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## Discussion

DR. DANA K. ANDERSEN (Chicago, Illinois): This study continues a productive line of investigation by this group in which they have examined the specific gene expression in normal and neoplastic tissue as a means of understanding control of growth of these tissues and this group is certainly to be congratulated for their expertise in this area.

This particular study extends a number of observations that this group has already made in other tumors such as the BON

cell line and in animal fetal and adult livers and suggests that these observations may have real clinical implications for all of us. I have just a couple of questions.

With regard to the role of neurotensin gene expression in the development of normal tissue growth, is this a nonspecific event that is perhaps only one of a large series of genes that are being expressed during a period of rapid growth, or is neurotensin a critical primary growth factor for the control of normal or neoplastic tissue? With regard to its role as a growth factor, was a neurotensin message translated for peptide synthesis in fetal liver tissue or, conversely, were neurotensin receptors present on either fetal tissue or regenerating tissue? This would suggest its primary role.

If neurotensin is to serve as a useful preoperative marker of fibrolamellar carcinoma, it must be detectable in the plasma or the serum of fibrolamellar carcinoma patients in levels higher than normal. Does this occur? Or, is neurotensin gene expression something that is only detectable in the tissue itself?

Finally, are there neurotensin receptors on fibrolamellar carcinoma to suggest that neurotensin is in fact an autocrine growth factor important for the control of tumor growth and development? If not, and in light of that titillating speculation at the end of the paper, I would like the authors to speculate in more detail how they might propose to use neurotensin gene expression to target specific strategy directed at the tumor and, as a corollary, how they might protect the small bowel, which normally expresses neurotensin, from that therapy.

DR. R. SCOTT JONES (Charlottesville, Virginia): Before I comment on the paper I would like to make an extraneous comment, if I may, by observing that the first discussant of this paper was an American Surgical Association Foundation Fellow in 1982 and 1984 and that the second author on the paper likewise was an American Surgical Association Foundation Fellow in 1990 and 1992. I point this out so the members of the Association will realize that they are getting returns on their investments in young investigators.

This was a very interesting paper. I would like to preface my questions by observing that on rare occasions primary liver cell tumors have the capacity to produce paraneoplastic syndromes. In particular, they can synthesize peptides. I think the experiments that we heard reported today give us insights into this question and also provide tools for the further investigation of those interesting phenomena.

My main question relates to the fact that we have now demonstrated we have the capacity to produce neurotensin in the fibrolamellar area of hepatocellular carcinoma, and it is important for us to realize before we use that as a marker how often that gene or this particular mechanism may exist in the ordinary variety of hepatocellular carcinoma. Therefore, I would like to ask the authors whether this gene expression also occurs in the ordinary liver cell cancer.

I would also like to point out that the third most common solid organ neoplasm in infants and children is the hepatoblastoma, many of which undoubtedly develop in utero. Since this gene occurs and is expressed in fetal tissue, would it also be recognizable in hepatoblastomas?

DR. WILEY W. SOUBA (Boston, Massachusetts): I will try to keep my comments brief as a number of the questions have already been asked by my predecessors. I'd like to start by congratulating Dr. Ehrenfried, who is a surgical resident at the University of Texas in Galveston.

Neurotensin is a gut-derived peptide. It is somewhat of interest in that it is only 13 amino acids long. It is released every time you eat a meal. It stimulates amino acid uptake by a number of tissues. The authors today have clearly shown the differential expression of the neurotensin gene in certain liver tissues.

The interesting feature of the work is that the gene is present in all tissues but its messenger RNA is not. And so the question that is raised is: What is the signal that controls the expression of the messenger RNA and then subsequently allows the neurotensin protein to be synthesized and released?

The first point is somewhat speculative. Why would hepatic tissue produce such a trophic peptide with apparently no benefit to itself? Is the fetal liver acting as an endocrine organ during development? Although the neurotensin gene is not apparently expressed in the regenerating rat liver it would be interesting to determine if its receptor is, given that the neurotensin compound can be a trophic factor.

The observation that fibrolamellar carcinoma expresses the neurotensin gene while fibronodular hyperplasia and normal liver tissue do not is significant and, as suggested by the authors, may provide a basis for the molecular diagnosis of liver cancer patients.

Two questions came to my mind. Has it been shown that malignant hepatocellular carcinoma expresses the gene, and does the fibrolamellar carcinoma express the neurotensin receptor? If this is the case, this may represent an autocrine loop that allows the tumor cells to stimulate their own growth.

Given the stem cell theory proposed in the paper, the question of hepatocellular tumor morphology must be raised. Since the fibrolamellar carcinomas express high amounts of mature collagen and do not express alpha fetal protein, the possibility exists that this form of hepatocellular tumor may arise from lipocyte or smooth muscle precursors as these cells have been shown to be the major source of collagen both in normal liver and in sclerotic liver.

In fact, it would be interesting to determine if either the neurotensin gene or neurotensin receptor was expressed in the cirrhotic liver, as this is a pathophysiologic state characterized by both nodular hyperplasia and smooth muscle cell proliferation.

DR. LESLIE H. BLUMGART (New York, New York): I enjoyed this paper very much. I perhaps can offer some data which would answer some of the questions that have been raised.

We first reported the presence of an elevated plasma neurotensin in the serum of patients with fibrolamellar hepatocellular cancer about 10 years ago and I've been using it as a diagnostic tool and a tumor marker during therapy.

There is no doubt that it is positive in a majority of patients with fibrolamellar hepatocellular cancer but not in all, and we have in fact detected raised plasma neurotensin levels in some patients with non-fibrolamellar hepatocellular carcinoma. So, in the future, it would be very interesting to see how gene expression in fact correlates with the plasma levels that one can or cannot record.

I would like to make one further point. In the summary it is suggested that this tumor has a better prognosis. I am not at all sure that this is so. It certainly seems to have a better prognosis in patients that are transplanted. However, in the studies that we have carried out both in London and now more recently in a retrospective survey in Memorial Hospital, I am not at all sure that the prognosis for this tumor is any better than in patients with a straightforward hepatocellular carcinoma.

It is tempting to speculate, and Marty Adson once suggested that there is a better resectability rate but not a better prognosis. Finally, I would put forward a suggestion and perhaps a question to the authors: Is this perhaps a form of neuroendocrine tumor? Is it in fact an hepatocellular carcinoma that has neuroendocrine characteristics?

DR. SEYMOUR I. SCHWARTZ (Rochester, New York): I think this is an exciting piece of work that offers us the opportunity to resolve a question that has recently arisen.

In the classic literature on hepatic surgery, focal nodular hyperplasia was deemed a nonsurgical lesion, one that rarely if ever bled, and should not be resected. More recently there have been perhaps three articles that suggested a transformation could occur from focal nodular hyperplasia to fibrolamellar carcinoma. That argument can readily be resolved, and this is the reason we have been supplying Dr. Ehrenfried and his lab with these specimens.

Is there a possibility, by providing a laboratory interested in determining neurotensin levels with sequential core biopsies of patients with established diagnoses of focal nodular hyperplasia, to determine over the course of time whether transformation can occur? I think if the group provides an interested laboratory with a series of specimens the answer can be resolved really quite quickly.

DR. B. MARK EVERS (Closing discussion): Drs. Andersen and Souba asked about the role of neurotensin in the liver and whether neurotensin represents a growth factor for normal liver tissue. We believe that our present study, as well as those of others, indicate that neurotensin is not a primary trophic factor for the liver. However, studies by Hasegawa and Carr from Pittsburgh suggest that neurotensin may play a secondary role in liver growth based on their *in vitro* studies demonstrating that neurotensin augments the trophic effect of EGF and TGFa. In our present study, we have analyzed these liver tumors using the human neurotensin receptor cDNA in sensitive RNase protection assays, and we have not detected expression of this receptor. We believe that absence of receptor expression further emphasizes the fact that neurotensin probably does not play a primary role in liver growth.

Dr. Andersen asked about possible treatment modalities using gene therapy. We think that this possibility represents the most exciting aspect of this work. It is clear from our studies that neurotensin is specifically expressed in the fibrolamellar tumor but not in the normal adult liver. Using this information, vectors could be designed using the 5′ flanking sequence of neurotensin to direct the delivery of various "suicide genes" so that directed enzyme drug therapy can be utilized to specifically target and kill tumor cells. This vector could be injected directly into the portal vein system so that normal neurotensin-expressing cells (e.g., in the small bowel) would not be affected.

Drs. Jones and Souba asked about the expression of neurotensin in standard hepatocellular carcinomas. We, as well as others, have not demonstrated neurotensin expression in these tumors. However, hepatoblastoma, a liver cancer that is found in children, expresses low levels of neurotensin. Therefore, neurotensin expression appears limited to fibrolamellar and hepatoblastoma, but not in the routine hepatocellular carcinoma. We have not yet analyzed livers from patients with cirrhosis; however, this is something that we are planning to study in the near future.

We appreciate the comments from Dr. Blumgart, who has performed the initial work on neurotensin peptide in fibrolamellar cancers. As he stated, neurotensin peptide levels are elevated in a majority of these patients with fibrolamellar carcinoma. We were trying to identify a rapid and accurate way of differentiating fibrolamellar carcinoma from the routine hepatocellular cancer and focal nodular hyperplasia. We hope that RNA extracted from needle biopsies of liver tumors may be utilized in sensitive RNase protection assays to differentiate these various tumors based on expression of neurotensin.

Finally, President Schwartz, who has been very instrumental in our present study, asked about neurotensin expression in focal nodular hyperplasia since some investigators regard this lesion as a possible precursor of fibrolamellar carcinoma, based on histologic similarities. Thus far, all that we can say is that neurotensin expression was not detected in either of the samples of focal nodular hyperplasia. Certainly, more samples need to be examined in order to better answer the question.