C-erb-B2 and Bcl-xl protein expression in Barrett´s oesophagus in correlation with morphological parameters

Barwijuk-Machała M, Reszeć J, Kemona A, Sobaniec-Łotowska M

Department of Pathology, Medical University of Bialystok, Poland

Abstract

The aim of the study was to evaluate the correlation of c-erb-b2 and Bcl-xl expression in biopsy specimens of Barrett´s oesophagus from 44 patients with morphological features. The examined group was subdivided into: negative for dysplasia, indefinite for dysplasia, positive for dysplasia - low grade, and adenocarcinoma with high grade dysplasia. Positive c-erb-B2 staining was found in 34.1% and Bcl-xl protein expression was observed in 96.9% of BE. The results showed increased c-erb-B2 and Bcl-xl protein expressions with progressive grades of dysplasia to adenocarcinoma. In conclusion, an evaluation of c-erb-B2 and Bcl-xl expression can be useful for the histopatologic diagnosis of BE and correct interpretation of dysplasia.

Key words: C-erb-B2, Bcl-xl, Barrett´s oesophagus.

Introduction

Barrett´s oesophagus (BE) is a complication of chronic gastroesophageal reflux disease and predisposes to dysplasia, which has been known as a precursor of adenocarcinoma [1, 2, 3]. Grading of dysplasia in BE is of great clinical importance and may serve to identify patients who are at high risk of developing cancer [4]. Attempts have been made to identify early markers of malignant transformation [5]. Bcl-xl is a protein, associated with cell survival, which prevents apoptosis [6]. The c-erb-B2 protooncogene encodes a transmembrane tyrosine kinase receptor that is homologous to EGF-R [7, 8]. The specimens of BE with severe dysplastic changes and adenocarcinoma showed an increased expression of c-erb-B2 protein on the cell membranes [8, 9]. The aim of our study was an evaluation of c-erb-b2 and Bcl-xl expressions in BE in correlation with morphological parameters.

ADDRESS FOR CORRESPONDENCE:
Małgorzata Barwijuk-Machała
Department of Pathology
Medical University of Bialystok
Waszyngtona 13; 15-269 Białystok, Poland

Material and methods

Paraffin sections from 44 BE-suffering patients with intestinal metaplasia, dysplasia and adenocarcinoma were retrospectively reviewed. The examined group was subdivided into negative for dysplasia - 19 cases - (I), indefinite for dysplasia, probably negative - 6 cases - (II), positive for dysplasia - low grade - 8 cases - (III) and adencarcinoma with high grade dysplasia - 11 cases - (IV). Immunostaining for c-erb-B2 and Bcl-xl was performed. using c-erb-B2 and Bcl-xl antibodies (Novocastra c-erb-B2 NCL - c-erb-B2 316, Santa Crus Biochemicals Bcl - xl No A 20, sc-7122). To visualize the antigen-antibody reaction, the LSAB technique was applied, using DAB (diaminobensidine) and ABC technique (working dilution 1:50). The stainability of c-erb-B2 was defined by the percentage of cells with a strongly stained cell membrane, often accompanied by cytoplasm staining as follows: 29% or bellow (-), between 30% and 59% (+) and 60% or over (++). The criteria for Bcl-xl reaction were as (++), when above 50% cells were immunopositive for the examined protein, (+) 50% -10% and as (-), when bellow 10% cells were immunopositive. Chi-squared test and Pearson correlation were used for statistical analysis. Values of $p< 0.05$ were considered as statistically significant.

Results and discussion

BE is defined as a replacement of the normal squamous epithelium of the lower oesophagus with metaplastic columnar

epithelium [2]. The diagnosis of BE should be made, based on the presence of goblet cells, which can easily be identified, using an Alcian blue PAS stain [10]. In our experience, this stain is very useful in the diagnostic procedure because of the necessity of discriminating goblet cells from reactive foveolar cells which may be very similar on H-E stain. Chronic oesophageal injury from gastroesophageal reflux may result in dysplasia, which is an intermediate step in the progression from metaplasia to adenocarcinoma [3]. Regular endoscopic surveillance, with histologic evaluation of biopsy specimens, has been recommended for patients with the diagnosis of BE [4]. The assessment of dysplasia in biopsy samples in BE may be difficult because of its multifocal distribution [3]. Bhargava et al. have stressed that only endoscopy with systematic mapping may provide better surveillance for an individual patient [5]. In our study, an initial microscopic examination revealed dysplasia in 57%. 8 patients with the diagnosis of BE had low grade dysplasia. High grade dysplasia was found adjacent to adenocarcinoma in the majority of examined cases. The coexistence of these abnormalites is very frequent in BE and is an evidence for a malignant transformation [3]. During microscopical examination, we had some difficulties with the diagnosis of dysplasia in 6 patients with BE. These cases were initially qualified to the indefinite for dysplasia, probably negative group. Staging of dysplasia is subjected to considerable inter and intra observer variations. Despite of the well defined pathological criteria for the diagnosis of BE, differentiating the degree of dysplasia, especially low-grade dysplasia from reactive changes may be difficult in the presence of inflammation [10]. Histological grading of dysplasia is important from the therapeutic point of view [4]. It may be helpful not only in the selection of therapy, but also in the monitoring of its effectiveness. Many investigators have tried to identify early markers of malignant transformation [5, 11]. The role for c-erb-B2 oncoprotein has not been clearly specified yet. In our study, positive c-erb-B2 staining was found in mucosa of BE patients with low grade dysplasia in 5 out of 8 cases (62.5%), in 8 out of 11 cases with high grade dysplasia (77.7%) and in 10 of 11 adenocarcinomas (90%), while it has not been found in any case of indefinite dysplasia. The reported prevalence of c-erb-B2 overexpression in esophageal adenocarcinomas varies from 10% to 64% [8, 9, 12]. Based on the large proportion of adenocarcinoma with c-erb-B2 expression and negative immunoreactivity in the dysplastic areas, it has been suggested that it is the late event in the dysplasia - carcinoma sequence [13]. The results of our study are in agreement with those obtained by other investigators, who have found positive c-erb-B2 staining in dysplastic epithelium but not in mucosa of BE without dysplasia [5]. In our study, the overexpression of c-erb-B2 increased significantly with the progressive grades of dysplasia to adenocarcinoma. Statistical analysis revealed significant ($p<0.05$) difference in c-erb-B2 expression between I - III, I-IV, II-III and III-IV groups. In Group I, 10.5% were (+) positive for c-erb-B2, in Group II, all the cases were negative for c-erb-B2, in Group III, 25% were (++) positive for c-erb-B2 and 81.8% tumours in Group IV were c-erb-B2 immunopositive ($p=0.00001$). Lesions, which overexpress c-erb-B2, may be associated with a higher degree of proliferation. Kim et al. found that the proliferation rate at the surface of BE gradually increased with progressive grades of dysplasia [12]. Those observations were also supported by Matsumoto et al., who noticed that enhanced cell proliferation correlated with morphological abnormalities in BE [3]. Chen et al. showed a shift of increased proliferation activity towards the upper crypt and the luminal surface with increasing severity of dysplasia [14]. The results, obtained by Whittles et al., revealed a significant increase in the glandular proliferation to apoptosis ratio in the progression of metaplasia through dysplasia to adenocarcinoma [15]. The authors observed the decrease in apoptosis in the upper crypt and luminal surface in dysplasia and adenocarcinoma, compared with metaplasia [15]. Those findings agreed with our results, concerning Bcl-xl protein that have shown an increase of expression with the progression of BE to adenocarcinoma. In our study, Bcl-xl protein expression was observed in 32, out of 44 cases with BE (96.9%), all the adenocarcinoma cases were Bcl-xl immunopositive. There were statistically significant differences of Bcl-xl protein expression between I and III, I and IV, as well as between II and IV groups. In Group I, 5.3% of al. cases were (++) positive for Bcl-xl, in Group II, 33.3%, in Group III, 62.5% and 90.9% tumours in Group IV were Bcl-xl immunopositive ($p=0.00063$). There is lack of data, concerning with apoptosis and BE, as well as adenocarcinoma development. Van der Woude et al. found an increased Bcl-xl expression through metaplasia to adenocarcinoma [6]. Soslow et al. observed a statistically significant linear association between Bcl-xl expression versus increasing histological severity in BE [16]. Our study support the hypothesis that neoplastic transformation through metaplasia, dysplasia to adenocarcinoma in BE may be associated with apoptosis proteins expression alterations and overexpression of some oncogenes, like c-erb-B2 protein, which can be useful for the correct interpretation of dysplasia in BE.

References

1. Shaheen N, Ransohoff DF. Gastroesophageal reflux, Barrett esophagus, and esophageal cancer: scientific review. JAMA. 2002; 287: 1972-81.

2. Kawakura Y, Tatsuzawa Y, Wakabayashi T, Ikeda N, Matsuda M, Nishihara S. Immunohistochemical study of p53, c-erbB-2, and PCNA in Barrett´s esophagus with dysplasia and adenocarcinoma arising from experimental acid or alkaline reflux model. J Gastroenterol. 2001; 36: 595-600.

3. Matsumoto Y, Arai N, Mieno H, Murakami K, Ishii K, Mitomi H. Adenocarcinoma comlicating Barrett´s esophagus: an analysis of cell proliferation. J Gastroenterol. 2001; 36: 410-14.

4. Cameron AJ. Management of Barrett´s esophagus. Mayo Clin Proc. 1998; 73: 457-461.

5. Bhargava P, Eisen GM, Holterman DA, Azumi N, Hartmann DP, Hanfelt JJ, Benjamin SB, Lippman ME, Montgomery EA. Endoscopic mapping and surrogate markers for better surveillance in Barrett esophagus. A study of 700 biopsy specimens. Am J Clin Pathol. 2000; 114: 552-63.

6. Van der Woude CJ, Jansen PL, Tiebosch AT, Beuving A, Homan M, Kleibeuker JH, Moshage H. Expression of apoptosis-related proteins in Barrett´s metaplasia-dysplasia-carcinoma sequence: a switch to a more resistant phenotype. Hum Pathol. 2002; 33: 686-92.

7. Al.-Kasspoles M, Moore JH, Orringer MB. Amplifi-

cation and overexpression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer. 1993; 54: 213-9.

8. Friess H, Fukuda A, Tang WH, Eichenberger A, Furlan N, Zimmermann A, Korc M, Buchler MW. Concomitant analysis of the epidermal growth factor receptor family in esophagel cancer: overexpression of epidermal growth factor receptor mRNA but not of c-erbB-2 and c-erbB3. World J Surg. 1999; 23: 1010-8.

9. Illueca C, Llombart-Bosch A, Ferrando Cucarella J. Prognostic factors in Barrett´s esophagus: an immunohistochemical and morphometric study of 120 cases. Rev Esp Enferm Dig. 2000; 92: 726-37.

10. Haggitt RC. Barrett´s esophagus, dysplasia, and adenocarcinoma. Hum Pathol. 1994; 25: 982-93.

11. Kleeff J, Friess H, Liao Q, Buchler MW. Immunohistochemical presentation in non-malignant and malignant Barrett´s epithelium. Dis Esophagus. 2002; 15: 10-15.

12. Kim R, Clarke MR, Melhem MF, Young MA, Vanbibber MM, Safatle-Ribeiro AV, Ribeiro U Jr, Reynolds JC. Expression of p53, PCNA, and C-erbB-2 in Barrett´s metaplasia and adenocarcinoma. Dig Dis Sci. 1997; 42: 2453-62.

13. Hardwick RH, Shepherd NA, Newcomb PV, Alderson D. C-erb-B2 overexpression in the dysphagia/carcinoma sequence of Barrett´s oesophagus. 1995; 48: 129-32.

14. Chen L, Hu C, Gaboury L, Pera M, Ferraro P, Duranceau AC. Proliferative activity in Barrett´s esophagus before and after antireflux surgery. Ann Surg. 2001; 234: 172-80.

15. Whittles CE, Biddlestone LR, Burton A, Barr H, Jankowski JA, Warner PJ, Shepherd NA. Apoptotic and proliferative activity in the neoplastic progression of Barrett´s esophagus: a comparative study. J Pathol. 1999; 187: 535-40.

16. Soslow RA, Remotti H, Baergen RN, Altorki NK. Suppression of apoptosis does not foster neoplastic growth in Barrett´s esophagus. Mod Pathol. 1999; 12: 239-50.