MICRORNA EXPRESSION PROFILING OF PAPILLARY THYROID CARCINOMA WITH LYMPH NODE METASTASIS

Mohamad Yusof, A[1], Ab Mutalib, NS[1], Mohamed Rose, I[2], Abdullah Suhaimi, SN[3], Muhammad, R[3], Jamal, R[1]

[1]UKM Medical Molecular Biology Institute (UMBI), [2]Department of Clinical Oral Biology, Faculty of Dentistry, [3]Department of Surgery, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

ABSTRACT

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy in thyroid which contributes to 70 – 80% of all cases. The incidence rates of PTC has rapidly increased in the recent decades, and microRNA (miRNA) is one of the most studied potential biomarkers in this cancer. This study aims to identify miRNAs which are differentially expressed in PTC with and without lymph node metastasis (LNM). Paired tumour and adjacent normal fresh frozen tissues from five patients diagnosed with PTC with LNM and PTC without LNM from Universiti Kebangsaan Malaysia Medical Centre (UKMMC) were collected and subjected to miRNA sequencing (miRNA-seq) using Illumina HiSeq 2500 system. The analysis was performed using BaseSpace miRNA analysis. miRNA-seq identified 80 upregulated and 94 downregulated miRNAs (FDR-adjusted p value <0.1, absolute fold change ≥1 or ≤-1) in PTC LNM-positive (PTC LNM-P) compared to normal samples. When we compared PTC lymph node negative (PTC LNN) with normal samples, we identified 37 upregulated and 17 downregulated miRNAs (FDR-adjusted p value <0.1, absolute fold change ≥1 or ≤-1). Integration of miRNA with their target genes may provide insight into carcinogenesis of PTC.

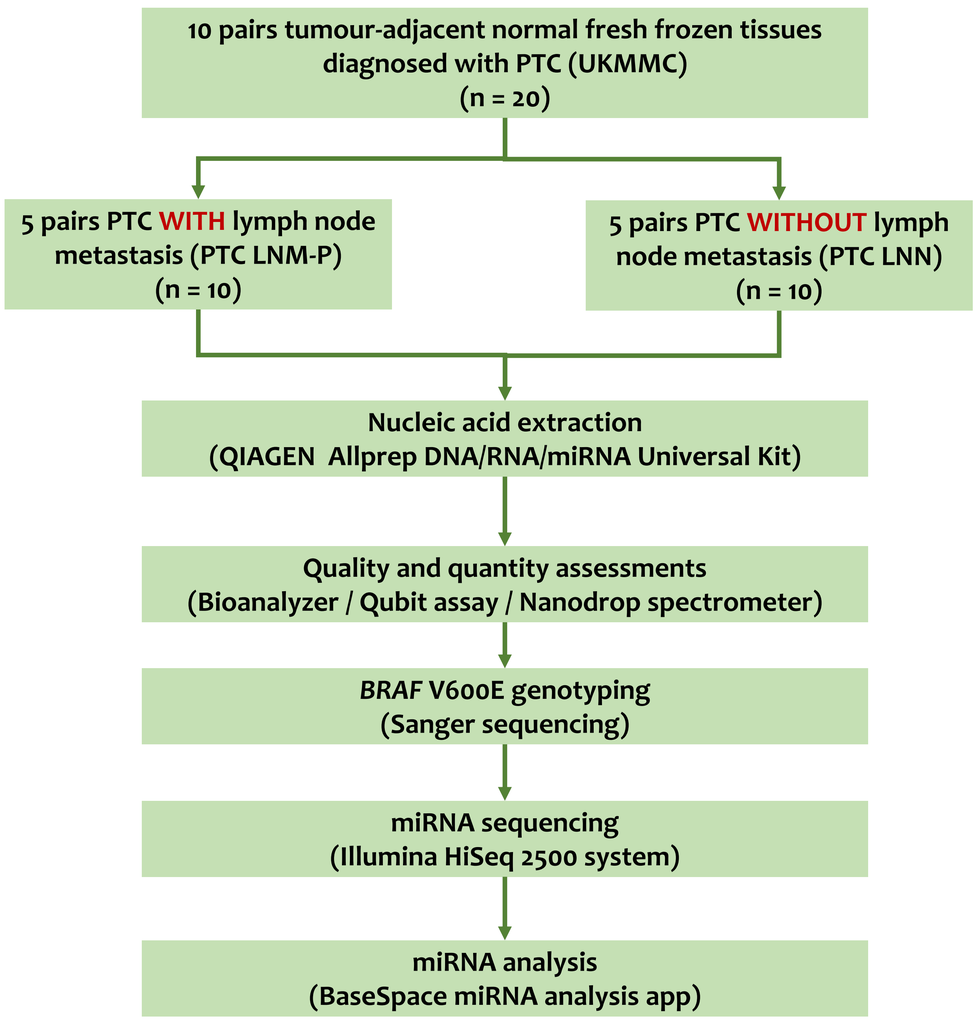
INTRODUCTION

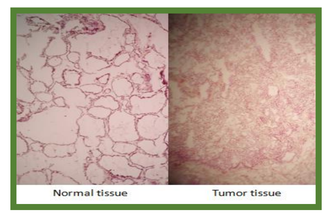
Nearly 40-60% of PTC patients suffer from LNM which increase their risk of recurrence especially among the elderly. Most of PTC recurrent cases are due to metastasis to the lymph node that remained after initial surgery. Therefore, finding the exact molecular biomarker is important in order to prevent from conducting the risky surgical procedure and to tailor treatment according to the patients. MiRNAs (one of the most studied potential biomarkers) have been shown to be involved in thyroid cancers whereby differential expression of miRNAs can distinguishes PTC from normal thyroid tissue. By performing miRNA-seq, we can accurately sequence the complete range of miRNA species due to higher multiplexing capability and sensitivity of this technology.

OBJECTIVE

To identify miRNAs which are differentially expressed in PTC with and without lymph node metastasis.

STUDY DESIGN





RESULTS AND DISCUSSIONS

Table 1: Patient's demographic data. *BRAF* V600E mutation status was determined in all patients using Sanger sequencing.

No	Patient's code	Gender	Age	Race	*BRAF* V600E mutation
1	TY2	Female	54	Malay	Mutated
2	TY5	Female	67	Chinese	Mutated
3	TY60	Female	54	Chinese	Mutated
4	TY135	Female	47	Malay	Mutated
5	TY146	Female	46	Malay	Mutated
6	TY3	Female	47	Malay	Non-mutated
7	TY29	Female	56	Malay	Non-mutated
8	TY42	Female	69	Malay	Non-mutated
9	TY148	Female	47	Chinese	Non-mutated
10	TY156	Female	18	Malay	Non-mutated

Table 2: The top deregulated miRNAs in **PTC LNM-P versus normal** with their absolute fold change value.

No	miRNAs	Absolute fold change
1	hsa-miR-146b	5.78
2	hsa-miR-221	4.28
3	hsa-miR-222	4.28
4	hsa-miR-486	-3.54
5	hsa-miR-873	-3.63

Table 3: The top deregulated miRNAs in **PTC LNN versus normal** with their absolute fold change value.

No	miRNAs	Absolute fold change
1	hsa-miR-146b	4.87
2	hsa-miR-551b	4.73
3	hsa-miR-375	3.63
4	hsa-miR-873	-3.7
5	hsa-miR-1179	-3.9

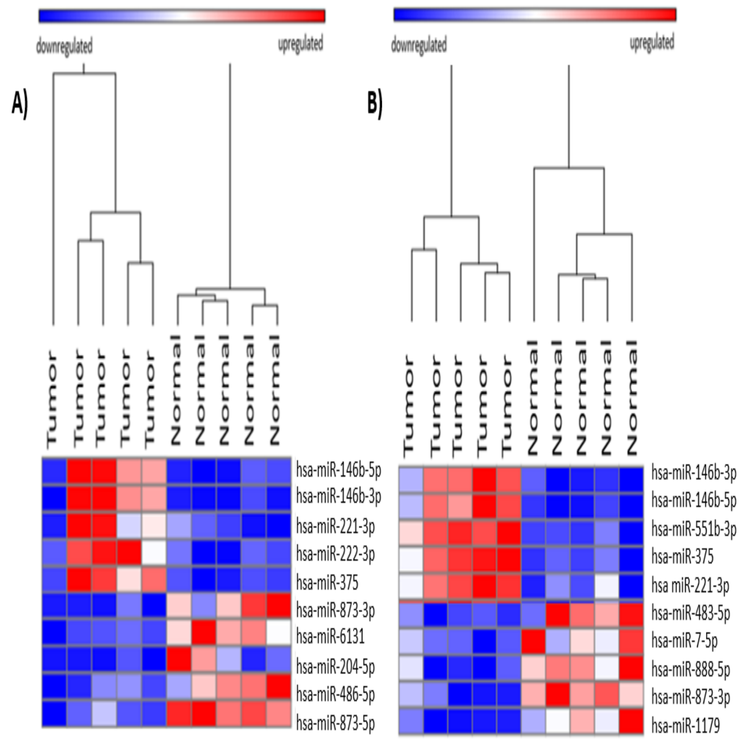


Figure 1. Hierarchical clustering of top 10 significantly dysregulated miRNAs. The list of differentially expressed miRNAs were filtered using FDR-adjusted p value <0.1, absolute fold change ≥1 or ≤-1. **(A)** In PTC with lymph node metastasis (PTC LNM-P) versus normal-adjacent tissues, miRNA-seq identified 174 differentially expressed miRNAs. Ninety-four (94) were under-expressed while 80 were over-expressed. **(B)** In PTC without lymph node metastasis (PTC LNN) versus normal-adjacent tissues, miRNA-seq identified 54 differentially expressed miRNAs, 17 were under-expressed while 37 were over-expressed.

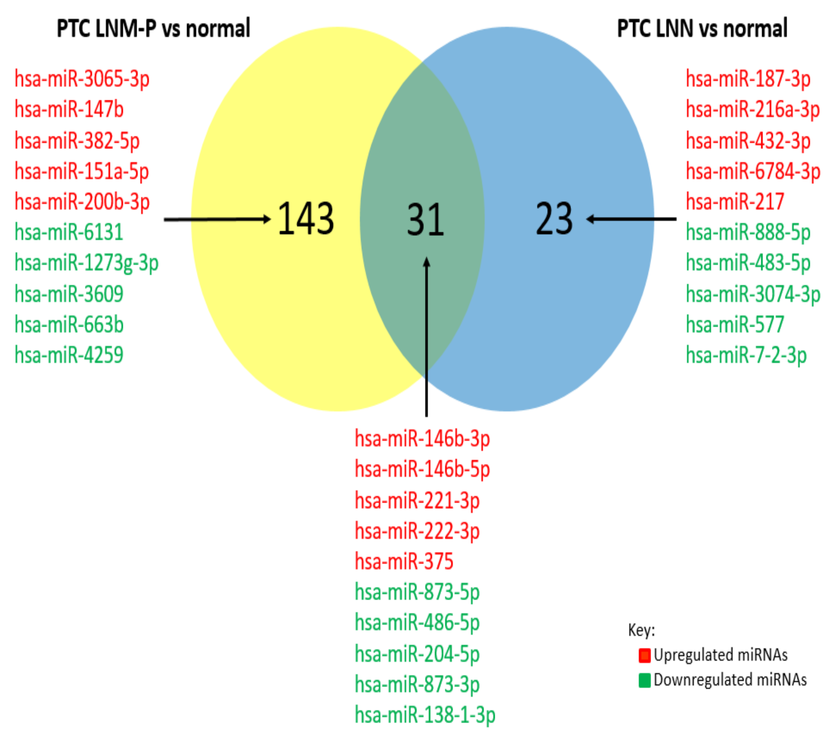


Figure 2. Venn diagram showing the intersection between differentially expressed miRNAs (FDR-adjusted p value <0.1, absolute fold change ≥1 or ≤-1) in PTC LNM-P versus their adjacent-normal samples and PTC LNN versus their adjacent-normal samples with the list of top 10 miRNAs for each group. There were 143 miRNAs which were uniquely significantly differentially expressed in PTC LNM-P.

CONCLUSION

- **Deregulation of microRNAs is another molecular feature occurring during PTC tumorigenesis.**
- **This deregulation can be expanded to understand miRNAs involvement in LNM.**
- **miR-151 is among the miRNAs exclusively upregulated in PTC with LNM.**
- **Integration of miRNA with their target genes can provide further insight into mechanism of LNM in PTC.**

ACKNOWLEDGEMENT

This research was funded by Fundamental Research Grant Scheme (FRGS) from the Ministry of Education Malaysia (FRGS/1/2014/SKK01/UKM/03/1)

REFERENCES

- **Ac't Hoen, P. et al. 2013. *Nature biotechnology* 31(11): 1015-1022.**
- **Gene-E (http://www.broadinstitute.org/cancer/software/GENE-E/index.html).**
- **Karakoc, D.et al. 2010. *International Surgery* 95(2): 142-146.**
- **Saiselet, M. et al. 2015. *BMC genomics* 16(1): 828.**
- **Swierniak, M. et al., 2013. *The Journal of Clinical Endocrinology & Metabolism* 98(8): E1401-E1409.**
- **Xing, M. 2013. *Nature Reviews Cancer* 13(3): 184-199.**
- **Yu, S. et al,. 2012. *The Journal of Clinical Endocrinology & Metabolism* 97(6): 2084-2092.**