Expression analysis of potentially MEN1-targeting microRNAs in sporadic and MEN-1 syndrome associated parathyroid adenomas and hyperplasias

Vince Kornél Grolmusz1,2, Katalin Borka1, Katalin Balogh1, Anna Szentpéteri1, Csaba Dékány3, András Kiss3, Zsuzsanna Valkus4, Miklós Tóth1, Anikó Somogyi1, János Horányi5, Károly Rácz1,6 and Attila Patócs1,2,6,7

1 2nd Department of Medicine, Semmelweis University, Budapest, Hungary
2 Lendület Hereditary Endocrine Tumours Research Group, Hungarian Academy of Sciences – Semmelweis University, Budapest, Hungary
3 1st Department of Medicine, University of Szeged, Szeged, Hungary
4 1st Department of Surgery, Semmelweis University, Budapest, Hungary
5 Molecular Medicine Research Group, Hungarian Academy of Sciences – Semmelweis University, Budapest, Hungary
6 2nd Department of Pathology, Semmelweis University, Budapest, Hungary
7 Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary

16 MEN-1 syndrome associated and 41 sporadic PHTP tissues were analyzed. MEN-1 associated PHTP occurred earlier at age (Table 1). Upon immunohistochemical analysis, all MEN-1 associated as well as 12/41 (29.3%) sporadic PHTP tissues lacked nuclear menin (Table 1). Mutations of various type were found in MEN-1 associated patients (Table 2). Upon n salico analysis, 6 miRNAs (hsa-miR-24, hsa-miR-28, hsa-miR-326, hsa-miR-484, hsa-miR-637 and hsa-miR-744) – all of these were predicted by at least two algorithms – were chosen for further investigations (Figure 2). MiRNA expression profiling revealed that hsa-miR-24 and hsa-miR-28 levels are elevated in sporadic compared to MEN-1 associated PHTP tissues (Figure 3, Panel A). Upon comparing MEN-1 associated and menin-negative sporadic PHTP tissues, this alteration was observed in only two other miRNAs – hsa-miR-484 and hsa-miR-744 – displayed elevated expression in menin-negative PHTP tissues (Figure 3, Panel B). Further analysis revealed that menin-negative PHTP tissues were larger compared to MEN-1 associated PHTP tissues (Table 3).

RESULTS

CONCLUSIONS

Lack of nuclear menin presence in MEN-1 associated and sporadic PHTP tissues confirms the tumour suppressor nature of menin. MiRNAs hsa-miR-24, hsa-miR-28, hsa-miR-484 and hsa-miR-744 may be involved in the tissue-specific downregulation of menin, contributing to sporadic parathyroid tumourigenesis.

REFERENCES


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Attila Patócs

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Immunohistochemical analysis of menin (Abcam, ab26005, 1:100 dilution) and Ki67 was performed in 16 MEN-1 associated and 41 sporadic PHTP tissues. In salico analysis using 5 different miRNA target prediction algorithms (Diana microT 3.0, mirWalk, microCosm Targets, PicTar, TargetScan) was performed to detect microRNAs potentially targeting MEN1. RNA was isolated from formalin-fixed, paraffin-embedded PHTP tissues using Ambion RecoverAll Total Nucleic Acid Isolation Kit (Life Technologies). MiRNA expression analysis of 6 chosen microRNAs was performed using predesigned TaqMan probes for quantitative PCR on Applied Biosystems Fast 7500 qRT-PCR instrument. Germine MEN1 mutation status was determined by Sanger sequencing. Statistical analysis was performed using IBM SPSS Statistics software. In all comparisons p < 0.05 was considered statistically significant.

METHODS

Figure 3 – MRNA expression changes between MEN-1 associated and sporadic (Panel A) and MEN-1 associated and menin-negative sporadic (Panel B) PHTP tissues. Data are given as mean ± S.E. Values indicate differentially expressed miRNAs. Statistical analysis is based on Student’s independent samples T-test. Statistically significant differences are highlighted with asterisks (**p<0.01, *p<0.05).

Figure 2 – 3’UTR of MEN1 mRNA with predicted binding sites of the 6 chosen miRNAs. Red frame highlights binding site for hsa-miR-24 confirmed earlier [2].

Table 3 – Characterization of MEN-1 associated and menin-negative sporadic PHTP tissues. Data are given as mean ± S.E. Values indicate differentially expressed miRNAs. Statistical analysis is based on Student’s independent samples T-test. Statistically significant differences are highlighted with yellow letters on blue background.

Table 2 – Germene MEN1 mutations found in 16 patients with MEN-1 syndrome patients harbouring the same mutation are close relatives and are displayed with same background colours.

Table 1 – Characterization of investigated PHTP tissues. Data are given as mean ± S.E. Values indicate differentially expressed miRNAs. Statistical analysis is based on Student’s independent samples T-test. Statistically significant differences are highlighted with yellow letters on blue background.

Figure 1 – Representative sites of immunohistochemical analysis of menin in MEN-1 associated (Panel A: MEN-1 adenoma, Panel B: MEN-1 hyperplasia) and sporadic (Panel C: adenoma with intensive presence of nuclear menin, Panel D: adenoma lacking nuclear menin presence) PHTP tissues. 600X magnification.