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Discussion

Dr. B. Debas (San Francisco, California): Thank you, Dr. Moody. Members and guests: It is a privilege to discuss this paper from the president's unit. It provides me with an opportunity to pay tribute to Jim Thompson and his colleagues.

We heard from Dr. Thompson about the individuals that had the most impact on his life. What he did not talk about was the dozens of individuals whose careers he himself has so powerfully impacted. I am privileged to count myself among the latter.

But no better example of that impact can be provided than by the study so ably presented just now by Dr. Evers. It epitomizes Dr. Thompson's contributions. It is evident that we have an inspired young man in Dr. Evers, who will go on to make his own contributions in the future. The international impact of Dr. Thompson is also evident. One of the co-authors is from Japan, the other from China.

But perhaps most significant is the collaboration with Courtney Townsend. What we are witnessing is the coming to a hot and probing point of Dr. Thompson's enormous interest and expertise in gastrointestinal peptides and Dr. Townsend's interest and experience in oncology.

Together, for some years now, they have made contributions that have been honored by being presented annually at this prestigious surgical meeting.

What the current study has shown is that neurotensin, one of the 40 or so gut peptides, satisfies all the criteria for an autocrine growth factor in colon cancer. It is elaborated by some colon cancers, the tumors have receptors for it, and that it binds to these receptors in a functional manner to stimulate signal transduction.

In this respect, neurotensin joins two other oncologically significant gut peptides: bombesin and gastrin. Bombesin has been shown to be an autocrine growth factor in small-cell cancers of the lung, in which both receptor antagonists and monoclonal antibodies have provided a new therapeutic approach. Gastrin, a peptide that this group has studied extensively for its trophic effects on colon cancer, recently has been shown to regulate release of histamine from enterochromaffin cells in the gastric corpus and to cause ECL cell proliferation in gastrinomas, and ECL cell carcinoid tumors in rats treated long-term with the proton-pump inhibitor, omeprazole. I have three questions for the authors.

First, how do you think the colon tumor that produces neurotensin comes to do so? There is not much neurotensin in the normal colon. Do you think there has been a differentiation in certain cells or derepression to allow them to express neurotensin, or did the tumors happen to arise in areas where some neurotensin-producing cells might be present?

Second, to prove that neurotensin is a true autocrine growth factor, you must not only show that the tumors produce it, and have receptors linked to signal transduction, but you also must show that it actually stimulates growth differentially either *in vitro* or in tumors implanted into nude mice. Have you done that?

And finally, do you see a potential for therapeutic intervention with neurotensin antagonists? I think you implied that in your last slide.

DR. THOMAS C. MOORE (Torrance, California): The authors have made some significant observations here today concerning an involvement of significance of vasoactive neurotransmitter/neuromodulator molecule neurotensin in human colon cancer cells as a possible growth factor. This is of particular interest because other vasoactive neurotransmitter molecules such as serotonin, bradykinin, substance P, bombesin, and others are being identified increasingly as "nonclassical" growth factors, in addition to their many other biologic actions.

Despite its discovery some 20 years ago, neurotensin remains somewhat of a mystery molecule in many respects, yet one of clear evolutionary importance and significance because it has been tracked down the phylogenetic tree to the most primitive of multicellular animals, the cnidarian coelenterate, *Hydra.* These are the most primitive animals to possess a nervous system. In *Hydra,* immunoreactive neurotensin has been identified in the primitive entrance to the primitive gut.

I have had a long interest in neurotensin as an immune response-influencing molecule and have observed a potent and depressant effect of neurotensin on lymphocyte traffic in the supported sheep lymphocyte model, as this slide demonstrates. Acute infusion of 50 μg neurotensin into a cannulated popliteal lymph node afferent lymphatic of an awake and unsedated sheep produces a prompt, sharp, and prolonged depression in lymphocyte traffic as monitored by the output of both blast and small recirculating lymphocytes into study node efferent lymph and lymphatics chronically cannulated. These responses have been interpreted as immunomodulatory and immunosuppressive.

In addition to the neurotensin-associated growth factor activity in human colon cancer, so impressively demonstrated by the authors, a metabolic shield deriving from neurotension-induced depression in lymphocyte traffic might provide an additional mechanism to diminish detection and facilitate the propagation of these malignant tumors. Have the authors employed *in situ* hybridization methodology to localize neurotensin messenger RNA in anatomic areas of human colon cancers and in the layers of lymphoid tissues draining these tumors? Also, have they investigated levels of neurotensin immunoreactivity in these areas?

I think it is of importance that many of these vasoactive neurotransmitter substances that are stimulatory in the central nervous system are also stimulatory in the immune response system. In this regard, neurotensin iontophoretically applied to the brain in certain areas has had a very strong inhibitory effect on both the dopaminergic and the noradrenergic nervous systems. It will be interesting to see what this effect is on the immune response as it relates to these tumors.

DR. DANA K. ANDERSEN (Chicago, Illinois): You were able to describe release of neurotensin by some but not other cell lines from human colon carcinomas, and I wonder if you can differentiate the responders in your study by any characteristic of these tumors, such as site or histology, DNA characteristics, or their ability to secrete other autocrine growth factors such as IGF-1 or 2?

My second question concerns the findings in the LoVo cells where both 6-bromocyclic adenosine monophosphate (AMP) and arginine were capable of stimulating neurotensin release. As you know, arginine is thought to stimulate peptide release by phosphorylation of inositides, and this suggests that there are two separate secretion coupling mechanisms that can release neurotensin from these cancer cells.

This is probably important because tumor cells are presumably being barraged by a variety of peptide hormones within the body, some of which might cause release of growth factors through stimulation of cyclic AMP and others presumably might cause the release of growth factors by mobilization of intracellular calcium stores. Your findings would suggest that neurotensin might be stimulated by both. It also would suggest that giving two kinds of secretagogues might show a potentiated release. Have you looked at the combination of arginine and 6-bromocyclic AMP to see if multiple secretagogues will in fact cause an exaggerated response of neurotensin from these cancer cells?

DR. B. M. EVERS (Closing discussion): Thank you, Dr. Debas, we appreciate your kind comments.

Your first question was how do these colon cancers produce neurotensin because, as you correctly point out, neurotensin is not normally expressed in the normal adult colon. Interestingly, we have found that neurotensin is expressed in the colon of human fetuses at relatively high levels; however, with maturation, neurotensin expression is repressed. This expression of neurotensin in colon cancers may reflect a de-repression of neurotensin and possibly a reversion to a more fetal-like pattern.

We are looking at the effects of neurotensin on the growth of these cell lines *in vitro.* Recently, we published the effect of neurotensin on LoVo xenografts in nude mice, in which we found that neurotensin stimulates the growth of these tumors *in vivo.*

You also speculate on the potential intervention using neurotensin antagonists. We certainly look forward to the future development of these agents; at this point, however, there are no specific neurotensin receptor antagonists.

Dr. Moore, you point out quite correctly that various neuroendocrine peptides, which include neurotensin, have immunomodulatory effects. We have not specifically looked at *in situ* hybridization studies in our group of colon cancers, but those experiments are planned.

Dr. Andersen, we have only looked at the release of neurotensin from LoVo cells in response to various secretagogues. We are evaluating the effects of these agents, either alone or in combination, on both the expression and release of neurotensin.