The Protein Expression of NDRG1 in Esophageal Squamous Cell Carcinoma and Its Relationship with Clinical Pathology Factors

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Abstract: Aim. To study the expression of N-myc downstream regulated gene 1(NDRG1) in esophageal squamous cell carcinomas(ESCC). **Methods.** The S-P immunohistochemical method was employed to detect the expression of NDRG1 in 49 cases of esophageal squamous cell carcinoma, mucosa adjacent to cancer and normal mucosa. **Results.** There were no differences in the positive expression rate of NDRG1 protein in cancerous tissues, mucosa adjacent to cancer and normal mucosa($P>0.05$), but protein expression levels were different. In normal mucosa, mucosa adjacent to cancer and cancerous tissues, the expression rate in low expression level(+) were: 8.2%, 65.3%, 81.6%; the expression rate of high expression level(++) were: 87.7%, 13.0%, 8.1%, respectively, and they had significantly differences($P<0.01$). There were no differences of NDRG1 protein expression in different differentiation levels of esophageal carcinoma and with or without lymph node metastasis of esophageal carcinoma. **Conclusion.** The expression of NDRG1 is lower in esophageal squamous cell carcinoma, and this may be related to the occurence of esophageal squamous cell carcinoma. [Life Science Journal. 2006; 3(1): 18 - 22] (ISSN: 1097 - 8135).

Keywords: esophageal squamous cell carcinoma; N-myc downstream regulated gene 1; immunohistochemistry; protein expression

1 Introduction

Esophageal squamous cell carcinoma(ESCC) is a common malignant tumor which seriously destroys people's health. A better understanding of the molecular events involved in the development of esophageal cancer will be helpful to its early diagnosis, treatment and prognosis. N-myc downstream regulated gene 1 (NDRG1) was found by different laboratories (van Belzen, 1997; Zhou, 1998) and was demonstrated to be related to cell differentiation(van Belzen, 1997; Guan, 2000). It caused great interest about the relationship between NDRG1 and cancers. Researchers found that the expression of NDRG1 was higher in human normal tissues, such as kidney, prostrate, colon, etc, and lower in prostrate cancer and colon cancer (Lachat, 2002). Treatment with differentiation agents to tumor cell lines can increase the expression of NDRG1 in colon cancer and prostrate cancer cells (Park, 2003; Piquemal, 1999). To date, the exact biological function of NDRG1 remains obscure. Scholars consider that it was related to cell differentiation and necessary but not sufficient for p53-induced apoptosis (Stein, 2004). There is little report about the expression of NDRG1 in esophageal carcinoma, so we detceted the protein expression in esophageal carcinoma to probe the role of NDRG1 in its occuring and developing in order to provide some clues for diagnosis and differentiation treatment of esophageal carcinoma.

2 Materials and Methods

2.1 Materials

Human esophageal tissue specimens of surgical resection were obtained from 49 patients with esophageal cancer at Anyang Tumor Hospital during the period from September to November,

2004. There were 25 men and 24 women ranging age from 40 to 76 years with a mean of 58.3 years. 21 cases had regional lymph node metastasis. The tumor tissue, tissue adjacent to cancer and normal mocusa were collected in each case. The diagnosis of squamous carcinoma was made according to the WHO's criteria, 2000. All patients had no chemical and radial therapy history before surgical operation.

All specimens were fixed in 4% polyformalin, embedded in paraffin and stained by routine HE for routine HE analysis and histochemistry. 49 cases of carcinoma were divided into three levels according to tumor differentiation grade: high differentiation in 14 cases, moderate differentiation in 23 cases, and poor differentiation in 12 cases.

2.2 Methods

Immunohistochemistry S-P method was used to detect NDRG1 protein. NDRG1 goat polyclonal antibody was purchased from SANTA CRUZE Corporation, US. and immunostaining S-P kit was purchased from ZYMED Corporation, US. Immunohistochemistry was performed as follows (1) Four-micron sections of human tissues were deparaffinized by xylene, dehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol at room temperature for 15 min. (2) To retrieve the immunoreactivity, tissue sections were boiled twice in 10 mM sodium citrate, pH 6.0 for 5 min in an 800W microwave oven. (3) Then, non-specific staining was blocked by incubating in normal non-immune serum at room temperature for 10 min. (4) The goat anti-NDRG1 was added to adjacent tissue sections and incubated overnight at 4℃. (5) Biotin-conjugated second antibody was added to the sections and incubated at room temperature for 10 min. (6) S-P complex was added at room temperature for 10 min and DAB was used for the color reaction, and then the slides were counterstained with hematoxylin. The tissue sections were washed with PBS (0.01M, pH 7.4) between each step. Positive and negative controls were simultaneously used to ensure the specificity and reliability of staining. The positive result showed yellow or brown coloration in cytoplasm and /or plasma membranes.

The degree of NDRG1 staining was estimated by semi-quantitative evaluation and categorized by the extent and intensity of staining as follows (Shen, 1995):

(1) The extent of positive cells was estimated as 0 = positive staining cells $\leqslant$5%, 1 = positive staining cells in 6% − 30%, 2 = positive staining cells in 31% − 70%, 3 = positive staining cells in 71% − 100%.

(2) The intensity of staining was scored as 0 = achromatic, 1 = light yellow, 2 = yellow, 3 = brown. Combined staining score was used to evaluate the results of NDRG1 staining. The extent of positive cells was multiplied by the intensity of staining and scored as follows: (−) = 0, (+) = 1 − 3, (++) = 4 − 6. Results of the immunohistochemistry were judged based on the intensity of staining and the grading of the NDRG1 was done by two independent persons without prior knowledge of the patient outcome.

2.3 Stastistical analysis

The results were calculated by analysis SPSS software 11.0. χ^2 test was used to analysis the difference between groups. $P < 0.05$ was considered stastistically significance. All reported P values were two-sides.

3 Results

3.1 The expression of NDRG1 protein in esophageal normal mucosa, atypical hyperplasia mucosa and squamous cell carcinoma

The NDRG1 protein was light yellow to brown localized in the cytoplasm and cell membrance. The nuclear stain was not observed. In normal esophageal mucosa, the surface epithelial cells mainly stained and the majorities were yellow micro-granules located in the cytoplasm. The basal cells and atypical hyperplasia cells didn't be stained. The esophageal carcinoma cell stained lighter than normal mucosa epithelial cell. The expression results of NDRG1 protein was summarized in Table 1 and showed in Figures 1 − 3.

Table 1. Comparison of NDRG1 protein expression in all groups

Group	Cases	Expression of NDRG1			Percentage (%)
		−	+	++	
Normal esophageal mucosa[A]	49	2	4	43	95.9
Atypical hyperplasia mucosa[B]	23	5	15	3	78.3
Esophageal carcinoma[C]	49	5	40	4	89.8

A to B; A to C, $P<0.01$; B to C, $P>0.05$

The results in Table 1 showed that the positive expression rate of NDRG1 protein had no differences in cancerous tissue, mucosa adjacent to cancer tissue and normal esophageal mucosa. But the protein expression levels were different. In normal mucosa, the positive expression rate in low expression level (+) were: 8.2%, 61.3%, 81.8%; the expression rate in higher expression level (++) were: 87.7%, 13%, 8.1%, and they had significant difference ($P<0.01$). These results showed

that the expression level of NDRG1 protein in normal esophageal mucosa was higher than atypical hyperplasia mocusa and esophageal carcinoma. The lower expression of NDRG1 in atypical hyperplasia mucosa was concerned with the unexpression of NDRG1 in basal cells.

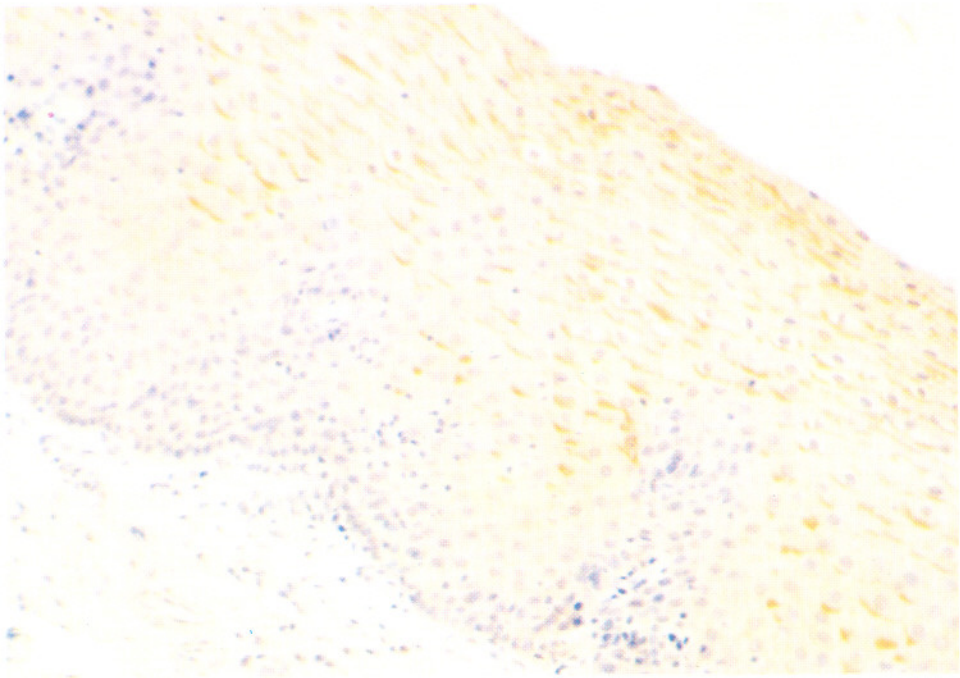

Figure 1. The expression of NDRG1 protein in normal esophageal tissue (S-P method, ×200)

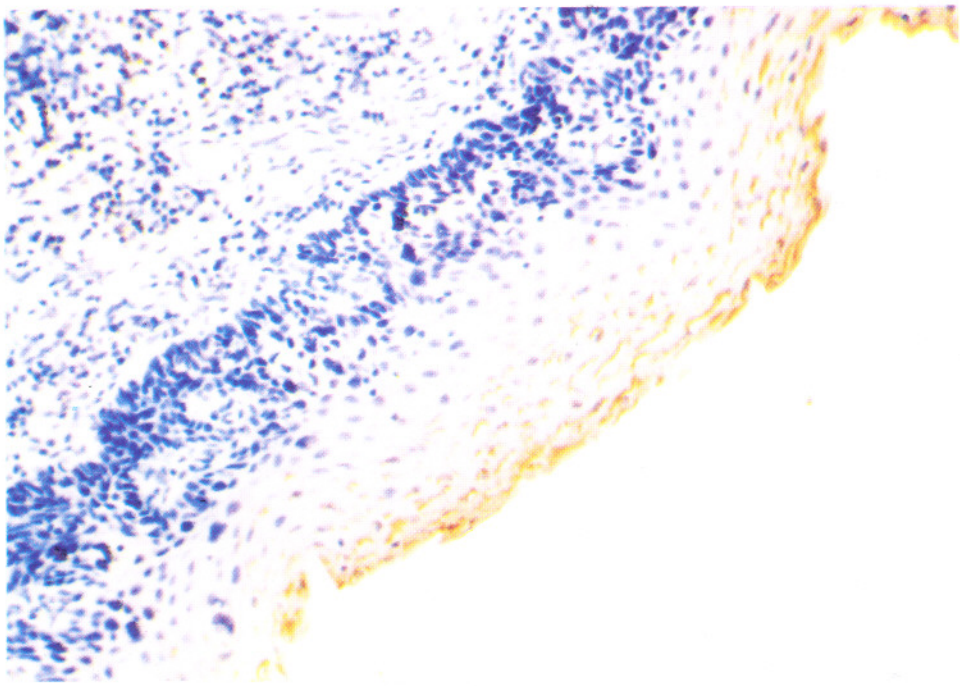

Figure 2. The expression of NDRG1 protein in esophageal atypical hyperplasia mucosa(S-P method, ×200)

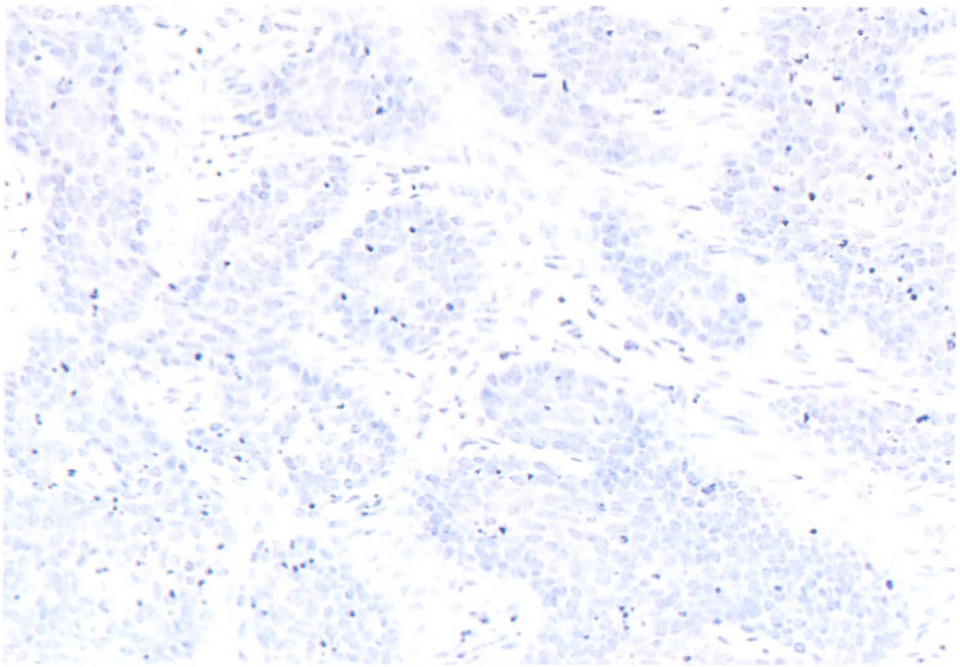

Figure 3. The expression of NDRG1 protein in ESCC(S-P method, ×200)

3.2 The relationship of NDRG1 protein expression and histological differentiations

The results of NDRG1 expression in esophageal carcinoma showed that there were no differences in different differentiation levels of esophageal squamous carcinomas ($P > 0.05$). The results were showed in Table 2.

Table 2. NDRG1 protein expression and histological differentiations in ESCC

Group	Cases	Expression of NDRG1			Percentage (%)
		−	+	++	
High differentiation	14	2	10	2	85.7
Moderate differentiation	23	2	19	2	91.3
Poor differentiation	12	1	10	1	91.7

Three groups compared, $P > 0.05$.

3.3 The relationship between NDRG1 protein expression and lymph node metastasis of esophageal carcinoma

The results showed that the expression of NDRG1 was lower and not concerned with the lymph node metastasis ($P > 0.05$). The results were showed in Table 3.

4 Discussion

NDRG1 has been mapped on chromosome 8q24.2 (van Belzen, 1997, 1998) and had a length of approximately 60 kb. NDRG1 contains 16 exons and 15 introns and NDRG1 mRNA has a length of 3 kb which contained an 1182 bp coding region, and its coding product contained 394 amino acids. Most studies showed that NDRG1 was involved in cellular growth (Kokame, 1998; Taketomi, 2003; Agarwala, 2000), cell differentiation (Piquemal, 1999; Qu, 2002; Piquemal, 1999), tumor genesis, metastasis (Kyuno, 2003) and poor clinical outcome of some tumor (Li, 2003).

Table 3. The relationship of NDRG1 protein and lymph node metastasis in ESCC

Group	Cases	Expression of NDRG1			Percentage (%)
		−	+	++	
Metastasis group	21	2	17	2	90.5
Non-metastasis group	28	3	23	2	89.1

Two groups compared, $P > 0.05$.

Our study showed that NDRG1 protein was mainly expressed on the surface of epithelial cells, and not expressed in basal cells. This result was in accordance with Lauchet P's (2002) result to digestive tract mucosa and urinary system mucosa. We found that the expression of NDRG1 protein was lower in esophageal squamous carcinoma cells. The positive rate was significantly lower than that of normal mucosa, and some squamous carcinoma even didn't express the NDRG1 protein. This showed NDRG1 was low expressed in esophageal carcinoma. Similar results were also found in colon cancer, prostrate cancer and renal cancer (Li,

2003). In addition, van Belzen et al (1997) reported that compared to normal colon mucosa, NDRG1 mRNA expression was decreased in colon adenomas and adenocarcinoma. The high expression of NDRG1 can inhibit tumor growth (Kurdistani, 1998; Kyuno, 2003). So our results may suggest that NDRG1 gene might play an important role in carcinogenesis and development of esophageal carcinoma.

Our study also found that there were no differences of NDRG1 expression between each differentiation levels of ESCC, which indicated that NDRG1 expression wasn't correlated to tumor differentiation level. Similar results also found in Wang Zhan's (2004) research on colon cancers.

There was little research on NDRG1 and tumor metastasis. Guan(2000) reported that NDRG1 stable transfection of SW620 metastatic colon cancer cell line with Drg1 cDNA induced morphological changes and down-regulated metastatic colon cancer cells to nearly undetectable levels when compared with primary colon cancer. Bandyopadhyay (2003) research also indicated that over expression of NDRG1 could inhibit the metastasis of prostrate cancer. But our results indicated there was no difference between lymph node metastasis group and no lymph metastasis group, suggesting that NDRG1 expression had no relation with ESCC metastasis. The reasons need further research.

Our results may provide some clues for ESCC carcinogenesis, prognosis and differentiation therapy.

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