MicroRNAs: a novel class of biomarkers for diagnosis of cancer and other diseases
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:

A reason breast cancer survival rates are so high is that there are good methods for early detection of tumors.

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%
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.

Moreover, the invasive, unpleasant, and inconvenient nature of current diagnostic procedures limits their application.
There is a great need for identification of novel non-invasive biomarkers for early tumor detection.
miRNAs, a class of naturally occurring small non-coding RNAs of 19-25 nucleotides (19-25 nt) in length, have recently been linked to cancer development.
Recently, altered miRNA expression has been reported in various cancers.
Inhibition of miRNA function by a microRNA antagonist
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.
Different cancer types have distinct miRNA profiles
A microRNA expression signature of human solid tumors defines cancer gene targets

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.
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 (ZAP-90) that is associated with a positive prognosis at low levels.
This emerging field of study has only just begun identifying biomarkers in serum.

**Blood plasma** is the yellow liquid component of blood, in which the blood cells in whole blood would normally be suspended. It makes up about 55% of the total blood volume.

**Blood serum** is blood plasma without fibrinogen or the other clotting factors.
Biomarkers that can be sampled from body fluids, such as serum or urine, are particularly desirable. In recent years it has become clear that both cell-free DNA and mRNA are present in serum, as well as in other body fluids, and that these CNAs represent potential biomarkers.
miRNAs are present in serum and plasma

qRT-PCR can be used to monitor low microRNA levels specifically and sensitively
miRNAs are present in serum and plasma

MicroRNAs are present at similar levels in serum samples from different unrelated individuals. This finding indicates that in general microRNA levels are similar among individuals.

This finding supports the idea that changes in the levels of specific microRNAs might allow detection of clinical conditions.

MicroRNAs are also detectable in other body fluids, such as urine, saliva, amniotic fluid and pleural fluid.
MiRNAs are very stable in blood plasma and serum: they are well protected from RNases and remain stable after being subjected to harsh conditions.

Serum miRNAs are resistant to RNase A digestion.
MiRNAs are very stable in blood plasma and serum: they are well protected from RNases and remain stable after being subjected to harsh conditions

MicroRNAs in serum are sufficiently robust to serve as practicable clinical biomarkers.

The levels of different microRNAs in unfrozen serum do not change substantially over a 4 hour period at room temperature
MiRNAs are very stable in blood plasma and serum: they are well protected from RNases and remain stable after being subjected to harsh conditions.

Plasma has been reported to contain high levels of RNase activity.

**Synthetic miRNAs rapidly degraded when added directly to plasma**

These results confirm the presence of RNase activity in plasma and the sensitivity of naked miRNAs to degradation. The levels of endogenous miRNAs were not significantly altered indicating that endogenous plasma miRNAs exist in a form that is resistant to plasma RNase activity.
One tantalizing hypothesis is that they are packaged inside exosomes that are secreted from cells. Exosomes are 50-to 90-nm, membrane-bound particles that have been reported to be abundant in plasma and that have recently been shown to contain miRNAs.
miRNAs are the major fraction of small nucleotide species in serum as determined by Solexa sequencing.

The distribution of small RNAs of various lengths (18-30 bp) sequenced by Solexa.

Small RNAs were isolated from the serum of healthy male subjects (MS), female subjects (FS), non-small cell lung cancer patients (LCS), colorectal cancer patients (CCS), and diabetes patients (DS), as well as blood cells of healthy male subjects (MC), female subjects (FC), non-small cell lung cancer patients (LCC), colorectal cancer patients (CCC), and diabetes patients (DC).
most of the miRNAs (91 out of 101) are detected in both serum and blood cells, whereas only a small number of miRNAs were uniquely present in either serum or blood cells. **Under normal conditions** most serum miRNAs are derived from circulating blood cells.
Serum microRNA profiles reflect physiological conditions

The three placental microRNAs (miR-527, miR-520d-5p and miR-526a) are highly abundant in the sera of pregnant women and their levels rise as pregnancy progresses.
Serum microRNA profiles reflect physiological conditions

Number and overlap of miRNAs between female serum (FS) and male serum (MS) samples.

Among 101 miRNAs, 90 miRNAs were detected in the serum of both male and female subjects, while 10 and 1 miRNAs were only present in the serum of male or female subjects, respectively.

Male-specific serum miRNAs included miR-100, miR-184, and miR-923, while miR-222 represented a female-specific serum miRNA.
Comparison of miRNA Levels Between Plasma and Serum

Measurements obtained from plasma or serum were strongly correlated, indicating that both serum and plasma samples will be suitable for investigations of miRNAs as blood-based biomarkers.

Clinical specimens of serum are more plentiful than plasma samples in many retrospective clinical sample repositories.
Serum microRNA profiles reflect pathological conditions

The expression profile of miRNA in lung cancer serum (LCS) was significantly different from that of normal subjects (NS). Compared to healthy subjects, 28 miRNAs were missing and 63 new miRNA species were detected in the lung cancer patients.
Surprisingly, the miRNA profile of lung cancer serum (LCS) was also remarkably different from that of lung cancer blood cell (LCC).

This is a striking contrast to that of healthy subjects, in which serum and blood cells essentially share the same miRNA profile.
Serum microRNA profiles reflect pathological conditions

Colorectal cancer patients also had a significantly different serum miRNA profile compared to healthy subjects.

Colorectal cancer patients shared a large number of serum miRNAs with lung cancer patients.
Serum microRNA profiles reflect pathological conditions

Compared to healthy subjects, diabetes patients also had a significantly altered expression profile of serum miRNAs, though the change was not as drastic as that in cancer patients.
Serum microRNA profiles reflect pathological conditions

Besides 84 common miRNAs shared by diabetes serum (DS) and diabetes blood cell (DC), there were 17 and 27 miRNAs that were only found in DS and DC, respectively. Pearson correlation scatter plots showed the comparison of miRNAs between DC and DS and between DC and NC, respectively: alteration of serum miRNAs is more sensitive than that of blood cell miRNAs in reflecting the diabetic condition.
The sources of circulating miRNAs

During diseases such as cancer, serum miRNAs are derived from not only circulating blood cells but also other tissues affected by ongoing diseases.

These disease-related miRNAs in the serum can serve as potential biomarkers.

Furthermore, different miRNA profiles in serum versus blood cells under the disease state again support the conclusion that the serum miRNA profile is not simply a default product of broken blood cells but serves as an indicator of biological...
The sources of circulating miRNAs

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.
The sources of circulating miRNAs
Of the panel of 95 miRNAs analyzed, 5 miRNAs were up-regulated both in plasma and tissue samples. All the 5 miRNAs were validated on the plasma of 25 CRC patients and 20 healthy controls. Both miR-17-3p and miR-92 were significantly elevated in CRC patients (p<0.0005).
MiRNA expression is altered in Alzheimer’s cerebro spinal fluid
Sixty miRNAs were detected as significantly different (p< 0.05) between the Braak stage 5 and Braak stage 1 samples.

Whether miRNAs are present in CSF as a result of cell destruction, production of exosomes is unknown.
While some miRNAs are expressed at high levels in the brain (e.g., miR-181) or are highly enriched in the choroid plexus at the interface between blood and CSF (e.g., miR-204), there was no obvious relationship between the miRNAs altered in CSF and the absolute levels insites of AD mediated destruction. Some evidences suggest that the CSF samples are derived from immune cells in the CSF.
REFERENCES


