

A Protein That Prolongs the Action of cAMP-Dependent Protein Kinases Is Overexpressed in Papillary Thyroid Cancer

Cantara S, D'Angeli F, Toti P, Lignitto L, Castagna MG, Capuano S, Prabhakar BS, Felicciello A, Pacini F. Expression of the Ring ligase praja2 in thyroid cancer. *J Clin Endocrinol Metab.* September 4, 2012 [Epub ahead of print]. doi: 10.1210/jc.2012-2360.

SUMMARY ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ●

Background

Praja2 is a ubiquitin ligase that can anchor cAMP-dependent protein kinases (PKAs) under basal conditions. When cAMP levels in a cell rise, however, the PKA catalytic subunits become free and phosphorylate praja2, activating it, promoting the ubiquitination and degradation of the regulatory subunits of PKA to which it is bound, thus prolonging the activity of free catalytic subunits (1). The current paper provides data showing that praja2 mRNA and protein levels are overexpressed in papillary but not anaplastic thyroid cancer.

Methods

Samples from 36 papillary thyroid cancers (PTCs), 6 anaplastic cancers and 14 benign nodules were obtained from patients who had been evaluated and treated according to ATA guidelines; 14 samples were also obtained from normal regions of the thyroids of some of these patients. Praja2 mRNA levels were assessed by (semi)quantitative RT-PCR on all 70 samples (expressed on the basis of two reference mRNAs). Praja2 protein was prepared from 8 PTCs, 4 benign nodules, and 2 anaplastic cancers as well as samples from 5 normal regions. Total praja2 levels were measured on Western blots using polyclonal antibodies, as described Lignitto et al. (1). Praja2 subcellular distribution was assessed by immunohistochemistry on samples using a monoclonal antibody from Sigma. Possible cross-reactivity of antibodies with the homologous protein praja1 was not discussed.

Results

The mean level of praja2 mRNA in the 36 PTCs was significantly greater ($P<0.001$) than the mean levels in the 6 anaplastic cancers, 14 benign nodules, or 14 “normal” samples, although the values found in 6 of the normal samples overlapped with the values seen in almost all of the PTC samples. The mean messenger RNA (mRNA) level in RET/PTC1 tumors ($n = 6$) was about 20% higher than in BRAF V600E tumors ($n = 26$), and about 5 times higher than in RET/PTC3 tumors ($n = 4$). mRNA from normal areas of the thyroids of 7 patients with PTCs was available for comparison with mRNA from the cancerous areas, and in each case the levels were higher in the cancer samples ($P<0.02$). On Western blots, the mean level of praja2 was significantly higher in 8 PTC samples than in any of the 2 anaplastic cancers, 4 benign nodules or 5 normal samples (when combined, $P<0.001$). Immunohistochemistry showed strong cytoplasmic staining in classical PTC, less in follicular variant and insular PTC, and hardly any in anaplastic cancer. No information was given concerning the level or subcellular distribution of immunoreactivity in normal thyroid tissue.

Conclusions

The mRNA and protein levels of praja2 are elevated in differentiated PTCs, and they correlate with PTC mutational status, whereas they are not elevated in anaplastic thyroid cancer.

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ANALYSIS AND COMMENTARY ● ● ● ● ●

The transcription factor CREB is a major substrate for free PKA catalytic subunits. When the free catalytic subunits phosphorylate CREB, it binds to the promoters of genes with CREB binding sites and increases their expression. Praja2 is such a gene. The substantial overexpression of praja2 presumably would provoke a cell to counter with some physiological homeostatic responses, however. In future studies, it will be important to determine the status of other factors known to affect PKA activity, such as other anchoring proteins, phosphodiesterases and heat-stable protein inhibitors, as well as the status of other proteins that praja2 binds and ubiquitinates, such as ELK and MAGE-D1. In other tissues, the level of praja2 expression changes in response to various stimuli, including neural differentiation, contact inhibition, hypothermia, experimental colitis, and estrogen replacement. Thus, it is quite possible that the overex-

pression of praja2 seen in PTC reflects a homeostatic response to changes that occur as papillary cancer develops. Nonetheless, it is still possible that praja2 does play a role in the development of PTCs, whereas this would not seem to be the case in the development of anaplastic cancer or benign adenomas. Such a role would presumably be more prominent in RET/PTC1 and BRAFV600E papillary tumors than in RET/PTC3 tumors, although the numbers of some variants studied were small.

Even if the level of praja2 should turn out to display so much physiological variability that it cannot be used for the diagnosis of PTC, its ability to regulate PKA activity may well prove important clinically for understanding anomalies in the regulation of normal thyroid function.

— Stephen W. Spaulding, MD

Reference

1. Lignitto L, Carlucci A, Sepe M, Stefan E, Cuomo O, Nisticò R, Scorziello A, Savoia C, Garbi C, Annunziato L, Feliciello A. Control of PKA stability and signalling by the RING ligase praja2. *Nat Cell Biol* 2011;13:412-22. Epub March 20, 2011.