

Immunocytochemical analysis of the tissue location of cytokines (IL-2 and IL-12) in neuroendocrine lung cancer

Aldona Kasprzak¹, Mariola Przewoźna¹, Maciej Zabel^{1, 2}, Juliusz Pankowski³,
Joanna Surdyk-Zasada¹

¹Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

²Department of Histology and Embryology, University of Medical Sciences, Wrocław, Poland

³Laboratory of Histopathology, Independent Health Care Institution, Szczecin, Poland

[Received 28 May 2003; Revised: 27 June 2003; Accepted 27 June 2003]

The study aimed at immunocytochemical evaluation of the cellular expression of two cytokines, IL-2 and IL-12 in lung carcinoids (n = 10), following the earlier demonstration of two markers of endocrine tumours (chromogranin A and NSE — neuron-specific enolase). In the immunocytochemical studies the classical avidin-biotinylated peroxidase (ABC) technique was used. Results of the tests were semiquantitatively appraised employing the IRS scale. In 9/10 cases intense reaction (score: 6–12 points) was noted for both lung neuroendocrine markers. In all cases of carcinoma co-expression of IL-2 and IL-12 was demonstrated in cells of the tumours. The cytokines showed a cytoplasmic localisation of mean (score: 3–4 points) or high (score: 6–12 points) intensity of reaction. Our studies point to a possible role of the two cytokines in the proliferation of lung neuroendocrine carcinomas but more detailed analysis is required on a broader material.

key words: lung carcinoids, IL-2 and IL-12 expression, immunocytochemistry

INTRODUCTION

Lung neuroendocrine tumours include carcinoids, large-cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC) [5]. Out of numerous markers of neuroendocrine tumours, chromogranin A, synaptophysin, neuron-specific enolase (NSE) and protein gene product 9.5 (PGP 9.5) [5, 6] have been most frequently detected. The role of lymphoid cell-secreted interleukin 2 (IL-2) and interleukin 12 (IL-12) in the activation of an anti-tumour immune response in humans has generally been recognised but few data exist on secretion of the cytokines by the tumour cells themselves [2, 7]. Tissue

expression of the two cytokines in neuroendocrine lung tumours remains unknown. The present study therefore aimed at immunocytochemical analysis of the expression of the two cytokines, i.e. IL-2 and IL-12, in selected neuroendocrine tumours of the lungs.

MATERIAL AND METHODS

The studies were performed on lung carcinoids of patients (n = 10) treated in the Independent Public Hospital for Lung Diseases in Zakopane. In each case the diagnosis of typical (8) and atypical (2) lung carcinoids was confirmed by histopathology. Tumour samples obtained during surgery were fixed in 10%

buffered formalin and embedded in paraffin blocks. In immunocytochemical tests, performed using ABC technique, the following monoclonal antibodies were used: a) rabbit anti-human chromogranin A antibody, (b) mouse anti-human NSE antibody (both from DAKO), (c) mouse anti-human IL-2 antibody, (d) mouse anti-human IL-12 antibody (the latter two from R&D Systems). The control reactions employed control sera of the respective species (negative control) (DAKO) and reactive lymph nodes ($n = 3$) of healthy patients (positive control). The intensity of the immunocytochemical reactions for neuroendocrine markers and both cytokines was evaluated employing the semi-quantitative IRS scale according to Remmele and Stegner [3], taking into account the intensity of the colour reaction and the number of positive cells. The final score represented a product of scores representing the two variables and ranged from 0 to 12 points (low reaction: 1 to 2 points, average reaction: 3 to 4 points, intense reaction: 6 to 12 points).

RESULTS AND DISCUSSION

The presence of chromogranin A and of NSE was demonstrated in all lung carcinoids, which con-

firmed the role of the markers in supplementing a diagnosis of lung neuroendocrine tumours [5, 6]. Intense reaction (score: 6–12) for both markers prevailed. In all the cases co-expression of IL-2 and IL-12 was also demonstrated. In two cases the intensity of reaction for IL-2 was low, in two cases it was moderate and in 6 cases it was high. Expression of IL-12 was moderate in half the cases and high in the other half. Cytoplasmic localisation of the cytokines was demonstrated, frequently of a granular character (Fig. 1). Immunoreactivity in mononuclear cells (lymphocytes, macrophages) for the two cytokines in the vicinity of the tumour was very seldom observed. Pronounced production of endogenous IL-2 by tumour cells *in vitro* and *in vivo* and its correlation with expression of Ki-67 and with histological tumour grade has already been demonstrated in cases of head and neck squamous cell carcinomas [4]. Our studies point to a possible role of IL-2 and IL-12 in tumour cell proliferation in cases of lung carcinoids also. However, the preliminary observations should be extended in studies on a larger material of patients and on other types of lung neuroendocrine tumours.

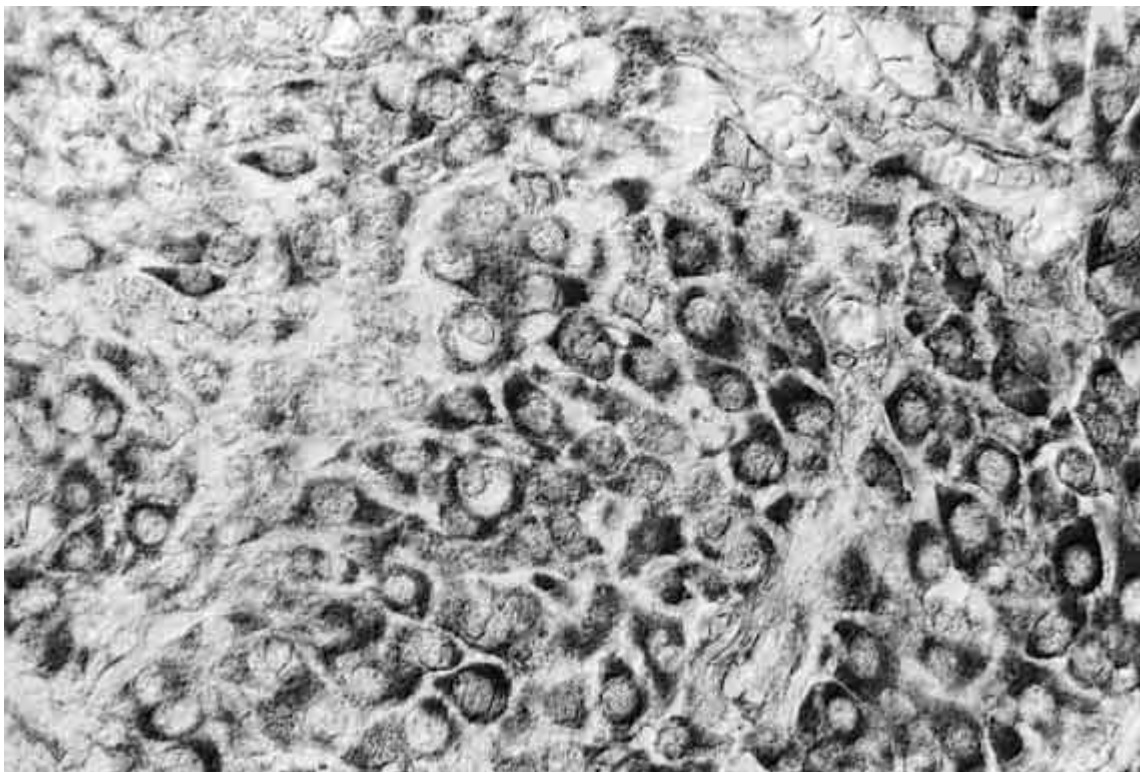


Figure 1. Immunocytochemical localisation of IL-12 in cancer cells of lung carcinoid; ABC method; $\times 400$.

REFERENCES

1. Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. *J Histochem Cytochem*, 29: 577–580
2. Lissoni P, Rovelli F, Giani L, Fumagalli L, Meregalli S, Comi G, Merlini D (1996) Correlation between IL-12 and IL-2 blood levels in the metastatic neoplastic disease: a possible inhibitory feedback system regulating their secretion. *J Biol Regul Homeost Agents*, 10: 92–94.
3. Remmele W, Stegner HE (1987) Vorschlag zur einheitlichen Definition eines immunoreactiven Score (IRS) für den Immunohistochemischen Östrogenrezeptor — Nachweis (ER-ICA) im Mammakarzinomgewebe. *Pathologe*, 8: 138–140.
4. Reichert TE, Watkins S, Stanson J, Johnson JT, Whiteside TL (1998) Endogenous IL-2 in cancer cells: a marker of cellular proliferation. *J Histochem Cytochem*, 46: 603–612.
5. Rydzewska-Rosołowska AE, Kasacka I, Sulewska A, Rudy A, Chyczewski L (2001) Pulmonary neuroendocrine cells in physiology and pathology. *Folia Histochem Cytobiol*, 39 (Suppl 2): 58–63.
6. Sheppard MN (2000) Specific markers for pulmonary tumours. *Histopathology*, 36: 273–276.
7. Yoshimura C, Nomura S, Kanazawa S, Kuwana M, Yamaguchi K, Fukuhura S (2002) Comparison of interleukin-12 with lung cancer and malignant lymphoma undergoing autologous peripheral blood stem cell transplantation. *J Cancer Res Clin Oncol*, 128: 29–36.