-GGCCACAGAGAACTGCGTAT-3'; PKP2-Reversed primer: 5'-TTCTACTCCGGCTGCCAAAA-3'; GAPDH-Forward primer: 5'-AGTGCCAGCCTCGTCTCATA-3'; GAPDH-Reversed primer: 5'-GATGGTGATGGGTTTCCCGT-3'.

PCR amplification of DSC2, DSG2, and PKP2 was conducted under the following conditions: 50 °C 2 min, 95 °C 10 min; 95 °C 15 s, 60 °C 1 min for 50 cycles. Raw data were exported from the Real-Time PCR System and the relative gene expression was calculated using the ΔΔ-Ct method. To ensure the reliability of the obtained results, all samples were processed in triplicate with negative control. The values were normalized to the control and a fold change was used as an expression level.

Western blot analysis

Western blot was performed to detect the protein expression of DSC2, DSG2, and PKP2 genes. The proteins were conventionally extracted by the RIPA Lysis Buffer (Beyotime, China; P0013B) and Protease inhibitor cocktail for general use (Beyotime, China; P1005). The total protein concentration was determined by the Bicinchoninic acid (BCA) method (Beyotime, China; P0010S). A similar quantity of tissue lysates (80ug) was electrophoresed on 10% polyacrylate gel electrophoresis (PAGE) Gel (EpiZyme, China; PG112) and then transferred to nitrocellulose filter (NC) membranes (Thermo Scientific, MA, USA). The membrane was blocked in 0.5 g/L skim milk for 2 hours followed by incubation with the corresponding primary antibody as follows: DSC2 antibody (1:250, biorbyt, United Kingdom; orb100766), DSG2 antibody (1:4000, Abcam, United States; ab150372), PKP2 antibody (1:2000, Abcam, United States; ab189323), and Rabbit anti-GAPDH (1:1000, GOODHERE, China; AB-P-R 001). The secondary antibodies conjugated with HRP were goat anti-rabbit (1:4000, ZSGB-BIO, Peking, China; ZB-2301) and rabbit anti-goat (1:4000, ZSGB-BIO, Peking, China; ZB-2306). Signals were developed using the Super ECL Detection Reagent (YEASEN, China, 36208ES60). Images were scanned by the Molecular Imager ChemiDoc™ XRS + and analyzed with the Image Lab™ Software (BIO-RAD, USA).

Statistical analysis

Statistical analyses were performed using the GraphPad Prism 7.0 software. Gray value analysis was performed with the Image Lab software (Version 5.0, Life Technologies Corporation, Carlsbad, CA). The ratio of the target band to the internal reference band represented the protein expression levels of the target gene.

The differential expression of DSC2, DSG2, and PKP2 among the groups were compared using the Kruskal–Wallis test or Shapiro-Wilk normality test, and the results of indicators between the four groups were analyzed using One-Way ANOVA multiple comparisons. A P-value of < 0.05 was considered statistically significant.

Results

Pathological analyses

Most mice (79.17%, 95/120) survived the surgery. The rest died of anesthesia, bleeding or unknown reasons during the surgery. All histopathological features were categorized into four: normal esophagus, esophagitis, dysplasia, and esophageal adenocarcinoma (EAC) (Fig. 1 and Table 1). As outlined in Table 1, there was no dysplasia or EAC observed in all groups at 8 weeks. In group A and group B, 80% and 100% of rats, respectively, had esophagitis type. However, at 32 weeks, 17.65% (3/17) in group A and 38.89% (7/18) in group B had EAC observed, while 29.41% (5/17) in group A and 44.44% (8/18) in group B developed dysplasia. These findings indicated that EGDA and EGDA in combination with iron promoted the occurrence of esophageal dysplasia and esophageal adenocarcinoma.

Table 1
Pathologic results of each time point after surgery n (%)

Group	8w				32w			
	Normal	Esophagitis	Dysplasia	EAC	Normal	Esophagitis	Dysplasia	EAC
Group A	2(20)	8(80)	0(0)	0(0)	0(0)	9(52.94)	5(29.41)	3(17.65)
Group B	0(0)	10(100)	0(0)	0(0)	0(0)	3(16.67)	8(44.44)	7(38.89)
Group C	10(100)	0(0)	0(0)	0(0)	10(100)	0(0)	0(0)	0(0)
Group D	10(100)	0(0)	0(0)	0(0)	6(60)	3(30)	1(10)	0(0)

Immunohistochemical analyses

The positive stainings of DSC2, DSG2, and PKP2 were localized in the esophageal epithelium. In normal esophagus and esophagitis, the staining background of DSC2, DSG2, and PKP2 appeared mild in the cell membrane and/or cytoplasm of the esophageal epithelium (Fig. 2A, 2B). Compared to that of normal esophagus and esophagitis, the staining was increased slightly in esophageal dysplasia (Fig. 2C). However, strong staining (dark brown) in the cell cytoplasm and/or nucleus was observed in esophageal adenocarcinoma (Fig. 2D), indicating the alterations in the levels of protein expression and locations of DSC2, DSG2, and PKP2 in esophageal carcinogenesis.

Gene expression analyses

The relative expression of the DSC2 gene was the highest in EAC and the lowest in esophagitis. Compared with the normal esophagus, the expression level of DSC2 was significantly higher in EAC ($P < 0.0001$), while no significant difference was observed in esophagitis and dysplasia. Additionally, there was a significant difference in the DSC2 gene expression between esophagitis and EAC ($P < 0.0001$) (Fig. 3A). For the DSG2 gene, the relative expression was the highest in EAC with significant differences when compared with other groups ($P < 0.0001$) (Fig. 3B), while no significant difference was observed between normal esophagus and esophagitis. Although the DSG2 expression was slightly higher in dysplasia compared with the normal esophagus, this difference was not statistically significant. Also, the expression of the PKP2 gene appeared progressively increased from the normal esophagus to EAC, with its expression significantly higher in EAC than normal esophagus and esophagitis ($P < 0.0001$) (Fig. 3C).

Western blot analyses

To validate the above findings, western blot was performed to detect the differential expression of DSC2, DSG2, and PKP2 protein expression in the normal esophagus, esophagitis, esophageal dysplasia, and EAC. After normalizing to the expression of GAPDH, the expression of DSC2, DSG2, and PKP2 were significantly upregulated in EAC compared with normal esophagus or esophagitis ($p < 0.05$), which were consistent with the results of qRT-PCR and IHC.

Discussion

Following the description and introduction of the surgically induced reflux model of esophagitis in rats, it has been extensively used to study esophageal carcinogenesis in order to develop novel preventive and treatment strategies[8, 21–23]. Among various surgical approaches available, EGDA is currently the most effective surgical method to induce animal reflux models[8, 24]. Different from other surgical approaches that encourage acid or alkaline reflux only, EGDA diverts both the gastric contents and some duodenal secretions to the esophagus, which is superior in promoting and simulating the process of human gastroesophageal reflux disease (GERD) from esophagitis to Barret' esophagus (BE), leading to esophageal dysplasia and consequently adenocarcinoma. In our study, the combination of EGDA with frequent iron injection (not as a carcinogenic agent) promoted tumorigenesis while the application of iron alone had a small influence, indicating that iron dextrin could potentiate further the oxidative stress in the inflamed esophageal epithelium[21]. Higher oxidative stress could in turn aggravate the inflammatory reaction, resulting in abnormal cell proliferation and transformation, with eventual esophageal carcinogenesis[3, 8, 25].

We reported previously DSG2, DSG2 and PKP2 were differently expressed in both esophagitis and esophageal cancer based on bioinformatics. DSC2 and DSG2 are members of the desmosomal cadherin family. Desmosomes not only contribute to an intercellular adhesive which is essential in the normal organization and stabilization of epithelial tissues, but also participate in cellular differentiation[26], transformation, and tumorigenesis[16]. Moreover, during malignant transformation of epithelial cells, alterations in the expression and function of intercellular junctions might result in tumor invasion and metastasis. There are four types of intercellular junction presenting in vertebrates: desmosomes (DSMs),

adherens junctions (AJs), tight junctions (TJs), and gap junctions (GJs),which are crucial for maintaining epithelial homeostasis[27]. All these complexes work as a single unit, interdependently rather than individually. The formation of DSMs is dependent on classic cadherin-mediated adhesion[28]. E-cadherin is the classical cadherins of adherens junctions. It has been reported that E-cadherin can associate with some Dsgs (DSG2), and this interaction may help initiate early stages of DSM assembly. Gap junctions metabolically and electrically connect the cytoplasm of adjacent cardiomyocytes. Meanwhile, some articles summarized the involvement of gap junctions in carcinogenesis [29]. Connexin 43 (Cx43), an important gap junction protein, has been reported associated with esophageal squamous cell carcinoma[30]. DSMs could contribute to Cx assembly and GJ function. The relationship between the DSM and Cxs was first studied in the heart, showing that PKP2 is critical for Cx43 expression and function. The above reports suggested that DSMs might impact gap junction. The connection between them will be explored further in the following study. As outlined above, there were tight connection among these intercellular junctions. The aim of our study is to elucidate the mechanism underlying the effect of DSG2, DSC2 and PKP2 on tumor progression in esophageal adenocarcinoma.

Our findings confirmed the differential expression of DSC2, DSG2, and PKP2 from benign esophagitis to esophageal adenocarcinoma. The upregulation of all these at mRNA expression levels in esophageal adenocarcinoma and the differences when compared to the normal esophagus or benign esophagitis were significant. Besides, the analyses of immunohistochemistry and western blot further validated the above observation, in which the protein expression levels of DSC2, DSG2, and PKP2 were all significantly elevated in EAC. Furthermore, in addition to quantitative changes of these protein expressions in the development of esophageal adenocarcinoma, immunohistochemical staining also demonstrated changes in their cellular localization. Several studies have described alterations of protein expression leading to structural changes of desmosomes, which can induce cell-transformation phenotype and promote carcinogenesis[19, 31, 32]. However, the mechanism involved in the changes of various desmosomal components remains unclear.

Both DSC2 and DSG2 are members of the desmosomal cadherin family. They are transmembrane proteins that maintain intercellular connectivity. The ectodomains of DSC are connected with the DSG at the extracellular region of apposing cells, and the intracellular regions of DSC and DSG directly or indirectly interact with the armadillo proteins including Plakophilins (PKPs), Junction plakoglobin (JUP), Desmoplakin (DSP), Intermediate filaments (IFs), and other desmosomal complexes, which mediate intercellular adhesion in epithelial tissues[12, 19] and may have a direct or indirect role in regulating cell differentiation[16] and tumorigenesis[14]. JUP, which is an homologous protein withβ-catenin, can replace β-catenin in adherens junctions and stimulate the transcription of WNT target genes, including oncogenic targets such as CCND1 (encoding cyclin D1). So, we inferred that desmosomes dysfunction can promote cancer by WNT-β-catenin signalling pathway indirectly. Additionally, PKP2 has been reported functioned as an intracellular inhibitor of the Wnt/β-catenin pathway in colon cance[52]. We will explore the connection between desmosomal cadherin family and Wnt/β-catenin pathway furtherly.

Through modified cell adhesive strength or changes in intracellular and intercellular signaling, the expression patterns of desmosomal cadherin may have been altered, which can affect cell behavior and drive proliferation under some circumstances[33]. In our study, the levels of mRNA and protein expression of DSC2

and DSG2 progressively increased from the epithelial of esophagitis to dysplasia and carcinoma, suggesting an important role of DSC2 and DSG2 overexpression in tumorigenesis of esophageal epithelia. These findings were consistent with previous studies, in which both the DSC2 and DSG2 were overexpressed in squamous cell cancers of the skin and head and neck (HNSCCs)[34]. This could enhance internalization, modulate extracellular vesicles (Evs) secretion and paracrine signaling, and increase local and distant mitogenic effects that encourage tumor progression[23, 35]. Besides, the loss of desmoglein-2 (DSG2) has been associated with decreased epithelial cell proliferation and suppressed xenograft tumor growth in mice[14]. In addition, DSC2-positive urothelial carcinomas (UC) have a more advanced stage disease than those tumors without DSC2[36]. Meanwhile, other studies have reported that DSG2 overexpression may promote tumorigenesis in basal cell carcinoma (BCC)[37] by activating Stat3 in the basal layer of the skin of mice[38], which potentiates the proliferation of CRC through up-regulation of PNN and activating EGFR/ERK signaling pathway[39]. Furthermore, DSG2 has been shown to induce and activate urokinase-type plasminogen activator receptor-related signaling cascade and accelerate cutaneous wound healing[40], which plays a critical role in the vasculature that is independent of its canonical role as a component of desmosomes in a distinct subpopulation of progenitor cells [41].

PKP2 is a member of the armadillo family generally localized in the nucleus and cytoplasm of epithelial tissues and cardiomyocytes, which binds the desmosomal cadherins and desmoplakin[42, 43]. Lately, PKP2 has been implicated in tumorigenesis and/or invasion and metastasis of cancer. For instance, in some soft tumors and highly proliferative colonies of cultured mesenchymal stem cells, the upregulation of PKP2 and its integration into adherent junctions (Ajs) have been observed[44]. PKP2 may excite Topflash activity, leading to the translocation of desmosomes[33]. Another study has revealed that PKP2 can promote actin recombination and regulate the assembly of the desmosomal complex by connecting Ras homolog A and protein kinase C-dependent pathways[45]. In our study, the mRNA and protein levels of PKP2 were significantly upregulated in esophageal adenocarcinoma tissues compared with tissues of normal esophagus and esophagitis in rats. Particularly, the expression of PKP2 progressively increased from esophagitis to esophageal adenocarcinoma, and significantly elevated in EAC. These findings were consistent with several previous studies and suggested that PKP2 might be essential for carcinogenesis. Indeed, reduced expression of PKP2 has been associated with inhibition of glioma cell proliferation and migration[46]. Moreover, decreased PKP2 expression reduces Epidermal growth factor (EGF)-dependent and EGF-independent epidermal growth factor receptor (EGFR) dimerization and phosphorylation, resulting in a significant decrease in proliferation and migration of cancer cells and tumor development[47, 48]. Besides, PKP2 has been implicated in the invasiveness of bladder cancer as a result of loose adhesion via the epithelial-mesenchymal transition (EMT) and β-catenin–mediated signaling pathways[49]. The redistribution of PKPs could promote adhesion, differentiation, and the localization of PKPs from desmosomes to the nucleus that contribute to tumorigenesis[20]. In a previous study, strong PKP2 immunoreactivity has been observed in 85.7% of metastatic tumors of oropharyngeal carcinoma[50]. Thus, an increase of plakophilin could promote carcinogenesis via the stimulation of translation and proliferation, while the loss of plakophilin may contribute to carcinogenesis and/or metastasis via loss of contact inhibition and increased motility[51].

Conclusion

In summary, this study has demonstrated that a combination of the EGDA method with frequent iron injection increases the incidence of EAC. Upregulations of DSC2, DSG2, and PKP2 expression have been observed in EAC, suggesting that these genes could be important in the development of EAC. Therefore, DSC2, DSG2, and PKP2 can be used as novel molecular biomarkers for early diagnosis and targeted therapy for esophageal adenocarcinoma. To further shed light on the complete complexities of these proteins in the progression of esophagitis to EAC, additional studies are certainly necessary. Human tissue is going to be collected to demonstrate the expression and contribution of desmosomal components to the development of esophageal cancer in the future.

Abbreviations

desmocollin2 (DSC2), desmoglein2 (DSG2), plakophilin2 (PKP2), esophageal adenocarcinoma (EAC), esophagogastroduodenal anastomosis (EGDA), esophageal squamous cell carcinoma (ESCC), gastroesophageal reflux disease (GERD), non-small cell lung cancer (NSCLC), diaminobenzidine (DAB), Bicinchoninic acid (BCA).

Declarations

Ethics approval and consent to participate:

This study was approved by the Medical Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

NL analyzed and interpreted the data regarding the operated animals and controls. YW performed the histological examination and was a major contributor in writing the manuscript. LZ and WB designed the study. All authors read and approved the final manuscript.

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Figures

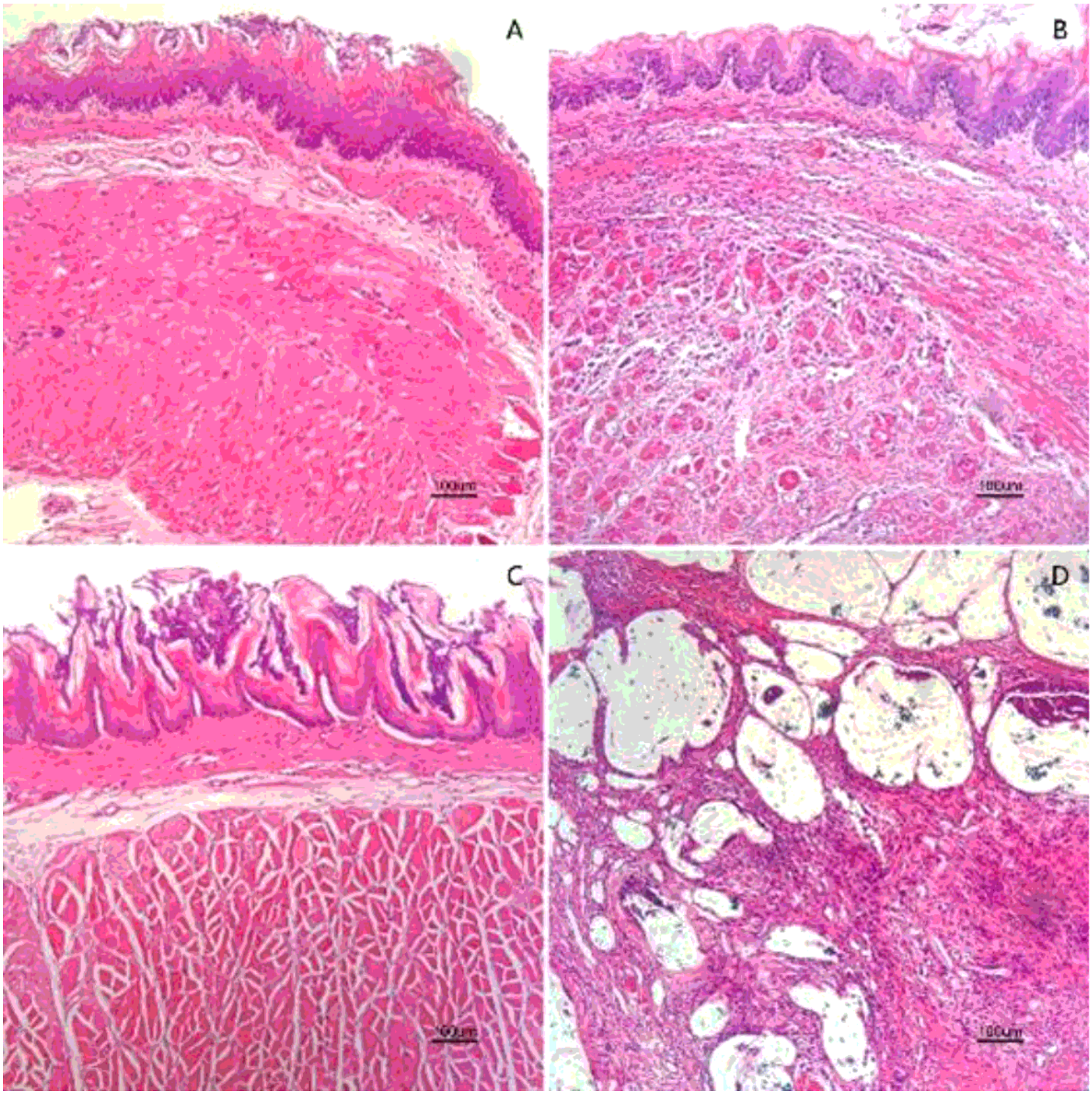

Figure 1

Hematoxylin and eosin staining of the esophagus in rats. A: Normal esophagus; B: Esophagitis; C: Dysplasia; D: Esophageal Adenocarcinoma. (10×10 magnification)

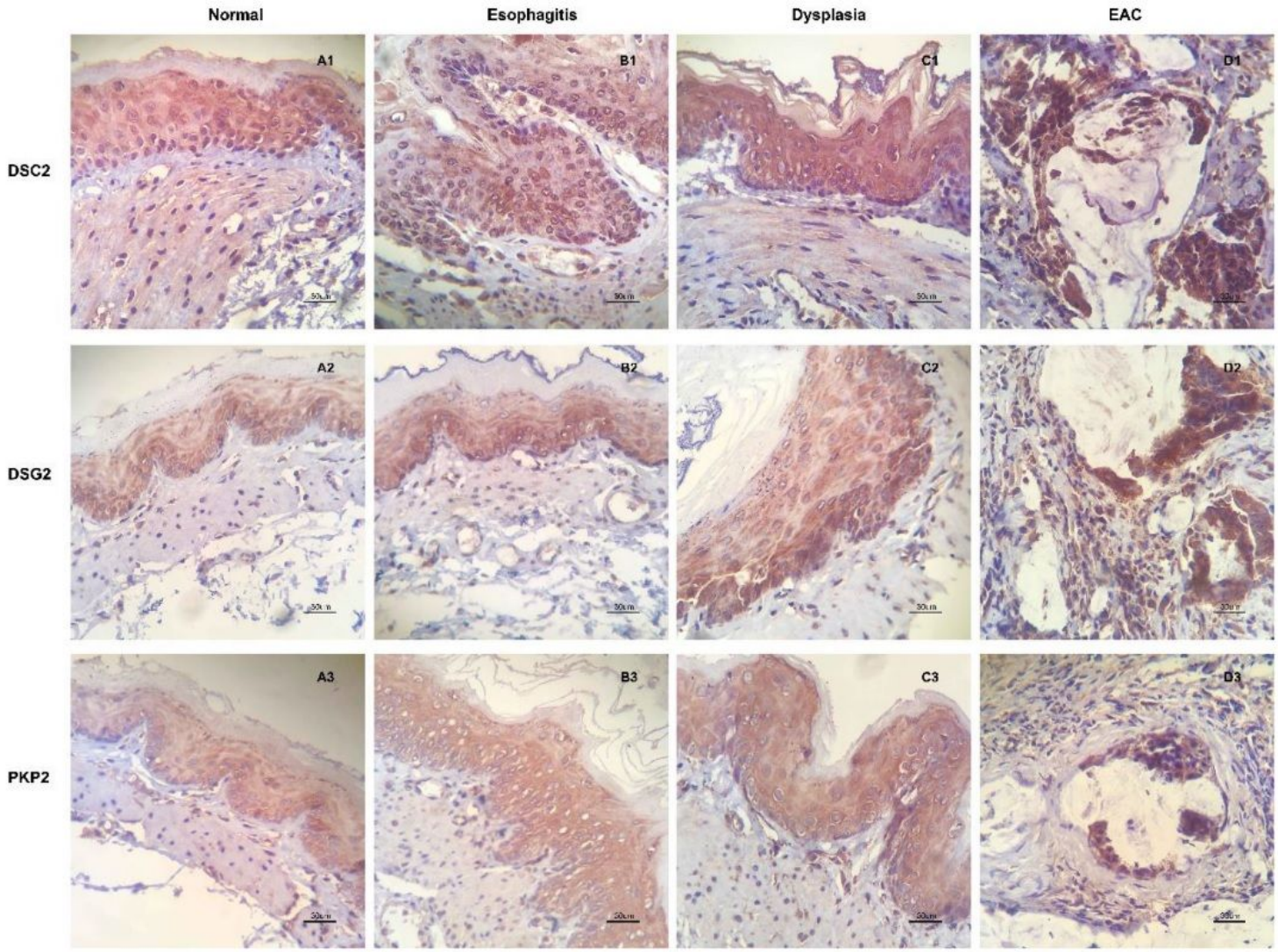

Figure 2

Immunohistochemical analyses: the expression levels of DSC2, DSG2, and PKP2 in the progression of the normal esophagus to EAC in the rat model of gastroesophageal reflux (10×40 magnification). Figure A1~D1, A2~D2, and A3~D3 depicted changes of DSC2, DSG2, and PKP2 expression in the normal esophagus, esophagitis, esophageal dysplasia, and EAC, respectively. The expressions of DSC2, DSG2, and PKP2 were the highest in EAC (stained dark brown). In normal esophageal tissues, DSC2 and DSG2 were mainly localized to the cytoplasm and membrane, while PKP2 was mainly localized in the cytoplasm in non-tumoral tissues. In tissues of esophageal adenocarcinoma, these proteins appeared primarily in the cytoplasm and nucleus.

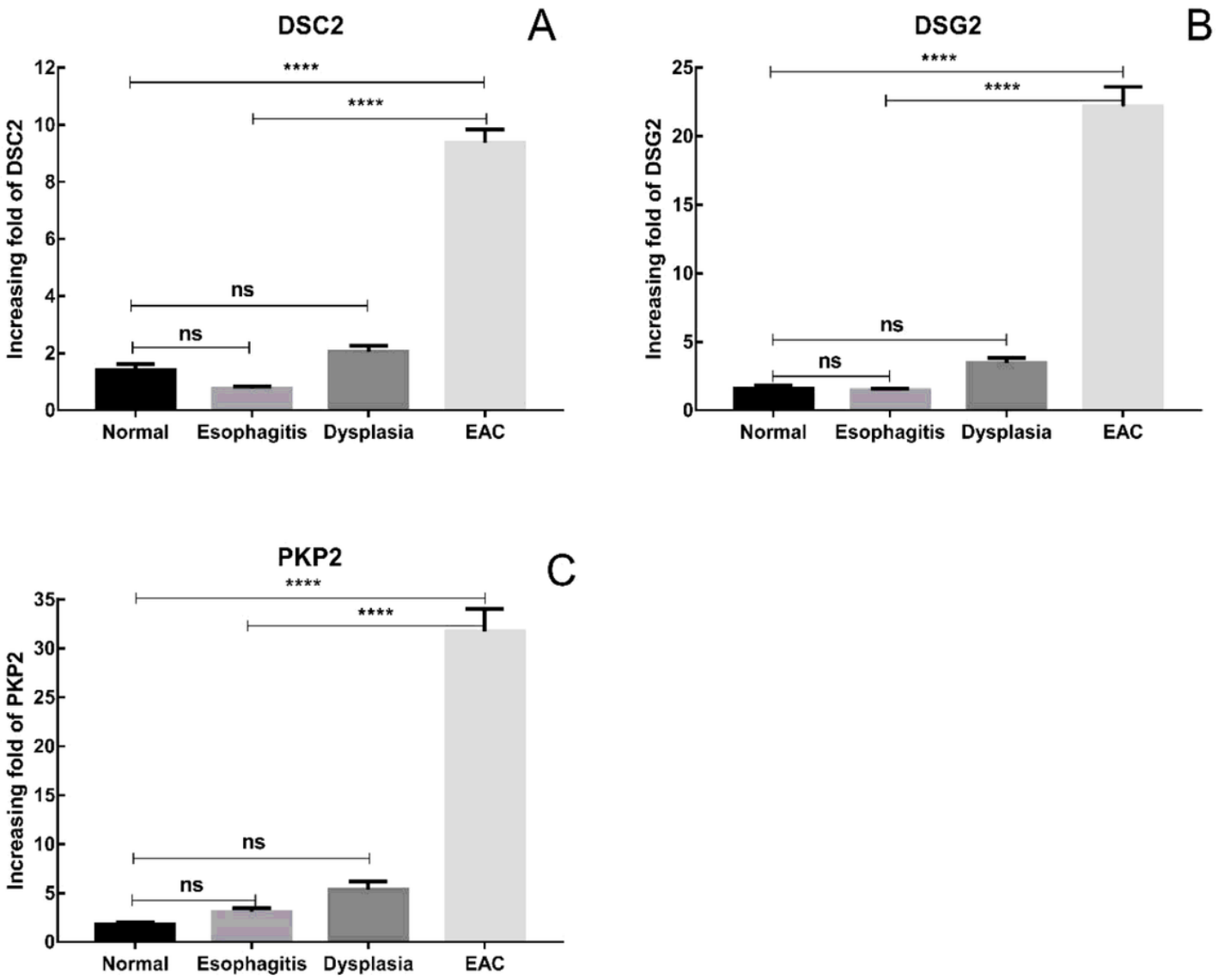

Figure 3

Histogram A to C illustrated the relative gene expression levels of DSC2, DSG2, and PKP2 at four histological stages (Normal esophagus, Esophagitis, Dysplasia, and EAC), respectively. Results were presented as mean±SE (ns $p > 0.05$, ****$p < 0.0001$).

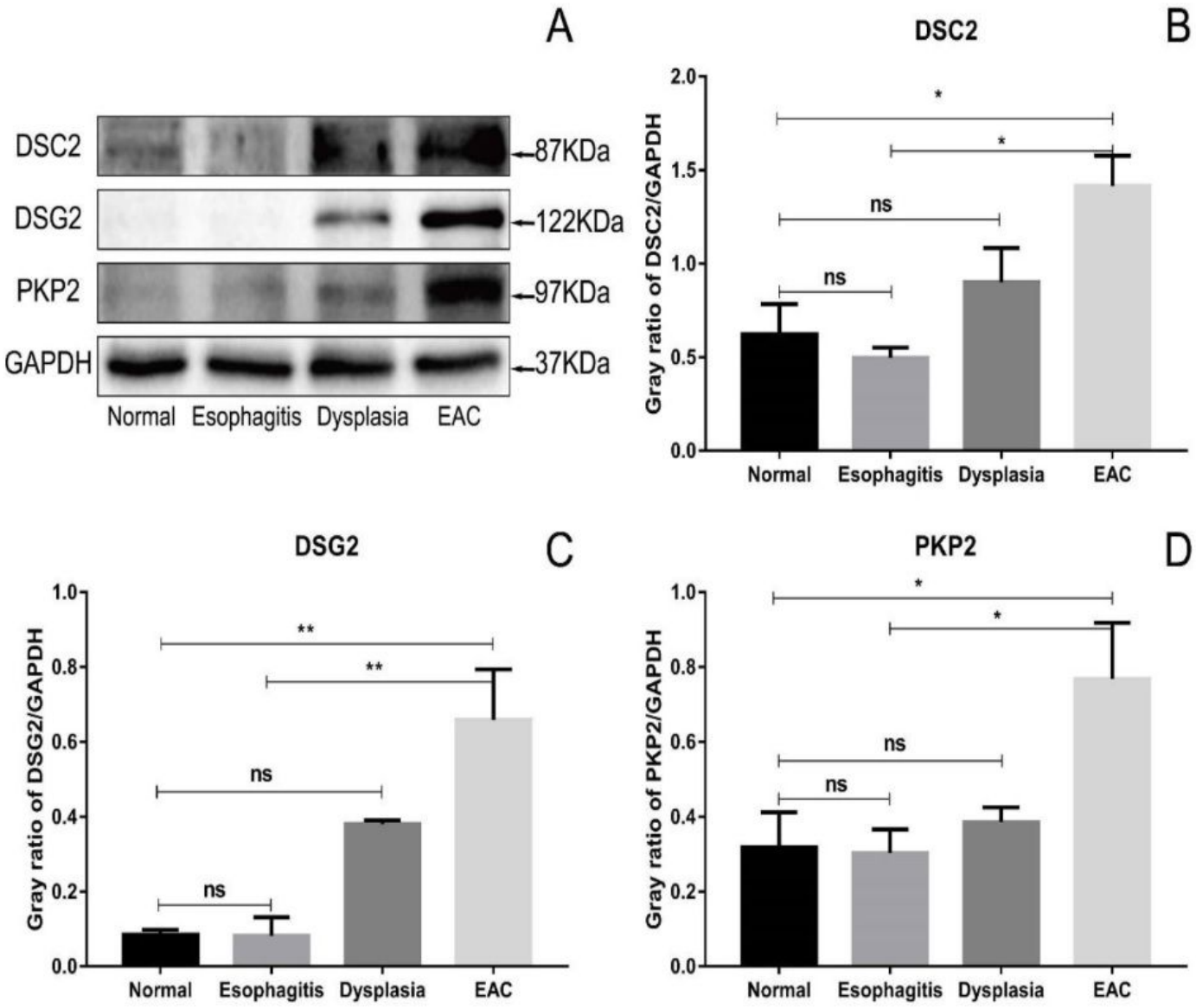

Figure 4

Immunoblotting analysis of DSC2, DSG2, and PKP2 expression in the normal esophagus, esophagitis, esophageal dysplasia, and EAC. The DSC2, DSG2, and PKP2 were normalized to the expression of GAPDH. Results were presented as mean±SE (* $p < 0.05$, ** $p < 0.001$).

Supplementary Files

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