Adenocarcinoma -- Pancreatic

Parathyroid Hormone-related Protein as a Novel Tumor Marker

Introduction: Parathyroid hormone-related protein (PTHrP) can act as an oncoprotein to regulate the growth and proliferation of many common malignancies, including pancreatic cancer. Previous studies have shown that PTHrP is produced by human pancreatic cancer cell lines, can be shown in the cytoplasm and nucleus of paraffin-embedded pancreatic adenocarcinoma tumor specimens, and is secreted into the media of cultured pancreatic adenocarcinoma cells. We hypothesized that PTHrP could serve as a tumor-marker for growth of pancreatic cancer in vivo.

Aim and Methodology: To test this hypothesis, we used an orthotopic model developed in our laboratory of the PTHrP-producing human pancreatic cancer line, BxPC-3. This tumor was stably transduced with green fluorescence protein (GFP) to facilitate visualization of tumor growth and metastases. At early (5 weeks) and late (13 weeks) time points after surgical orthotopic implantation, serum PTHrP was measured and primary and metastatic tumor burden was determined for each mouse by assessing GFP expression.

Results: By 5 weeks after surgical orthotopic implantation (early group), the mean serum PTHrP level was 33.3 pg/mL. In contrast, by 13 weeks after surgical orthotopic implantation (late group), the mean serum PTHrP level increased to 158.5 pg/mL. These differences were highly significant ($p < 0.001$, Student t test). Numerous metastatic lesions were readily visualized by GFP in the late group. Serum PTHrP levels measured by immunoassay correlated with primary pancreatic tumor weights and serum calcium levels ($p < 0.01$). PTHrP levels were not detectable (<21 pg/mL) in any of the 10 control mice with no tumor. Western blotting of BxPC-3-GFP tumor lysates confirmed the presence of PTHrP. BxPC-3-GFP tumor tissue stained with antibody to PTHrP.

Conclusion: These results indicate that PTHrP can serve as a tumor marker in animal models of pancreatic cancer and may be a useful tumor marker for clinical pancreatic adenocarcinoma.

Key Words: Pancreas—Pancreatic cancer—Parathyroid hormone-related

protein.

Parathyroid hormone-related protein (PTHrP) is an oncoprotein that regulates the growth and proliferation of essentially every tissue in which it is expressed, including many common malignancies such as breast, colon, gastric, melanoma, and prostate cancer. In these tumors, PTHrP is processed into distinct peptides that mediate its unique biologic effects through intracrine (nuclear localization) and endocrine (autocrine and para- crine cell surface receptor) pathways. In addition to its endocrine effects, several studies have shown that increased expression of PTHrP in cancer is associated with accelerated tumor growth and a more malignant phenotype, suggesting that PTHrP may play a role in promoting tumor progression.

Recently, we showed that PTHrP is produced by human pancreatic adenocarcinoma cell lines and is present in the cytoplasm and nucleus of paraffin-embedded pancreatic adenocarcinoma tumor specimens. We also observed that PTHrP is secreted into the media of cultured pancreatic adenocarcinoma cell lines and could be measured by radioimmunoassay. Because of these findings, we hypothesized that PTHrP could serve as a tumor marker for growth of pancreatic cancer. To test this hypothesis in vivo, we used an orthotopic model, developed in our laboratory, of the PTHrP-producing pancreatic cancer cell line, BxPC-3, which expresses the green fluorescence protein (GFP) to facilitate visualization of tumor growth and metastases.

Calcium measurements

We measured serum calcium levels in the available remaining samples for the mice in the study. Serum calcium was measured using a commercial calcium kit 587-M (Sigma Diagnostics, St. Louis, MO, U.S.A.). Briefly, 5 jjlL mouse serum was added to an alkaline solution containing o-cresolphthalein complex one in a 96-well plate. A purple complex formed and was quantitated at 570 nm in a plate spectrophotometer, and a reference calcium standard curve was generated at the same time to convert the sample optical density values into calcium concentrations.

RESULTS

PTHrP serum levels increased chronologically with metastatic progression
Parathyroid hormone-related protein levels were not detectable (<21 pg/mL) in all 10 control mice with no tumor. PTHrP levels increased in a chronologic manner in the mouse pancreatic cancer model. By 5 weeks after SOI (early group), the mean serum PTHrP level was a mean of 33.3 pg/mL. In contrast, by 13 weeks after SOI (late group), the mean serum PTHrP level increased to 158.5 pg/mL. These differences were highly significant ($p < 0.001$, test) for the 5-week and 13-week groups compared with the control group. Numerous metastatic lesions were easily visualized by GFP in the late group. The most common sites were to the retroperitoneum and the spleen, followed by the mesentery, liver, and colon. PTHrP serum levels correlate with tumor weight
Serum PTHrP levels measured in 47 tumor-bearing mice correlated with primary pancreatic tumor weights. The correlation was significant ($p < 0.01$, $n = 47$). The correlation coefficient was 0.38832 for $n = 47$ at >99% confidence limits.
Serum calcium levels correlate with tumor weight and serum PTHrP.
Serum calcium levels correlated with tumor weight ($p < 0.01$, $r = 0.643$ for $n = 39$ at >99% confidence limit), and serum calcium correlated with serum PTHrP ($p < 0.01$, $r = 0.481$ for $n = 37$ at >99% confidence limit).

PTHrP immunohistochemistry in human pancreatic adenocarcinoma tumors

The BxPC-3-GFP tumor was stained with PTHrP antibody. PTHrP staining of the BxPC-3 tumor was strong. In all cases of positive staining, incubation of sections with the antibody-antigen control mixture showed no positive staining.

DISCUSSION

In this study, we have shown that PTHrP is secreted into the blood of mice orthotopically implanted with human pancreatic adenocarcinoma and that serum PTHrP levels correlate with tumor burden. The use of GFP helped visualize the extensive metastases that develop in the later stages of this pancreatic cancer.

This study shows that PTHrP can serve as a tumor marker for this model of

pancreatic cancer and raises the possibility that PTHrP may be a useful clinical marker for pancreatic cancer. Secretion of PTHrP into the serum has been described in many case reports for various pancreatic tumors. However, most of these are endocrine neoplasms. Ratcliffe et al described a patient with a neuroendocrine tumor of the pancreas associated with hypercalcemia, which was attributed to production of PTHrP by the tumor. Plasma PTHrP levels were significantly increased and decreased after surgical resection of the tumor. Mitlak et al described a patient with an islet cell carcinoma and hypercalcemia in whose serum PTHrP was measured during the course of therapy by an immunoradiometric assay directed toward the mid portion of the molecule. The concentration of PTHrP increased with time and decreased after the patient received chemotherapy directed toward the islet cell tumor. Wu et al measured PTHrP levels in 17 patients with islet cell carcinoma and 110 healthy subjects. PTHrP levels were significantly higher in 10 patients with hypercalcemia and islet cell carcinoma than in 7 patients with eucalcemia and islet cell carcinoma or in the 110 healthy subjects. Despite these reports in endocrine pancreatic tumors, serum PTHrP levels have not been extensively evaluated in patients with exocrine pancreatic adenocarcinoma.

Our recent studies have shown that PTHrP is commonly expressed in exocrine pancreatic cancer. To study PTHrP in pancreatic exocrine cancer, we studied its expression in eight pancreatic cancer cell lines and 14 surgical specimens. Cellular PTHrP was detected in all cell line extracts by Western blotting and immunoassay. PTHrP secretion measured by a PTHrP 38-64 assay was highest for BxPC-3, which is consistent with our use of the PTHrP 38-64 immunoassay to measure the serum PTHrP in BxPC-3 orthotopic mice.

This assay is also our most sensitive and technically reliable PTHrP immunoassay. CFPAC-1, derived from a pancreatic liver metastasis, had the highest concentration of PTHrP, and MIA PaCa-2, derived from primary pancreatic adenocarcinoma, had the lowest. PTHrP was localized by immunocytochemical staining in the cytoplasm in all but one cell line, and nuclear and cytoplasmic immuno staining was observed in the MIA PaCa-2 and PANC-1 cells. Secretion of PTHrP into cell media was also observed for each cell line and paralleled intracellular PTHrP levels. Evidence for differential processing of PTHrP expression was provided by studies showing different patterns of

PTHrP among the cell lines when assessed by PTHrP immunoassays directed against different PTHrP peptides. Growth of AsPC-1 cells was stimulated in a dose-dependent manner by PTHrP 1-34. Immunostaining from archival tissue of patients with pancreatic adenocarcinoma showed robust PTHrP expression in 14 of 14 specimens. These results showed that PTHrP is commonly expressed in pancreatic cancer.

Although the function of PTHrP in pancreatic cancer is unknown, it appears to regulate growth in other tumor types (28). We have previously shown that PTHrP 1-34 stimulated thymidine uptake in prostate cancer cells more than threefold over control under scrum-free and steroid-free conditions; in addition, the PTHrP induced DNA synthesis was completely neutralized by our mouse monoclonal antibody against PTHrP 1-34. We also have shown that PTHrP 1-34 regulates the growth of cultured type II epithelial cells. Recent preliminary studies from our laboratory indicate that PTHrP may similarly regulate the growth of pancreatic cancer. Future studies in orthotopic models of pancreatic cancer will also address the growth properties of PTHrP.

In our study, PTHrP levels were highest in late stages of the disease, when multiple metastatic lesions were present in the mice. Some of these metastases, such as those of the liver, were hematogenous, whereas other metastases, such as those to the retroperitoneum, were by direct extension. This may indicate that PTHrP may be a useful tumor marker for clinical pancreatic adenocarcinoma. However, serum levels of PTHrP of patients with pancreatic cancer must be examined and correlated with clinical stage, prognosis, and pathologic tumor features before PTHrP can be validated as a useful clinical tumor marker for pancreatic cancer.

In summary, we have shown that PTHrP is secreted into the blood of mice that have undergone surgical orthotopic implantation of PTHrP-expressing human pancreatic tumors. Furthermore, serum PTHrP correlated with the primary pancreatic tumor weight and the extent of metastatic burden. Future studies will determine whether there is differential expression of PTHrP in the primary tumor and specific metastatic sites. Additional studies are needed to elucidate the role of PTHrP in the development and progression of pancreatic cancer and to determine whether PTHrP could be useful in the

early detection or clinical treatment of patients with this disease.