More Accurate Markers Identified for Detecting Response to Epigenetic Drugs for Myelodysplastic Syndromes

- The currently used marker has demonstrated inaccuracy.
- Researchers identified and verified two biomarkers.
- Findings could lead to a simple urine test to detect the effectiveness of epigenetic drug treatment.

WASHINGTON, D.C. — Researchers have identified and validated two DNA methylation markers that could help physicians to more accurately determine a patient’s response to epigenetic drugs for treatment of myelodysplastic syndromes (MDS), according to Xiaojing Yang, Ph.D., a postdoctoral fellow at the University of Southern California, Los Angeles, who presented the data at the AACR Annual Meeting 2013, held in Washington, D.C., April 6-10.

“The current feedback from physicians is that they cannot tell if a patient is really getting the epigenetic drug they are being treated with or not, which makes it difficult for them to decide whether to stop treatment or increase the dosage of the drug,” said Yang. “This pushed us to think, why? Why didn’t the current marker work and should we try to seek a better one?”

These drugs, called DNA methyltransferase inhibitors (DNMTi), work by turning on genes that suppress cancer development, according to Yang. In patients with MDS, these genes are often silenced by the attachment of chemicals called methyl groups to the DNA backbone of the gene (an epigenetic modification made through a process called methylation), and DNMTi prevent methyl group attachment to DNA.

Currently, measuring methylation changes in DNA sequences known as LINE-1 elements is widely used as a predictor of whether or not DNMTi are working, but recent research has found that LINE-1 remethylation after a DNMTi is withdrawn occurs faster than in other regions. This implies that LINE-1 methylation changes may not reflect overall demethylation effects of DNMTi, according to Yang.

Yang and colleagues sought to find improved markers of DNA methylation status. Using the Infinium DNA methylation platform, they assessed the methylation profile of 27,000 genomic regions. The team tested this methylation profile on both normal and tumor bladder tissue
samples and on white blood cells from healthy donors. They identified 1,429 regions that were consistently methylated in all three samples.

They then tested the methylation profile of these 1,429 regions in T24 bladder cancer and HL60 leukemia cell lines treated with a DNMTi for 24 hours. Of these, 79 significantly responded to demethylation treatment and remained demethylated beyond 30 days. Further analysis focused on the top two regions, which showed consistent hypermethylation in normal and tumor samples.

To verify their findings, Yang and colleagues studied the DNA demethylation levels of those two markers in urine samples from seven patients with MDS treated with the DNMTi azacitidine. They found that the two markers were significantly demethylated, in contrast to LINE-1 methylation, which showed no clear decreasing trend.

According to Yang, these findings could lead to the use of a simple urine test for detecting response to a DNMTi.

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Presenter: Xiaojing Yang, Ph.D.

Title: Identification of novel DNA methylation markers to track patient’s response to DNA demethylation agents

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The successful use of DNA methyltransferase inhibitors (DNMTis) in Myelodysplastic Syndromes (MDS) therapy has brought epigenetic drugs to the forefront of cancer management. However, it has been difficult to find a consistent association between patients’ outcome and DNA methylation changes. This could be attributed to a variety of factors including the choice of DNA methylation markers to track the response to treatment. LINE-1 methylation changes are widely studied as a marker predictive of genome-wide DNA methylation changes; however, recent reports have shown that its methylation levels are variable across tissues. In addition, we have found that LINE-1 remethylation after DNMTis withdrawal occurs faster than that of other regions, suggesting that LINE-1 methylation changes might not reflect the overall demethylation effects of DNMTis. Thus, it is imperative to find more sensitive DNA methylation markers to accurately track the methylation change that occurs after DNMTi treatment. Ideally, such markers could be applied to various tumor types and to samples collected by invasive and non-invasive methods. To find improved DNA methylation markers, we took advantage of the well-established Infinium DNA methylation platform and found 1429 probes, which were consistently methylated in both normal/tumor bladder tissue samples as well as white blood cells from healthy donors. The remethylation pattern of those 1439 probes was investigated in the T24 bladder cancer and HL60 leukemia cell lines treated with 5-Aza-CdR for 24 hours. Probe consensus clustering yielded a group of 79 probes that significantly responded to the demethylation treatment and remained demethylated beyond 30 days. In silico analysis of the DNA methylation patterns of the top two probes show consistent hypermethylation in both normal and tumor samples. As a proof of principle, we tested the DNA demethylation levels of these two markers in urine sediments from 7 MDS patients treated with Azacitidine using pyrosequencing. Our results showed that the two markers were significantly demethylated; in contrast, LINE-1 methylation showed no clear decreasing trend. Demethylation of these two markers was also observed in peripheral blood samples from MDS patients treated with Azacitidine. We are in the process of testing more samples to find the association between marker demethylation and patient’s outcome.
In summary, we have identified and validated two DNA methylation markers, which unlike LINE-1, show consistent demethylation in response to the DNMTi treatment irrespective of the type of sample tested. The wider range of demethylation provided by these markers may offer a more accurate representation of the patient’s response to treatment.