KIDNEY CANCER

RCC classification using miRNA signatures

A microRNA (miRNA)-based classification system that can differentiate between types of kidney tumor has been developed by a team in Toronto, Canada.

Renal cell carcinoma (RCC) encompasses a number of tumor types, including clear cell RCC, papillary RCC, chromophobe RCC and collecting duct carcinoma. These subtypes are usually distinguished by histological assessment of morphology. This method is not always accurate, however, because some morphological features are common to more than one type of RCC, and to the benign renal neoplasm oncocytoma.

"The identification of many new entities, like clear cell papillary RCC [and] translocation carcinoma, [further] challenges our morphology-based classification of renal tumors and its validity to guide therapy, and [highlights] the need for a more accurate molecular-based classification," says George Yousef from St Michael's Hospital in Toronto.

As dysregulated miRNAs are known to be involved in the pathogenesis of kidney cancer, the team focused their efforts on these short molecules. Total RNA was extracted from fresh frozen nephrectomy specimens; 20 clear cell RCCs, 20 normal tissue samples from the same patients, 10 papillary RCCs, 10 chromophobe RCCs and 10 oncocytomas. The harvested RNA was then hybridized to microarrays so that relative levels of miRNA expression could be determined. 91 miRNAs were found to be differentially expressed. The expression of eight individual miRNAs was measured using quantitative reverse transcription PCR, with the results mimicking those of the microarray analysis.

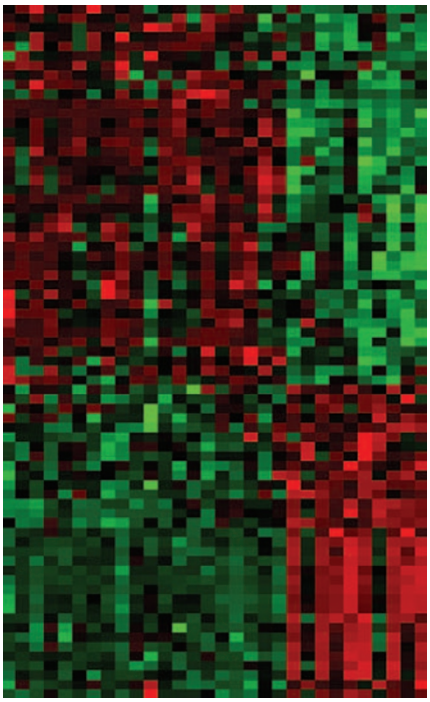
The investigators used the derived miRNA signatures to construct a 'decision tree' that can accurately discriminate between normal tissue, oncocytoma, and the three malignant subtypes. First, the expression level of six specific pairs of miRNAs was used to distinguish between cancerous and noncancerous samples, with expression levels between each pair compared and a 'majority vote' used for classification. Each subtype of tumor was then isolated from the others in a similar manner, with all subtypes distinguishable in a maximum of four steps.

The decision tree correctly classified 96% and 100% of samples at each step. Cross-validation using an independent test set yielded an accuracy of about 90%. Clear cell RCC and papillary RCC were shown to be closely related to each other, as were chromophobe RCC and oncocytoma.

Interestingly, the majority of the 65 miRNAs that were most distinctly differentially expressed on microarrays are located within chromosomal regions that are known to harbor mutations related to kidney malignancy (for example, *VHL* in clear cell RCC and fumarate hydratase in papillary RCC).

The decision tree developed by Yousef *et al*. could offer an alternative, or adjunct, to the current gold-standard histological analysis, the accuracy of which can be compromised by interobserver variability and human error. Importantly, Yousef and colleagues showed their miRNA-based technique to be equally accurate when formalin-fixed paraffin-embedded—as opposed to fresh frozen—tissue was used.

miRNA profiling also requires less tissue than histological examination. "A practical application of these classifiers is to use them on a small biopsy with limited material that is not usually enough for histological diagnosis," suggests Yousef. Reliable determination of tumor subtype at this early stage of patient management would help to individualize treatment plans, potentially avoiding unnecessary interventions.



Courtesy of G. Yousef

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