Assessment of use of DcR 3 in diagnosis of dysplastic lesions and adenocarcinoma of the esophagus

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ABSTRACT

Background: Because of confusion to gastric cancers arising at the gastro-esophageal junction, true esophageal adenocarcinoma was thought to be unusual. Esophageal adenocarcinoma (EAC) is becoming more common worldwide with increasing incidences.

Material and Methods: Overexpression of decoy receptor (DcR) 3 protein, - a recently discovered member of the tumor necrosis factor receptor super-family, was examined in 60 esophagogastrectomy specimens containing areas of Barrett esophagus (n = 27), low-grade dysplasia (n = 40), high-grade dysplasia or carcinoma in situ (n = 33), and esophageal adenocarcinoma (EAC; n = 42) with immunohistochemical analysis. All cases were retrieved from the pathology files of Damanhour national medical institute hospital.

Results: The results of this study revealed more overexpression of DcR3 in high-grade dysplasia or carcinoma in situ and EAC than in benign esophageal mucosa (both $P < 0.0001$), Barrett esophagus (both $P < 0.001$), and low-grade dysplasia ($P < 0.01$ and $P = 0.033$, respectively) significantly. Low-grade dysplasia also showed significant overexpression of DcR3 compared with benign esophagus ($P < 0.05$) but not with Barrett esophagus ($P > 0.05$). DcR3 overexpression seems negatively correlated with the grade of EAC.

Conclusion: Results of this study suggest that overexpression of DcR3 protein might be an aid in the diagnosis of high-grade dysplasia or carcinoma in situ and EAC and also might serve as a potential therapeutic target.

Keywords: Esophageal adenocarcinoma, Barrett esophagus

INTRODUCTION

Esophageal cancer poses several general diagnostic tasks for the surgical pathologist: establishment of the diagnosis of malignancy, classification of the histological type and assessment of prognostic factors (1). More than 13,900 new cases with 13,000 deaths were anticipated in the United States in 2003(2). With increase in recognition of Barret mucosa, it is apparent that most adenocarcinomas in the lower third of esophagus are true esophageal cancers, rather than gastric cancers straddling the esopago-gastric junction. Barrett esophagus is considered the key precancerous lesion with a strong association with development of dysplasia and subsequent EAC, but the pathogenic mechanisms of this process remain specifically unclear. However, genetic alterations are well-documented (3).

Decoy receptor (DcR) 3 is a recently discovered member of the tumor necrosis factor receptor superfamily (4). The DcR group consists of 4 members, DcR1, DcR2, and DcR3 and osteoprotegerin (5,6). Although osteoprotegerin has very low physiological affinity for TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), DcR1 and DcR2 are capable of binding TRAIL but incapable of transducing the death signal because of the lack of a death domain in their structures (6,7). In fact, DcR1 and DcR2 compete with the TRAIL receptor for the ligands and subsequently inhibit apoptosis. In addition, DcR can inhibit T-cell activation via the repression of actin polymerization and pseudopodium formation by T cells (8).

DcR3 is a unique member in the DcR group because it is secreted rather than membrane-bound like DcR1 and DcR2. In addition, DcR3 binds to the Fas ligand, not the TRAIL, as other DcRs do (9). DcR3 also can modulate immune cell interaction by down-regulating functions of macrophages and T cells (8,10). DcR's functions of blocking apoptosis and escaping immune surveillance are important in carcinogenesis. The gene amplification and overexpression of DcR3 messenger RNA (mRNA) and protein have been demonstrated in lymphoma, glioblastoma, and cancers of the lung, esophagus, colon, and

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pancreas (9,11-14). The serum level of DcR3 also was significantly elevated in 56.2% of a variety of tumors, including cancers of the digestive system, thyroid, lung, and breast (15).

The aim of this work was to study the expression of DcR3 in 60 cases of Barrett esophagus-associated lesions by immunohistochemical analyses and the potential application of DcR3 overexpression in the diagnosis of EAC.

MATERIALS AND METHODS

A total of 60 cases of Barrett esophagus-associated lesions in the surgical pathology files of Damanhour national medical institute hospital during the period at April 2005 to May 2006 were included in this study. Sixty esophagogastrectomy specimens that contained areas of normal esophageal mucosa (n = 60), Barrett esophagus (n = 40), low-grade dysplasia (n = 40), high-grade dysplasia or carcinoma in situ (CIS; n = 33), and EAC (n = 42) were selected. The diagnoses of low- and high-grade dysplasia were based on the World Health Organization criteria (16). Of 42 EACs, 10, 20, and 12 were well-, moderately, and poorly differentiated, respectively. Two measures were taken to ensure that all lesions were Barrett esophagus but not gastric cardiac-associated: (1) All cases were selected from patients who had a history of Barrett esophagus and/or Barrett esophagus-associated problems. (2) Strict topographic criteria were used to select only cases in which the main tumors and/or lesions were clearly in the esophagus.

The patients were 50 men and 10 women whose ages ranged from 42 to 84 years (mean, 66.2 years). All patients had a history of Barrett esophagus ranging from 2 to 12 years.

One of the most representative sections (slides) from each case was selected and stained with a monoclonal antibody against DcR3. Briefly, 5m-thick sections were deparaffinized, quenched with 3% hydrogen peroxide for 6 minutes, and heated for 40 minutes at 95-99 °C in DAKO antigen retrieval solution (citrate buffer, pH 6.1, DAKO, Carpinteria, CA) in a Black and Decker steamer. The slides were stained for 60 minutes with antihuman DcR3 monoclonal antibody (clone MD3B1, 1:1,000 dilution), followed by 30-minute incubations; each in goat antimouse IgG (Vector Laboratories, Burlingame, CA) and streptavidin-horseradish peroxidase (Jackson Laboratories, West Grove, PA). The positive reaction was visualized with 3-amino-9-ethylcarbazole (DAKO) which showed cytoplasmic staining (17). One hundred cells from 5 representative areas in each lesion and normal esophageal mucosa were counted. An average of 10% or more tumor cells which were stained, considered positive. The degree of staining was subdivided as follows: 1+, focal or fine granular, weak staining; 2+, linear or cluster, strong staining; and 3+, diffuse, intense staining. Overexpression of DcR3 was defined as staining of 2+ or more.

The Fisher exact test was used for comparison of overexpression of DcR3 among different groups. A *P* value of less than 0.05 was considered statistically significant. Multivariate analysis was used to identify correlation of DcR3 overexpression with patients' age and survival, tumor size, and clinical and pathological stages. A *P* value of less than 0.05 (for 1 side) or less than 0.025 (for 2 sides) was considered statistically significant.

RESULTS

DcR3 Expression in Normal Esophagus and Stomach:

Focal DcR3 overexpression was observed in 3 out of 57 cases of normal esophageal (squamous) or gastric (glandular) mucosa, although more often it was observed in the esophageal or gastric mucosa adjacent to Barrett esophagus (4/40), dysplasia (low-grade, 12/40; high-grade, 12/33), or EAC (21/42). DcR3 also stained plasma cells.

DcR3 Overexpression in Barrett-Associated Lesions

DcR3 antibody showed similar granular stains in Barrett esophagus and Barrett-associated lesions, but the staining patterns were different. In Barrett esophagus, DcR3 staining was predominantly in the apical surface or basal portion of the columnar epithelium (Figure 1A) and (Figure 1B), but the mucin of goblet cells. In dysplastic and malignant cells was not stained, more cytoplasmic staining of DcR3 were observed (Figure 1C), (Figure 1D), (Figure 1E), (Figure 1F), (Figure 1G), and (Figure 1H). The results revealed significant over-expression of DcR3 in high-grade dysplasia or carcinoma in situ and EAC than in benign esophageal mucosa (both $P < 0.0001$), Barrett esophagus (both $P < 0.001$), and low-grade dysplasia ($P < 0.01$ and $P = 0.033$, respectively). Low-grade dysplasia also showed significant overexpression of DcR3 compared with benign esophagus ($P<0.05$) but not with Barrett esophagus ($P>0.05$). DcR3 overexpression seems to correlate negatively with the grade of EAC.

DISCUSSION

EAC is becoming more common worldwide with its incidences tripling in the United States from 1976 to 1990 and an annual increase of approximately 10% (1). Esophageal cancer is most common in Northern China (with an

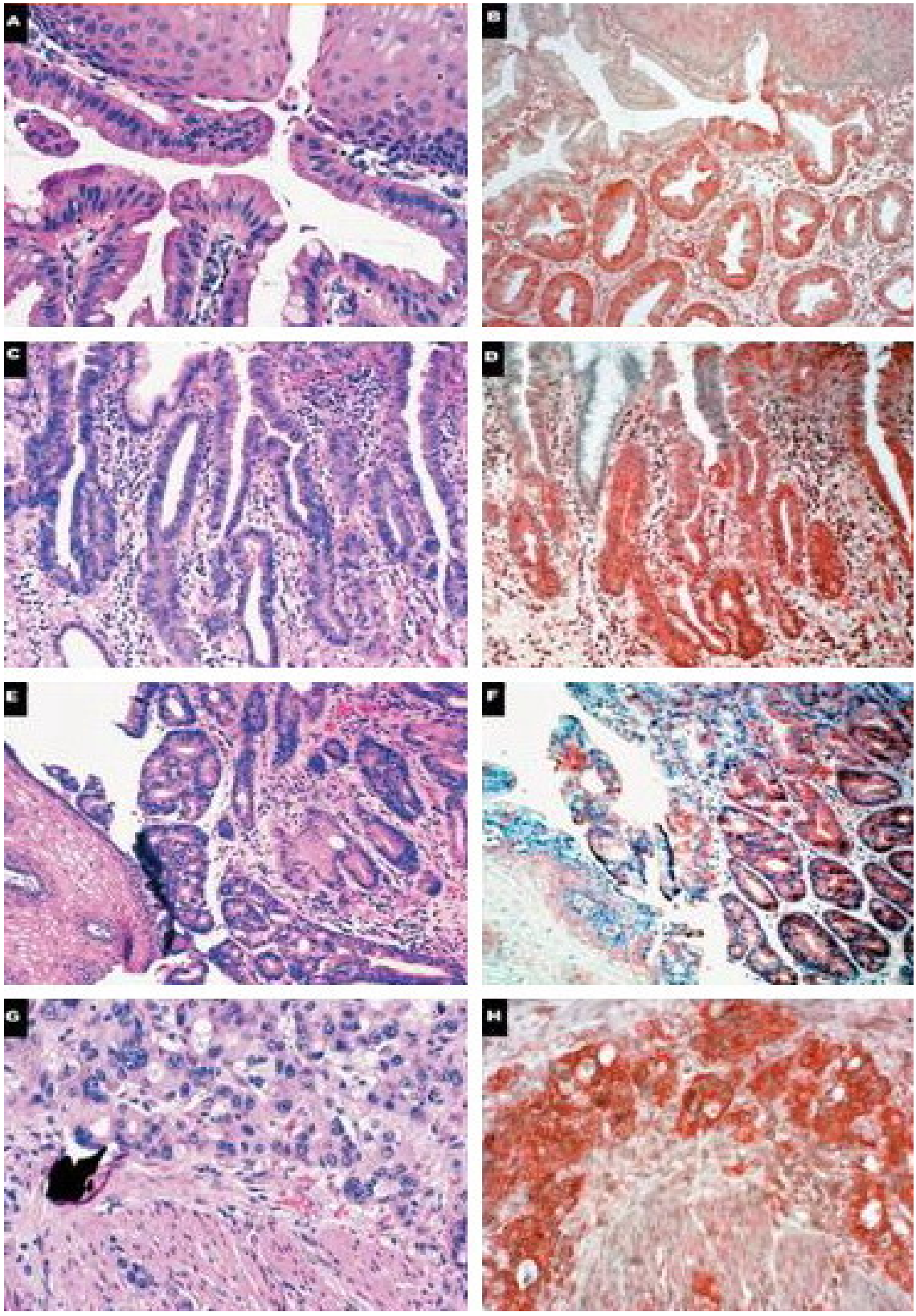

Figure 1. Overexpression of decoy receptor (DcR) 3 in Barrett esophagus and its associated lesions. A & B, Barrett esophagus (A, H&E, ×100; B, DcR3, ×100). C & D, Low-grade dysplasia (C, H&E, ×100; D, DcR3, ×100). E & F, High-grade dysplasia (E , H&E, ×100; F, DcR3, ×100). G & H, Esophageal adenocarcinoma (G, H&E, ×200; H, DcR3, ×200).

incidence of 300 per 100,000), Northern Iran, South Africa and India (23). In spite of rapid increase in its incidences (1,2) EAC remains one of the least studied and deadliest cancers in the world. The precursor lesion of EAC is thought to be Barrett esophagus, which might lead to dysplasia and eventually EAC. The pathogenesis of EAC remains unclear and the diagnoses of dysplasia and early EAC also remain a challenge. Overexpression of DcR3 has been demonstrated in several human malignant neoplasms, including esophageal adenocarcinoma (9,11-14). The present study was designed for better understanding of the pathogenesis and more accurate diagnosis of esophageal dysplasia and EAC.

As for most human cancers, it is true that complete removal has been the most effective treatment for EAC. Thus, early and accurate diagnosis is essential. The finding of significant overexpression of DcR3 in high-grade dysplasia and EAC in the present study suggests its possible role as a biomarker for the diagnoses of high-grade esophageal dysplasia and/or EAC. The percentage of DcR3 protein overexpression in esophageal adenocarcinoma in the present study was higher than that previously reported (14) (79% vs 63%). Such variation might result from a difference in the number of samples.

It is critical to differentiate high-grade dysplasia from low-grade dysplasia in Barrett esophagus because the former might result in an esophagectomy. Many biomarkers have been tested to characterize Barrett-associated dysplasia, and some progress has been achieved (18-21,24). Expression of the bcl-xl protein and human telomerase reverse transcriptase mRNA is significantly higher in Barrett-associated dysplasia than in Barrett esophagus (19,21,24,25). However, its correlation with grade of dysplasia is not known. The significant overexpression of DcR3 in high-grade or CIS vs low-grade dysplasia arising in Barrett esophagus in this study might provide some clue but not a specific marker to help diagnose of high-grade dysplasia. Moreover, the positive correlation of DcR3 overexpression with degree of dysplasia might be helpful in monitoring the progression of esophageal dysplasia.

Many genes, including oncogenes, tumor suppressor genes, growth factors and their receptors, apoptosis genes, DNA mismatch repair genes, and the *p53* gene show aberrant transcription and translation in EAC (2-3,22,24). It is known that EAC may result from the metaplasia-dysplasia-carcinoma sequence, i.e., Barrett esophagus → low-grade dysplasia → high-grade dysplasia → EAC. However, the molecular mechanisms of these processes remain unclear. The present study demonstrates a correlation between overexpression of DcR3 and the progressive severity of Barrett-associated lesions, indicating that DcR3 might be involved in the progression of dysplasia and subsequent development of EAC.

DcR3 might serve as an index to monitor the regression or progression of cancer because its serum level dramatically decreased after removal of tumors.(15). Our data showed a tendency of negative correlation of DcR3 overexpression with the grade of EAC. Such tendency might results from loss of ability to produce and/or secrete DcR3 in poorly differentiated tumor cells. However, multivariate analysis did not show any correlation of DcR3 overexpression in EAC with the prognosis or other clinicopathologic factors. Further study with more cases might be required to determine whether DcR3 protein overexpression has a role in predicting the prognosis of EAC.

CONCLUSION

From the results of this study might concluded that the positive correlation of DcR3 overexpression with degree of dysplasia might be helpful in monitoring the progression of esophageal dysplasia as DcR3 might be involved in the progression of dysplasia and subsequent development of EAC and may also serve as a potential therapeutic target for the gene therapy of EAC in the future.

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