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RESEARCH HIGHLIGHT

A truth serum for cancer — microRNAs have major potential as cancer biomarkers

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Identification of biological markers of cancer is a major area of research. Biomarkers could identify the presence of a tumor before it could otherwise be easily detected, and the ability to detect cancers at early stages is a key factor in increasing survivability. For example, the American Cancer Society finds that a reason breast cancer survival rates are so high is that there are good methods for early detection of tumors. However, this is not the case for most cancers. For lung cancer, the five-year survival is 15%, but for the 16% of lung cancer cases diagnosed at early stages, the five-year survival rate is 49% [1]. While this is just one example, the ability to identify a cancer while it is still localized is clearly beneficial. Currently, most methods for discovering and testing tumor biomarkers are difficult and labor-intensive procedures, and at most, only several markers can be tested for at one time. However, due to the simplicity of getting a blood sample, easily testable biomarkers found in blood serum would be especially useful.

Just recently, scientists have begun identifying microRNAs (miRNAs) as cancer biomarkers [2-4]. MiRNA genes code for a relatively new class of regulatory RNAs that are ~22 nucleotides long. miRBase (Release 12.0), the central database for miRNAs, lists over 8 000 miRNAs from plants, animals and viruses. Many miRNAs are well conserved across species, suggesting an important role for them. MiRNA biogenesis and regulation of gene expression have been extensively covered in numerous reviews [5-7]. Briefly, most mature miRNAs are the products of RNA polymerase II-transcribed transcripts that have been processed by two RNase III enzymes, Drosha and Dicer. The mature miRNA is incorporated into an RNA-induced silencing complex that binds to a target messenger RNA (mRNA). In animals, miRNAs bind with imperfect complementarity to the 3′ untranslated region of their targets to inhibit gene expression through several possible mechanisms, including degradation of the mRNA, inhibition of the initiation or elongation steps of translation, and localization to cytoplasmic P-bodies. Because animal miRNAs bind with imperfect complementarity, miRNAs are though to be capable of targeting numerous mRNAs; thus, misexpression of one miRNA can disrupt the expression of hundreds of proteins.

Although first discovered in *C. elegans*, miRNAs have since been shown to be involved in human cancers [5]. A number of miRNA genes were shown to be located in fragile regions of the human genome that are associated with cancer [8]. Furthermore, miRNAs are continuously being shown to act as both oncogenes and tumor suppressors [5]. With the development of technologies to look at the expression levels of hundreds of miRNAs at a time and the clear role of miRNAs in cancers, groups began looking at miRNA profiles of different cancers. Calin *et al.* were the first to show that their microRNA microarray could differentiate between B cell chronic lymphocyte leukemia (CLL) cells and normal cells. Furthermore, they classified CLL samples into two different groups based on their miRNA profiles, and these profiles corresponded to high or low levels of a protein that is associated with a positive prognosis at low levels [9]. Shortly after, Lu *et al.* developed a method for bead-based miRNA profiling that they found to be more sensitive since the hybridizations were taking place in solution. Employing this technique on twenty different cancers, they found that each cancer had a specific miRNA profile and that most poorly differentiated tumors could be classified to their tissues of origin based on their miRNA expression levels [10]. Through various techniques, numerous groups have gone on to show that different cancer types have distinct miRNA profiles. Cancer-specific miRNA signatures can be useful for classifying tumor origin of poorly differentiated tumors, and being able to determine the tissue of origin of these tumors may be valuable in determining a patient's course of treatment.

While cancer-specific miRNAs are also important for shedding light onto the molecular basis of cancer, being

able to identify cancer-specific miRNAs in the blood to be used as biomarkers of cancers could be vital in detecting early-stage cancers. This emerging field of study has only just begun identifying biomarkers in serum. MiRNAs are very stable in blood plasma and serum: they are well protected from RNases and remain stable after being subjected to harsh conditions [2, 4]. Therefore, their stability makes miRNA levels well suited for being tested in patient samples. The first serum-miRNA biomarker discovered was miR-21. Lawrie *et al.* determined that patients with diffuse large B cell lymphoma had high serum levels of miR-21, which associated with increased relapse-free survival [3]. Around the same time, Mitchell *et al.* used a mouse xenograft model where a human prostate cancer cell line was implanted into mice to show that there were tumor-derived miRNAs circulating in blood. They then found that in the sera of human metastatic prostate cancer patients, miR-141 was very highly overexpressed. In fact, miR-141 levels could identify prostate cancer patients with high sensitivity and perfect accuracy [4]. Furthermore, in a report published in this issue of *Cell Res*, Chen *et al.* looked at serum miRNAs from lung cancer, colorectal cancer, and diabetes patients and determined that each of these diseases had specific serum-miRNA profiles. In the sera of lung cancer patients, there were 63 miRNAs that were not found in normal sera. Interestingly, they also saw differences between the miRNAs found in the sera and blood cells of lung cancer patients, while there were no differences between sera and blood cells of healthy individuals. A sera-miRNA fingerprint was determined for colorectal cancer as well. While some of these miRNAs were in common with ones found in lung cancer sera, there were ~10 miRNAs specific to just lung or colorectal cancers. More specifically, this latest study determined that miR-25 and miR-223, miRNAs previously shown to be involved in tumor formation, were highly expressed in lung cancer sera, as compared to normal sera, by both Solexa sequencing and quantitative reverse transcription-PCR (qRT-PCR) and therefore biomarkers of non-small cell lung cancer [2]. These reports clearly show that cancers affect miRNA levels in the bloodstream, although it is still unclear how tumor miRNAs are making their way into the bloodstream. Tumor miRNAs may be present as a result of tumor cells dying and getting lysed or tumor cells releasing miRNAs into the surrounding environment.

While individual miRNA biomarkers are important discoveries, the variability among different patients, even with the same type of cancer, likely makes using just one marker an unreliable method of determining cancer status. However, if a miRNA biomarker fingerprint can be established, a more accurate assessment would be more likely. As technologies continue improving, miRNA profiling will get even easier, faster, and cheaper. In order to show their usefulness for detecting cancers early, it would also be interesting to determine how early these biomarkers become significant – when a cancer is at its earliest stages or perhaps even before a tumor has fully developed? At most, each of the previously mentioned studies identifying serum biomarkers only looked at ~150 cancer patients from very limited populations; therefore, further testing on larger populations will be necessary before any such markers can be proven to be useful in a clinical setting. Meanwhile, these studies show that analyzing miRNA levels in serum is a promising area for identifying biomarkers of cancer.

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