VIA MEDICA

SHORT COMMUNICATION

Folia Morphol.
Vol. 62, No. 4, pp. 497–499

ISSN 0015–5659
www.fm.viamedica.pl

The expression of selected neuroendocrine markers and of anti-neoplastic cytokines (IL-2 and IL-12) in lung cancers

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[Received 17 September 2003; Accepted 23 October 2003]

We have continued our studies by detecting three markers of neuroendocrine tumours of the lungs, including chromogranin A, NSE and synaptophysin, to confirm the neuroendocrine origin of lung tumours and by examining the content of two anti-neoplastic cytokines, IL-2 and IL-12 in the tumours. The studies were performed on paraffin sections of lung carcinoids (n = 13) and small cell lung carcinomas (SCLC) (n = 15). Pronounced expression of all 3 markers of neuroendocrine tumours was detected in most of the pulmonary carcinoids and in 5/15 of SCLC. Co-expression of the two cytokines (IL-2 and IL-12) in tumour cells was detected in 12/13 patients with lung carcinoid and expression of at least one cytokine in 12/15 patients with SCLC. Significantly lower numbers of cells immunoreactive to both cytokines were detected in SCLC as compared to lung carcinoids. The studies have confirmed the literature data on the lowered secretion of IL-2 in SCLC and extend the data by supplying information on the expression of IL-12. The lowered expression of the two cytokines at the time of diagnosis may represent a prognostic factor for survival in SCLC.

key words: smallcell lung carcinoma, lung carcinoid, IL-2 and IL-12 expression, immunocytochemistry

INTRODUCTION

Activation of an anti-neoplastic immune response in humans is generally known to involve 2 cytokines, interleukin 2 (IL-2) and interleukin 12 (IL-12), but few data are available on the production and secretion of the cytokines by the tumour cells themselves [3, 4]. Inhibited secretion of IL-2 in SCLC was considered to represent a prognostic index of survival in patients with the tumour type [1]. Tissue expression of the 2 cytokines in neuroendocrine lung tumours remains unknown. The present study therefore aimed at extending our previous studies on immunocytochemical detection of 2 cytokines, including IL-2 and IL-12, in neuroendocrine tumours of the lungs of various degrees of histological malignancy and various clinical patterns. The studies were preceded by immunocytochemical detection of 3 generally recognised markers of pulmonary neuroendocrine tumours (chromogranin A, NSE, synaptophysin) [6].

MATERIAL AND METHODS

The studies were performed on pulmonary carcinoids (n = 13) and on small cell lung carcinomas (n = 15) obtained from patients treated in the Independent Specialist Public Hospital for Pulmonary Diseases in Zakopane. Tumour samples obtained during surgery were fixed in 10% buffered formalin and embedded in paraffin blocks. Immunocytochemical

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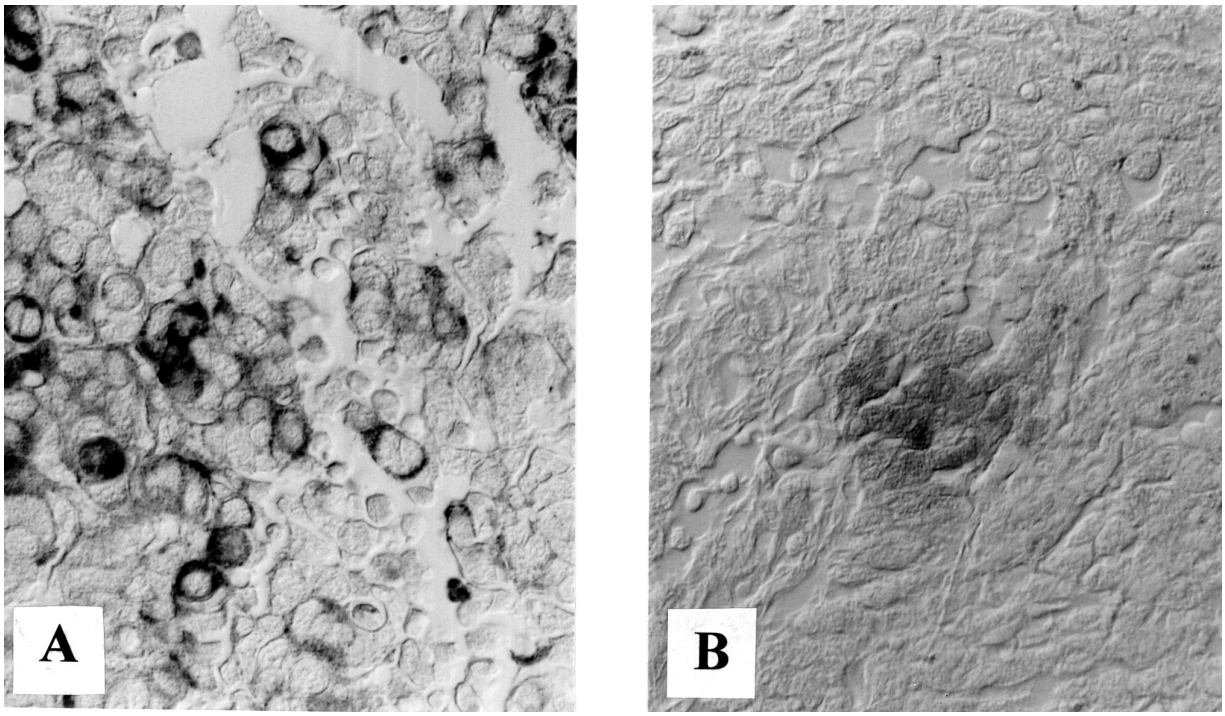

Figure 1. Immunocytochemical localisation of chromogranin A (**A**) and IL-2 (**B**) in cancer cells of smallcell lung carcinoma. ABC method. Original magn. × 400.

studies according to ABC technique [2] employed the following monoclonal antibodies: (a) rabbit anti--human chromogranin A antibody (b) mouse anti--human NSE antibody (c) mouse anti-human synaptophysin antibody (all from DAKO), (d) mouse anti-human IL-2 antibody, (e) mouse anti-human IL-12 antibody (two latter from R&D Systems). Control reactions employed control sera of the respective species (negative control) (DAKO) and reactive lymph nodes (n = 3) from healthy controls (positive control). The intensity of the immunocytochemical reactions for neuroendocrine markers and both cytokines was evaluated employing the semiquantitative IRS scale, according to Remmele and Stegner [5], taking into account the intensity of the colour reaction and the number of positive cells. The final score represented a product of the scores representing the 2 variables and ranged from 0 to 12 points (low reaction: 1–2 points, average reaction: 3–4 points, intense reaction: 6–12 points).

RESULTS AND DISCUSSION

In all the lung carcinoids (n = 13) and in 14/15 cases of SCLC the presence of chromogranin A, NSE and synaptophysin was demonstrated. In the carcinoids an intense reaction for all the 3 markers dominated (score: 6–12). In SCLC the reactions for individual markers were more variable and intense reactions were seen only in 5/15 cases (Fig. 1A). Co-expression of IL-2 and IL-12 in tumour cells was observed in 12/13 lung carcinoids and expression of at least one cytokine was seen in 12/15 SCLC. The cytokines demonstrated a cytoplasmic localisation. As compared to the carcinoids, intensity of the reactions for the two cytokines was generally lower in SCLC (Fig. 1B). Immunoreactivity to both cytokines was seen very rarely in mononuclear blood cells (macrophages, lymphocytes) in tumour-surrounding tissues. Our results demonstrated a decreased expression of the 2 cytokines in SCLC cells as compared to cells of lung carcinoids, which confirmed some literature data on the lowered secretion of IL-2 in SCLC, which was thought to represent an independent prognostic index of survival in SCLC [1]. The earlier findings and our present results have indicated also that cells of the true tumour and not the mononuclear lymphoid cells in the vicinity of tumour cells represent the main source of the cytokines [4]. Detection of the lowered expression of IL-2 and/or IL-12 in neoplastic cells of neuroendocrine tumours may be regarded as representing a prognostic factor, in line with clinical observations and literature data on the much higher malignancy of SCLC as compared to that of lung carcinoids. This finding may also provide an indication for a more radical therapy. In ad-

dition, our studies have confirmed earlier reports on the high sensitivity of studies on markers such as chromogranin A, NSE and synaptophysin in the diagnosis of the neuroendocrine origin of lung tumours [4, 6].

REFERENCES

1. Fischer JR, Schindel M, Bulzebruck H, Lahm H, Krammer PH, Drings P (1997) Decrease of interleukin 2 secretion is a new independent prognostic factor associated with poor survival in patients with small-cell lung cancer. Ann Oncol, 8: 457–461.
2. Hsu S-M, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem, 29: 577–580.
3. Kasprzak A, Przewoźna M, Zabel M, Pankowski J, Surdyk-Zasada J (2003) Immunocytochemical analysis of the tissue location of cytokines (IL-2 and IL-12) in neuroendocrine lung cancer. Folia Morphol, 62: 301–303.
4. 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.
5. Remmele W, Stegner HE (1987) Vorschlag zur einheitlichen Definition eines immunoreactiven Score (IRS) fur den Immunohistochemichen Ostrogenrezeptor — Nachweis (ER-ICA) im Mammakarzinomgewebe. Pathologe, 8: 138–140.
6. Sheppard MN (2000) Specific markers for pulmonary tumours. Histopathology, 36: 273–276.