Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas

MM Moura*,1, BM Cavaco1, AE Pinto2, R Domingues1, JR Santos3, MO Cid3, MJ Bugalho1,4,5 and V Leite1,4,5

1Centro de Investigação de Patobiologia Molecular (CIPM), Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E., Rua Prof. Lima Basto, 1099-023 Lisboa, Portugal; 2Serviço de Anatomia Patológica, Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E., Rua Prof. Lima Basto, 1099-023 Lisboa, Portugal; 3Serviço de Cirurgia Cabeça e Pescoço, Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E., Rua Prof. Lima Basto, 1099-023 Lisboa, Portugal; 4Serviço de Endocrinologia, Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E., Rua Prof. Lima Basto, 1099-023 Lisboa, Portugal; 5Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Campo Mártires da Pátria 130, 1150-228 Lisboa, Portugal

Screening of RET rearranged during Transfection (RET) gene mutations has been carried out in different series of sporadic medullary thyroid carcinomas (MTC). RET-positive tumours seem to be associated to a worse clinical outcome. However, the correlation between the type of RET mutation and the patients’ clinicopathological data has not been evaluated yet.

We analysed RET exons 5, 8, 10–16 in fifty-one sporadic MTC, and found somatic mutations in thirty-three (64.7%) tumours. Among the RET-positive cases, exon 16 was the most frequently affected (60.6%). Two novel somatic mutations (Cys630Gly, c.1881del18) were identified. MTC patients were divided into three groups: group 1, with mutations in RET exons 15 and 16; group 2, with other RET mutations; group 3, having no RET mutations. Group 1 had higher prevalence (P = 0.0051) and number of lymph node metastases (P = 0.0017), and presented more often multifocal tumours (P = 0.037) and persistent disease at last control (P = 0.0242) than group 2. Detectable serum calcitonin levels at last screening (P = 0.0119) and stage IV disease (P = 0.0145) were more frequent in group 1, than in the other groups.

Our results suggest that, among the sporadic MTC, cases with RET mutations in exons 15 and 16 are associated with the worst prognosis. Cases with other RET mutations have the most indolent course, and those with no RET mutations have an intermediate risk.

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MDCTyrmsion culcwhm oocy aticpcn trefor od a casysre of thyesy and an acounts fo a disportionate number of thyroid cancer deaths (Hundahl et al, 1998). Except for surgery, therapy for MTC is generally ineffective. MTC may occur sporadically (in about 75% of cases) as a part of the autosomal dominantly inherited cancer syndrome, known as multiple endocrine neoplasia type 2 (MEN 2) (Mulligan et al, 1993; Eng, 1999; Frank-Raue et al, 2007). MTC is the most common cause of death in patients with MEN 2 (Skinner et al, 2005). This familial type of thyroid carcinoma usually originates as multifocal C-cell hyperplasia, its progression to MTC is extremely variable, and may take several years (Carling, 2005). In sporadic cases, the mean age at presentation is 50 years, with a slight female predominance (Matias-Guiu et al, 2004).

Activating germline mutations in the RETarranged during Transfection (RET) gene are detected in over 95% of MEN 2 cases (Mulligan et al, 1993; Marx, 2005). The oncogenic potential of different RET mutations seems to be dependent on the site of the amino acid change, and may account for the diverse phenotypes observed in MEN 2 patients (Asai et al, 1995).

The screening of RET mutations has been carried out in different series of sporadic MTC, however the observed frequencies are variable (12–100%) (Hofstra et al, 1994; Zedenius et al, 1999; Jhiang et al, 1996, 2003; Romei et al, 1996; Wohllk et al, 1996; Bugalho et al, 1997; Scurini et al, 1998; Shan et al, 1998; Uchino et al, 1998, 1999; Bockhorn et al, 1999; Dvorakova et al, 2008; Elisei et al, 2008). Met918Thr RET mutation is the most common somatic mutation in sporadic forms of MTC, and its detection rate varies greatly (5–66%) in the published literature (Zedenius et al, 1994; Marsh et al, 1996; Romei et al, 1996; Wohllk et al, 1996; Bugalho et al, 1997; Scurini et al, 1998; Uchino et al, 1998, 1999; Dvorakova et al, 2008; Elisei et al, 2008). However, in some of these studies, the authors have screened sporadic MTC for only a few specific mutations, mostly in codon 918 (Hofstra et al, 1994; Shan et al, 1998; Bockhorn et al, 1999; Marsh et al, 2003). Therefore, the number of exons screened, as well as the sizes of the analysed series, may explain some of the reported differences in the prevalence of RET mutations in sporadic MTC. In addition, ethnic or environmental factors, differences in detection or in sampling methods may also account for the reported differences (Uchino et al, 1998; Dvorakova et al, 2008). In some cohorts, besides the Met918Thr mutation, other
somatic mutations were also detected, at a lower frequency in exons 10, 11, 12, 13 and 15 (Bugalho et al., 1997; Scurini et al., 1998; Uchino et al., 1999).

The major somatic mutation (Met918Thr) localised in the tyrosine kinase domain in exon 16 of RET (Marini et al., 2006) has been associated to a worse clinical outcome in sporadic MTC when compared with tumours that did not harbour this mutation (Zedenius et al., 1994, 1995; Wohlk et al., 1996; Schilling et al., 2001).

Several reports have presented contradictory results concerning the ploidy pattern in MTC. Schröder et al. (1988) found that most MTC have a diploid DNA pattern, and that a benign disease course was twice as frequent in patients with diploid tumours compared with aneuploid tumours. Conversely, the results presented by Lindsay (1970) seemed to be more consistent with aneuploidy in MTC.

In this study, we carried out a comprehensive analysis of exons 5, 8 and 10–16 of RET to evaluate the prevalence of somatic mutations in a series of fifty-one sporadic MTC and to correlate with clinicopathological characteristics of the patients, including tumour ploidy pattern.

MATERIALS AND METHODS

Patients

A total of fifty-two unrelated patients with MTC without family history of the disease were studied for RET mutations. A detailed personal history was obtained from all patients. All individuals were of Caucasian origin (34 females and 18 males). Each patient underwent total thyroidectomy, with the exception of two patients who were submitted to partial thyroidectomy. The diagnosis of MTC was confirmed by histopathology of the surgically removed tumours. The Tumour-Node-Metastases (TNM) classification of all tumours was carried out after the criteria described in the WHO classification of thyroid tumours (DeLellis et al., 2004). Stage grouping was addressed according to the TNM classification (Sobin and Wittekind, 2002), namely, stage I (T1N0M0), stage II (T2N0M0), stage III (T3N0M0 or T1–T3N1aM0) and stage IV (T1–T4N1bM0, T4N0–N1M0 or T1–T4N0–N1M1).

The number of truly sporadic MTC patients was reduced to fifty-one (Table 1, patients 10, 15, 34, 35, 36, 47) following standard protocols. Otherwise, DNA was isolated from formalin-fixed paraffin-embedded tumour tissues (n = 5), as described earlier (Imyanitov et al., 2001). Exons 5, 8 and 10 through 16 of RET were amplified by PCR. Sequences of the oligonucleotide primers and amplification conditions are available on request. Sequencing was carried out in both sense and antisense directions, using the same primers as for PCR amplification and the ABI PRISM BigDye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), in an automated DNA sequencer (ABI PRISM 310 Genetic Analyser, Applied Biosystems). All the mutations identified were confirmed by two independent experiments (restriction enzyme analysis, or repeated sequence analysis). To support somatic origin of the mutations, constitutional DNA from peripheral blood or non-tumourous tissue from the same patient was also analysed.

Statistical analysis

The statistical analysis was accomplished using GraphPad Prism version 4.0 statistical software (GraphPad Software Inc., San Diego, CA, USA) and SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Values were expressed as mean ± s.e.m. The χ² or Fisher’s exact tests, and one-way analysis of variance or Kruskal–Wallis test were used according to the studied variables. Survival curves were analysed using the Kaplan–Meier method, and the statistical significance was assessed by the logrank test. Values of P < 0.05 were considered statistically significant.

RESULTS

Genetic analysis

One out of the fifty-two (1.9%) cases of clinically apparently sporadic MTC carried a new germline mutation (Cys515Trp) located in exon 8 (manuscript under preparation). This case was excluded from further analysis.

In the remaining fifty-one sporadic MTC cases, thirty-three (64.7%) had mutations in RET exons 10, 11, 15 and 16 (Table 2). The absence of these mutations in the constitutional DNA excluded a germline origin.

Among the RET-positive cases, exon 16 was the most frequently affected (60.6%) by the same specific Met918Thr mutation, followed by exon 11 (21.2%). RET mutations were also detected in exons 10 (9.1%) and 15 (9.1%). In the present series, two novel RET mutations (Cys630Gly and c.1881del18) located at exon 11 were identified. The novel Cys630Gly variant creates a restriction site for the enzyme BsrI, facilitating its independent confirmation (data not shown). The other novel variant (c.1881del18) is expected to lead to the replacement of seven amino acid residues by a glutamic acid residue. In this case, PCR amplification originated two fragments: the expected wild-type product (322 bp) and a smaller mutant product (304 bp), allowing the independent sequencing of both alleles (data not shown).

In the MTC tissue of patient 51, with a Cys618Arg mutation (Table 1), the wild-type allele was not detected. The finding of allelic loss at flanking markers D10S141 and ZNF22 showed hemizygosity for this mutation (data not shown). No mutations were identified in the other analysed exons, namely, exons 5, 8, 12, 13 or 14 (Table 2).

Fourty-four (86.3%) tumours displayed a diploid DNA content and seven (13.7%) were aneuploid. No correlation between the presence or type of RET mutation and the ploidy pattern was observed (Table 3).
Clinical evaluation

Table 1 describes the clinical and pathological data of the fifty-one patients with sporadic MTC.

In the thirty-three MTC patients (19 females and 14 males) carrying somatic RET mutations, the mean age at surgery and mean follow-up time were 52.9 years (median 55, range 26 – 71) and 90.8 months (median 100.2, range 4 – 303), respectively. Lymph node and distant metastases were present in 11/18 (61.1%) and 3/18 (16.7%) cases, respectively. According to the TNM classification, four patients (12.1%) had stage I disease, three (9.1%) had stage II, two (6.1%) had stage III and twenty-four (72.7%) had stage IV. At the time of the last clinical screening, nine patients (29.0%) were free of disease, and twenty-two (71.0%) were non-disease free (seven of them were deceased from MTC). The status at last control from two patients was not available. Among the twenty-two patients with persistent disease, ten (47.6%) showed a biochemical persistence of the disease with detectable levels of serum CT, but no evidence of distant metastases, whereas eleven patients (52.4%) were affected by metastatic disease. Clinical data from one case were not available.

As regard to the eighteen MTC patients (14 females and 4 males) without somatic RET mutation, the mean age at surgery and mean follow-up were 55.8 years (median 59.5, range 27 – 82) and 90.8 months (median 100.2, range 4 – 303), respectively. Lymph node and distant metastases were present in 11/18 (61.1%) and 9/18 (50%) cases, respectively. According to the TNM classification, two patients (11.1%) had stage I disease, nine (50.0%) had stage II, four (22.2%) had stage III and three (16.7%) had stage IV. At the time of the last clinical screening, seven patients (38.9%) were free of disease, and eleven (61.1%) were non-disease free (seven of them were deceased from MTC). The status at last control from two patients was not available.

Clinical data from one case were not available.

Abbreviations: A = aneuploid; CT = calcitonin; D = diploid; Dec = deceased from MTC; DF = disease free; Extragl. ext. = extraglandular extension; F = female; LN = lymph node; M = male; MTC = medullary thyroid carcinoma; Multif. = multifocality; NA = not available; NDF = non-disease free; Neg = negative for RET mutations; No. = number; Post-op. = post-operative; RET = RET rearrangement during transfection; TN = thyroid nodule; TNM = Tumour-Node-Metastases; Undetect. = undetectable; Vasc. inv. = vascular invasion; *(the biggest); ¼Partial thyroidectomy.
There was no statistical significant difference between patients of the different groups regarding the remaining clinicopathological characteristics (Table 3). Furthermore, when the survival curves of MTC patients from the three groups were evaluated, no significant differences were observed between each group (data not shown).

**DISCUSSION**

Medullary thyroid carcinoma is clinically diagnosed as sporadic when the patient does not present other endocrine tumours, and when no other cases of MTC, pheochromocytoma or parathyroid disease are identified in the patient’s family. However, only the exclusion of germline mutations in the RET proto-oncogene allows a definitive diagnosis of sporadic MTC.

The herein reported cohort is one of the largest single-country studies. Fifty-one sporadic MTC were analysed and somatic mutations were found in thirty-three (64.7%) cases. Two novel mutations were identified in exon 11 of the RET proto-oncogene, in two sporadic MTC cases: a heterozygous point mutation at codon 630 (Cys630Gly), and a 18 bp deletion at nucleotide c.1881 associated in the same allele with a silent nucleotide substitution at codon 634 (Cys634Cys). Both mutations are located in the cysteine-rich domain coding sequence, which, when mutated, has been shown to constitutively activate RET (Santoro et al, 1995, 2002).

In this study, eight earlier described missense changes in RET were detected in exons 10, 11, 15 and 16. In accordance with other studies, the most common mutation was Met918Thr with a detection rate of 39.2%, which represents 60.6% of all detected mutations. In one case, loss of heterozygosity at RET caused by a deletion of a 2.5 kb segment at nucleotide 1881 associated in the same allele with a silent nucleotide substitution at codon 634 (Cys634Cys). Both mutations are located in the cysteine-rich domain coding sequence, which, when mutated, has been shown to constitutively activate RET (Santoro et al, 1995, 2002).

As RET mutations other than Met918Thr are rare, most of the reported series did not compare the clinicopathological characteristics of Met918Thr vs other RET mutations (Dvorakova et al, 2008; Elisei et al, 2008). In our study, 13/51 (25.5%) cases had a RET mutation other than Met918Thr, which allowed such comparison. On the basis of the recent literature, RET mutations have been stratified into three risk levels, regarding the predisposition to originate MTC, as well as their in vitro transforming activity. Patients with germline mutations in RET codons 883 (exon 15) and 918 (exon 16), for which thyroidectomy is recommended at an early age, have the highest risk for the early development and the most aggressive MTC growth (Evans et al, 2007). Likewise, these two mutations (which are considered as level 3) have the highest in vitro transforming activity. Therefore, in this study, cases with Ala883Phe or Met918Thr mutations were analysed in the same group (group 1), and compared with cases with other somatic RET mutations (group 2) and cases with no RET mutation (group 3).

A statistically significant correlation was shown between group 1 and the presence of lymph node metastases, as well as the number of positive lymph nodes at the time of surgery, multifocality and a non-disease free status, compared with group 2. This correlation may account for the significantly higher frequency of patients from group 1 in stage IV and with detectable serum CT at last control, compared with groups 2 and 3, and also for the significantly increased levels of serum CT at the last control in group 1 cases in comparison with group 2. Furthermore, such correlation supports the hypothesis that mutations in RET exons 15 and 16 are related to a more aggressive behaviour of MTC. This could be explained by an earlier dissemination of Met918Thr and Ala883Phe cases to lymph nodes (Table 3). Indeed, 26.1% of MTC cases came to clinical attention because of lymph nodes in group 1, whereas this

**Table 2 RET mutations identified in sporadic MTC cases**

| Exon | Type of alteration | Number of patients | % of patients carrying a RET somatic mutation |
|------|-------------------|--------------------|---------------------------------------------|
| 10   | TGC>CGC (Cys618Arg)* | 1                  | 9.1%                                        |
|      | TGC>CGC (Cys620Arg) | 1                  |                                             |
|      | TGC>TCC (Cys620Ser) | 1                  |                                             |
| 11   | TGC>CGC (Cys630Arg) | 2                  | 21.2%                                       |
|      | TGC>GGC (Cys630Gly) | 1                  |                                             |
|      | TGC>CGC (Cys634Arg) | 2                  |                                             |
|      | TGC>TAC (Cys634Tyr) | 1                  |                                             |
|      | c.1881del18+       | 1                  |                                             |
|      | TGC>TGT (Cys634Cys) | 1                  |                                             |
| 15   | GCT>TTT (Ala883Phe)+ | 1                  | 9.1%                                        |
|      | GTA>GTG (Val882Val) | 1                  |                                             |
|      | GCT>TTT (Ala883Phe) | 2                  |                                             |
| 16   | ATG>ACG (Met918Thr) | 20                 | 60.6%                                       |

Abbreviations: MTC = medullary thyroid carcinoma; RET = RET rearranged during Transfection. *This mutation was in the hemizygous status. Mutations were present only in tumour DNA. The two novel RET proto-oncogene variants are represented in bold.
| Characteristics                  | Group 1 Met918Thr and Ala883Phe RET mutation (n = 23, 45.1%) | Group 2 Other RET mutation (n = 10, 19.6%) | Group 3 No RET mutation (n = 18, 35.3%) | P-value |
|---------------------------------|------------------------------------------------------------|--------------------------------------------|----------------------------------------|---------|
| **Sex**                         |                                                            |                                            |                                        |         |
| Female                          | 52.2% (1/23)                                               | 70.0% (7/10)                               | 77.8% (14/18)                          | 0.2175<sup>a</sup> |
| Male                            | 47.8% (1/11)                                               | 30.0% (3/10)                               | 22.2% (4/18)                           |         |
| **MTC presentation**            |                                                            |                                            |                                        |         |
| Thyroid nodule                  | 39.1% (9/23)                                               | 90.0% (9/10)                               | 64.7% (11/17)                          | 0.099<sup>b</sup> |
| Lymph node                      | 26.1% (6/23)                                               | 0.0% (0/10)                                | 11.8% (2/17)                           |         |
| Thyroid nodule and lymph node   | 34.8% (8/23)                                               | 100.0% (1/1)                               | 23.5% (4/17)                           |         |
| **Age at surgery (years), mean ± s.e.m.** | 50.00 ± 2.80                                        | 59.60 ± 4.19                               | 55.78 ± 3.85                           | 0.1835<sup>c</sup> |
| **Tumour size (cm), mean ± s.e.m.** | 3.59 ± 0.42                                      | 3.77 ± 0.84                                | 4.68 ± 0.81                            | 0.4240<sup>c</sup> |
| **Post-operative serum calcitonin** |                                               |                                            |                                        |         |
| Undetectable                    | 9.5% (2/21)                                               | 30.0% (3/10)                               | 37.5% (6/16)                           | 0.132<sup>b</sup> |
| Detectable                      | 90.5% (19/21)                                             | 70.0% (7/10)                               | 62.5% (10/16)                          |         |
| **Serum calcitonin at last control** |                                               |                                            |                                        | 0.0119<sup>a</sup> |
| Undetectable                    | 14.3% (3/21)                                              | 66.7% (6/9)                                | 47.1% (8/17)                           |         |
| Detectable                      | 85.7% (18/21)                                             | 33.3% (3/9)                                | 52.9% (9/17)                           |         |
| **T categories**                |                                                            |                                            |                                        |         |
| T1                              | 13.0% (3/23)                                              | 40.0% (4/10)                               | 17.6% (3/17)                           | 0.664<sup>b</sup> |
| T2                              | 39.1% (9/23)                                              | 200.0% (2/10)                              | 29.4% (5/17)                           |         |
| T3                              | 13.0% (3/23)                                              | 200.0% (2/10)                              | 23.5% (4/17)                           |         |
| T4                              | 34.8% (8/23)                                              | 200.0% (2/10)                              | 29.4% (5/17)                           |         |
| **T categories grouping**       |                                                            |                                            |                                        | 0.694<sup>a</sup> |
| T1–T3                           | 65.2% (15/23)                                             | 80.0% (8/10)                               | 70.6% (12/17)                          |         |
| T4                              | 34.8% (8/23)                                              | 200.0% (2/10)                              | 29.4% (5/17)                           |         |
| **Lymph node metastases**       |                                                            |                                            |                                        | 0.0051<sup>a</sup> |
| N1                              | 87.0% (20/23)                                             | 30.0% (3/10)                               | 61.1% (11/18)                          |         |
| N0                              | 13.0% (3/23)                                              | 70.0% (7/10)                               | 38.9% (7/18)                           |         |
| **Distant metastases**          |                                                            |                                            |                                        | 0.0588<sup>a</sup> |
| M1                              | 45.5% (10/22)                                             | 11.1% (1/9)                                | 16.7% (3/18)                           |         |
| M0                              | 54.5% (12/22)                                             | 88.9% (8/9)                                | 83.3% (15/18)                          |         |
| **Presence of extraglandular extension** | 36.4% (8/22)                                   | 200.0% (2/10)                              | 35.3% (6/17)                           | 0.631<sup>c</sup> |
| **Presence of vascular invasion** | 52.9% (9/17)                                     | 33.3% (2/6)                                | 50.0% (7/14)                           | 0.823<sup>b</sup> |
| **Presence of multifocality**   | 40.9% (9/22)                                              | 0.0% (0/9)                                 | 17.6% (3/17)                           | 0.037<sup>b</sup> |
| **Ploidy pattern**              |                                                            |                                            |                                        |         |
| Diploid                         | 87.0% (20/23)                                             | 80.0% (8/10)                               | 88.9% (16/18)                          | 0.758<sup>b</sup> |
| Aneuploid                        | 13.0% (3/23)                                              | 20.0% (2/10)                               | 11.1% (2/18)                           |         |
| **Stage**                       |                                                            |                                            |                                        | 0.065<sup>b</sup> |
| I                               | 4.3% (1/23)                                               | 30.0% (3/10)                               | 16.7% (3/18)                           |         |
| II                              | 4.3% (1/23)                                               | 200.0% (2/10)                              | 22.2% (4/18)                           |         |
| III                             | 4.3% (1/23)                                               | 100.0% (1/10)                              | 5.6% (1/18)                            |         |
| IV                              | 87.0% (20/23)                                             | 40.0% (4/10)                               | 55.6% (10/18)                          |         |
| **Stage grouping**              |                                                            |                                            |                                        | 0.0145<sup>a</sup> |
| I–III                           | 13.0% (3/23)                                              | 60.0% (6/10)                               | 44.4% (8/18)                           |         |
| IV                              | 87.0% (20/23)                                             | 40.0% (4/10)                               | 55.6% (10/18)                          |         |
| **Number of positive lymph nodes, mean ± s.e.m.** | 11.48 ± 2.09                                         | 1.20 ± 0.63                                | 5.24 ± 1.64                           | 0.0017<sup>d</sup> |
| **Follow-up (months), mean ± s.e.m.** | 91.8 ± 15.1                                      | 101.3 ± 16.9                               | 90.8 ± 17.6                           | 0.9180<sup>d</sup> |
| **Status at last control**      |                                                            |                                            |                                        | 0.0242<sup>a</sup> |
| Disease free                    | 14.3% (3/21)                                              | 60.0% (6/10)                               | 44.4% (8/18)                           |         |
| Non-disease free                | 85.7% (18/21)                                             | 40.0% (4/10)                               | 55.6% (10/18)                          |         |

Abbreviations: MTC = medullary thyroid carcinomas; RET = REarranged during Transfection. *2-test, bFisher’s exact test, cOne-way analysis of variance and dKruskal–Wallis test. P-values in italics and bold are statistically significant.
occurred in only 0.0% and 11.8% of the cases in groups 2 and 3, respectively.

The results presented in Table 3 show a trend towards the stratification of the three groups of sporadic MTC patients into risk levels on the basis of the statistically significant clinico-pathological characteristics. Group 1 patients are at the highest risk for aggressive MTC, followed by group 3 at intermediate risk, and group 2 patients, which present the lowest risk for a worse clinical outcome. Therefore, our study shows that RET mutations in exons 15 and 16 are associated with a more aggressive behaviour of sporadic MTC than other RET mutations, as it has been shown in vitro, as well as in the hereditary variants of MTC.

Taken together, these results suggest that the screening of RET somatic mutations may be helpful in the management of patients with MTC, according to the presence and type of RET somatic mutation.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

André S, Pinto AE, Laranjeira C, Quaresma M, Soares J (2007) Male and female breast cancer differences in DNA ploidy, p21 and p53 expression reinforce the possibility of distinct pathways of oncogenesis. Pathobiology 74: 325–327

Asai N, Iwashita T, Matsuyma M, Takahashi M (1995) Mechanism of activation of the ret proto-oncogene by multiple endocrine neoplasia 2A mutations. Mol Cell Biol 15: 1613–1619

Bockhorn M, Frilling A, Kalinin V, Schröder S, Broelsch CE (1999) No correlation between RET immunostaining and the codon 918 mutation in sporadic medullary thyroid carcinoma. Langenbecks Arch Surg 386: 60–64

Bugalho MJ, Coelho I, Sobrinho LG (2000) Somatic trinucleotide change encompassing codons 882 and 883 of the RET proto-oncogene in a patient with sporadic medullary thyroid carcinoma. Eur J Endocrinol 142: 573–575

Bugalho MJ, Frade JP, Santos JR, Limbert E, Sobrinho L (1997) Molecular analysis of the RET proto-oncogene in patients with sporadic medullary thyroid carcinoma: a novel point mutation in the extracellular cysteine-rich domain. Eur J Endocrinol 136: 423–426

Carling T (2005) Multiple endocrine neoplasia syndrome: genetic basis for clinical management. Curr Opin Oncol 17: 7–12

de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM (2006) RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. Endocr Rev 27: 535–560

Del·lès RA, Lloyd RV, Heitz PU, Eng C (2004) World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs. Vol. 8, IARC Press: Lyon, p 50

Dvoráková S, Vláclavíková E, Sýkorová V, Dusková J, Vlcek P, Ryska A, Novák Z, Bendlova B (2006) New multiple somatic mutations in the RET proto-oncogene associated with a sporadic medullary thyroid carcinoma. Thyroid 16: 314–316.

Dvoráková S, Vláclavíková E, Sýkorová V, Vlcek J, Novak Z, Dusková J, Ryska A, Laco J, Cap J, Kodetova D, Kodet R, Krskova L, Vlcek P, Astl J, Vesely D, Bendlova B (2008) Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinoma. Mol Cell Endocrinol 284: 21–27

Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, Agate L, Vivaldi M, A, Fusco A, Vecchio G, Matoskova B, Kraus MH, Di Fiore PP (1995) Prognostic significance of somatic RET oncoproteins mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. J Clin Endocrinol Metab 93: 682–687

Eng C (1999) RET proto-oncogene in the development of human cancer. J Clin Oncol 17: 380–393

Evans DB, Shapiro SE, Cote GJ (2007) Invited commentary: medullary thyroid cancer: the importance of RET testing. Surgery 141: 96–99

Frank-Raue K, Horbach R, Mole JF, Galg G, Alares-Sauvad J, Bussolati G, Kaserer K, Williams ED, Balloch Z (2004) Medullary thyroid carcinoma. In: Del·lès RA, Lloyd RV, Heitz PU, Eng C (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs. Vol. 8, IARC Press: Lyon, pp 86–91

Hundahl SA, Fleming ID, Fremgen AM, Menc H (1998) A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995. Cancer 83: 2638–2648

Imanuyten EV, Grigoriev MY, Gorodinskaya VM, Kuligina ES, Pozharisskiy RM, Togo AV, Hansen KP (2001) Partial restoration of degraded DNA from archival paraffin-embedded tissues. Biotechniques 31: 1000, 1002

Jiang SM, Fithian L, Weghorst CM, Clark OH, Falk JM, O’Dorisio TM, Mafzaferri EL (1996) RET mutation screening in MEN2 patients and discovery of a novel mutation in a sporadic medullary thyroid carcinoma. Thyroid 6: 115–121

Jindrichova S, Kodet R, Krskova L, Vlcek P, Bendlova B (2003) The newly detected mutations in the RET proto-oncogene in exon 16 as a cause of sporadic medullary thyroid carcinoma. J Mol Med 81: 819–823

Lindsay S (1970) Microspectrophotometric measurements of deoxyribo-nucleic acid in human thyroid carcinomas. Surg Gynecol Obstet 131: 905–913

Marini F, Falcetti A, Del Monte F, Carbonell Sala S, Tognarini I, Luzi E, Brandi ML (2006) Multiple endocrine neoplasia type 2. Orphanet J Rare Dis 1: 45

Marsh DJ, Learoyd DL, Andrew SD, Krishnan L, Pojer R, Richardson AL, Delbridge L, Eng C, Robinson BG (1996) Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinoma. Clin Endocrinol (Oxf) 44: 249–257

Marsh DJ, Theodosopoulos G, Martin-Shulte K, Richardson AL, Philips J, Röther HD, Delbridge L, Robinson BG (2003) Genome-wide copy number imbalances identified in familial and sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab 88: 1866–1872

Marx SJ (2005) Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer 5: 367–375

Matias-Guiu X, DeLellis RA, Mole JF, Gagel RF, Albares-Sauveda J, Bussolati G, Kaserer K, Williams ED, Balloch Z (2004) Medullary thyroid carcinoma. In: Del·lès RA, Lloyd RV, Heitz PU, Eng C (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs. Vol. 8, IARC Press: Lyon, pp 86–91

Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardiner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnacliffe A, Fusco A, Vecchio G, Matoskova B, Kraus MH, Di Fiore PP (1995) Prognostic significance of somatic RET oncoproteins mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. J Clin Endocrinol Metab 93: 682–687

Romei C, Elisei R, Pinchera A, Ceccherini I, Molinaro E, Mancusi F, Martino E, Romeo G, Pacini F (1996) Somatic mutations of the ret proto-oncogene in sporadic medullary thyroid carcinoma are not restricted to exon 16 and are associated with tumor recurrence. J Clin Endocrinol Metab 81: 1619–1622

Santoro M, Carlonmago F, Romano A, Bottaro DP, Dathan NA, Grieco M, Fusco A, Vecchio G, Matoskova B, Kraus MH, Di Fiore PP (1995) Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science 267: 381–383

Santoro M, Melillo RM, Carlonmago F, Fusco A, Vecchio G (2002) Molecular mechanisms of RET activation in human cancer. Ann N Y Acad Sci 963: 116–121

Schilling T, Bürck J, Sinn HP, Clemens A, Otto HF, Höppner W, Herfarth C, Ziegler R, Schwab M, Raue F (2001) Prognostic value of codon 918 (ATG→AGT) RET proto-oncogene mutations in sporadic medullary thyroid carcinoma. Int J Cancer 95: 62–66
Schröder S, Böcker W, Baisch H, Bürk CG, Arps H, Meiners I, Kastendieck H, Heitz PU, Klöppel G (1988) Prognostic factors in medullary thyroid carcinomas. Survival in relation to age, sex, stage, histology, immuno-cytochemistry, and DNA content. Cancer 61: 806 – 816

Scurini C, Quadro L, Fattoruso O, Verga U, Libroia A, Lupoli G, Cascone E, Marzano L, Paracchi S, Busnardo B, Girelli ME, Bellastella A, Colantuoni V (1998) Germline and somatic mutations of the RET proto-oncogene in apparently sporadic medullary thyroid carcinomas. Mol Cell Endocrinol 137: 51 – 57

Shan L, Nakamura M, Nakamura Y, Utsunomiya H, Shou N, Jiang X, Jing X, Yokoi T, Kakudo K (1998) Somatic mutations in the RET protooncogene in Japanese and Chinese sporadic medullary thyroid carcinomas. Jpn J Cancer Res 89: 883 – 886

Sobin LH, Wittekind C (2002) TNM Classification of Malignant Tumours (UICC), 6th edn. John Wiley & Sons: Hoboken, New Jersey, pp 52–56

Uchino S, Noguchi S, Adachi M, Sato M, Yamashita H, Watanabe S, Murakami T, Toda M, Murakami N, Yamashita H (1998) Novel point mutations and allele loss at the RET locus in sporadic medullary thyroid carcinomas. Jpn J Cancer Res 89: 411 – 418

Zedenius J, Larsson C, Bergholm U, Bovée J, Svensson A, Hallengren B, Grimelius L, Bäckdahl M, Weber G, Wallin G (1995) Mutations of codon 918 in the RET proto-oncogene correlate to poor prognosis in sporadic medullary thyroid carcinomas. J Clin Endocrinol Metab 80: 3088 – 3090

Zedenius J, Wallin G, Hamberger B, Nordenskjöld M, Weber G, Larsson C (1994) Somatic and MEN 2A de novo mutations identified in the RET proto-oncogene by screening of sporadic MTCs. Hum Mol Genet 3: 1259 – 1262