YALE JOURNAL OF BIOLOGY AND MEDICINE 70 (1997), pp. 501-508.

Biochemical Diagnosis of Neuroendocrine GEP Tumor

Kjell Öberg

Endocrine Oncology Unit, Department of Internal Medicine, Uppsala, Sweden

(Received January 30, 1997; accepted June 15, 1998)

Neuroendocrine gut and pancreatic tumors are known to contain and secret different peptide hormones and amines. During the last two decades, many radioimmunoassays and Elizas have been developed to analyze these substances in blood and urine, which has enabled clinicians to improve the diagnosis and monitoring of patients with various neuroendocrine tumors. Due to cost constraints in medical care, it is important to try to define the most useful biochemical markers from the clinical point of view.

The glycoprotein chromogranin A has been shown to be a useful marker for diagnosing various neuroendocrine tumors, both by histopathology and circulating tumor markers. In patients with demonstrable endocrine tumors, about 90 percent of the patients present high circulating levels of chromogranin A. A hundred-fold increase of plasma chromogranin is seen in patients with midgut carcinoid tumors and liver metastases. The plasma levels of chromogranin A reflect the tumor mass and can be used for monitoring the patient during treatment and follow-up, although the day-to-day variation might be 30-40 percent. High circulating levels of the chromogranin A might be an indicator of bad prognosis in patients with malignant carcinoid tumors.

Besides analyzing plasma chromogranin A, specific analyses such as urinary 5-HIAA in midgut carcinoid patients, serum gastrin in patients with Zollinger-Ellison syndrome and insulin/proinsulin in patients with hypoglycemia should be performed. In patients with small tumor masses or intermittent symptoms, provocative tests such as a meal stimulation test, secretin test or pentagastrin stimulation of tachykinin release can supplement the basal measurements of peptides and amines. To fully evaluate the growth potential in neuroendocrine tumors, traditional biochemical markers should be supplemented with indicators of growth proliferation (Ki-67, PCNA) and immunohistochemical staining for the adhesion molecule CD44 and the PDGF-α receptor. Finally, analysis of somatostatin receptor subtypes and induction of the enzymes 2-5A syntethase and PKR are of clinical value.

INTRODUCTION

Neuroendocrine gut and pancreatic tumors are known to contain and secrete different peptide hormones and amines. During the last two decades, many radioimmunoassays and Elizas have been developed for analyzing peptide hormones and amines in the blood and urine. Such assays have enabled clinicians to improve the diagnosis and monitoring of patients with various neuroendocrine tumors.

There are several well-known clinical syndromes related to specific hormone production in patients with endocrine pancreatic tumors. The most frequent type of endocrine pancreatic tumor, which also can be confined in the duodenum and rarely outside the gut, is the gastrinoma, producing high amounts of gastrin causing the Zollinger-

[a] *To whom all correspondence should be addressed:* Kjell Öberg, M.D., Ph.D., Head Endocrine Oncology Unit, Department of Internal Medicine, S-751 85 Uppsala, Sweden.
[b] *Abbreviations:* MEN I, multiple endocrine neoplasia type 1; MAO, maximal acid output; BAO, basal acid output.

Ellison syndrome. The second most common clinical syndrome is the hypoglycemic or insulinoma syndrome, due to an endocrine pancreatic tumor producing insulin or proinsulin. More rare clinical syndromes are the so-called glucagonoma syndrome and WDHA syndrome. In the former, a glucagon-producing tumor is found in the pancreas with production of glucagon or proglucagon with a typical skin rash, the so-called necrolytic migratory erythema, but also anemia, weight loss and diarrhea. The WDHA syndrome, or VIP-oma syndrome, is caused by a tumor producing VIP or PHI, which might be located either in the pancreas, in the lung or in the adrenals. Somatostatin-producing tumors are found in a small number of patients with endocrine pancreatic tumors or duodenal carcinoids. These patients might present gall stones, diarrhea and diabetic glucose tolerance. Most of these patients show very mild clinical symptoms and are often considered to have a "nonfunctioning" islet cell tumor. It should be noted that most malignant tumors produce concomitantly several peptide hormones and amines and might shift from one clinical syndrome to another during the course of the disease. About one-third of the patients with endocrine pancreatic tumors do not present any clinical hormone related syndrome and are so called "non-functioning" islet cell tumors. These tumors are, from a biochemical point of view, functioning because they are secreting various hormones such as pancreatic polypeptide, chromogranin A and B, HCG-alpha and beta subunits, neurotensin, PYY, and so forth. Sometimes they are also producing biologically inactive forms of other peptide hormones [1].

The most common type of neuroendocrine GEP tumor is the midgut carcinoid, which presents the carcinoid syndrome when liver metastases are present. This syndrome is related to secretion of serotonin, tachykinins such as neurokinin A and substance P, as well as bradykinins and prostaglandins. Foregut carcinoids are confined to the lung or gastric or duodenal mucosa and might produce a lot of different peptide hormones and amines. Lung carcinoids might secrete ACTH, GHRH, CRF, ADH, gastrin, VIP, pancreatic polypeptide, HCG-alpha and beta subunits. The patients might present various clinical syndromes related to this hormone production such as Cushing's syndrome, acromegaly and even the Zollinger-Ellison syndrome. Gastric neuroendocrine tumors, for example the ECL-oma, may produce histamin, serotonin as well as chromogranin A. Duodenal carcinoids might produce gastrin as well as somatostatin and also chromogranin A. Hindgut carcinoids with the primary tumors located in the distal part of colon or rectum are mostly non-functioning from the clinical point of view, but they may produce peptide hormones such as PYY, PP, HCG-alpha and beta as well as chromogranin A [2].

For the biochemical work-up in patients with neuroendocrine GEP tumors, all these peptides mentioned above can be analyzed both by histopathology as well as by radioimmunoassays and Elizas for detection in the plasma or serum using antibodies against the different hormones and amines. Today, however, due to increased costs of medical care, it is important to attempt a cost-benefit analysis when trying for the ultimate biochemical diagnosis of these tumors. When the patients suffer small tumors or present intermittent symptoms, provocative tests might supplement basal measurements of peptides and amines. Today it is also of interest to combined the biochemical characterization of neuroendocrine tumors with markers for cell proliferation, cell adhesion, growth factor/receptor expression, content of somatostatin receptor subtypes and also induction of specific enzymes induced by interferon such as P 68 kinase (PKR) and 2-5A synthetase.

This overview will discuss a general tumor marker for neuroendocrine tumor, chromogranin A and also more specifically biochemical diagnosis of patients with midgut carcinoids, the Zollinger-Ellison syndrome and hypoglycemia. Useful stimulatory tests will be presented, and in the end, markers of tumor biology.

CHROMOGRANIN A

Chromogranins or secretogranins constitute a family of water-soluble glycoproteins, widely distributed in the secretory granules of the neuroendocrine tissues. The physiological role of the molecules is unclear, but a number of possible biological functions have been postulated including regulation of secretory granule function and serving as a precursor molecule for biologically active peptides [3].

Chromogranins can serve as cytochemical markers for neurendocrine tissues and as a diagnostic tool for neuroendocrine tumors. Because of their water-soluble properties, chromogranins are secreted together with peptide hormones and can be detected in the blood by radioimmunoassay techniques. Accordingly, chromogranins can serve as circulating tumor markers for neuroendocrine tumors of different origin [4, 5].

Chromogranin A is a 48 kd glycoprotein, which is stored in the large dense core vesicles containing protein and peptide hormones. The gene on chromosome 14 encode for a single amino acid chain with an terminal signal peptide that directs the protein into the trans-Golgi regulated pathway [6]. Chromogranin A contains several di-basic amino acid positions that serve as potential cleavage sites for production of biological active peptides [7]. Identified peptides with established biological functions derived from chromogranin A are pancreastatin (CgA 249-301), β-granin (CgA 1-114), chromostatin (CgA 124-343) and vasostatin CgA 1-77 [7] (Figure 1). Other members of the chromogranin or secretogranin family are chromogranin B or secretogranin I and chromogranin C or secretogranin II. These two members share similar properties but are stored in different tissues. Chromogranin A as well as chromogranin B are originally isolated from chromaffin cells in the bovine adrenal medulla, whereas chromogranin C was isolated from the anterior pituitary. Chromogranin A and B are present in the human adrenal medulla in about equal amounts, but chromogranin C is mainly found in the pituitary [8, 9]. The main source of circulating chromogranin A is the adrenal medulla, but other neuroendocrine tissues contribute [10].

CHROMOGRANINS AS TUMOR MARKER

In a recent study, we developed specific antibodies against chromogranin A, B and C as well as pancreastatin [11]. The antibodies were used for immunohistochemical staining of normal and neoplastic neuroendocrine tissue and development of reliable radioimmunoassays for chromogranin A, B and C as well as pancreastatin. In 44 patients with

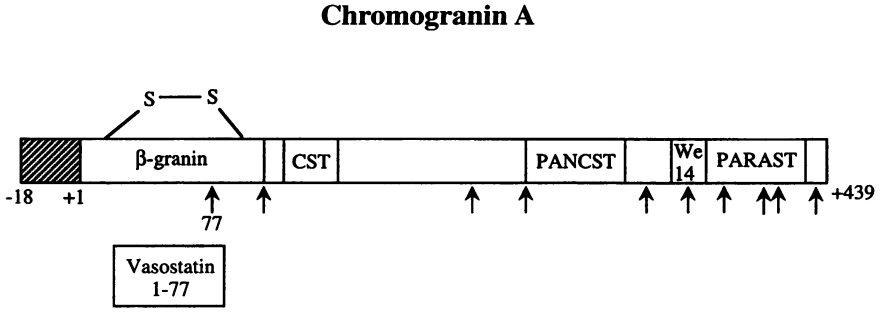

Figure 1. The chromogranin A molecule. Arrows indicate di-basic cleavage sites CST, chromostatin; PANCST, pancreastatin; PARAST, parastatin.

carcinoid tumors, 17 patients with sporadic endocrine pancreatic tumors and 11 patients with endocrine pancreatic tumors and the MEN I syndrome, plasma measurements revealed elevated chromogranin A levels in 99 percent, chromogranin B in 88 percent, chromogranin C in just six percent and pancreastatin in 46 percent of the patients (Figure. 2). Urinary measurements of the same peptides revealed lower numbers of patients with elevated levels in 39 percent, 15 percent, 14 percent and 33 percent, respectively. Gel permetion chromatography of plasma and urine from nine patients showed that the circulating chromogranin A has a higher molecular weight than chromogranin A excreted to the urine [12].

Chromogranin A is increased in about 90 percent of patients with various neuroendocrine tumors, however, somewhat lower number of patients with increased levels have been found in insulinomas. In those patients, chromogranin B might be a more useful marker. Although pancreastatin is a fragment of chromogranin A, it is a less sensitive

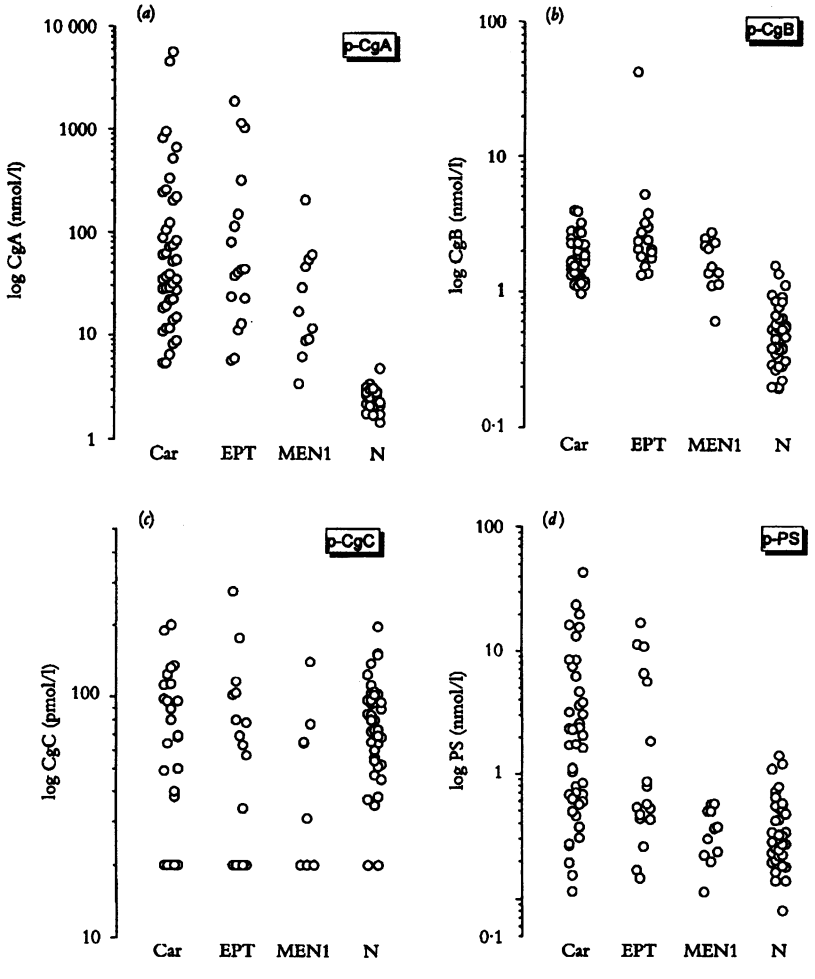

Figure 2. Plasma levels of chromogranin A, B, C and pancreastatin in patients with carcinoids (Car), endocrine pancreatid tumors (EPT) or multiple endocrine neoplasia type I (MEN 1). N, normal individuals.

marker than chromogranin A for neuroendocrine tumors [12]. One reason might be that many tumors are lacking necessary enzymes that can cleave off the peptide at di-basic cleavage sites. Therefore, a specific pancreastatin antibody does not recognize such a fragment. In patients with Multiple Endocrine Neoplasia type 1 (MEN I), measurements of chromogranin A is a less sensitive marker than in sporadic cases of endocrine pancreatic tumors or patients with carcinoid tumors. The sensitivity was 76 percent in a study we recently performed (to be published), and, therefore, this analysis should be supplemented with other diagnostic tests, such as a meal stimulation with a sensitivity of 85 percent. Falsely elevated levels of chromogranin A can be found in patients with impaired renal function, essential hypertonia and some patients with inflammatory bowel disease. In general, the levels of chromogranin A are significantly lower in these patients, usually less than 10 nmol/l. However, this might generate problems in the early diagnosis of endocrine pancreatic tumors since patients with only primary tumors can provide chromogranin A levels between 5 and 10 nmol/l. Patients with metastatic disease usually present 10-fold higher levels, and, in particular, patients with classical carcinoid tumors present 100-fold higher levels when liver metastases have developed. There is a good correlation between chromogranin A levels and the tumor size in neuroendocrine tumors, which has particularly been shown for classical midgut carcinoids. In a multivariate analysis, we could demonstrate that high levels of chromogranin A correlated to a significantly worse prognosis than patients with lower levels of chromogranins A (to be published). This might indicate that chromogranin A or fragments of it might have growth promoting effects. Plasma chromogranin A levels are suitable for monitoring patients during treatment. However, day-to-day variation in patients with endocrine tumors is median 38 percent, which should be taken into consideration.

SPECIFIC BIOCHEMICAL MARKERS

In patients with classical midgut carcinoid tumors, chromogranin A in plasma together with urinary 5-HIAA are the corner stones for biochemical diagnosis. In a study of 301 carcinoid patients (Table 1), urinary 5-HIAA was increased in 76 percent of the patients, chromogranin A in 87 percent and plasma neuropeptide K in 46 percent. With respect to foregut carcinoids, urinary 5-HIAA was only increased in 31 percent, plasma

Table 1. Tumor markers in 301 carcinoid patients.

	Increased (percent)	Median level	Refs. range
Midgut			
U-5HIAA	76	285 μmol/24 hr	<80 μmol/24 hr
p-Chromogranin A	87	2.300 μg/l	<350 μg/l
p-Neuropeptide K	46	23 pmol/l	<16 pmol/l
Foregut			
U-5HIAA	31	85 μmol/24 hr	
p-Chromogranin A	79	847 μg/l	
p-Neuropeptide K	9	20 pmol/l	
Hindgut			
U-5HIAA	0	33 μmol/24 hr	
p-Chromogranin A	100	727 μg/l	
p-Neuropeptide K	0	7.8 pmol/l	

chromogranin A in 79 percent and neuropeptide K in nine percent. In patients with hindgut tumors, only chromogranin A was increased among these three markers, and it was increased in 100 percent of the patients. In patients with the Zollinger-Ellison syndrome, measurements of basal (BAO) and maximal acid output (MAO) is mandatory, and levels of BAO above 15 mekv/hour raises a suspicion of a Zollinger-Ellison syndrome. This test is then supplemented by measurement of serum gastrin and chromogranin A. In some patients, analyses of progastrin might be of value since some patient's tumors are not able to process progastrin to amidated forms of gastrin [13]. In patients with insulin-producing tumors, measurement of blood glucose together with serum insulin and proinsulin forms the basic biochemical diagnosis. Non-functioning neuroendocrine tumors are often diagnosed in the late stage of the disease, and the most useful biochemical markers are chromogranin A, pancreatic polypeptide and HCG-alpha and beta subunits. Some patients can also be diagnosed by measurements of C-A 19-9 and C-A 50.

STIMULATORY TEST

There are some useful stimulatory tests for the diagnosis of neuroendocrine tumors. One is a meal stimulatory test, with a standardized meal containing totally 563 kcal (66 g carbohydrates, 18 g proteins and 22 g lipids) [14]. Measurements of pancreatic polypeptide and gastrin are performed during the meal and have been particularly useful in patients with MEN I with endocrine pancreatic tumors. The biochemical diagnosis of an endocrine pancreatic tumor can be obtained four to five years before radiological detection of the tumor. Another important stimulatory test is the secretin test for patients with suspected gastrinomas, where 2 CU of secretin is infused intravenously and gastrin sampling occurs during 20 minutes after the infusion. Serum gastrin increments of about 200 pg/ml or 50 percent increase of basal gives a sensitivity of 75-85 percent [15]. Calcium infusion test with measurement of gastrin can be used in selected cases but with lower sensitivities (40-70 percent) [15, 16]. Pentagastrin stimulation of tachykinin release in patients with carcinoid tumors might be of value for early detection of classical midgut carcinoids. Pentagastrin 0.6 μg/kg is injected with measurements of tachykinin for 20 minutes. A positive test shows a peak within five minutes after injection [17].

MARKERS OF TUMOR BIOLOGY

Besides a characterization of the hormone production in different neuroendocrine tumors, it is of value to try to evaluate the growth potential of the tumors. This can be done by staining tissue sections for proliferation markers such as Ki67 or PCNA [18]. Other informative markers might be the adhesion molecule CD44 where expression of higher molecular forms of CD44 is related to worse prognoses in many malignancies [19]. Another marker of bad prognosis is the expression of PDGF-α receptor, which has been shown both for ovarian tumor as well as carcinoids [20]. A complete work-up in these patients should also include analyses of subtypes of somatostatin receptors and induction of 2-5A syntethase as well as PKR. The last two being enzymes that are induced by α-interferon and can be used as prognostic markers [21].

In conclusion, the basis for biochemical diagnoses in neuroendocrine tumors is measurements of chromogranin A, which can be used as a general screening marker but should be supplemented in the various syndromes with the more specific analyses such as urinary 5-HIAA in patients with carcinoid tumors, gastrin in Zollinger-Ellison patients and insulin/pro-insulin in hypoglycemic patients. For monitoring patients during treatment, chromogranin A is a good marker to follow, although the day-to-day variation might be as

high as 38 percent. Plasma levels of chromogranin A might be of prognostic value for carcinoid patients. Assessments of parameters for tumor growth potential and tumor biology should be included in the future to be able to improve the therapeutic outcome.

REFERENCES

1. Eriksson, B., Arnberg, H., Lindgren, P.G., Lorelius, L.E., Magnusson, A., Lundqvist, G., Skogseid, B., Wide, L., Wilander, E., and Öberg, K. Neuroendocrine pancreatic tumors: clinical presentation, biochemical and histopathological findings in 84 patients. J. Int. Med. 228:103-13, 1990.
2. Norheim, I., Öberg, K., Theodorsson-Norheim, E., Lindgren, P.G., Lundqvist, G., Magnusson, A., Wide, L., and Wilander, E. Malignant carcinoid tumors: an analysis of 103 patients with regard to tumor localization, hormone production and survival. Ann. Surg. 206:115-25, 1987.
3. Winkler, H. and Fischer-Colbrie, R. The chromogranin A and B: the first 25 years and future perspectives. Neuroscience 49:497-528, 1992.
4. O'Connor, D.T. and Deftos, U. Secretion of chromogranin A by peptide-producing endocrine neoplasms. N. Engl. J. Med. 314:1145-1151, 1986.
5. Eriksson, B., Arnberg, H., Öberg, K., Hellman, U., Lundqvist, G., Wernstedt, D., and Wilander, E. Chromogranins—new sensitive markers for neuroendocrine tumors. Acta Oncol. 28:325-329, 1989.
6. Wiedenmann, B. and Huttner, W.B. Synaptophysin and chromogranins/secretogranins—widespread constituents of distinct types of neuroendocrine visicles and new tools in tumor diagnosis. Virchows Arch. (B). 58:95-121, 1989.
7. Huttner, W.B., Gerdes, H.H., and Rosa, P. The granin (chromogranin/secretogranin) family. Trends Biochem. Sci. 16:27-30, 1991.
8. Blaschko, H., Comline, R.S., Schneider, F.H., Silver, M. and Smith, A.D. Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. Nature. 215:58-59, 1967.
9. Fischer-Colbrie, R. and Frischenschlager, T. Immunological characterization of secretory proteins of chromaffin granules: chromogranin A, chromogranin B and enkephlin-containing peptides. J. Neurochem. 44:1854-1961, 1985.
10. Takiyyuddin, M.A., Cervenka, J.H., Pandian, M.R., Stuenkel, C.A., Neumann, H.P.H., and O'Connor, D.T. Neuroendocrine sources of chromogranin-A in normal man: clues from selective stimulation of endocrine glands. J. Clin. Endocrinol. Metab. 71:360-369, 1990.
11. Stridsberg, M., Hellman, U., Wilander, E., Lundqvist, G., Hellsing, K., and Öberg, K. Fragments of chromogranin A are present in the urine of patients with carcinoid tumours: development of a specific radioimmunoassay for chromogranin A and its frequency. J. Endocrinol. 134:329-337, 1993.
12. Stridsberg, M., Öberg, K., Li, Q., Engstrom, U., and Lundqvist, G. Measurements of chromogranin A, chromogranin B (secretogranin 1), chromogranin C (secretogranin 11) and pancreastatin in plasma and urine from patients with carcinoid tumours and endocrine pancreatic tumours J. Endocrinol. 144:49-59, 1995.
13. Bardram, L. Progastrin in serum from Zollinger-Ellison patients: non indicator of malignancy? Gastroenterology 98:1420-1425, 1990.
14. Skogseid, B., Öberg, K., Benson, L., Lindgren, P.G., Lörelius, L.E., Lundqvist, G., Wide, L. and Wilander, E. A standardized meal stimulation test of the endocrine pancreas for early detection of pancreatic endocrine tumors in multiple endocrine neoplasia type I syndrome: five years experience. J. Clin. Endocrinol. Metab. 94:1233-1240, 1987.
15. Frucht, H., Howard, J.M., Slaff, J.E., Wank, S.A., McCarthy, D.M., Maton, P.N., Vinayek, R., Gardner, J.D., and Jensen, R.T. Secretin and calcium provocative tests in patients with Zollinger-Ellison syndrome: a prospective study. Ann. Intern. Med. 111:713, 1989.
16. Lamers, C.B.H. and van Tongeren, J.H.M. Comparative study of the value of calcium, secretin and meal stimulated increase in serum gastrin in the diagnosis of the Zollinger-Ellison syndrome. Gut 18:128, 1979.
17. Norheim, I., Theodorsson-Norheim, E., Brodin, E., and Öberg, K. Tachykinins in carcinoid tumors: their use as a tumor marker and possible role in carcinoid flush. J. Clin. Endocrinol. Metab. 63:605-612, 1986.
18. Chaudhry, A., Öberg, K., and Wilander, E. A study of biological behaviour based on the expression of a proliferating antigen in neuroendocrine tumors of the digestive system. Tumor Biol. 13:27-35, 1992.

19. Chaudhry, A., Gobl, A., Eriksson, B., Skogseid, B., and Öberg, K. Different splice variants of CD44 are expressed in gastrinomas but not in other subtypes of endocrine pancreatic tumors. Cancer Res. 54:981-986, 1994.
20. Henriksen, R., Funa, K., Wilander, E., Bäckström, T., Ridderheim, M., and Öberg, K. Expression and prognostic significance of platelet derived growth factor and its receptors in epithelial ovarian neoplasms. Cancer Res. 53:4550-4554, 1993.
21. Grandér, D., Öberg, K., Lundqvist, M.L., Janson, E.T., Eriksson, B., and Einhorn, S. Interferon-induced enhancement of 2′-5′-oligoadenylate synthetase in mid-gut carcinoid tumors. Lancet 336:337-340, 1990.