Nothing To Disclose

Molecular Diagnosis of Medullary Thyroid Carcinoma

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BACKGROUND

- Medullary thyroid carcinoma (MTC) is a rare neuroendocrine cancer originating from parafollicular C-cells that produce calcitonin
- MTC accounts for 5-10% of all thyroid carcinomas
- Age and Stage of disease at the time of diagnosis are the main prognostic indicators
- Diagnosis of MTC can be challenging in thyroid fine needle aspiration (FNA) cytology specimens
- Cytologic appearance of MTC can overlap with other thyroid neoplasms, thereby preventing a definitive diagnosis
- This study was designed to identify distinguishing molecular characteristics of MTC versus other thyroid neoplasms, for use in limited specimens such as FNA biopsies

DESIGN

- Twelve specimens from eleven previously diagnosed (primary and metastatic) cases of MTC were identified
- Unstained FFPE slides were micro-dissected and analyzed retrospectively using:
  - Next generation sequencing to identify oncogene point mutations (including *BRAF*, *KRAS*, *HRAS*, *NRAS*, *PIK3CA*) and gene rearrangements / translocations (including PAX8/PPARɣ, RET/ PTC1 and RET/ PTC3)
  - MicroRNA (miR) classifier analysis utilizing a 10 miR panel trained on 257 reactive, benign, malignant specimens
  - Quantitative evaluation of PAX8 and NKX2.1 messenger RNA (mRNA) levels; results were compared to papillary thyroid carcinomas (PTC)

RESULTS

- *HRAS* mutation was the only oncogene point mutation detected in 3 of the 11 MTC cases (27.3%)
- While the mRNA level of PAX8 was consistently greater relative to NKX2.1 in PTC, the relationship was reversed in all MTCs
- Compared to PTC, the MTCs showed a strikingly differential expression of four out of the ten miR markers (miRs 375, 551, 146 and 31) tested
- All MTCs could be distinguished from PTCs using this combination molecular approach

Case	miRNA 375 as most abundant marker	NKX2.1 mRNA level	PAX8 mRNA level	Oncogene mutation
1	Yes	15145	8397	*HRAS Q61R*
2	Yes	21531	10368	-
3	Yes	14149	12663	-
4	Yes	17850	61	*HRAS Q61R*
5	Yes	13495	3293	-
6	Yes	19231	8224	-
7	Yes	18395	16247	-
8	Yes	22398	17925	*HRAS Q61R*
9	Yes	8923	5692	-
10	Yes	16401	5609	-
11	Yes	29206	10016	-

ThyraMIR Idcs
Include On Report
Diagnosis: Positive
P5 Score: 0.99779
P5 Diagnosis: Positive
P10 Score: 0.82715
P10 Diagnosis: Positive
L5 Score: 6.11217
L10 Score: 1.56553

Medullary Thyroid Carcinoma

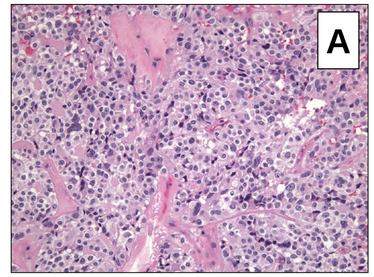
Marker	CT1	CT2	Avg CT
miR-204-5p	29.602	29.834	29.718
miR-139-5p	29.89	29.976	29.933
miR-375	18.028	18.191	18.1095
miR-29b-1-5p	27.919	28.073	27.996
miR-155-5p	28.486	28.592	28.539
miR-551b-3p	32.188	34.56	33.374
miR-146b-5p	29.023	29.166	29.0945
miR-31-5p	32.416	33.002	32.709
miR-222-3p	21.224	21.4	21.312
miR-138-1-3p	30.161	30.055	30.108

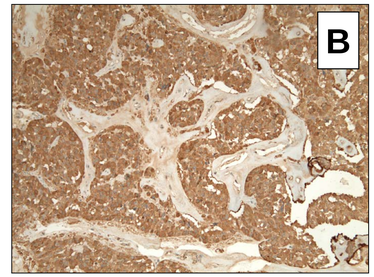
ThyraMIR Idcs
Include On Report
Diagnosis: Not Required
P5 Score: 0.97565
P5 Diagnosis: Positive
P10 Score: 0.96376
P10 Diagnosis: Positive
L5 Score: 3.6904
L10 Score: 3.28074

Papillary Thyroid Carcinoma

Marker	CT1	CT2	Avg CT
miR-204-5p	30.512	30.924	30.718
miR-139-5p	28.424	28.495	28.4595
miR-375	25.175	25.219	25.197
miR-29b-1-5p	28.567	28.495	28.531
miR-155-5p	24.078	24.024	24.051
miR-551b-3p	26.843	27.015	26.929
miR-146b-5p	21.269	21.203	21.236
miR-31-5p	23.551	23.456	23.5035
miR-222-3p	21.232	21.101	21.1665
miR-138-1-3p	30.608	30.214	30.411

- miR classifier testing is recommended for nodules that are mutation negative and nodules that show weak driver mutations
- Down-regulation of miRs- 204, 139, 29b, 155, 138 and up-regulation of miRs- 375, 551, 146b, 31, 222 acts as a predictor of malignant risk. A relatively differential miR expression is seen in MTC as compared to PTC





Histological sections of MTC **(A)** are characterized by round, polygonal or spindle cells in nests, cords or follicles, defined by sharply outlined fibrous bands. Tumor cells have granular cytoplasm and uniform round / oval nuclei with punctate chromatin;

(B) Tumor cells show positive immunohistochemical staining with calcitonin

CONCLUSIONS

- MTC can be differentiated from PTC based on mutational genotype, relative mRNA expression levels of NKX2.1 versus PAX8 and differential miR expression profile
- Molecular tests based on these findings can be applied to FNA of thyroid nodules when cytology evaluation is indeterminate or when a MTC is considered in the differential diagnosis
- Molecular abnormalities could also provide potential therapeutic guidance in the current era of targeted therapy

REFERENCES

- Mian C, et al. MicroRNA Profiles in Familial and Sporadic Medullary Thyroid Carcinoma: Preliminary Relationships with RET Status and Outcome. Thyroid. 2012 Sep; 22(9): 890–896.
- Labourier E. Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology. J Clin Endocrinol Metab, July 2015, 100(7):2743–2750.