Case Report

Hirschsprung disease of the colon, a vaginal mass and medullary thyroid cancer – a RET oncogene driven problem

Romy Pandey[1], Tiffany Thurow[2], Robert de W Marsh[3]

[1]Departments of Internal Medicine; [2]Pathology, North Shore University Health System and University of Chicago Pritzker School of Medicine, Chicago; [3]Department of Gastrointestinal Oncology, North Shore University Health System, Evanston, Illinois, USA

ABSTRACT   This case report emphasizes the fact that all patients with Hirschsprung disease should be screened for RET Oncogene mutation as there is a well known association between Hirschsprung Disease and Multiple Endocrine Neoplasia (MEN) Type 2A. It also reminds us that Medullary Thyroid Carcinoma is known to cause elevated levels of CEA which does not originate from gastrointestinal tract.

KEY WORDS   Hirschsprung Disease, RET Oncogene, Medullary Thyroid Cancer

Case Report

A 55 year old postmenopausal woman presented with vaginal spotting which rapidly progressed to more severe bleeding.

On examination she was found to have a mass in the vaginal vault which was close to, but not attached to, the cervix. Excisional biopsy of the lesion in the vaginal wall and biopsies of the endometrium, along with cervical conization revealed adenocarcinoma in the vaginal lesion only (Figure 1). Immunostains were performed and these showed a pattern which was most compatible with intestinal differentiation (CK20 and CDX-2 positive, CK7 focally positive (less than 5% of cells), ER and PR both negative, P16 and CEA variably positive) (Figure 2). The Tissue of Origin Test®, run on micro dissected tumor tissue, showed the highest similarity score of 91.1 for a colo-rectal origin. The 14 other tissue types in the panel had similarity scores of less than or equal to 5. CT scan and MRI of the abdomen and pelvis showed several cavernous hemangiomas and

cysts in the liver but there was no evidence of any residual or metastatic disease. PET scan was also unremarkable. Additional history of Hirschprung disease (HD) as a child, which had required surgical correction (with complications of obstruction and fistula formation at age 19 which were addressed with additional surgery), was obtained. Anorectal examination was grossly unremarkable and random biopsies showed mucosa consistent with a squamous papilloma but with no evidence of malignancy. Colonoscopy was normal. Of note, her CEA level at this time was found to be elevated at 35 ng/ml (normal range 0-5 ng/ml).

Family history was significant for colorectal cancer in her mother and grandfather and endometrial and appendiceal cancer in a cousin. Her brother had also been treated for HD.

At this time she was referred to medical oncology. Physical examination, including a pelvic exam and careful exam of the thyroid, was unremarkable. A repeat PET scan now showed slightly irregular, moderately increased radionuclide accumulation in both lobes of the thyroid. Ultrasound showed a diffusely heterogeneous gland mimicking confluent nodules. TSH was normal but unstimulated serum calcitonin was elevated at 121 pg/mL (Reference Value - Basal: <8) and CEA remained abnormal but stable at 37 ng/ml. Ultrasound guided fine needle biopsy of the thyroid was consistent with medullary thyroid carcinoma (MTC). As a result, she underwent total thyroidectomy and paratracheal lymph node dissection. A 22 gram thyroid revealed a 1.5 cm yellow-tan, firm nodule in the left superior lobe and a 0.7 cm yellow-tan, firm nodule

No potential conflict of interest.
Corresponding author: Dr Robert de W Marsh. Department of Gastrointestinal Oncology, 2650 Ridge Ave, Evanston, Illinois 60201, United States. Tel: (847) 570-2112; Fax: (847) 570-2336. Email: rmarsh@northshore.org
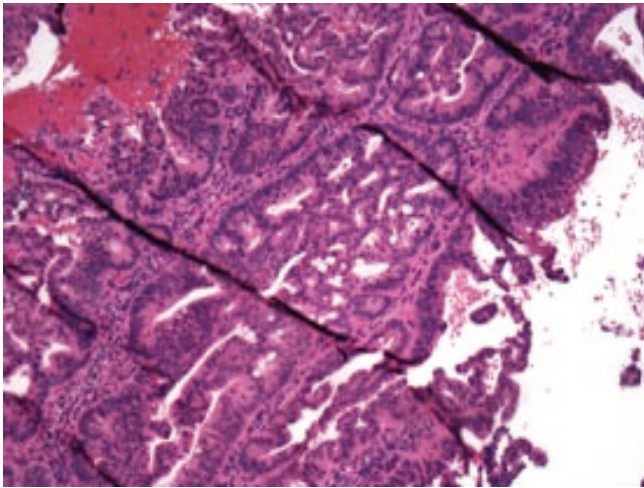
Figure 1 Section shows high power view of a well-differentiated adenocarcinoma displaying a complex glandular arrangement with some micro-papillary architecture. The cells lining the glands are columnar and have mucinous cytoplasm. There are scattered goblet cells.
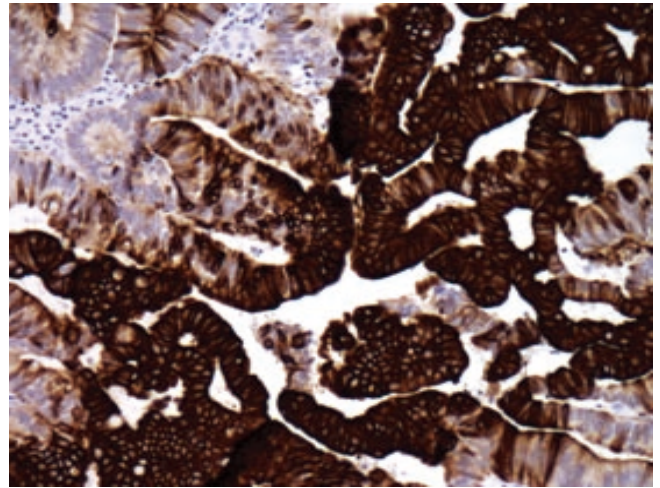

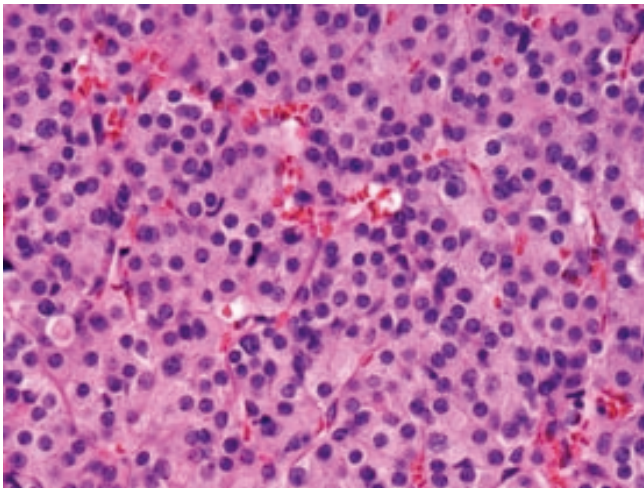**Figure 2** Positive CK20 immunohistochemical (IHC) stain.

Figure 3 Medullary carcinoma at high power with sheets and nests of monomorphic cells with abundant granular cytoplasm and uniform nuclei with stippled chromatin.
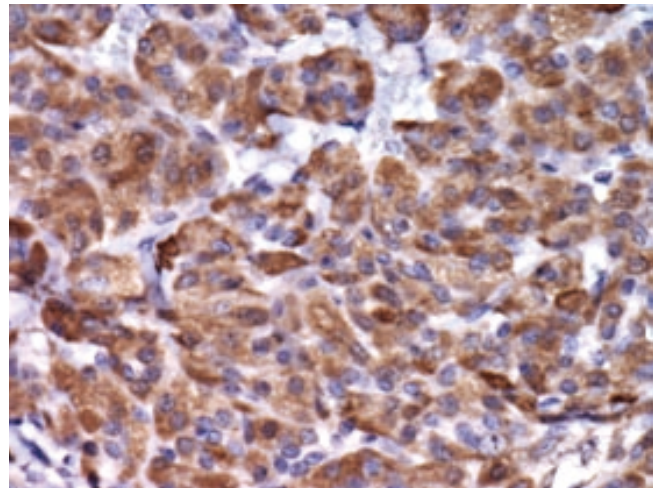

Figure 4 Positive Calcitonin IHC stain.

in the right inferior lobe. Histologic examination of each of the nodules revealed sheets and nests of monomorphic cells with abundant granular cytoplasm and uniform nuclei with stippled chromatin (Figure 3). Immunohistochemical evaluation of these cells revealed positive staining with calcitonin (Figure 4), chromogranin, and synaptophysin. Staining was negative with thyroglobulin. There was no lymph node involvement. The diagnosis of T1b N0 MTC was thus confirmed. Both CEA and calcitonin levels

normalized following surgery.

A subsequent evaluation for MEN (Multiple Endocrine Neoplasia) syndrome included a 24-hour urine collection for metanephrine and normetanephrine and both metanephrine 47 mcg/24hrs (reference range 30-180 mcg/24hrs) and normetanephrine 126 mc/24hrs (reference range 128 -484 mcg/24 hrs) were found to be normal. Despite these normal findings, a high suspicion for RET oncogene mutation persisted, given her history of MTC

256

Pandey et al. Hirschsprung disease of the colon

as well as a history of HD, with the result that genetic consultation was requested.

Following appropriate counseling, she was tested and found to be positive for a specific RET mutation, C620W, diagnostic of MEN2A. Her sister then also tested positive for the same RET mutation. This particular mutation is known to be associated with familial HD, but in contrast to other RET gene mutations, is less strongly correlated with parathyroid and adrenal disease.

She has continued to have physical examination, blood tests and serial imaging in follow up, and thus far there has been no evidence of recurrent or new disease. The origin of the adenocarcinoma in the vaginal vault is still unclear. Given the definitive diagnosis of medullary thyroid carcinoma, immunohistochemical staining for calcitonin was performed on the tumor cells and was negative. Therefore, a diagnosis of adenocarcinoma of unknown origin remains and any relationship to the MEN syndrome or the RET germline mutation is undefined. Continued surveillance for a possible primary site continues.

Discussion

Germline mutation of the RET (REarranged during Transfection) proto-oncogene (10q 11.2) may result in constitutively activated RET protein. RET protein consists of an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain, which contains two tyrosine kinase subdomains (TK1 and TK2) that are involved in the activation of several intracellular signal transduction pathways. There is a correlation between specific mutations and specific disease phenotypes (1). Mutations in RET exons 10 (codons 609, 611, 618, and 620) or 11 (codons 630 or 634), are seen in the majority of MEN2A and FMTC (Familial medullary thyroid cancer) cases resulting in alterations in the cysteine-rich region of the RET protein's extracellular domain. A mutation in codon 634 in exon 11 is the most common genetic defect in this disorder and is strongly associated with hyperparathyroidism and pheochromocytoma (PC) in MEN2A. Mutations in codons 768 (exon 13), 804 (exon 14) and 891 (exon 15), which result in changes in the intracellular tyrosine kinase domains, are found only in FMTC (2). In MEN 2B patients, the mutation involves codon 918 in exon 16 in 95% of cases and, rarely, codon 883 in exon 15 with resultant change in either methionine or alanine, respectively, in the tyrosine kinase domain of RET (3).

Germaine to our patient and her family, in the rare cases where MEN 2A and HD co-exist, germline RET mutations most often involve exon 10 (1,4), especially codon 618 or 620 (1,5). This association poses a scientific dilemma, as the mutations in MEN are gain of function mutations with RET acting as a dominantly acting oncogene (6,7) and those of HD result in loss of function (8,9). However, a unifying hypothesis has been suggested in that mutations in exon 10 result in a relatively weaker activation of the RET protein kinase, perhaps just sufficient to cause MTC. A concurrent decrease in the total number of receptor molecules on the cell surface possibly results in insufficient numbers of receptors for normal gangliogenesis and migration and/or for the prevention of inappropriate apoptosis, with HD as a result (10,11).

This case teaches us a number of important lessons. Firstly, that all patients with a history of HD should consider screening for RET mutations (it should be noted that RET mutations are the predominant but only one of a number of possible causes of HD) (12,13), as there is a well established association between HD and MEN2A. If present, this could facilitate early diagnosis of MEN2A with resultant thyroidectomy prior to the onset of MTC or at least prior to the development of metastatic disease. Equally, it is desirable that all patients with MTC should be tested for germline RET mutations in accordance with 2009 American Thyroid Association Guidelines for Management of MTC (14). While somatic mutation of RET gene are limited to C cells with no additional risk of neoplasia in other tissues (approximately 50% of patients with sporadic MTC have somatic RET mutations), germline mutations affect all the tissues derived from neural crest such as neural cells, neuroendocrine cells and urogenital cells, causing MEN syndromes and rarely HD. Secondly, it reminds us that MTC is a potential cause of elevated CEA which does not have its origins in bowel cancer. Unlike calcitonin levels, which are susceptible to stimulation and hence tend to fluctuate on serial measurements, CEA levels are more stable and can be used as a tumor marker for MTC. Elevated CEA levels have been associated with increased tumor aggressiveness, tumor recurrence, and poor prognosis. Thirdly, it illustrates the value of a thorough genetic evaluation in all patients suspected of having a genetic component to their disease. This could have profound implications not only for the index patient but also for family members. Finally, it reconfirms the value of a good history and physical examination, and the therapeutic challenges presented by cancer of unknown origin, even with the sophisticated genome based diagnostics available today (15).

References

1. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 1996;276:1575-9.

2. Margraf RL, Crockett DK, Krautscheid PM, Seamons R, Calderon FR, Wittwer CT, et al. Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. Hum Mutat 2009;30:548-56.

3. Gimm O, Marsh DJ, Andrew SD, Frilling A, Dahia PL, Mulligan LM, et al. Germline dinucleotide mutation in codon 883 of the RET proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. J Clin Endocrinol Metab 1997;82:3902-4.

4. Decker RA, Peacock ML, Watson P. Hirschsprung disease in MEN 2A: increased spectrum of RET exon 10 genotypes and strong genotype-phenotype correlation. Hum Mol Genet 1998;7:129-34.

5. Caron P, Attié T, David D, Amiel J, Brousset F, Roger P, et al. C618R mutation in exon 10 of the RET proto-oncogene in a kindred with multiple endocrine neoplasia type 2A and Hirschsprung's disease. J Clin Endocrinol Metab 1996;81:2731-3.

6. Asai N, Iwashita T, Matsuyama M, Takahashi M. Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations. Mol Cell Biol 1995;15:1613-9.

7. Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, et al. Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 1995;267:381-3.

8. Pasini B, Borrello MG, Greco A, Bongarzone I, Luo Y, Mondellini P, et al. Loss of function effect of RET mutations causing Hirschsprung disease. Nat Genet 1995;10:35-40.

9. Pelet A, Geneste O, Edery P, Pasini A, Chappuis S, Atti T, et al. Various mechanisms cause RET-mediated signaling defects in Hirschsprung's disease. J Clin Invest 1998;101:1415-23.

10. Eng C. RET proto-oncogene in the development of human cancer. J Clin Oncol 1999;17:380-93.

11. Arighi E, Popsueva A, Degl'Innocenti D, Borrello MG, Carniti C, Perälä NM, et al. Biological effects of the dual phenotypic Janus mutation of ret cosegregating with both multiple endocrine neoplasia type 2 and Hirschsprung's disease. Mol Endocrinol 2004;18:1004-17.

12. Parisi MA, Kapur RP. Genetics of Hirschsprung disease. Curr Opin Pediatr 2000;12:610-7.

13. Martucciello G, Ceccherini I, Lerone M, Jasonni V. Pathogenesis of Hirschsprung's disease. J Pediatr Surg 2000;35:1017-25.

14. American Thyroid Association Guidelines Task Force, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 2009;19:565-612.

15. Pillai R, Deeter R, Rigl CT, Nystrom JS, Miller MH, Buturovic L, et al. Validation and reproducibility of a microarray-based gene expression test for tumor identification in formalin-fixed, paraffin-embedded specimens. J Mol Diagn 2011;13:48-56.