Circulating microRNAs may help to differentiate malignant from benign thyroid nodules

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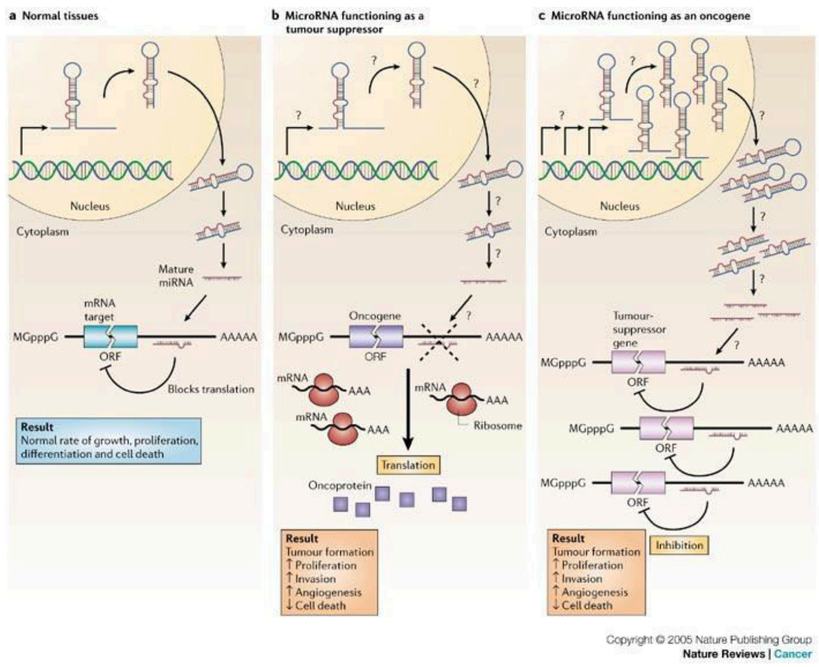
Introduction

❖MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs that act as negative regulators of gene expression.



❖The miRNA expression is impaired in many types of human cancer including thyroid cancer.

❖The tissue profile of miRNAs has been shown to be useful for differentiating benign from malignant thyroid nodules, however attainment of tissue samples requires an invasive procedure while blood sampling is minimally invasive and easy to obtain.

Objective

The aim of this study was to evaluate the circulating levels of a series of miRNAs in 46 patients with nodular goiter in order to identify those that might be useful in the differential diagnosis of thyroid nodules.

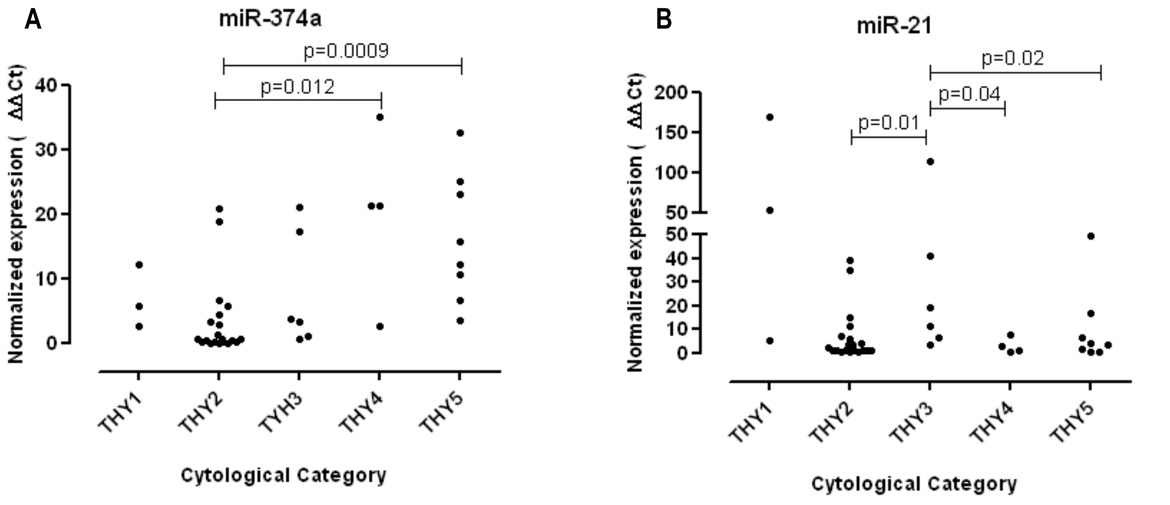
Materials and Methods

❖13 miRNAs (miR-222, miR-221, miR-146a, miR-146b, miR-21, miR-155, miR-181a miR-181c, miR-7, miR-30d, miR-126, miR-374th, miR-let7g) were extracted from serum, reverse transcribed, subjected to Real-Time PCR and then analyzed by the ΔΔCt method.

❖10/13 miRNAs were evaluated post-surgically in a subset of patients undergone thyrodectomy.

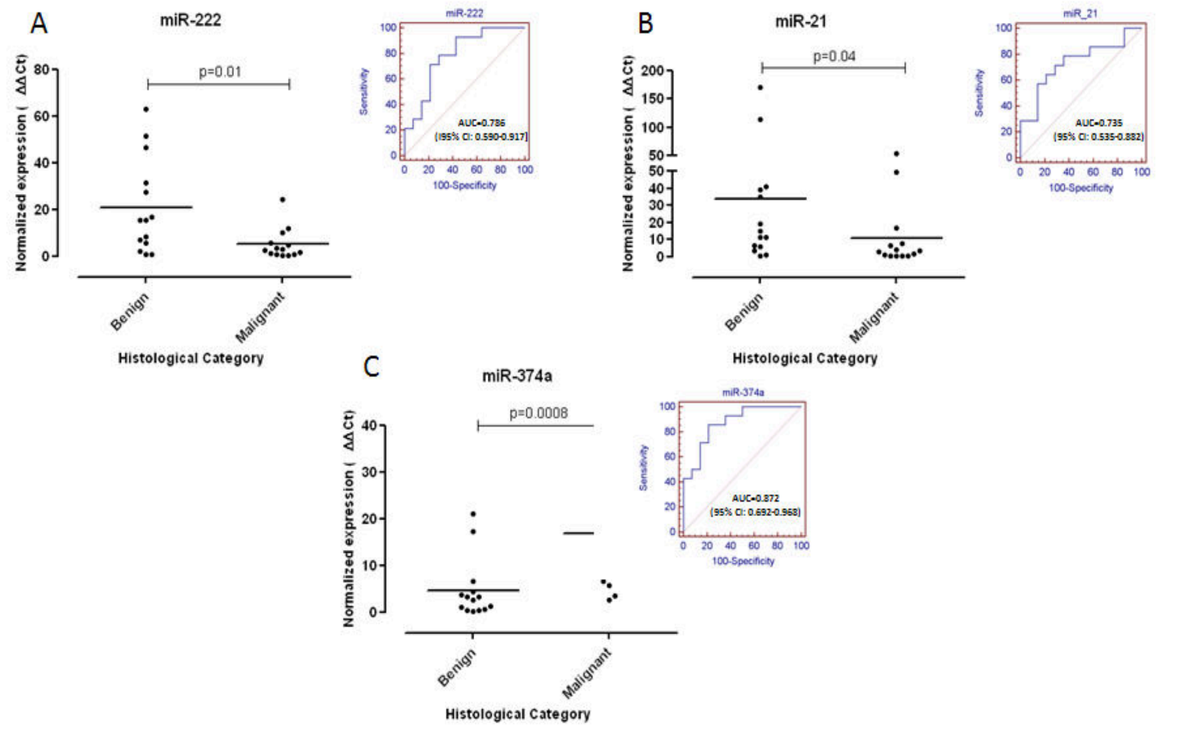
Results

Serum levels of miR-374a (A) and -21 (B) in patients with different cytological diagnosis



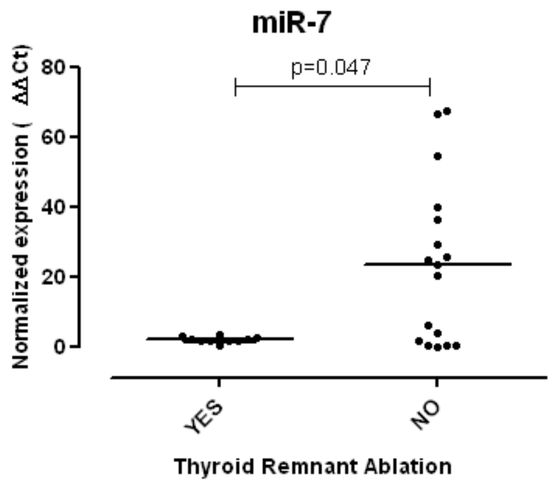
1.A MiR-374a levels were significantly lower in patients with cytologically benign nodules compared to patients with nodules suspicious for malignancy or malignant B MiR-21 levels were significantly higher in patients with cytologically indeterminate nodules compared to patients with benign nodules, suspicious for malignancy or malignant. The values of expression levels of miR are presented as normalized with respect to the 18S ribosomal subunit with the ΔΔCt method. The P value was calculated with the Mann-Whitney U test. THY1: non diagnostic nodules; THY2: benign nodules; THY3: indeterminate nodules; THY4: suspicious for malignancy nodules; THY5: malignant nodules.

Serum levels of miR-222 (A) -21 (B) and-374a (C) and corresponding ROC curves in patients with benign and malignant nodular disease



2 MiR-222 (A) and miR-21 (B) levels were significantly reduced in patients with malignant compared with benign nodular disease, while the levels of miR-374a (C) were significantly higher in patients with malignant compared to benign nodular disease. The ROC curve analysis (top right corner of each graph) showed the best sensitivity (78.57%) and specificity (57.14%) of miR-222 at the cut-off of 8.35 (A); the best sensitivity (78.57%), specificity (64.29%) of miR-21 at the cut-off 7.51 (B) and the best sensitivity (85,717%) and specificity (78.57%) of the miR-374a at the cut-off of 4.4 (C). The values of expression levels of miR are presented as normalized with respect to the 18S ribosomal subunit with the ΔΔCt method. The P value was calculated with the Mann-Whitney U test. The ROC curve analysis was performed with the statistical software MedCalc version 9.4.0.0.

Serum levels of miR-7 in patients treated only surgically versus patients treated with thyroidectomy+thyroid remnant ablation with 131-I



3 MiR-7 levels were significantly higher in patients treated only with surgery compared to patients who were also ablated with 131-I. The values of expression of miR-7 are presented as normalized levels compared to the 18S ribosomal subunit with the ΔΔCt method. The P value was calculated with the Mann-Whitney U test.

Conclusions

❖Our data, although preliminary, suggest the utility of circulating miRNAs (miR-374a showing the best diagnostic accuracy) in the differential diagnosis of thyroid nodules, and the lower expression of miR-7 in patients ablated suggests its potential use as a tumor marker.

❖Both in patients with benign and malignant thyroid disease, after thyroidectomy, the majority of miRNAs decreased while a minority of miRNAs increased or remained unchanged. The significance of this behaviour should be further evaluated.