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Involvement of Signal Transduction Pathways in Lung Cancer Biology

Jean Viallet and Edward A. Sausville

Departments of Medicine and Oncology Center, Hôpital Notre-Dame and Université de Montréal, Montreal, P.Q., Canada H2L 4M1 (J.V.); Laboratory of Biological Chemistry, Developmental Therapeutics Program, DCT, NCI, Bethesda, Maryland 20892 (E.A.S.)

Abstract *Pathways* involved in the transduction of biological signals within cells overlap with those involved in oncogenesis. Previous studies have identified a number of discrete disturbances of some elements of these pathways in human lung cancer cells, by virtue of the overexpression or the mutation of certain key molecules. The sequence of biochemical events triggered by a mitogenic stimulus such as the exposure to bombesin-like peptides are being unravelled. The opportunity exists to identify additional changes involving regulatory proteins which may contribute to the regulation of these systems and which may function as suppressors of the malignant phenotype. Furthermore, the understanding of these pathways may identify targets for the pharmacological regulation of tumor cell response to mitogens which may be usable in the clinic. © 1996 Wiley-Liss, Inc.

Key words: transduction, biological signals, oncogenesis, lung cancer, bombesin

Over the last decade, investigations into the basic mechanisms of neoplasia have identified a number of events which contribute to cellular transformation. Cellular oncogenes were first identified as the normal cellular homologues of the transforming genes isolated from animal retroviruses. Their exhaustive study has converged with expanded knowledge about the pathways which normally govern cellular responses to external mitogenic stimuli [1,2]. These pathways have been diverted towards oncogenesis by quantitative and qualitative changes in growth factors, growth factor receptors, membrane associated guanine nucleotide binding proteins (G-proteins[1]), membrane associated non receptor tyrosine kinases, cytoplasmic serine-threonine kinases, and nuclear transcription factors. Clearly, the understanding of the biochemical pathways generating these cascades of signal transduction events have helped us better understand the implications of the many genetic lesions found in cancer cells. These interactions between the disciplines are continuing at a vigorous rate and are likely to lead to further important insights. However, as the known components of signal transduction cascades are becoming continuously more complex, there is a significant lag between our understanding of normal cellular controls and how they may be involved in the genesis of specific malignancies.

Few cellular models have been exhaustively evaluated, among which the murine Swiss 3T3 cell and the human small cell lung cancer (SCLC) cell have been the most exploited [3]; as we will see, significant similarities and distinctions are to be found in the cellular responses to the same mitogens in these two models. Importantly, many growth factors appear to influence the growth of lung cancer cells, often through autocrine growth regulatory loops [4]. It has been proposed that interruption of autocrine/paracrine growth stimulatory circuits may be exploitable therapeutically [5]. However, such efforts must take into account the fact that a given cell type may produce and respond to multiple growth factors [6,7] and that the blockade of any single one may well be insufficient to achieve clinically significant tumor regression.

Abbreviations used: βAR, β adrenergic receptor; βARK, β adrenergic receptor kinase; CT, cholera toxin; G-protein, guanine-nucleotide binding protein; HGF, hepatocyte growth factor; NSCLC, non-small cell lung cancer; $[Ca^{2+}]_i$, intracellular free calcium concentration; PKA, cAMP dependent protein kinase; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; PT, pertussis toxin; SCLC, small cell lung cancer.

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Address reprint requests to Dr. Jean Viallet, Oncology Center, Hôpital Notre-Dame, 1560 rue Sherbrooke Est, Montreal, (Québec), CANADA H2L 4M1.

Whereas these obstacles can potentially be circumvented by the use of a combination of growth factor antagonists or of antagonists with broad specificities [8], another approach is to identify the intracellular pathways activated by specific growth factor stimulation, some of which will be shared by distinct ligands, and to develop pharmacological tools to manipulate them. Alternatively, the modulation of specific biochemical pathways can antagonize the action of oncogenic events and partially revert the malignant phenotype [9]. The full exploitation of these strategies requires a description of the different levels involved in the signal transduction cascade, of their regulation and relation to each other as well as to the events activated by other stimuli (cross-talk). It is worth pointing out that clinically significant manipulations of signal transduction pathways are commonplace in other areas of medicine, as exemplified by the use of calcium channel blockers or that of inhibitors of cyclic AMP catabolism such as aminophylline.

ALTERED PROTEIN PHOSPHORYLATION: THE KEY BIOCHEMICAL RESULT OF SIGNALING (Fig. 1)

Phosphorylation at serine/threonine or tyrosine residues is a common mode of regulation of protein function. A wide variety of kinases, with restricted or promiscuous substrate specificities, and a more limited spectrum of protein phosphatases, regulate cycles of phosphorylation and dephosphorylation.

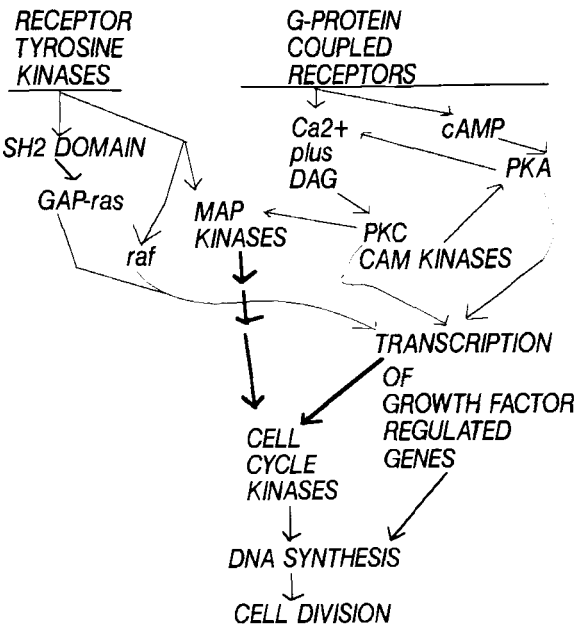

Fig. 1. Summary of signal transduction pathways. Activation of either receptor-linked tyrosine kinases or G protein-coupled receptors activate "second echelon" kinases which then result in activation of progress through the cell cycle.

Protein Tyrosine Kinases as Part of Signal Reception

Protein tyrosine kinases exist as ligand activated transmembrane receptors or as membrane associated cytoplasmic enzymes who share homology with the *src* oncogene. One of these cytoplasmic tyrosine kinases, $p56^{lck}$, suggests a model for the mode of action for this family. $p56^{lck}$ is known to participate in T cell activation by forming a reversible complex with the cell surface protein CD4 which binds class *II major* histocompatibility complex molecules expressed on antigen presenting cells. This functional complex appears to play the same role as a transmembrane tyrosine kinase receptor [10]. CD4 provides the ligand binding function and $p56^{lck}$ the effector function.

Second Messenger-Activated Kinases

A second broad class of protein kinases involved in signal transduction are the serine/threonine kinases many of which are activated by intermediary molecules called second messengers. Second messengers are generated by the activation of a different class of membrane receptors which are characterized structurally by the presence of seven hydrophobic domains which are thought to span the surface membrane. These receptors have no recognized enzymatic activity of their own but, once activated by ligand binding, interact with other membrane associated proteins to transfer and amplify the signal. These intermediary proteins are characterized by their ability to bind and hydrolyze guanine nucleotides (G proteins). Activated G proteins in turn reversibly activate effectors that generate second messengers such as cAMP (activating cAMP-dependent protein kinases), calcium (activating calcium/calmodulin dependent protein kinases), or diacylglycerol (activating protein kinase C).

Second Echelon Kinases: S6, MAP, and raf Kinases

Complex cascades of kinases are now known to contribute to mitogen induced cellular responses [11]. These kinases include the normal cellular homologue of the *raf* oncogene as well as

others. One of the first signature events of mitogenesis to have been identified is the phosphorylation of the S6 ribosomal protein. Certain S6 kinases are themselves activated through phosphorylation by mitogen-activated protein kinases (MAPK) which are themselves phosphorylated by a MAP kinase kinase (MAPKK), itself a substrate for MAP kinase (MAPK) and a MAP kinase kinase kinase (MAPKKK) [12], indicating complex regulatory interactions between the partners of this cascade.

Interactions Between Signaling Pathways and Molecules

Activation of receptor tyrosine kinases can affect other pathways through phosphorylation of GTPase-activating protein, phosphatidylinositol 3′-kinase, or phospholipase C-γ [2]. Non receptor tyrosine kinases also can phosphorylate multiple substrates which have remained anonymous until recently.

Another major insight is the discovery that many proteins that are tyrosine kinases or substrates for tyrosine kinases share certain amino acid domains first identified by analogy with the protooncogene *src*. These *src* homology domains (SH2 and SH3) appear to function as intermolecular recognition sites, allowing the association of these proteins and mutual modulation of their function [13]. Analogous events appear to occur by other mechanisms in the interaction of *ras* proteins and their partners (GTP'ase activating proteins and guanine nucleotide exchange factors), which alter their function by reversible physical association.

Thus signal transduction involves both events which act at a distance through the diffusion of soluble second messengers and phosphorylation cascades and others which require physical interaction between protein molecules. This hierarchy of functional compartments contributes to the control of activation in time and space and suggests that relatively subtle changes in the overall balance of the system may have profound phenotypic consequences.

KNOWN INVOLVEMENT OF SIGNAL TRANSDUCING MOLECULES IN LUNG CANCER BIOLOGY

A number of growth factors, growth factor receptors, and oncogenes with signal transducing functions have been found to be expressed and/or mutated in human lung cancer cells. These include the expression of EGF and its receptor in non-small cell lung cancer (NSCLC), multiple neuropeptides in SCLC and NSCLC, the expression of *src* and *lck*, the expression of raf, and the presence of mutated ras proteins in NSCLC [14].

An area of particular interest is the description of the pathways activated by mitogenic neuropeptides which may act as autocrine growth factors in lung cancer cells. Evidence suggests that these pathways are shared by several peptide receptors. The remainder of this review will thus focus on the events triggered by bombesin-like peptides and their regulation as a model for neuropeptide dependent cellular responses.

PATHWAYS ACTIVATED BY BOMBESIN, A PROTOTYPICAL NEUROPEPTIDE GROWTH FACTOR

Involvement of G Proteins in Bombesin Signaling

GRP and bombesin-like peptides have been extensively studied as mitogens in the Swiss 3T3 system where the signaling events they activate have been delineated [3]. In this murine system of immortal but inefficiently tumorigenic cells, bombesin analogues bind to high affinity specific receptors which have been cloned and sequenced. The bombesin receptor is a member of the seven transmembrane domains family of receptors and is linked to phospholipase C by one or more G proteins which have been functionally characterized but not formally identified [15–17].

PKC Activation by Bombesin

Binding of bombesin to its receptor activates phosphoinositide turnover with the generation of inositol trisphosphate and diacylglycerol and the subsequent transient elevation of intracellular free calcium concentration ($[Ca^{2+}]_i$) and activation of protein kinase C (PKC). Some studies have suggested that PKC activation can result in feedback desensitization of the bombesin response [18], as well as causing a transmodulation of the EGF receptor, the phosphorylation of an 80 kd substrate protein (MARCKS protein [19]), and activation of the Na^+/H^+ transporter with the consequent elevation in intracellular pH.

Involvement of Second Echelon Kinases in Bombesin Signaling

The stimulation of quiescent Swiss 3T3 cells with bombesin results in the activation of a

number of serine/threonine protein kinases [20]. Among these are found several S6 peptide protein kinases, two MAP kinases, as well as casein kinase 2. The latter can phosphorylate a number of nuclear phosphoproteins involved in transcription control (myc, p53, $p67^{SRF}$), thus providing a continuum between binding of bombesin to its receptor and the changes in gene expression eventually leading to cellular division.

Activation of the FAK Kinase by Bombesin

Recent work has shown that a p125 phosphoprotein associated with focal adhesion points is in fact a novel tyrosine kinase (focal adhesion kinase, FAK) that is phosphorylated in cells harboring an activated *src* gene or stimulated by mitogenic neuropeptides including bombesin [21]. These observation serve to link cellular skeleton components with mitogenesis and oncogenesis.

An understanding of the functional interrelationships of these complex cascades of biochemical events may be of practical significance. They provide targets for pharmacological manipulation at many stages of lung carcinogenesis. As an example of such targets, we will focus the remainder of this review on the signals generated by bombesin in lung cancer cells and how their manipulation may influence the rate of cellular growth.

RESPONSES TO NEUROPEPTIDES AS A MODEL FOR THE STUDY OF SIGNAL TRANSDUCTION IN LUNG CANCER CELLS

Lung cancer cells produce and respond to a wide variety of peptides, some of which, such as gastrin-releasing peptide (a homologue of bombesin), are known to function in autocrine growth stimulatory loops [4]. The description of the pathways activated by bombesin in Swiss 3T3 cells has motivated similar studies in SCLC cell lines for which bombesin can function as an autocrine growth factor. The classical SCLC cell line, NCI-H345, has been the most extensively studied in this regard. One must keep in mind that the responses of cells that are chronically exposed to the effects of an autocrine growth factor may be different from those of cells which are not. The differences of behavior of Swiss 3T3 cells when exposed to bombesin acutely or chronically illustrate this point [22] (see below).

Early work in SCLC cell lines demonstrated that stimulation by a variety of bombesin analogues resulted in a transient elevation of free $[Ca^{2+}]_i$, just as in Swiss 3T3 cells [23]. This response was maximal after exposure to 100 nM [Tyr^4]-bombesin was derived from both extracellular fluid and intracellular calcium stores and was desensitized to further addition of the peptide after the initial exposure. Cellular response to [Tyr^4]-bombesin recovered 90 min after the initial peptide was washed out. Further work showed that in SCLC NCI-H345, stimulation by [Tyr^4]-bombesin caused an increase of phosphoinositide turnover, demonstrating the coupling of the bombesin receptor to phospholipase C in this cell line, and implicating generation of inositol trisphosphate as a contributing factor in the previously described increase in $[Ca^{2+}]_i$ [24]. Of interest, the bombesin responses in this SCLC cell line were shown to be modulated by different compounds including cholera toxin and phorbol esters.

In addition to phospholipase C dependent pathways, bombesin is known to lead to the activation of collateral pathways (second echelon kinases and FAK kinase), at least in Swiss 3T3 cells. Previous reports have shown bombesin stimulated tyrosine phosphorylation in SCLC cells [25,26]. It is tempting to presume that this may be related to activation of the FAK kinase but this issue has not yet been directly studied. The mechanism of activation of these collateral pathways is not known, nor is their relative contribution to the mitogenic potential of bombesin. Microinjection of antibodies directed against phosphatidylinositol bisphosphate, which effectively block phosphoinositide turnover generated by bombesin, efficiently inhibits bombesin stimulated mitogenesis [27]. This suggests that these alternative pathways are either not involved in bombesin stimulated mitogenesis or are themselves activated by the products of phosphoinositide hydrolysis.

Ultimately, the mitogenic potential of the original stimulus will translate into a change in the pattern of gene expression in preparation for DNA synthesis and cell division. Bombesin stimulation of Swiss 3T3 is known to transiently increase the expression of the nuclear oncogenes and transcription factors c-*fos* and c-*myc*. However, pretreatment of Swiss 3T3 cells with pertussis toxin effectively inhibits bombesin induced DNA synthesis and c-*myc* expression without affecting the phospholipase C dependent responses of inositol phosphate generation, elevation of $[Ca^{2+}]_i$ and PKC activation [28,29]. Other evidence shows that desensi-

tization of acute events such as $[Ca^{2+}]_i$ responses can be dissociated from desensitization of DNA synthesis provoked by chronic exposure of Swiss 3T3 cells to bombesin [22]. This suggests that not all the cellular events activated by bombesin are intimately or directly linked to mitogenesis. Further work needs to be done to delineate which of the distal pathways involved in Swiss 3T3 responses to bombesin are also operative in lung cancer cells.

NEWER ISSUES IN LUNG CARCINOMA RELATED SIGNALS

Desensitization to Bombesin Peptides

Cells exposed to bombesin remain refractory to a subsequent addition of bombesin for at least 90 min after the initial bombesin molecules are washed away [23]. Rapid desensitization after an acute exposure to their cognate ligand is an ubiquitous feature of G protein coupled receptors and deserves to be understood as perturbations of this pathway may lead to amplification or undue persistence of the mitogenic response to the growth factor. The best understood mechanisms of desensitization are those responsible for the regulation of β2 adrenergic receptor (βAR) function [30]. In this case, desensitization is dependent on phosphorylation of the receptor and its removal from the cell membrane.

Phosphorylation of βAR is known to occur through the action of two kinases, cAMP dependent protein kinase (PKA) and the β adrenergic receptor kinase (bARK). Phosphorylation by PKA directly interferes with the receptor activation of G protein function. In contrast, βARK phosphorylates only the agonist occupied receptor in a way which still allows interaction of the receptor with the G protein. The agonist occupied βARK phosphorylated receptor-G protein complex is recognized by another protein, β arrestin, which blocks the receptor-G protein interaction. In addition to desensitization from receptor phosphorylation, after exposure to ligand, β adrenergic receptors are rapidly sequestered and may also undergo a more prolonged processing, resulting in their destruction.

It was noted that pretreatment of NCI-H345 with the phorbol ester and PKC activator phorbol 12-myristate-13 acetate (PMA) acutely desensitized cells to the subsequent addition of [Tyr⁴]-bombesin, abrogating the $[Ca^{2+}]_i$ and phosphoinositide responses. Prolonged (48 h) pretreatment of NCI-H345 with PMA, thus depleting the cells of PKC, blocked the effects of acute addition to PMA while preserving the intensity of the response to [Tyr⁴] bombesin [24,31].

These observations tentatively implicated PKC as a possible negative regulator of bombesin induced signals, a notion which has been reinforced by the description of canonical PKC phosphorylation sites in the intracytoplasmic domains of the cloned human bombesin receptors [32]. In different systems, down-regulation of PKC by a chronic treatment with phorbol esters has been associated with an amplification of response [33] or alternatively to no change in primary response or in homologous desensitization [34]. In addition, the PKC dependent desensitization has been associated with a decrease in receptor affinity [35] or to a decrease in receptor/G protein coupling as suggested by a loss of GTP modulation of receptor affinity [36]. However, the activation of PKC by bombesin in SCLC cells has yet to be directly demonstrated. Cells depleted of PKC by prolonged pretreatment with PMA and which retain a normal response to acute exposure to [Tyr⁴]-bombesin also retain the homologous desensitization to further addition of the peptide [31]. Thus, desensitization of bombesin responses in SCLC cannot be clearly and uniquely attributable to PKC activation. Nonetheless, modulators of PKC activity have the potential to act as desensitizers of a number of pathways and may be of therapeutic utility.

It is possible that mechanisms analogous to those involved in β adrenergic regulation or others such as effects on phospholipase C substrates or phospholipase C itself could contribute to desensitization of bombesin responses in human lung cancer cells. Current evidence suggests that in fact the mechanisms of desensitization of bombesin responses may be distinct in Swiss 3T3 cells and SCLC cells (see Table I). For instance, exposure of Swiss 3T3 cells to radioiodinated ligand at 37°C results in rapid internalization (50% after 5 min), suggesting that receptor sequestration is an important component of acute desensitization in this cell type [37]. In contrast, in the SCLC cell line NCI-H345, most of the radioligand was still acid-extractable from the cell surface after a 2 h exposure at 37°C, conditions under which the cell shows no further $[Ca^{2+}]_i$ response to repeated additions of bombesin [38]. This suggests that, unlike the situation in Swiss 3T3 cells, receptor sequestra-

TABLE I. A Comparison of Some Features of Bombesin Responses Between Swiss 3T3 and SCLC Cells

	Swiss 3T3	SCLC
Homologous desensitization	Yes	Yes
Desensitization by PKC activators	Yes	Yes
Receptor internalized	Yes	No
Heterologous desensitization in response to vasopressin	Yes	No

tion is not an important component of the acute desensitization of the bombesin response in SCLC cells.

Acute homologous desensitization to bombesin occurs in Swiss 3T3 cells with abrogation of early signals such as calcium mobilization at least partly through receptor sequestration. Chronic exposure to bombesin results in chronic desensitization through down-regulation of bombesin cell surface receptors [22]. This raises interesting questions about the maintenance of a sustained mitogenic signal in the context of an autocrine growth stimulatory loop. Could the mechanism underlying desensitization to bombesin stimuli be impaired in human lung cancer cells?

We have noted another significant difference in the behavior of bombesin signals in Swiss 3T3 and SCLC cells. Heterologous desensitization, i.e., desensitization to structurally unrelated ligands after the acute or chronic exposure to another growth factor, is present in Swiss 3T3 cells. The exposure of cells to bombesin inhibits the subsequent $[Ca^{2+}]_i$ responses to vasopressin, and vice versa [22,39]. In addition, chronic exposure to vasopressin inhibits DNA synthesis in response to bombesin without altering the binding characteristics of the bombesin receptor [40]. This heterologous desensitization, at least in terms of acute $[Ca^{2+}]_i$ responses, is not present in human SCLC cells [7] and Viallet and Sausville, unpublished observations).

Some authors have reported that certain bombesin producing SCLC cell lines, despite eliciting a $[Ca^{2+}]_i$ response to bombesin related peptides, showed no enhanced DNA synthesis [41]. These data would appear to contradict others, indicating that bombesin is an autocrine growth factor for SCLC [4]. Although these conditions may be related to cell culture conditions, one possible interpretation is that the tonic mitogenic potential of autocrine bombesin production can only be realized if an additional lesion, i.e., a defect in the desensitization process, is also present. One may postulate the existence of a bombesin receptor kinase and its arrestin which may be the target for mutations or deletions which may serve to enhance the mitogenicity of bombesin and perhaps other neuropeptides. This notion is reinforced by the recent demonstration that under certain conditions, the absolute amount of β-arrestin and β-adrenergic receptor kinase are limiting in β2-adrenergic receptor desensitization [42]. Thus kinases involved in the desensitization of mitogenic neuropeptide responses may demonstrate tumor suppressor functions.

Interruption of Pathways Stimulated by Several Mitogens as a Possible Strategy for the Inhibition of Lung Cancer Cell Growth

Since G proteins couple many receptor subtypes to their effectors, they may be an attractive target for attempts at simultaneously interrupting signals generated by multiple growth factors. As in other cellular systems, [Tyr^4]-bombesin stimulated activation of phospholipase C is modulated by guanine nucleotides [17], thus clearly implicating one or more G proteins in the coupling of the bombesin receptor to phospholipase C in SCLC cells.

Emerging evidence suggests that combinations of the known 16 α, 4 β, and 5 γ subunit genes can produce a large enough variety of heterotrimers to selectively couple specific receptors to specific effectors [43,44]. However, bacterial toxins such as pertussis (PT) and cholera (CT) toxins have the ability to ADP-ribosylate certain G proteins, thus altering their function. These toxins have been important pharmacological probes in the elucidation of the involvement of G proteins in cellular responses. Prior work had implicated PT as an inhibitor of some but not other bombesin stimulated responses in Swiss 3T3 and NIH 3T3 cells [28,29]. When studied, CT appeared to have no action in these cell systems. However, because we had observed a major inhibitory effect of CT on [Tyr^4]-bombesin stimulated phosphoinositide turnover and $[Ca^{2+}]_i$ elevation in SCLC NCI-H345 [24], we studied the effects of CT on the growth of this cell line and found that CT was profoundly growth inhibitory at nanomolar concentrations [38]. We found that CT pretreatment could inhibit the $[Ca^{2+}]_i$ response to multiple ligands without affecting the binding characteristics of I^{125}-[Tyr^4]-bombesin. This latter observation in-

dicated that the effects of CT on bombesin activated signal transduction in SCLC cells was not through an interaction with a G protein coupling the bombesin receptor to phospholipase C. We generalized our findings to a wide selection of SCLC and NSCLC cell lines and demonstrated that CT growth inhibition could be found in both cell types [45]. Whereas CT sensitivity in SCLC appears to correlate faithfully with expression of the cellular receptor for CT, the ganglioside G_{M1}, in SCLC, many CT resistant NSCLC cell lines were found to express abundant G_{M1} ganglioside. Accumulated evidence strongly suggests that elevation of intracellular cAMP generated by CT cannot account for the cellular effects of CT in this system. Current attention is directed at the possibility that CT could act by depleting the membrane of PIP_2, the immediate substrate for phospholipase C [46–48]. However, other possible mechanisms include redistribution of G-βγ subunits, degradation of G-α subunits, and alteration of intracellular membrane transport and handling [49].

These experiments demonstrate that CT is a probe for a growth inhibitory pathway present in lung cancer cells which may act through inhibition of signal transduction stimulated by a wide variety of ligands. Whether it is the cumulative effect of the deprivation of these trophic influences or as yet uncharacterized cellular effects of CT which account for the growth inhibition awaits further study. However, the fact that the inhibitory actions of CT can be demonstrated in vivo (see Fig. 2) as well as in vitro, emphasizes that the understanding of these mechanisms may be rewarding.

Clarification of the Role of the Known and Newer Tyrosine Kinases

Human NSCLC cells express a variety of tyrosine kinase surface receptor such as the EGF receptor and the *c-met*/hepatocyte growth factor (HGF) receptor. A recent study has suggested that whereas HGF appeared to act as an autocrine growth inhibitor of normal human bronchial epithelial cells, it stimulated the growth of 45% of the NSCLC cell lines tested [50]. The HGF receptor of these cells was constitutively phosphorylated on tyrosine residues. Whether these differences in responses to HGF between the normal and malignant cells of bronchial origin are related to perturbations in the

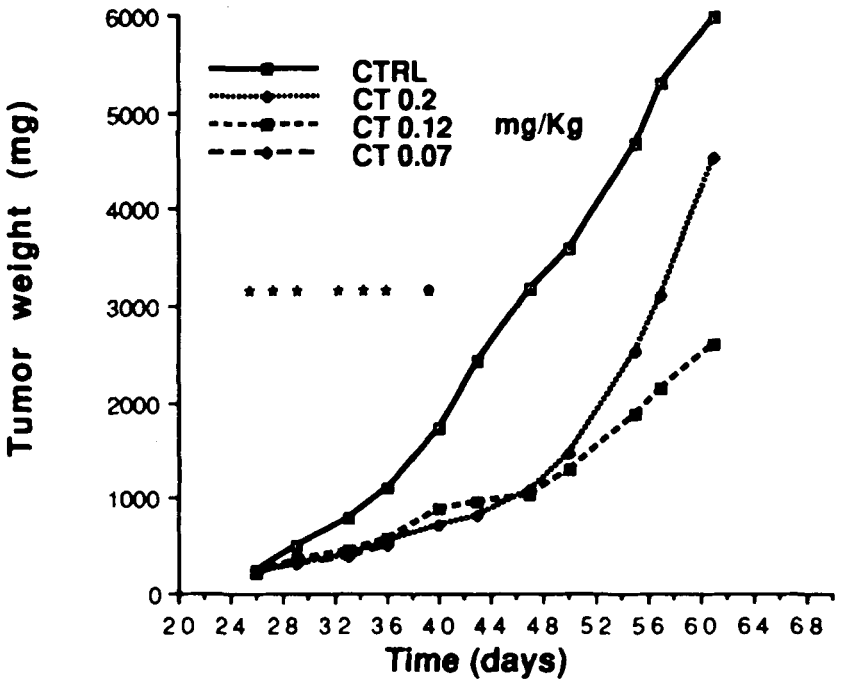

Fig. 2. Cholera toxin inhibits NCI-N592 growth in vivo. N-592 xenografts in athymic mice were treated on days 26, 28, 30, 33, 35, 37, and 40 with the indicated amount of cholera toxin per dose, and tumor weight scored. The 0.07 mg/kg/dose was accompanied by excessive toxicity, while the two higher dose regimens were well tolerated (minimum T/C = 28% on day 43 with a dose of 0.2 mg/kg/injection). Data courtesy of Dr. J. Plowman.

regulation of intracellular signalling or to other factors cannot be determined at this point. However, it is of interest that a novel receptor protein-tyrosine phosphatase has been mapped to an area of chromosome 3 frequently deleted in lung cancer cells [51].

CONCLUSIONS

The regulation of signal transduction pathways in cancer cells offers opportunities for the development of sorely needed novel therapeutic approaches for lung cancer. Already, animal models have shown promise for cAMP analogues, Ca^{2+}/calmodulin inhibitors [52,53], and cholera toxin. Additional studies should delineate whether specific functional abnormalities in these pathways contribute to the malignant phenotype and constitute discrete targets for developmental therapeutics. The human lung cancer cell lines that have been studied and are now broadly available should constitute a valuable resource for further investigations.

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