1783-101A
MB:pah:20

COMPOSITIONS AND METHODS FOR TREATING SMALL CELL AND NONSMALL CELL LUNG CANCERS

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates generally to the treatment of lung cancer. More particularly, it relates to certain biological response modifiers and their use in treating neuroendocrine small cell lung cancers and non-neuroendocrine nonsmall cell lung cancers.

Description of the Related Art

Lung cancer is a serious public health problem that kills approximately 150,000 people in the United States annually. Minna, J.D., et al. in Cancer, Principles and Practice of Oncology, eds., DeVita, V.T., Jr., et al., Lippincott, Philadelphia (1985), pp. 507-599.

Lung cancers can be grossly divided into small cell lung cancer (SCLC) which accounts for approximately 25% of lung cancer cases and nonsmall cell lung cancer (NSCLC). NSCLC can be further subdivided into adenocarcinoma, lung cell carcinoma and squamus cell carcinoma, each of which account for about 25% of lung cancer cases.

SCLC is caused by a neuroendocrine tumor that undergoes remission generally after chemo- or radiation-therapy but that generally recurs after a few months, after which the patient is usually refractory

to these therapeutic modalities. The tumor is initially localized to the lung, but undergoes metastasis to other organs such as the liver, lymph nodes, bone and/or brain. The median survival time of SCLC patients is 1 year.

NSCLC is treated by surgical resection, as well as by chemotherapeutic agents such as cyclophosphamide, methotrexate, ifosfamid and cis-platin. For NSCLC patients, only 25% survive for 5 years after diagnosis.

Clearly, there is a need for newer, more imaginative and more effective therapeutic approaches, such as an approach based upon endogenous biochemical factors that regulate the growth of lung cancer cells that is the subject matter of this invention.

Lung cancer cells make and secrete endogenous growth factors that regulate tumor proliferation. Moody, T., Science, 214:1246 (1981). For example, bombesin/gastrin releasing peptide (BN/GRP), members of a class of peptides referred to as "biological response modifiers" (BRM), are synthesized in SCLC cells as a high molecular weight protein that is processed to a 27-amino acid, biologically active form. Sausville, E.A., et al., J. Biol. Chem., 261:2451 (1985). SCLC cells use BN/GRP as an autocrine growth factor (Cuttitta, F., et al., Nature, 316:823 (1985); these peptides stimulate the clonal growth of such cells. Carney, D.N., et al., Cancer Res., 47:821 (1987). Thus, SCLC cells synthesize and secrete BN/GRP, and BN or GRP bind to cell surface receptors and stimulate the growth of SCLC cells. The autocrine growth cycle of BN/GRP can be blocked using either monoclonal antibodies against BN (Cuttitta et al., 1985) or a BN receptor antagonist, $Psi^{13,14}$ (Leu^{14} BN). Mahmoud, S., et al., Life Sci., 44:367 (1989)).

In contrast, NSCLC cells secrete transforming growth factor α (TGFα), but not BRMs such as BN/GRP. TGFα binds to cell surface receptors for epidermal growth factor (EGF), and stimulates growth of NSCLC cells. Imanishi, L., et al., J. Natl. Cancer Inst., 81:220 (1989). Also, EGF stimulates the clonal growth of established NSCLC cell lines such as adenocarcinoma cell line 5M2 (Lee, M., et al., J. Cell Biochem., 1991, in press), and the growth of such NSCLC cells is inhibited by an EGF receptor monoclonal antibody. Moody, T.N., J. Cell. Biochem., 43:139 (1990).

Vasoactive intestinal polypeptide ("VIP"), a 28-amino acid basic peptide BRM of Mr 3324, with the sequence shown in (1) below, is synthesized in normal lung cells, and functions as a bronchodilator. Morice, A.H., et al., Lancet 1:457 (1984). These actions of VIP are mediated by cell surface receptors localized to alveoli and smooth muscle cells. Robberecht, P., et al., Regul. Peptides, 4:241 (1982). VIP receptors have also been identified on human B-lymphocyte cell lines and cells in the peripheral circulation. O'Dorisio, S., et al., J. Immunol., 15:142 (1989). It has recently been found that SCLC and NSCLC cells bind VIP with high affinity. Shaffer, M.M., et al., Peptides, 8:1101 (1987). These observations suggest that VIP, acting through cell surface receptors on normal and malignant lung cells, regulates the growth of such cells.

N-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-
Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-
Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH2 (1)

"Thymosin" is the name given to a class of polypeptide "hormones", isolated from the thymus glands of mammals, that exert profound effects as potentiators of the growth and differentiation of T-lymphocytes. Smith, E.L., et al. Principles of Biochemistry, McGraw-Hill, New York, 1983, Chapter 19. Thymosins can thus be considered to be BRMs. Members of this class include thymosin α1 ("THNα1"), a 28-amino acid peptide of 3,108 molecular weight with the following structure (2):

```
                        5                   10
Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-
                    15                  20
Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-          (2)
                25
Val-Val-Glu-Glu-Ala-Glu-Asn-OH
```

(Goldstein, A.L., et al., U.S. patent no. 4079127), and with substantial sequence homology to VIP at amino acid positions 4, 5, 7, 20 and 28; thymosin β4 (a 43-amino acid peptide, MW 4982, U.S. patent no. 4395404); thymosin α11 (a 35-amino acid peptide, U.S. patent no. 4614731); thymosin α7 (U.S. patent no. 4517119); and, thymosin β3 (a 50-amino acid peptide, U.S. patent no. 4395404).

THNα1 is known to restore cellular immunity to patients with primary immunodeficiency (Frasca, D., et al., Eur. J. Immun., 17:727 (1987)), and, in patients with NSCLC, THNα1 has been reported to accelerate the reconstitution of thymic dependent immunity. Schulof, R.S., et al., J. Biol. Response Modifiers, 4:147 (1985).

Clinical trials with cancer patients using thymosin Fraction V (a crude mixture of thymosins

isolated from thymus glands - Goldstein, A.L., et al., Proc. Nat'l Acad. Sci. USA, 56:1010 (1966), which contains about 0.6% of THNα1) and THNα1 itself have yielded equivocal results with regard to the value of immunopotentiation by these polypeptides in the course of the cancers. Thymosin Fraction V in combination with intensive remission-induction chemotherapy in patients with small cell bronchogenic carcinoma reportedly significantly prolonged survival times relative to other treatment groups. Cohen, M.H., et al. J. Am. Med. Assoc., 241:1813 (1979); Johnston-Early, A., et al., J. Am. Med. Assoc., 244:2175 (1980). However, more recently it has been reported that thymosin Fraction V did not influence the outcome in patients with SCLC who were being treated concurrently with induction chemotherapy and radiation therapy (Scher, H.I., et al., Cancer Res., 48:1663 (1988)).

In patients with NSCLC, thymosin Fraction V did not improve the results with vindesine-adriamycin-cis-platin chemotherapy. Valdivieso, M., et al., ASCO Abstracts, C-636 (1981); Bedikian, A.Y., Am. J. Clin. Oncol. (CCT) 7:399 (1984). THNα1 α1 itself also failed to influence the course of NSCLC in a small group of patients. Dillman, R.D., et al., J. Biol. Resp. Med., 6:263 (1987). In contrast, in a randomized trial to evaluate the immunorestorative properties of synthetic THNα1 in postradiation therapy patients with NSCLC, thymosin normalized T-cell function and was associated with significant improvements in relapse-free and overall survival. Schulof, R.S., et al., J. Biol. Resp. Med., 4:147 (1985).

Because of these uncertainties regarding the effects of the thymosins in patients with advanced lung cancers, we have sought, and have found, a more basic

and biochemical approach to the inhibition of proliferation of SCLC and NSCLC cells, an approach based on the roles of growth regulation factors operative in lung cancer cells. A new modality for treating SCLC and NSCLC is disclosed and claimed below.

SUMMARY OF THE INVENTION

It has been found that the growth and clonal proliferation of lung cancer cells in vitro and in vivo is reduced by agents that interfere with the functional activities in such lung cancer cells of the biological response modifier VIP.

It is thus an object of this invention to disclose thymosin preparations and analogues, derivatives and fragments thereof as therapeutic modalities that will inhibit the growth and proliferation of lung cancer cells.

It is also an object of this invention to disclose polyclonal and monoclonal anti-VIP receptor and anti-VIP antibody preparations that will inhibit the growth and proliferation of lung cancer cells.

It is yet another object of this invention to provide methods for screening thymosin and antibody preparations for efficacy as inhibitors of growth and clonal proliferation of lung cancer cells.

These and other objects of the invention will become clear by reference to the specification and to the appended claims.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 is a plot of the specific binding of ^{125}I-labeled VIP to lung cancer cell plasma membranes as a function of the log of the concentration of varying amounts of unlabeled VIP (•) and THNα1 (o).

Figure 2 is an autoradiogram showing ^{125}I-labeled VIP cross-linked to its 82 kDa receptor protein from lung cancer cell plasma membranes in the absence (b) and presence (c) of 1 μM THNα1, as well as 1 uM VIP (a).

Figure 3 shows colonization of NCI-H838 lung cancer cells in the absence (top) and presence (bottom) of 10 μM THNα1.

Figure 4 shows the size of lung cancer cell xenografts in mice in the absence (o) and presence (•) of THNα1.

DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that the growth and proliferation of lung cancer cells in vitro and in vivo can be inhibited by agents that interfere with the biological, functional activities of the biological response modifier VIP in such cells. Such agents include specific thymosins, such as THNα1 and related N-terminal and C-terminal peptides, analogues and derivatives, and anti-VIP receptor antibodies, presumably acting either to inhibit the binding of VIP to its cell surface receptors on lung cancer cells or interfere with the subsequent activities of VIP. Such agents also include anti-VIP antibodies capable of inhibiting the binding of endogenous or exogenous VIP to cell surface receptors on SCLC and NSCLC lung cancer cells and, in so doing, inhibiting the growth and proliferation of these cells. These discoveries will be reported in a future publication. Moody, T.W., et al., Cancer Research Proceedings, 1991, in press.

Specific in vitro interactions of thymosins and related fragments with cell surface binding sites on lung cancer cells may be studied by standard binding assay procedures using intact lung cancer cells or plasma membranes derived therefrom. See, e.g., Blecher, M., ed., Methods In Receptor Research, Parts I and II, Marcel Dekker, N.Y., 1976; Posner, B., ed., Polypeptide Hormone Receptors, Vol. 4, Marcel Dekker, N.Y., 1985; Journal of Receptor Research, vols. 7 and 8 (1988), which are incorporated herein by reference to the extent that they disclose binding assay procedures.

SCLC and NSCLC established cell lines may be obtained through the National Cancer Institute, Bethesda, MD (USA) (e.g., NCI-H157, NSCLC cells; NCI-H345, SCLC cells; NCI-H292, NSCLC squamous cell

carcinoma; NCI-H838, adenocarcinoma; NCI-H417, SCLC cells; H720, lung carcinoid cells; NCI-H727, lung carcinoid cells; NCI-N592, SCLC cells; EPLC-65H, squamous cell carcinoma) or from the American Type Culture Collection, Rockville, MD (USA).

Plasma membranes from such cells may be isolated according to Lee, M., et al., Peptides, 11:1205 (1990), which is incorporated herein by reference.

Generally, binding competitions are set up between a tracer concentration of labeled ligand and varying concentrations of either the unlabeled counterpart of the labeled ligand or an unlabeled peptide suspected of binding to the receptor for the ligand. For example, SCLC or NSCLC cells or plasma membranes isolated therefrom are incubated with a tracer concentration of ^{125}I-labeled VIP and a range of concentrations of unlabeled VIP or an unlabeled thymosin or fragments thereof, or an anti-VIP receptor antibody, and the amount of specific binding of the tracer at each concentration of the unlabeled ligands determined.

It has now been found that ^{125}I-labeled VIP binds with high affinity to SCLC and NSCLC cell lines, and that VIP markedly stimulates the adenylate cyclase activity of these cells. It has also now been found that THNα1 inhibits the binding of labeled VIP to lung cancer cells, although it does so with a potency only a twentieth that of unlabeled VIP. In addition, THNα1 inhibits the stimulatory effect of VIP on second messenger cAMP production in lung cancer cells.

Direct, i.e., noncompetitive, binding of a labeled ligand to intact cells or plasma membranes may be determined. Attempts to carry out such analyses using, for example, (^{125}I-Tyr)-labeled THNα1 or N-terminal or C-terminal fragments demonstrated that such molecules

bind with poor affinity to lung cancer cells or plasma membranes derived therefrom. These and other experiments not detailed here indicate that the thymosins may not have specific receptors. Palaszynski, E., Biochemical Studies on Thymosin α1, Ph.D. Thesis, George Washington University, Washington, D.C., 1981. It is thus all the more suprising and unexpected that certain thymosins have such profound inhibitory effects on the binding of VIP to its specific receptors and on the growth promoting effects of VIP in lung cancer cells.

Although ^{125}I is a frequently used label for peptide binding studies, other markers such as chemiluminescent compounds and fluorescent molecules may be used.

Cross-linking procedures may also be used to identify ligand-receptor interactions with SCLC or NSCLC cells or plasma membranes. Generally, the tissue is incubated with a tracer concentration of a labeled ligand, without and with varying concentrations of an unlabeled ligand, until steady state binding is attained. At that point, unbound labeled ligand is removed by washing the tissue at ice bath temperature, and the bound labeled ligand is covalently cross-linked to its receptor protein by the addition of a cross-linking reagent. Thereafter, the cells or membranes are solubilized with a detergent, and the samples analyzed by SDS-PAGE electrophoresis, followed by autoradiography to determine the molecular weight of the receptor protein to which the labeled ligand had bound. A wide variety of peptide-peptide cross-linking reagents are known, such as those available from Pierce Co., Rockford, Il (USA), 1989 Handbook and General

Catalog, pages 283-311, which is incorporated herein by reference.

It has been found from such cross-linking studies that THNα1 inhibits the cross-linking of VIP to its receptor protein of about 82 kDa located in the plasma membrane of lung cancer cells of both SCLC and NSCLC types.

Candidate anti-lung cancer peptides and antibodies may be screened in vitro in a soft agar colonization assay such as that described by Mahmoud, S., et al., Life Sci., 44:367 (1989) which is herein incorporated by reference. In general, single viable lung cancer cells are plated in soft agar. After an appropriate growth period, such as 14 days, cell colonies may be stained with an appropriate cytochemical stain, and the stained cells viewed microscopically. Cytochemical stains are well known to those skilled in the art of histology.

It has been found from such in vitro colonization experiments that THNα1 dramatically reduces colony formation of lung cancer cells in vitro under conditions in which THNβ4 and VIP have little effect.

The aforementioned binding, cross-linking and colonization experiment lead to the unexpected finding that thymosins inhibit the growth of lung cancer cells by an action mediated by cell surface receptors for a BRM active in such cells.

Candidate anti-lung cancer peptides and anti-VIP receptor antibodies may be tested in vivo in a mouse model of lung cancer. For example, when SCLC cell lines such as NCI-N592 or NCI H69, or NSCLC cell lines such as NCI-H157 or NCI-H292 are injected subcutaneously into nude mice, xenografts will form. The test peptide may then be injected adjacent to the

tumor, and the tumor volume measured periodically. Control mice will not receive the test peptide or will receive a placebo peptide in the same pharmaceutically acceptable vehicle. Tumor growth in experimental and control animals are compared, and test peptides ranked according to efficacy in reducing tumor size.

It has been found that THNα1 and its C-terminal (such as amino acids 4-28 and 15-28) and N-terminal (such as amino acids 1-8, 1-14, and 1-20) fragments inhibit lung cancer growth in vivo in tumors of both SCLC and NSCLC types of lung cancer cells. No toxic side effects were observed. Taken together with the binding and in vitro experiments, the data of the invention demonstrate a novel modality for treating lung cancers of both the small cell and nonsmall cell types without radiation treatment and without the need for concurrent use of the toxic classical chemotherapeutic agents, although the use of such additional modalities is not precluded by the present invention.

Anti-EGF receptor antibodies are known to inhibit the binding of this peptide hormone to its specific cell surface receptor macromolecule. Lee, M. et al, Cancer Res. Proc., 1990. Monoclonal antibodies to a human colonic adenocarcinoma cell line VIP receptor have been prepared (Kummer, W., et al., Histochem. J., 22:249 (1990)) and used for histochemical identification of cells bearing this receptor.

Polyclonal and monoclonal antibodies directed against the VIP receptors on SCLC and NSCLC cells may be used to inhibit the binding of VIP to its receptors on these cells, thereby reducing the proliferative effects of VIP. These antibodies may be prepared by raising antisera and/or monoclonal antibodies against

detergent-solubilized, purified forms of the SCLC and NSCLC VIP receptor macromolecules. Monoclonal antibody-producing hybridoma cells can be produced by injecting mice, such as Balb/c mice, with purified VIP receptor antigen, fusing spleen cells of hyperimmunized mice with myeloma cells, such as NSO myeloma cells, using the polyethyleneglycol technique, selecting hybridomas using the HAT growth medium, screening hybridoma supernatant fluids for the ability to immunoprecipitate cross-linked VIP receptors as well as the ability to inhibit the binding of labeled VIP to lung cancer cells, and cloning positive hybridomas. For details of these techniques, see Cuttitta, F., et al. Nature, 316:823 (1985); Engleman, E., et al., eds., Human Hybridomas and Monoclonal Antibodies, Plenum Publishing Co., N.Y., 1985; Harrell, J.G.R., ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, 1982; Larrick, J.W., et al., BioTechniques, 6-14 (Jan./Feb. 1984); Oi, V.T., et al., in Mishell, B.B., et al., eds., Selected Methods In Cellular Immunology, Chapter 17, pp. 351-372; and Kennett, R.H., et al., Monoclonal Antibodies, Plenum Press, N.Y., 1980, Appendix, Goding, ____, in Monoclonal Antibodies: Principles and Practices, Academic Press, N.Y., 1983, pp. 118-124, which are incorporated herein by reference. Also, ascites fluids may be produced from positive clones to generate large amounts of monoclonal antibodies.

Also, a VIP receptor fragment may be conjugated to a carrier such as keyhole limpet hemocyanin, edestin, thyroglobulin or albumins, if it is not sufficiently large to be adequately immunogenic, as is understood in the art. The method of preparation of and administration of the antigenic VIP receptor varies

with its nature and abundance in the particular cells employed, and general approaches depending on these particulars are well within the skill of those practicing this art. Any effective mode of preparation and administration is acceptable.

The VIP receptor preparation in suitable form is then administered to an experimental animal for generation of the antibody-producing cells. To obtain the desired polyclonal antibodies, the antisera may be harvested and the antibodies purified by standard techniques and used directly. If monoclonal antibodies directed to specific VIP receptor epitopes are desired, the procedures described above can be employed.

The desired anti-receptor antibodies can be conveniently purified using affinity chromatography, taking advantage of the ability of the desired antibodies to link tightly to the VIP receptor moiety. Anti-receptor antibodies may also advantageously be purified by cytological purification, that is, by contacting the impure receptor preparation with SCLC or NSCLC cells, washing the cells free of unbound impurities, then eluting the purified anti-receptor antibodies from the cell surface.

It is desired that the anti-VIP receptor monoclonal or polyclonal antibody be administered to mammalian subjects in substantially pure form. As used herein, the expression "substantially pure" means that, within serologically detectable limits, only one specie of antibody combining site capable of binding the VIP receptor is present. Thus, while a substantially pure antibody molecule preparation may contain more than one species of antibody combining site, such a preparation displays a single binding affinity for a VIP receptor antigen epitope. An antibody molecule in substantially

pure form is typically designated a "monoclonal antibody" by those skilled in the art because such molecules are usually produced using monoclonal hybridoma cultures.

Methods for preparing paratope-containing portions of anti-VIP receptor immunoglobulin molecules such as Fab, Fab', $F(ab')_2$ and F(v) from substantially intact antibodies are well known. See, for example, U.S. patent no. 4,342,566, Inbar et al., PNAS (USA), 69:2659 (1972), and Goding, 1983, ibid.

An anti-VIP antibody molecule of the present invention is an antibody molecule that immunoreacts with an VIP epitope and thereby neutralizes the molecule, that is to say, the immunoconjugate is incapable of acting as VIP molecule biologically.

The term "cellular receptor" as used herein for VIP refers to a protein or glycoprotein macromolecule contained within the plasma membrane of one or more types of cells in the host organism, including abnormal cells such SCLC and NSCLC cells, and which receptor macromolecule, when bound to a ligand (herein, VIP), initiates the chain of responses and events that lead to what is referred to as the physiological effect of the ligand (herein, the biological response modifier action(s) of VIP).

The examples that follow are designed merely to exemplify various embodiments of this invention and are not intended to in any way limit the scope of the invention which is set forth in the specification and appended claims.

EXAMPLE 1

INTERACTION OF THYMOSIN α1 WITH VIP RECEPTORS ON LUNG CANCER CELLS

Lung cancer cells (EPLC-65H squamous cell carcinoma) were cultured in serum supplemented growth medium (RPMI 1640 containing 10% fetal bovine serum). Plasma membranes were isolated (Lee, M., et al., 1990, above) and the binding potency of THNα1 determined.

The ability of unlabeled VIP (•) and THNα1 (o) to inhibit specific binding of ^{125}I-labeled VIP to plasma membranes was determined in 3 experiments. The means and ranges of specific binding values as a function of unlabeled peptide concentration are plotted in Figure 1.

The data show that the specific binding of a tracer concentration of labeled VIP was inhibited in a concentration-dependent manner by unlabeled VIP and THNα1. Little specific binding of ^{125}I-VIP to VIP receptors was inhibited by 1 nM VIP, whereas almost all specific binding was inhibited by 1000 nM VIP. The IC_{50} (concentration causing 50% inhibition) for VIP was about 10 nM.

In contrast, the IC_{50} for THNα1 was about 200 nM, indicating that the affinity of the VIP receptor for THNα1 is about one-twentieth that for VIP itself.

EXAMPLE 2

INHIBITION BY THNα1 OF CROSS-LINKING OF LABELED VIP TO ITS RECEPTORS ON LUNG CANCER CELLS

Plasma membranes derived from squamous cell carcinoma cell line EPLC-65H were incubated with a tracer concentration (2 nM) of ^{125}I-labeled VIP in the absence (control) and presence of THNα1. Membranes were then washed with ice cold buffer to remove unbound

labeled VIP and the bound labeled VIP covalently cross linked to its membrane receptor protein using 2 mM disuccinimidylsuberate. Membranes were solubilized with 10% SDS in electrophoresis buffer, and the protein mixture separated by SDS-PAGE electrophoresis. Gels were then radioautographed to produce X-ray films showing radioactive proteins.

As shown by the autoradiogram of Figure 2, lane c, 1 μM THNα1 inhibited the binding of ^{125}I-labeled VIP to a 83 kDa glycoprotein, to an extent similar to that accomplished by unlabeled VIP itself (lane a). This glycoprotein presumably is the VIP receptor in this cell line, and it also presumably is this glycoprotein by which thymosins and anti-VIP receptor antibodies inhibit the lung cancer growth promoting effects of VIP.

EXAMPLE 3

COLONIZATION OF LUNG CANCER CELLS IN VITRO

The effects of THNα1 on the growth of lung cancer cell lines was studied. Single viable cells were plated in soft agar as previously described (Mahmoud, S., et al., Life Sci., 44:367 (1989)), and, after two weeks, the colonies were stained with 0.1% p-iodonitrotetrazolium violet.

The pattern of colony staining shown in Figure 3 demonstrates that numerous large colonies of NCI-H838 cells had formed by two weeks (top), whereas in the presence of 10 μM THNα1 (bottom) the number and size of the colonies were dramatically reduced.

The number of colonies larger than 50 μm in diameter were counted. The data of Table I shows that 89 colonies of NCI-H157 (squamous cell carcinoma) formed. The number of colonies did not change when

either 1 nM or 10 nM VIP was added, although there was a slight increase at 100 nM or 1000 nM VIP. In contrast, 10 nM THNα1 produced no change in colony number, 100 nM or 1,000 nM THNα1 produced a slight decrease, but 10,000 nM THNα1 significantly decreased colony formation.

TABLE I

Dose Response Curve of VIP and THNα1 on NCI-H157 (Squamous Cell Carcinoma) Growth

Peptide	Colony Number	N/No
None	89 ± 10	1.00
1 nM VIP	89 ± 7	1.00
10 nM VIP	89 ± 7	1.00
100 nM VIP	113 ± 6	1.27
1000 nM VIP	123 ± 16	1.38
10 nM THNα1	89 ± 8	1.00
100 nM THNα1	82 ± 3	0.92
1000 nM THNα1	74 ± 10	0.83
10000 nM THNα1	61 ± 7	0.69

The mean ± S.D. of 3 determinations is indicated. Also, the colony fraction (N/No) is calculated where No and N are the number of colonies in the absence or presence of additions respectively.

EXAMPLE 4

EFFECT OF THNα1 AND THN β4 ON GROWTH OF LUNG CANCER CELL LINES

The experimental conditions of Example 3 were repeated with NCI-H720 (lung carcinoid cancer cells) and NCI-N417 (SCLC lung cancer cells) cell lines. Colonies were counted as before. The absolute and relative number of colonies of greater than 50 μm diameter are shown in Table II.

VIP at 100 nM increased colony formation for both SCLC and lung carcinoid cell lines. THNβ4 at 1 μM did

not influence colony number significantly. In dramatic contrast, THNα1 at 1 μM greatly reduced colony formation for both types of lung cancer cells.

TABLE II

Specificity of Peptides on Lung Cancer Growth

Agent	NCI-H720 (carcinoid)	N/No	N417	(SCLC)N/No
control	73 ± 24	1.00	201 ± 16	1.00
VIP 100 nM	100 ± 26	1.37	216 ± 40	1.08
THNα1 1 μM	28 ± 14	0.39	99 ± 16	0.49
THN β4 1 μM	68 ± 20	0.93	240 ± 68	1.20

The mean value ± S.E. of 3 determinations is indicated.

EXAMPLE 5

EFFECTS OF THYMOSINS ON LUNG CANCER IN ANIMALS

Squamous cell carcinoma (NCI-H157, 10^7 cells) was injected subcutaneously into female Balb/c nude mice. Xenografts formed after about one week, and tumor size (mm^3) was followed weekly.

THNα1, 10 μg, in a sterile pharmaceutical vehicle, was injected subcutaneously adjacent to the tumor. No toxic effects on the animals were observed. Figure 4 shows that after two weeks a palpable mass (28 mm^3) was observed in control mice which received only placebo injections. Tumor growth increased exponentially over the next several weeks, and at week 6, control mice that had very large tumors (2430 mm^3) were killed. In dramatic contrast, mice receiving THNα1 developed a palpable mass (3 mm^3) only at week 3, and the tumor growth slowly increased until week 5 (187 mm^3). At this point, withdrawal of THNα1 resulted in the resumption of rapid tumor growth (at week 6 the tumor volume was

585 mm^3, still only 25% of the size of the control xenografts).

This experiment shows that THNα1 may function as a reversible inhibitor of NSCLC growth.

EXAMPLE 6

The experiment of Example 5 was repeated, except that xenografts were produced with NCI-H292 squamous cell lung carcinoma and fragments of THNα1 were compared for efficacy with the parent polypeptide.

The data of Table III show that the squamous cell carcinoma xenografts produced by NCI-H292 cells grew more slowly than did xenografts produced by NCI-H157 squamous cell carcinoma cells. After three weeks, measurable tumors (2-5 mm^3) were observed. By week 8, the control tumor volume had increased about 60-fold to 305 mm^3. THNα1 and the C- and N-terminal fragments thereof reduced tumor growth by approximately 50%. The C-terminal fragment was composed of amino acids 15 to 28 of THNα1, and the N-terminal fragment composed of amino acids 1 to 14 of THNα1.

TABLE III

Xenograft Formation of Cell Line NCI-H292

Addition at week	3	4	5	6	7	8
C-fragment	4	15	36	65	123	141
Control	5	31	48	150	237	305
THNα1	2	23	40	72	128	135
N-fragment	3	27	45	72	85	162

NCI-H292 was injected into nude mice and the tumor volume (mm3) was indicated. Peptides (10 μg) were injected daily subcutaneously. The mean value of 3 determinations is indicated. Routinely the S.E. was 15% of the mean value.

We claim:

1. A method for treating lung cancer in mammals in need of such treatment, comprising administering to said mammals an anti-cancer effective amount of a composition comprising one or more inhibitors of the lung cancer cell growth promoting action of vasoactive intestinal polypeptide ("VIP") in a pharmaceutically acceptable injectable vehicle.

2. A method of claim 1, wherein said lung cancer is a small cell lung cancer or nonsmall cell lung cancer.

3. A method of claim 1, wherein said inhibitor is a polypeptide.

4. A method of claim 3, wherein said polypeptide is a thymosin polypeptide.

5. A method of claim 4, wherein said thymosin polypeptide is selected from the group consisting of thymosin α1, desacetyl thymosin α1, and analogues, derivatives and fragments thereof.

6. A method of claim 4, wherein said polypeptide is thymosin α1.

7. A method of claim 4, wherein said polypeptide is the desacetyl analogue of thymosin α1.

8. A method of claim 3, wherein said polypeptide is a carboxy-terminal fragment of a thymosin.

9. A method of claim 8 wherein said thymosin is thymosin α1.

10. A method of claim 9 wherein said fragment is amino acids 4 to 28 or 15 to 28.

11. A method of claim 3, wherein said polypeptide is an amino-terminal fragment of a thymosin.

12. A method of claim 11, wherein said thymosin is thymosin α1.



13. A method of claim 12, wherein said fragment is selected from the group consisting of amino acids 1 to 8, 1 to 14, and 1 to 20.

14. A composition for treating lung cancer in mammals in need of such treatment, comprising one or more inhibitors of the lung cancer cell growth promoting action of vasoactive intestinal polypeptide in a pharmaceutically acceptable vehicle.

15. A composition of claim 14, wherein said lung cancer is a small cell lung cancer or nonsmall cell lung cancer.

16. A composition of claim 14, wherein said inhibitor is a polypeptide.

17. A composition of claim 16, wherein said polypeptide inhibitor is a thymosin polypeptide or a pharmaceutically acceptable salt thereof.

18. A composition of claim 17, wherein said thymosin polypeptide is selected from the group consisting of thymosin α1, Nα-desacetyl thymosin α1, and analogues, derivatives and fragments thereof.

19. A composition of claim 17, wherein said polypeptide is thymosin α1.

20. A composition of claim 17, wherein said polypeptide is Nα-desacetyl thymosin α1.

21. A composition of claim 18, wherein said fragment is a carboxy-terminal fragment of a thymosin.

22. A composition of claim 21, wherein said thymosin is thymosin α1.

23. A composition of claim 22, wherein said fragment comprises amino acids 4 to 28 or 15 to 28.

24. A composition of claim 18, wherein said fragment is an amino-terminal fragment of a thymosin.

25. A composition of claim 24, wherein said thymosin is thymosin α1.

* C L M S *

26. A composition of claim 25, wherein said fragment is selected from the group consisting of amino acids 1 to 8, 1 to 14, and 1 to 20.

27. A method of claim 1, wherein said inhibitor comprises an antibody directed against a receptor for vasoactive intestinal polypeptide.

28. A method of claim 27, wherein said antibody is a polyclonal or monoclonal antibody.

29. A method of claim 1, wherein said inhibitor comprises an antibody directed against vasoactive intestinal polypeptide.

30. A method of claim 29, wherein said antibody is a polyclonal or monoclonal antibody.

31. A composition of claim 16, wherein said polypeptide inhibitor comprises an antibody directed against a receptor for vasoactive intestinal polypeptide.

32. A composition of claim 31, wherein said antibody is a polyclonal or monoclonal antibody.

33. A composition of claim 16, wherein said polypeptide inhibitor comprises an antibody directed against vasoactive intestinal polypeptide.

34. A composition of claim 33, wherein said antibody is a polyclonal or monoclonal antibody.