Measurement of Four MicroRNAs Accurately Differentiates Malignant from Benign Indeterminate Thyroid Lesions

Keutgen XM, Filicori F, Crowley MJ, Wang Y, Scognamiglio T, Hoda R, Buitrago D, Cooper D, Zeiger MA, Zarnegar R, Elemento O, Fahey TJ 3rd. A panel of four microRNAs accurately differentiates malignant from benign indeterminate thyroid lesions on fine needle aspiration. Clin Cancer Res. February 20, 2012 [Epub ahead of print].

SUMMARY ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ●

Background

A plethora of papers has recently appeared on the use of microRNAs for the diagnosis of thyroid cancer. As discussed in the article by Stephen Spaulding in the December 2011 issue of Clinical Thyroidology (pp 14–15), miRNAs are single-stranded noncoding small RNA segments, 19 to 23 nucleotides in length, that bind to multiple mRNAs by sequence-specific interaction with the 3' untranslated region of the mRNA targets. They cause suppression of translation and increased degradation of the specific mRNA. In cancers, a number of miRNAs have been shown to be tumor suppressors and are often down-regulated.

The current paper is a study of specific miRNAs in lesions cytologically classified to be indeterminate. Such lesions have a possibility of approximately 25% of being malignant.

Methods

A "derivation" group, classified by FNA as indeterminate lesions, consisted of 15 benign and 14 malignant specimens from which RNA was obtained at FNA biopsy. Based on an extensive search of the literature on miRNAs that were differentially expressed in thyroid cancer versus benign tissue, the authors decided to measure the levels of six miRNAs: mir-328, mir-222, mir-197, mir-181a, mir-146b, and mir-21. Measurements were performed on carefully prepared miRNAs using quantitative RT-PCR and 45 cycles of amplification; results were compared in terms of cycle number with the housekeeping gene RNU6B. They evaluated several methods for building statistical models that can predict benign versus malignant status based on expression profiles of the six miRNAs.

After developing the model, they applied it to a validation group of 72 consecutive FNA specimens that were classified as indeterminate and in which all the patients had agreed to have surgery.

Results

Using nonparametric analysis in the derivation group of 29 specimens, they found that four miRNAs were differentially expressed between malignant and benign lesions; these included miR-222 (P<0.005), miR-21 (P<0.03), miR-181a (P<0.04), and miR-146b (P<0.03). MicroRNA expression of mir-197 (P = 0.3) and mir-328 (P = 0.6) was not significantly different. By highly sophisticated statistical analysis, they developed a model that gave 85% sensitivity and 85% specificity for a diagnosis of malignancy using the four miRNAs. The model was then applied to the validation samples.

There were 50 benign lesions and 22 malignant lesions in the validation group based on final histopathology. The model that used the four miRNAs correctly classified 65 of the 72 FNA samples. The sensitivity was 100% and the specificity 86% for the diagnosis of cancer. Five of the seven lesions that the model predicted incorrectly to be malignant were Hürthle-cell neoplasms on FNA and hyperplastic nodules or adenomas on final pathology.

Conclusions

This study shows that that the expression of miR-222, miR-328, miR-197, and miR-21 combined in a predictive model is reasonably accurate for differentiating malignant from benign indeterminate thyroid lesions on FNA.

ANALYSIS AND COMMENTARY ● ● ● ● ● ● ●

This field of measuring miRNA in FNA specimens is moving rapidly and competes with the measurement of oncogenes (1) or the measurement of an array of proteins (2) for diagnosis of thyroid cancer in the specimens. It may have great utility when applied to specimens classified as indeterminate or even those deemed to be inadequate based on cytology. Unfortunately, there has not been uniformity in the choice of which miRNAs are most useful to measure. In contrast with this paper, another recent paper reported measurements of miR-7, miR-126, miR-374a, and let-7g and found that miRNA-7 was most useful for the diagnosis of cancer in inconclusive FNA specimens (3). Yet another report found that miRNA-138 was the most useful for the diagnosis of cancer in a series of 125 FNA biopsies that had been classified as indeterminate (4). Still another recent report in Thyroid focused on miRNA analysis of FNA specimens classified as atypia of undetermined significance (5). The results showed that a panel of miR-146b, -221, -187, and -30d had good accuracy for identification of papillary thyroid cancers but not follicular cancers. Although each of these four reports of miRNA measurements resulted in a choice of the best miRNA for the diagnosis of cancer, it is worth emphasizing that each report concluded that a different panel of miRNA was the most useful.

It is important to note that a single miRNA may modify the expression of several genes. In the paper studied here, the authors point out that the four miRNAs they used for the predictive model were all involved in control of the cell cycle or in cell proliferation.

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References

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