Adding Methylation Data to the Mix

How methylation profiling is emerging as a powerful new omic tool

Long after the dust was cleared from the World Trade Center (WTC) site after Sept. 11, medical scientists have been trying to understand the wider range of health effects from the disaster on survivors. Now a sharp new tool, DNA methylation analysis, is helping with this effort and other major scientific challenges, including cancer diagnosis.

Methylation has long been considered a promising target linking environmental exposure and cancer. Now specialized tools, such as Illumina's Infinium™ MethylationEpic arrays, are helping advance the field at a fast pace. This array allows scientists to interrogate more than 850,000 methylation sites quantitatively across the genome at single-nucleotide resolution. It can analyze multiple sample types in parallel, including FFPE, for high throughput while minimizing cost per sample and providing highly reproducible results.

The research mentioned above used this platform to study differences in DNA methylation between WTC-exposed and unexposed survivors.[1]

That was a pilot study whose primary goal was to assess the feasibility of research to "address the hypothesis that complex exposures to the World Trade Center dust and fumes resulted in long-term epigenetic changes," the authors explained in their paper.

They used blood samples from 18 WTC-exposed cancer-free women and compared results to those of 24 age-matched cancer-free women. Those subjects were from an existing prospective cohort and had donated their samples before Sept. 11, 2001. The functional genomic analyses included mapping the top 5000 differentially expressed CpG sites to the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway database.

The study found "substantial" differences between WTC-exposed and unexposed women. The top 15 differentially methylated gene probes included BCAS2, OSGIN1, EEF1A2, SPTBN5, CHD8, CDCA7L, AIDA, DDN, SNORD45C, ZFAND6, ARHGEF7, UBXN8, USF1, and USP12. The WTC-exposed subjects showed several enriched cancer-related pathways, including endocytosis, mitogen-activated protein kinase (MAPK), viral carcinogenesis, Ras-associated protein-1 (Rap1), and mammalian target of rapamycin (mTOR) signaling.

Cracking hard-to-diagnose tumors

While research like this WTC-related project is helping expand the field, cancer is currently the major focus for work using DNA methylation arrays.

For example, a high percentage of brain tumors cannot easily be distinguished using the traditional histopathology approach. As a result, "for many years some of us have been on a quest to improve diagnosis of cancer," says Matija Snuderl, M.D., a neuropathologist at New York University Langone Medical Center in New York. "DNA methylation is unique because it takes into account epigenetic changes, as well as unique signatures caused by driver mutations," he says.

Scientists around the world have set out to collect big enough libraries of methylation data to create diagnostic signatures. Langone collaborated with the German Cancer Research Center (DKFZ), in Heidelberg, on the creation of a landmark epigenetic map of brain tumors, as reported in Nature in 2018.[2]

"That study showed that 10% to 14% of brain tumors may be misdiagnosed using traditional diagnostics. We knew that DNA methylation can provide us with additional information not available by traditional techniques," said Snuderl, who is also director of Molecular Pathology and Diagnostics at NYU Langone and member of Perlmutter Cancer Center.

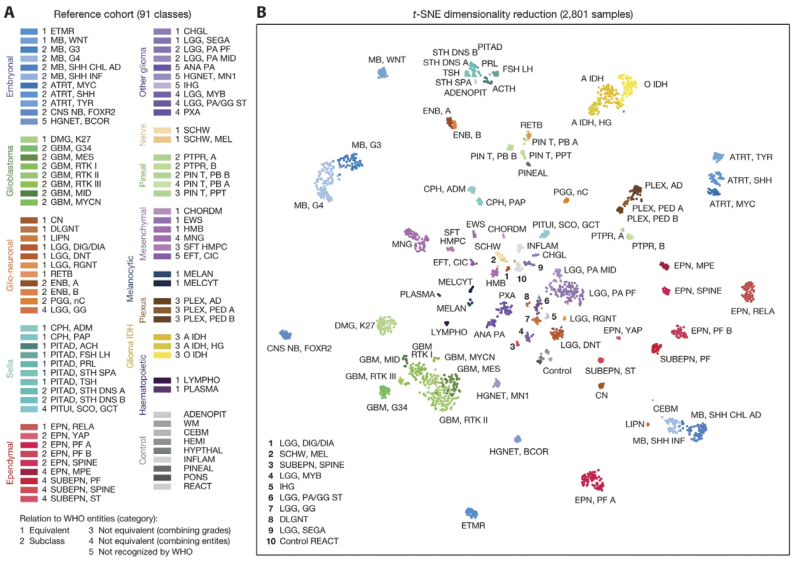
Snuderl and his colleagues went on to use machine learning to develop a methylation classifier using this data, and performed the clinical validation to be able to use the test at NYU Langone. He and his colleagues at Langone now test any patient's brain tumor sample for DNA methylation at no cost to the patient. Other scientists are also contributing to the database and using it as a research tool. All the new data helps the Langone team make their diagnostic algorithm even more robust.

The research database, Snuderl and his colleagues hope, will lead to further clinical gains. He says that in his experience, "DNA methylation can make the diagnosis more accurate, or even flat out change it."

A new angle on brain tumors

Likewise, Gelareh Zadeh, M.D., Ph.D., FRCPC, FRCSC, is also looking at DNA methylation to diagnose brain tumors. Zadeh is the Head of the Division of Neurosurgery and Medical Director for Krembil Neuroscience and senior scientist at UHN's Princess Margaret Cancer Centre in Toronto. She and her colleagues have also developed a methylation-based molecular signature.[3]

Zadeh points to the 2018 Nature paper[2], which was based on several thousand samples, as a key advance in the field. "The methylation signature is distinct between the wild type and the mutant," she says.



A. Overview of the 82 CNS tumor methylation classes and nine control tissue methylation classes of the reference cohort. The methylation classes are grouped by histology and color-coded. Category 1 methylation classes are equivalent to a WHO entity, category 2 methylation classes are a subgroup of a WHO entity, category 3 methylation classes are not equivalent to a unique WHO entity with combining of WHO grades, category 4 methylation classes are not equivalent to a unique WHO entity with combining of WHO entities, and category 5 methylation classes are not recognized as a WHO entity. Full names and further details of the 91 classes are included in Supplementary Table 1. Embryonal tumors, shades of blue; glioblastomas, shades of green; other gliomas, shades of violet; ependymomas, shades of red; glio-neuronal tumors, shades of orange; IDH-mutated gliomas, shades of yellow; choroid plexus tumors, shades of brown; pineal region tumors, shades of mint green; melanocytic tumors, shades of dark blue; sellar region tumors, shades of cyan; mesenchymal tumors, shades of pink; nerve tumors, shades of beige; haematopoietic tumors, shades of dark purple; control tissues: shades of grey. **B.** Unsupervised clustering of reference cohort samples (n = 2,801) using t-SNE dimensionality reduction. Individual samples are color-coded in the respective class color (n = 91) and labelled with the class abbreviation. The color code and abbreviations are identical to A.

Reprinted by permission from Nature: *Capper, D., Jones, D., Sill, M. et al. DNA methylation-based classification of central nervous system tumours.* Nature *555, 469–474 (2018). https://doi.org/10.1038/nature26000*

One of the most common types of tumors are meningiomas. These are often benign and require nothing more than surgery, but they can also be aggressive and require further treatment. As a result, it's important to be able to distinguish between the two types. However, traditional histopathology often gives ambiguous results, and there are 15 established subtypes alone by the WHO classification scheme. Researchers like Zadeh want to layer epigenetic data over the available molecular data. Additional layers, they hope, will provide finer distinctions between tumors.

Zadeh and her colleagues used DNA methylation profiles of clinically annotated tumor samples from multiple institutions to develop a methylome model of five-year recurrence-free survival (RFS) in meningioma patients. They then generated a five-year recurrence score using a nomogram that integrated the model with established prognostic clinical factors. Both models were then evaluated and compared with standard-of-care models using multiple independent cohorts.

The researchers concluded that their models "provide important prognostic information not captured by previously established clinical and molecular factors," and that they could be used to individualize decisions about treatment, "in particular, whether to treat patients with adjuvant radiotherapy versus observation alone"

"We are probably one of the few sites in the world that has such a robust model in hand," Zadeh says.

DNA methylation analysis, Zadeh adds, "is catching on here, because we typically have 100 or so cases per year. Considering we've been handling COVID-19 patients, that's a significant number." She hears interest in the platform from peers as well as patients. "It's reassuring that we can demonstrate the value of this technology by showing results."

References

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