New technique detects trace levels of new class of cancer biomarkers

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(Phys.org) -- In less than a decade, a new type of RNA — microRNA (miRNA) — has gone from curiosity to one of the most important sets of regulatory molecules in the body. And because these short pieces of RNA are associated with specific tissues and functions, and because they circulate stably in the bloodstream, they have great potential as diagnostic biomarkers for cancer and other diseases. The major obstacle to realizing that potential is that miRNAs are present in blood at levels too low to detect reliably with today's applicable biomolecular detection technology.

That obstacle is no more. A team of investigators from the Northwestern University Center for Cancer Nanotechnology Excellence (Northwestern CCNE) has developed a rapid, array–based technology using gold nanoparticles that is capable of detecting miRNAs at levels as low as 1 femtomolar (about 30,000 molecules in a drop of blood). In contrast, standard fluorescence–based detection schemes are unable to detect 88 percent of the miRNAs in blood at concentrations 10 times higher than the limit of detection of the new assay. The Northwestern CCNE team, led by Chad Mirkin and Shad Thaxton, published the details of their new assay in the journal *Analytical Chemistry*.

The new assay, which the researchers call the Scanometric miRNA platform, can detect hundreds to thousands of miRNAs simultaneously. After isolating miRNA from human blood or a tissue sample, the Northwestern CCNE team uses an enzyme to attach a universal linker to every molecule of miRNA. They then add the modified miRNA mixture

to an array comprising individual spots of DNA, each of which is designed to bind to a known miRNA sequence. After allowing the miRNAs in the sample to bind to their particular spot on the array, the investigators wash the array to remove unbound miRNAs and then treat it with the key reagent – a spherical nucleic acid–gold nanoparticle conjugate that binds very tightly to the universal linker that had been added to the miRNA mixture. Finally, the array is treated with a solution that adds an extra shell of gold onto the bound spherical nucleic acid–gold nanoparticles. This last step enhances the nanoparticle's ability to scatter light and greatly increases the assay's sensitivity. The treated array is then imaged with a commercial array scanner.

To test the performance of their system, the Northwestern CCNE team used their array to detect miRNAs isolated from prostate cancer cells. The Scanometric miRNA platform 88 percent more miRNAs than did the standard fluorescence–based assays now used to measure miRNA content in biological samples. The team then used their assay to detect miRNAs from human prostate cancer tissue samples and found distinct differences between tissue samples obtained from slow–growing versus aggressive prostate cancers, and were able to identify the aggressive tumors with 98.8 percent accuracy.

Further analysis of the data revealed a small set of miRNAs that appear to be deregulated and may play a role in the progression of prostate cancer. Some of these miRNAs may prove to be useful as novel biomarkers for prostate cancer, though the investigators note that a larger clinical trial is needed to confirm this finding.

This work, which is detailed in a paper titled, "Scanometric microRNA array profiling of prostate cancer markers using spherical nucleic acid–gold nanoparticle conjugates," was supported in part by the NCI Alliance for Nanotechnology in Cancer, a comprehensive initiative designed to accelerate the application of nanotechnology to the

prevention, diagnosis, and treatment of cancer. An abstract of this paper is available at the journal's website.

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