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CAN THE MEASUREMENT OF MICRORNA LEVELS IN "INDETERMINATE" FINE-NEEDLE ASPIRATION (FNA) BIOPSIES HELP IN DIAGNOSING THYROID MALIGNANCY?

Vriens MR, Weng J, Suh I, Huynh N, Guerrero MA, Shen WT, Duh Q-Y, Clark OH, Kebebew E. **MicroRNA expression profiling is a potential diagnostic tool for thyroid cancer.** Cancer. October 17, 2011. [Epub ahead of print]. doi:10.1002/cncr.26587.

SUMMARY

BACKGROUND

Cytopathologists and clinicians alike feel disappointed when a thyroid fine-needle aspiration (FNA) biopsy, that seemed to have plenty of cells must be given an "indeterminate" reading. The discovery of microRNAs (miRNAs), which are transcribed from regions of DNA previously thought to be silent, has revealed a new level of complexity in genetic regulation. MicroRNAs are small (approximately 22 nucleotides long), noncoding single-stranded RNAs that constitute a novel class of gene regulators. Each miRNA regulates the expression of hundreds of different messenger RNAs (mRNAs) by blocking their translation and promoting their degradation, and in turn, each mRNA is targeted by multiple miRNAs. Several groups studying thyroid malignancy have measured the levels of many of the 1000-plus miRNAs, looking for miRNA patterns that would identify thyroid cancer in FNA biopsies by nonhistologic means. Although miR-121, -122, -146a&b have been reported in some studies to be over-expressed on average in thyroid cancers, it remains to be shown in large prospective studies whether they consistently establish that an indeterminate FNA is benign or malignant (1). If a specific pattern of miRNAs that discriminated between all thyroid cancers and all benign tissues on FNA biopsies were found consistently, it would be an excellent diagnostic tool.

METHODS

The authors compared the levels of about 850 miRNAs in two RNA pools, one containing total RNA from 56 "benign" thyroid tissues (7 normal glands 14 multinodular goiters, 15 follicular adenomas, and 12 Hürthle-cell adenomas) and the other pool from 48 "malignant" thyroid tissues (8 papillary thyroid carcinomas [PTCs], 12 follicular variant PTCs, and 12 follicular, 20 Hürthle–cell, and 4 anaplastic carcinomas). The miRNA probes were made of nucleic acid analogs whose sugar-ring structure was chemically locked to strengthen binding and reduce cross-hybridization. The levels of the five most overexpressed and the five most underexpressed miRNAs found on this screen were then measured in all the individual tissue samples making up the two pools, by reverse transcription using primers specific for each of the 10 miRNAs, followed by polymerase-chain-reaction amplification. Control miRNAs were also measured, and the most stable one was used to normalize the measurements. The levels of the 10 miRNAs were also measured in a series of 125 FNA biopsies that had been read as "indeterminate," and the miRNA levels were compared to the histopathology findings after surgery.

RESULTS

When the levels of the five most underexpressed miRNAs (0.06 to 0.20 times lower than in the benign pool), and the five most overexpressed (4 to 8 times higher than in the benign pool) were measured in the 94 tissue samples individually, only 4 of 10 were still significantly different, all being down-regulated (miR100, miR125b, miR138, and miR768-3p; $P<0.002$).

The level of miR138 gave a theoretical diagnostic accuracy of 79% in distinguishing all benign neoplasms from all malignant tissue samples. The level of miR768-3p apparently was lower in the 12 follicular variant of PTC samples than in all 56 benign samples. When the 125 indeterminate FNA samples were assayed for the levels of the 10 miRNAs, only the miR138 level was significantly different between the 37 malignant and the 78 benign FNA samples, with a theoretical accuracy rate of 75% and a negative predictive value of 81%.

CONCLUSIONS

If the level of an individual miRNA like miR138 or even a pattern of miRNAs were to consistently distinguish malignancy on indeterminate thyroid FNA biopsies, it would certainly deserve to be studied in prospective clinical trials.

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COMMENTARY

Trying to analyze the significance of differences in a study that has many variables—but only a small number of samples of each variable—can be tricky. In this paper, it was not always clear how many comparisons were made, whether significance levels were corrected for the total number of comparisons performed, or whether a "difference" observed was an increase or a decrease. There is also the ever-present concern about whether the small amount of tissue obtained by FNA is representative of disease present elsewhere in the thyroid. In thyroid cancer, the relative level of expression of different miRNAs differs not only from normal tissue, but also between malignant regions (2), presumably reflecting environmental differences such as hypoxia or inflammation, as well as genetic changes. Malignancy can alter the covalent modification and processing of miRNA precursors, thus altering the level of the mature miRNAs in the cytoplasm. Upon binding to one of the Argonaute proteins in the cytoplasm, an miRNA is not only protected from nuclease degradation, but can also then form complexes with the other proteins needed for it to attack its target mRNAs. However, free mature miRNA also can bind directly to target sequences found in long noncoding RNAs and transcribed pseudogenes, as well as in mRNAs. This nonspecific binding can "buffer" miRNA levels. Thus, the finding that an miRNA appears to be substantially down-regulated in indeterminate FNAs from diverse thyroid malignancies, but not in diverse benign samples, does suggest that the metabolism of some miRNAs is altered with some consistency in thyroid cancer. The true utility of specific miRNAs for diagnosis of thyroid cancer will require additional studies.

— Stephen W. Spaulding, MD

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