Medullary Thyroid Carcinoma Arises in the Absence of Prolactin Signaling

Cécile Kedzia, Ludovic Lacroix, Nabahet Ameur, Thierry Ragot, Paul A. Kelly, Bernard Caillou, and Nadine Binart

1Institut National de la Sante et de la Recherche Medicale U884, Faculte de Medecine Necker, Paris, France; 2Unite de Genomique Fonctionnelle, UMR 8121, Vectorologie et Transfert de Genes; and 3Departement d'Histopathologie, Institut Gustave Roussy, Villejuif, France

Abstract

Prolactin, a pituitary hormone, exerts pleiotropic effects in various cells. These effects are mediated by a membrane receptor highly expressed in many tissues. To analyze prolactin effects on the thyroid gland, we first identified prolactin receptor (PRLR) mRNAs by in situ hybridization. To further evaluate the physiologic relevance of PRLR actions in the thyroid in vivo, we used PRLR knockout mice. Whereas the histologic structure of thyroid of PRLR-null mice was not disturbed, we show that T4 levels are lower in null animals (13.63 ± 2.98 versus 10.78 ± 2.25 pmol/L in null mice), confirming that prolactin participates in the control of thyroid metabolism. To further investigate thyroid effects in mice, we measured body temperature and thyroid-stimulating hormone in young and adult male and/or female PRLR-null mice and their normal siblings. Surprisingly, in null animals, we saw medullary thyroid carcinoma (MTC) arising from parafollicular C cells producing calcitonin. The incidence of these carcinomas attained 41% in PRLR-null mice, whereas this malignant tumor occurs sporadically or as a component of the familial cancer syndrome in humans. This finding suggests that PRLR-null mice could represent a valuable animal model for MTC, which could be compared with existing MTC models. These observations suggest a possible link between the appearance of this carcinoma and the absence of prolactin signaling. (Cancer Res 2005; 65(18): 8497-503)

Introduction

Prolactin is synthesized by the anterior pituitary and to a lesser extent by numerous extrapituitary tissues. This hormone influences various physiologic processes among which are the regulation of mammary gland development, initiation and maintenance of lactation, reproduction, omosregulation, immunomodulation, and metabolism (1). These actions are mediated via the binding of prolactin to its receptor, a member of the class I cytokine receptor superfamily, which activates the Janus-activated kinase/signal transducers and activators of transcription signaling pathway (2).

The prolactin receptor (PRLR) is expressed as short and long forms, differing in sequence of their cytoplasmic tails due to alternative splicing of a single PRLR gene (3).

Null mutation of the PRLR functions (4) has mainly highlighted its essential role in reproduction and mammary gland development. Most of the actions, other reported target tissues are presumably modulated by rather than strictly dependent on prolactin. It is known that prolactin plays a role in carbohydrate metabolism via effects on pancreatic insulin production and peripheral insulin sensitivity; however, its role in lipid metabolism is poorly understood (5). Progressive reduction in body weight associated with a reduction in total abdominal fat mass and in leptin concentrations was observed in null females (6). Moreover, PRLR deficiency is accompanied by islet and β-cell hypoplasia, reduced pancreatic insulin mRNA levels, a blunted insulin secretory response to glucose, and mild glucose intolerance establishing a physiologic role for prolactin in islet and β-cell maturation, development, and function. Moreover, we have shown bone alterations in null mice representing a delay in bone formation leading to minor osteopenia (7). The observed mineral abnormalities could be explained by an elevated level of parathyroid hormone.

Medullary thyroid carcinoma (MTC) is an endocrine neoplasm of C cells (for review, see refs. 8, 9). This thyroid tumor is sporadic in about 75% of cases and familial in 25% (10). Hereditary germ line mutations or somatic mutations of the RET proto-oncogene are involved in the carcinogenesis of familial or sporadic MTCs (11, 12) and multiple endocrine neoplasia 2 syndromes (MEN2A and MEN2B). RET is a membrane-associated tyrosine kinase receptor involved in neural crest development. Activating mutations in codon 634 are present in 80% of MEN2A patients and a part of familial MTC. On the other hand, 95% of MEN2B syndrome patients display a gain of function mutation in codon 918. In sporadic tumors, somatic mutations in codon 918 of RET proto-oncogene have been identified in 25% to 33% of case, whereas other mutations are not frequent (13). Moreover, MTC synthesizes and secretes large amounts of calcitonin (14), the most specific marker that can be measured in plasma or by immunohistochemistry. Levels of plasma calcitonin are generally correlated with tumor size (15).

With the aim of better understanding the precise role of parathyroid and thyroid function in PRLR knockout mice, we analyzed, in detail, numerous biochemical, histologic, and hormonal variables to explore in vivo the function of prolactin in the thyroid. Our results show that a high incidence of MTC occurs in both genders at about 1 year of age in the absence of the PRLR, suggesting that this cytokine receptor could play some protective role against carcinogenesis.

Materials and Methods

Prolactin receptor knockout mice. Wild-type, PRLR+/− and PRLR−/− mice on a pure 129Sv genetic background were generated by heterozygous crossing breeding (4). PCR analysis of tail DNA determined the genotypes of the offspring as described previously (16).
In situ hybridization. Microdissected thyroids were snap-frozen and sectioned at 8 µm using a cryocut (Leica, Deerfield, IL). Sections were mounted on Superfrost gold slides (CML, Nenmours, France) and fixed in 4% paraformaldehyde for 10 minutes at room temperature. After rinsing in PBS, slides were acetylated for 10 minutes in triethanolamine/acetic anhydride (0.25%, 0.1 mol/L) and dehydrated. Slides were prehybridized for 2 hours at 50°C in the hybridization solution [50% formamide, 10% sulphate dextran, 1× Denhardt’s solution, 10 mmol/L Tris-HCl (pH 7), 1 mmol/L EDTA (pH 8), 200 µg/mL yeast tRNA, 600 mmol/L NaCl, and 50 mmol/L DTT]. Slides were then hybridized at 55°C for 18 hours in the same solution containing the labeled probes. [35S]-UTP-labeled sense and antisense probes were synthesized using SP6 or T7 RNA polymerase (Kit riboprobe system, Promega, Madison, WI) and used at 4 × 105 cpm/µg/mL. Sections were then washed in high stringency, treated with RNase A (20 µg/mL) for 15 minutes at 37°C, and washed in SSC 2×, 50% formamide for 30 minutes at 65°C, in SSC 2× then in 0.1× at 37°C for 20 and 10 minutes, respectively. After drying in ethanol series, the slides were dipped in photographic NTB-2 emulsion (Kodak, Rochester, NY) and exposed for 2 weeks. Slides were developed and fixed (D19 developer, Kodak, Rochester, NY) and counterstained by hematoxylin.

Free T4 and calcitonin measurements. Free T4 was measured in individual serum samples from 1- to 14-month-old females using a human RIA kit (Immunotech, Marseille, France). Calcitonin was measured in individual serum with a commercially available chemiluminescence immunoassay (immunoluminometric assay, ILMA, Nichols Institute Diagnostics, Nijmegen, the Netherlands). The ILMA mainly recognizes the mature monomeric form of human calcitonin (calibration to WHO 2nd IS 634 under the control of rat CGRP/calcitonin promoter (18)). The transgene contained the cDNA coding the human RET 9 isoform carrying the MEN2A-specific mutation at codon 5319 founder animal (18). The transgene was finally obtained after a s.c. passage of the CMT-125 C cell line was finally obtained after a s.c. passage of the individual calcitonin-secreting clones in cell culture. Total RNA was extracted from C cells by modified guanidine isothiocyanate phenol chloroform (Trizol); 1 µg was reverse transcribed using 1 µg oligo-dT (Amersham Pharmacia Biotech, Orsay, France).

Measurement of body temperature. Body temperature was measured every day between 9:30 and 10:00 am for 10 days, using a rectal probe equipped with a digital thermometer (model 508 BR, Bioseb, Chaville, France) in PRLR+/+ and PRLR−/− females. The median temperature was calculated for each animal and the average of median temperatures was determined for 7- to 10-month-old animals.

Thyroid dissection and weight. Thyroids with trachea were extracted from PRLR+/+ and PRLR−/− animals. Thyroid glands were microdissected with needles under a dissecting microscope to discard all contaminant tissue; glands were immediately weighed to avoid drying and to quantitate mass (Mettler, Hightstown, NJ). Thyroid glands were fixed in 4% paraformaldehyde in PBS for few hours, processed for paraffin embedding and sectioning (5 µm), or they were embedded in cryocompound Tissue-Tek and then frozen in −80°C, cooled isopentane for cryocut sections.

Histology and anticalcitonin immunohistochemistry. Histologic sections were deparaffinized, rehydrated, and processed for either histology sections by H&E staining or for immunohistochemistry. Tissue sections were pretreated with microwaves heating in citrate buffer. After washing in PBS and blocking in bovine serum albumin and goat serum, rabbit polyclonal to calcitonin (DAKO, Trappes, France) was used at 1:700 dilution overnight at 4°C. Immunostaining was visualized by using Texas red-conjugated anti-rabbit IgG. Sections were counterstained by Hoechst solution (Sigma, St. Louis, MO).

Reverse transcription-PCR of mRNA prolactin receptor. The C cell line used here was initially derived from a medullary thyroid carcinoma tumor arisen in a 19.5-month-old transgenic animal deriving from the 5319 founder animal (18). The transgene contained the cDNA coding the human RET 9 isoform carrying the MEN2A-specific mutation at codon 634 under the control of the rat CGRP/calcitonin promoter (18). The CMT-125 C cell line was finally obtained after a s.c. passage of the dissociated tumor cells in nude mouse and isolation from the dissociated nude s.c. tumor of individual calcitonin-secreting clones in cell culture.5 Total RNA was extracted from C cells by modified guanidine isothiocyanate phenol chloroform (Trizol); 1 µg was reverse transcribed using 1 µg oligo-dT (Amersham Pharmacia Biotech, Orsay, France).

5 T. Ragot et al., in preparation.
Identification of prolactin receptor in thyroid cells. The cause of the increase in serum calcium levels observed in PRLR−/− animals remains obscure, but it is well known that mineral homeostasis is under the control of hormones such as parathyroid hormone, estradiol, or calcitriol, which have been suggested to be directly or indirectly involved with prolactin metabolism (19–21). All mineral abnormalities could be explained by estrogen and progesterone deficiency or/and high level of parathyroid hormone. Such elevated levels of parathyroid hormone in PRLR−/− mice could also explain the increased calcium concentration and bone abnormalities (7). Thus, the hormonal status of PRLR−/− mice is perturbed, resulting in several potential consequences, not only on bone formation but also for metabolic status. To analyze potential parathyroid dysfunctions, we first did a study of PRLR mRNA expression in parathyroid sections of PRLR−/− sibling mice. A representative section of parathyroid included in thyroid tissue is shown in Fig. 1A. We revealed for the first time, by in situ hybridization analysis (Fig. 1C), a moderate expression of PRLR transcripts in the parathyroid glands but a very high level of expression in the thyroid gland, mostly in follicles of young mice (6-week-old females). The hybridization signal is clearly specific compared with the sense probe (Fig. 1D).

To determine whether the thyroid C cells also express PRLR mRNA, we did reverse transcription-PCR on a population of isolated C cell line derived from a transgenic mouse developing MTC (18). As shown in Fig. 1B, the C cells express an intense band representing PRLR mRNA that was also detected in the thyroid gland, mostly in follicles of young mice (6-week-old females). The hybridization signal is clearly specific compared with the sense probe (Fig. 1D).

Weight and function of thyroid glands in PRLR+/+ and PRLR−/− mice. To evaluate the possible phenotypic differences between PRLR+/+ and PRLR−/− thyroids, the weight of microdissected glands was measured at different ages (2-, 4-, and 14-month-old mice). A significant increase (P < 0.05) in PRLR−/− thyroid gland weight was observed in animals at 2, 4, and 5 months of age (Fig. 2A), although at 14 months of age, this did not quite reach statistical significance (P = 0.06). However, the regression curve corresponding to PRLR−/− thyroid weights (y = 0.3634x + 3.3735) was systematically above that of PRLR+/+ (y = 0.3881x + 2.0208) animals.

Because thyroid weight was increased in PRLR−/− mice, the presence of abnormal thyroid function was considered. Serum-free T4 (FT4) was significantly decreased compared with that in
PRLR+/+ mice (Fig. 2B). The mean FT4 concentration was 13.63 ± 2.98 and 10.78 ± 2.25 pmol/L in PRLR+/+ and PRLR−/− mice, respectively corresponding to a reduction of 21% in PRLR−/− mice. The serum TSH level is one of the most sensitive indicators of thyroid function state and is therefore useful in understanding the action of prolactin in our model mouse. TSH levels were not statistically different in PRLR+/+ and PRLR−/− animals [16.58 ± 1.99 ng/ mL (n = 12) versus 17.58 ± 1.16 ng/mL (n = 18)]. These results suggest only a moderate hypothyroid status, which led us to measure body temperature, a good index of thyroid function.

Throughout the 2-week period of recording, body temperature in PRLR−/− mice was significantly lower than in PRLR+/+ mice (Fig. 2C). Over this period, mean body temperature in PRLR−/− mice was 0.55°C lower than controls. At 7 to 10 months, it was 36.91 ± 0.18°C and 37.46 ± 0.36°C in PRLR−/− and PRLR+/+

Figure 3. Histology of longitudinal sections of thyroid gland from PRLR+/+ (A-C) and PRLR−/− (B-D) mice. At high magnification (C-D), one can note the presence of mucus-like cells (arrow), which are more abundant in PRLR−/− than in PRLR+/+ thyroid. Bar, 100 μm.

Figure 4. A, macroscopic observation of MTC in a 14-month-old PRLR−/− mouse (left). The size of the contralateral lobe (right) is normal compared with the size of PRLR−/− lobe which does not develop tumor. B, histologic section of MTC with massive invasion of one lobe. Arrows, remnants of thyroid follicles at the periphery of the tumor mass (*). C, details of MTC exhibiting numerous mitoses (arrows). D, positive calcitonin staining (red) of the section described in (B) shows definitively that the tumor is medullary thyroid carcinoma. Nuclei (blue) are detected by Hoechst staining. Bar, 1 mm (A), 100 μm (B), and 50 μm (C, D).
mice, respectively. The same two groups, analyzed 5 months later, exhibited a similar significant difference (36.94 ± 0.26°C and 37.24 ± 0.14°C in PRLR−/− and PRLR+/+ mice, respectively).

### Histologic analysis

Longitudinal sections and H&E staining of 14- to 16-month-old PRLR−/− and PRLR+/+ thyroid gland revealed that the follicular structure was regular and uniform in both genotypes (Fig. 3). At low magnification (Fig. 3A-B), the global histology was comparable between the two genotypes. At high magnification, the aspect and size of the follicles of PRLR−/− mice were not different from that observed in PRLR+/+ mice. The abundance and structure of the different organelles were normal (Fig. 3C-D). In addition, the average area of the follicular lumen was not different between PRLR−/− and PRLR+/+ mice (4,418 ± 624 μm², n = 7 and 4,603 μm² ± 791, n = 6), respectively. However, we observed much more mucus cell–like structures in thyroid gland from PRLR−/− than in PRLR+/+ mice (Fig. 3C-D).

**Appearance of medullary thyroid carcinoma in PRLR−/− mice.** We examined thyroid of older animals and were surprised to discover the presence of tumors in 5 of 17 animals around 1 year of age. Gross thyroid morphology of a large unilateral tumor is illustrated in Fig. 4A. The definitive diagnosis of MTC rests on thyroid sections, whereas all five animals presenting a tumor or an early tumor were highly positive for calcitonin immunostaining. The serum calcitonin level in these five animals was extremely high in accordance with tumor size. Two additional PRLR−/− animals (T40 and T50) exhibited >25 positive C cells on thyroid sections suggesting a hyperplasic phenotype, these cells were able to produce sufficient quantities of calcitonin to be measured in sera of these animals (Table 1). In addition, the analysis of the distribution of thyroid C cells in PRLR−/− and PRLR+/+ of 14-month-old mice by immunohistochemical calcitonin labeling was done (Fig. 5). The number of positive calcitonin cells by section was <25 of total cells in both PRLR−/− (Fig. 5A) and PRLR−/− sections (Fig. 5B). However, >25 positive C cells were observed in the 14-month-old PRLR−/− thyroid corresponding to T40 mouse evocative of C-cell hyperplasia (CCH). Taken together, these data including five bona fide tumors and two CCH lead to a tumor incidence of 41% in a total of seventeen 1-year-old PRLR−/− animals.

**Search of characterized Ret mutations.** The characterized mutations currently found in MEN2A and MEN2B corresponding to the human amino acid 634 (extracellulare cysteine-rich domain, syndrome MEN2A) and amino acid 918 (tyrosine kinase 2 intra-cellular domain, syndrome MEN2B) after alignment of mouse and human sequences were analyzed in all five CMT. A perfect homology at the nucleotide level is evident between mouse and human species (codons 633-635 and codons 917-919).

No mutation was found in any of the tumors compared with wild-type thyroid and PRLR−/− nontumoral thyroid. This result tends to exclude the known human mutations that occur at high frequency in the CMT.

### Discussion

The development of the PRLR knockout mouse model has largely contributed to a better understanding of prolactin physiology. Results of the present study reveal that PRLR−/− mice share a number of characteristics with growth hormone receptor (GHR)−/− mice (22). PRLR−/− mice have mild hypothyroidism, generally reduced body temperature, and

#### Table 1. Serum calcitonin measurement and immunohistochemical staining in PRLR+/+ and PRLR−/− mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>[Calcitonin], pg/mL</th>
<th>Anticalcitonin IHC</th>
<th>Gross morphology of thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 10</td>
<td>++/−</td>
<td>&lt;25 cells</td>
<td>Normal</td>
</tr>
<tr>
<td>n = 9</td>
<td>−/−</td>
<td>&lt;25 cells</td>
<td>Normal</td>
</tr>
<tr>
<td>T44</td>
<td>−/−</td>
<td>+++</td>
<td>Tumor (48.2 mg)</td>
</tr>
<tr>
<td>T45</td>
<td>−/−</td>
<td>+++</td>
<td>Tumor (129 mg)</td>
</tr>
<tr>
<td>T63</td>
<td>−/−</td>
<td>+++</td>
<td>Tumor (31 mg)</td>
</tr>
<tr>
<td>2076</td>
<td>−/−</td>
<td>+++</td>
<td>Tumor (ND)</td>
</tr>
<tr>
<td>2146</td>
<td>−/−</td>
<td>++</td>
<td>Beginning tumor</td>
</tr>
<tr>
<td>T40</td>
<td>−/−</td>
<td>&gt;25 cells</td>
<td>C-cell hyperplasia</td>
</tr>
<tr>
<td>T30</td>
<td>−/−</td>
<td>&gt;25 cells</td>
<td>C-cell hyperplasia</td>
</tr>
</tbody>
</table>

NOTE: First row refers to the wild type thyroid; second row refers to the PRLR−/− mice that did not present with thyroid tumors; and succeeding rows refer to PRLR−/− that presented with hyperplasic or tumor C cells. All animals were 10- to 16-month-old except sample 2146 that was 8-month-old. The zero value of calcitonin concentrations corresponds to the undetectable measurement limit. Parentheses indicate the tumor weight when it occurs. Abbreviations: IHC, immunohistochemistry; ND, not determined.
both compartments: thyroid follicles and C cells, the site of hormone synthesis. Pro lactin could be a potential negative regulator of parathyroid abnormalities have been reported in null animals (7). Thus, hormone, an increased calcium concentration, and bone interesting observation because high serum levels of parathyroid the appearance of this cancer.

In 1-year-old animals, there was a high incidence of MTC that arises from C cells of the thyroid that produce calcitonin. Hereditary MTC either as a single entity, familial MTC or as MEN syndrome (MEN2A or MEN2B) represents 20% to 30% of all MTCs. The identification of hereditary MTC has been facilitated by the direct analysis of the RET proto-oncogene. We observed 41% of MTC in PRLR−/− mice; this very high incidence was not linked to the most frequent RET gain-offunction mutations involved in the etiology of hereditary MTC and up to 30% of sporadic MTC (13, 18, 27). Even if mutation out of codons 634 and 918 are not frequent in human-inherited MTC (28), these results do not exclude the presence of other RET genetic abnormalities. These negative results might also suggest that oncogenic pathway could be independent from RET proto-oncogene. An “old” animal model for inherited C-cell carcinoma, the WAG/Rij rat strain, has a similar behavior: a high incidence of spontaneous C-cell tumors without any of the RET-activating mutations (29). This may suggest there must be additional mechanisms for the genesis and progression of the WAG/Rij rat and PRLR−/− mouse MTCs. The problem is to know if they are also relevant in humans.

In accord with the development of MTC, we have previously shown the development of prolactinomas in PRLR−/− mice by direct and indirect mechanisms. In fact, we previously found pituitary hyperplasia and adenoma formation in PRLR−/− mice (30). In the majority of tissues where it has been evaluated mutation of IFN-γ and interleukin-4 (25). Moreover, it has been suggested that hyperprolactinemia increases the release of calcitonin by thyroid cells in rats through a cyclic AMP–dependent pathway caused an indirect effect of prolactin (26).

We showed also that PRLR mRNA expression was present in both compartments: thyroid follicles and C cells, the site of calcitonin production. The apparent morphology of follicles was not altered in the absence of prolactin signaling and the C-cell compartment did not seem affected because the calcitonin immunostaining was not modified. No clear effect of prolactin on thyroid has been reported until now, although some authors described that prolactin can exert indirect effects on CD40 expression on thyocytes by antagonizing the modulatory actions of IFN-γ and interleukin-4 (25). Moreover, it has been suggested that hyperprolactinemia increases the release of calcitonin by thyroid cells in rats through a cyclic AMP–dependent pathway caused an indirect effect of prolactin (26).

Reduced insulin and glucose levels (23). This is an interesting point because both knockout mouse models are the result of the deletion of a hormone receptor belonging to the same family, class 1 cytokine receptor. Lower body temperature in PRLR−/− mice resulting, perhaps, from reduced metabolic rate would potentially lead to the production of fewer free radicals, which are known to be related to the aging process (24). Measurements of plasma T4 and TSH revealed that PRLR−/− mice are moderately hypothyroid when compared with their normal littermates with no major alterations in thyroid function. Furthermore, these data show that some similarities exist between GHR- and PRLR-deficient mice.

We show for the first time the presence of PRLR mRNA in thyroid and parathyroid tissues in wild-type mice. This was an interesting observation because high serum levels of parathyroid hormone, an increased calcium concentration, and bone abnormalities have been reported in null animals (7). Thus, prolactin could be a potential negative regulator of parathyroid hormone synthesis. We observed 41% of MTC in PRLR−/− mice; this very high incidence was not linked to the most frequent RET gain-of-function mutations involved in the etiology of hereditary MTC and up to 30% of sporadic MTC (13, 18, 27). Even if mutation out of codons 634 and 918 are not frequent in human-inherited MTC (28), these results do not exclude the presence of other RET genetic abnormalities. These negative results might also suggest that oncogenic pathway could be independent from RET proto-oncogene. An “old” animal model for inherited C-cell carcinoma, the WAG/Rij rat strain, has a similar behavior: a high incidence of spontaneous C-cell tumors without any of the RET-activating mutations (29). This may suggest there must be additional mechanisms for the genesis and progression of the WAG/Rij rat and PRLR−/− mouse MTCs. The problem is to know if they are also relevant in humans.

In accord with the development of MTC, we have previously shown the development of prolactinomas in PRLR−/− mice by direct and indirect mechanisms. In fact, we previously found pituitary hyperplasia and adenoma formation in PRLR−/− mice (30). In the majority of tissues where it has been evaluated prolactin is mitogenic (31), with only a few tissues showing an antiproliferative role (32). In PRLR−/− mice, there are two factors that contribute to the release of the lactotroph from its usual secretory and proliferative controls: a decrease in the inhibitory dopaminergic control and a second direct effect at the level of the pituitary, which is more consistent with an antiproliferative action of prolactin on lactotroph. No causes of sporadic MTC in absence of RET mutation has been reported for now. However, in one human case report, hyperparathyroidism has been associated to sporadic MTC (33). Hypercalcemia and increased parathyroid hormone level found in our mouse model could constitute a potential link to be investigated.

This new mouse model described here may therefore provide a suitable model system in which to identify and elucidate the effects of undiscovered genes in the etiology of MTC and to establish a link between the absence of prolactin signaling and the appearance of this cancer.

Acknowledgments

Received 11/2/2004; revised 2/11/2005; accepted 5/13/2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Gérard Pivert and Monique Talbot for excellent technical assistance and Dr. Gabor Szinnai for helpful discussion.
Medullary Thyroid Carcinoma Arises in the Absence of Prolactin Signaling

Cécile Kedzia, Ludovic Lacroix, Nabahet Ameur, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/18/8497

Cited articles
This article cites 32 articles, 5 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/18/8497.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/65/18/8497.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.