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Q&A

By Eleftherios P. Diamandis[1]

Epigenomics-Based Diagnostics

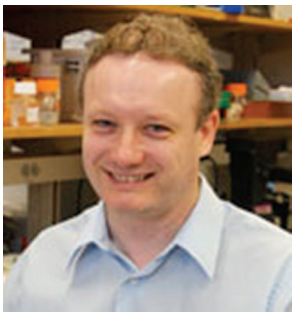
The term "epigenetics," first coined in 1942, refers to heritable traits of cells (over many rounds of cell division) that do not involve changes to the underlying DNA sequence. The 2 predominant epigenetic mechanisms are DNA methylation and histone modification.

Epigenetic changes, especially methylation of DNA at the 5 position of cytosine, which occurs in cytosine/guanine-rich regions (CpG islands), may have major effects on gene transcription. For example, it is known that extensive methylation at GC-rich regions of gene promoters or other areas can dramatically affect gene transcription and consequently the whole biology of a particular cell. Epigenetic changes are preserved when cells divide. Although it is well-known that cancer may be caused by the loss of tumor suppressor genes (e.g., through mutation or deletion), another major mechanism of silencing critical genes is through methylation. There are numerous examples of genes that can be silenced by methylation. It is thus reasonable to suggest that DNA methylation may be a marker of gene silencing and that such modifications may correlate with cancer initiation and progression. For this reason, it is likely that epigenetic changes in DNA may carry diagnostic, prognostic, or predictive information. It is thus not surprising that epigenomic/epigenetic changes have attracted increased attention recently for their possible application in cancer and other disease diagnostics. In this Q&A, 4 experts in the field discuss the present and future of epigenomics as they apply to diagnostic applications.

Could you briefly describe the field of epigenomics?

David Sidransky[2]**:** The field of epigenomics is focused on finding genomic alterations beyond changes in DNA sequence.

Peter W. Laird[3]**:** Epigenomics is the genome-scale study of the distribution of epigenetic marks, which are stable modifications to DNA or DNA packaging that convey nongenetically encoded heritable information. Although there is some debate as to which marks are faithfully preserved during cell division, it is generally accepted that epigenomic marks include cytosine-5 DNA methylation, modifications of histone tails, and nucleosome positioning.



Paul Cairns[4]**:** The study of DNA methylation, histone modifications, the chromatin state, and RNA interference, and the related effect on gene expression in different types of normal cells that share an identical genome, between the normal progenitor cell and tumor cell, and in other human diseases.

Bharati Bapat[5]**:** Epigenomics refers to the regulation of genome by heritable changes in gene expression mediated by non–DNA sequence factors. These include DNA methylation, regulation of chromatin structure and function, and small noncoding RNA–mediated regulation. A unique feature is that unlike mutations in DNA sequences, many of

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[1] Eleftherios P. Diamandis, Head of Clinical Biochemistry, Department of Clinical Biochemistry, Mount Sinai Hospital and University Health Network, and Professor and Head, Division of Clinical Biochemistry, Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Ontario, Canada.
[2] David Sidransky, Director, Head and Neck Cancer Research, Johns Hopkins Sidney Kimmel Cancer Center, and Professor, Otolaryngology and Oncology, Johns Hopkins University, Baltimore, MD; and Chair, Early Detection Research Network, National Cancer Institute, Rockville, MD.
[3] Peter W. Laird, Director, USC Epigenome Center, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, and Associate Professor, Departments of Surgery and of Biochemistry and Molecular Biology, University of Southern California, Keck School of Medicine, Los Angeles, CA.

these alterations are potentially reversible in nature and thus provide attractive targets for therapeutic approaches.

What techniques are used for epigenomic diagnostics? Are these easily adaptable to diagnostic labs?

David Sidransky: There are many techniques used to find epigenomic changes in cancer cells. For promoter methylation, these include sequencing of modified DNA, array-based approaches, and quantitative PCR-based assays. Yes, all of these approaches could be adapted into diagnostic laboratories. Some, like quantitative PCR, are already integrated into many laboratories.

Peter W. Laird: Genome-scale techniques for epigenetic analysis are technically challenging and unlikely to be adapted by diagnostic laboratories in the near future. Gene-specific assays based on real-time PCR are more easily adapted to commercial platforms present in diagnostic laboratories and offer the advantage of increased technical sensitivity and specificity.

Paul Cairns: Methylation-specific PCR is the predominant technology used for epigenomics-based diagnostics. The availability of commercial bisulfite-modification kits and quantitative real-time PCR technology means that methylation is more readily adaptable to diagnostic laboratories. More recently, quantitative reverse-transcription PCR has been used for detection of microRNA expression as a diagnostic tool.

Bharati Bapat: Several techniques are available to detect epigenomic changes. DNA methylation is detected by sequence analysis of bisulfite-modified DNA. For epigenetic diagnostics, DNA methylation and microRNAs are detected by quantitative PCR–based assays and high-throughput microarray–based techniques. PCR-based assays are easily adaptable to diagnostic laboratories and are already implemented in cancer diagnostics.

What are the distinct advantages of epigenomics over other techniques for diagnostics? What do you think are the most serious disadvantages of epigenomics-based diagnostics?

David Sidransky: In cancer, promoter methylation yields a positive signal, so finding methylated alleles makes the job of finding rare cells or molecules easier than looking for events that are absent in cells. As opposed to genetic mutations, where a set of tumors may have hundreds of different mutations, defining dense methylation at one locus is easier and more amenable to high-throughput techniques.

The disadvantages of this approach are that epigenetic changes are diverse and require various types of materials and assays. Also, one still has to test several loci or alterations to cover a tumor type.

Peter W. Laird: Epigenomic profiles are fairly stable and relatively impervious to fluctuations in physiological state and sample-collection conditions. DNA-methylation assays have an advantage that the DNA analyte is resistant to degradation, both in vivo and after obtaining the sample. The epigenetic marks at a region of the genome determine the potential for gene expression, rather than the actual expression state. Therefore, the epigenome conveys information not fully captured by gene expression profiles. The most serious disadvantage of epigenomics-based diagnostics is the technical difficulty of epigenomic analysis.

Paul Cairns: For diagnosis of cancer the distinct advantages are that aberrant hypermethylation is frequent and early in tumorigenesis, that it is a positive change, and that sensitive detection technology is available. The disadvantages of methylation are that a relatively large amount of biospecimen is needed (as with any nucleic acid–based target of detection) and that the robustness of the assay is dependent upon careful design and operation.

Bharati Bapat: The detection of epigenetic changes, particularly DNA-methylation changes, has several advantages over conventional genetic markers. For a given gene, point mutations often occur at several sites in individual tumors. In contrast, DNA methylation usually occurs over the same region of the gene (e.g., promoter), and this greatly simplifies the design and interpretation of screening tests. DNA methylation constitutes a positively detectable signal, as opposed to a loss of signal, such as chromosomal deletions. Abnormal DNA methylation usually does not occur in normal cells. Therefore, tumor-derived DNA can be de-

[4] Paul Cairns, Associate Professor, Departments of Surgical Oncology and Pathology, Fox Chase Cancer Center, Philadelphia, PA.
[5] Bharati Bapat, Scientist, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and Professor, Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

tected with a high degree of specificity, particularly in body fluids such as sputum, serum, or urine samples.

The main disadvantage of epigenomics-based diagnostics is that several different assays may be required to detect different types of epigenetic changes in a cancer.

What are the most preferred cancers for diagnosis by epigenomics?

David Sidransky: All cancers have epigenomic alterations. Lung, breast, prostate, head and neck, and colon cancer may also be amenable to early-detection approaches.

Peter W. Laird: Epigenomic abnormalities have been described for a wide variety of cancer types. Epigenomic profiles can be used to identify molecular subtypes of a particular cancer, with associated clinical outcomes. An example of this would be CpG island methylator phenotypes, described for several types of cancer. Abnormal DNA-methylation patterns can be used to detect the presence of preneoplastic or malignant cells in luminal samples or tissue biopsies for cancers in accessible sites. Abnormal DNA methylation can also be detected in free-floating tumor-derived DNA in the bloodstream of some cancer patients. Such assays are best applied to cancers with well-established follow-up detection procedures, such as colonoscopy for colorectal cancer.

Paul Cairns: There are feasibility studies of diagnosis of all types of cancer in the literature. Cancers where tissue biopsies are obtained probably have the most traction because they generally contain a higher proportion of neoplastic cells compared to a body fluid specimen.

Bharati Bapat: All cancers carry epigenetic alterations. At present, DNA-methylation detection assays are offered for the detection of prostate, lung, and brain cancers, and also certain subtypes (mismatch-repair deficient) of colon cancer. MicroRNA detection assays are also currently being developed for the detection of pancreatic and other cancers.

Are there epigenomic diagnostic applications besides cancer that have been considered?

David Sidransky: I am not an expert in this area.

Peter W. Laird: Epigenomic characterizations are under way for a large number of chronic conditions, including psychiatric and neurodegenerative diseases, cardiovascular disease, and diabetes. Two major problems confront the use of epigenomic diagnosis for such diseases. First, these diseases generally do not have clonal expansion of cells with a defined epigenetic abnormality, and, second, the disease target tissue may not be readily accessible for diagnostic assays. To the extent that the disease is reflective of systemic epigenomic alterations, surrogate tissues such as white blood cells may be appropriate diagnostic sources of DNA or chromatin.

Paul Cairns: Yes, for two diseases, Prader–Willi and Angelman syndromes, where a minority of cases arise from a defect in imprinting.

Bharati Bapat: Other epigenetic diagnostic applications are not as common as those seen for cancer diagnostics. However, DNA-methylation analysis is one of the diagnostics tests offered for inherited genetic disorders associated with "genomic imprinting," such as Angelman syndrome, Prader–Willi syndrome, and Beckwith–Wiedemann syndrome. The last two disorders are associated with tumor development.

Are there any commercial assays for epigenomic diagnostics at the moment? Are these used at the clinic?

David Sidransky: *MGMT*[6] (*O*-6-methylguanine-DNA methyltransferase) methylation in glioblastoma and *GSTP1* (glutathione *S*-transferase pi 1) methylation in prostate cancers have well-worn studies and are offered now in CLIA laboratories. Both are likely to be approved relatively soon by the US Food and Drug Administration.

Peter W. Laird: I have a conflict of interest in answering this question.

Paul Cairns: Hypermethylation of the *MGMT* gene promoter for prediction of response to temozolomide in glioma tissue, *GSTP1* and *APC* (adenomatous polyposis coli) hypermethylation in prostate biopsy tissue for detection of cancer, methylation of vimentin in stool-based screen for colorectal cancer, and *SEPT9* (septin 9) methylation in serum DNA for colorectal cancer. There are also assays for Prader–Willi and Angelman syndromes.

Bharati Bapat: Yes. Detection of *GSTP1* methylation in prostate cancer, *MLH1* [mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*)] methylation for

[6] Human genes: *MGMT*, *O*-6-methylguanine-DNA methyltransferase; *GSTP1*, glutathione *S*-transferase pi 1; *APC*, adenomatous polyposis coli; *SEPT9*, septin 9; *MLH1*, mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*); *BRCA1*, breast cancer 1, early onset.

mismatch-repair–deficient colon cancer, and *MGMT* methylation in glioblastoma are a few examples of such assays used in the clinic.

What is your view of the future of epigenomic-based diagnostics over the next 10 years?

David Sidransky: Epigenomic tests will be incorporated into most cancer types for early detection, prognosis, and prediction for therapeutic response.

Peter W. Laird: The clinical application of epigenetic diagnostics is likely to be restricted to DNA-methylation markers in the near future. The rapidly declining cost of genomic sequencing may enable genome-wide epigenomic profiling within the next decade. However, it is more likely that genome-wide approaches will be used for marker discovery in research settings and that epigenetic clinical tests will be based on individual genes, or on panels of genes.

Paul Cairns: For cancer, it will depend upon our understanding of early tumorigenesis and in particular the interface between aging and cancer. It is possible that aberrant methylation becomes a marker of risk of cancer. For other common diseases, as Andy Feinberg has pointed out, alterations to the epigenome accumulated over life are at least as compelling an underlying cause for different susceptibilities to disease between individuals as, say, single-nucleotide polymorphisms, and epigenomics in disease other than cancer is becoming an area of intense study.

Bharati Bapat: Epigenomics-based diagnostics is an emerging area with great potential for clinical application. In the future, DNA-methylation and microRNA-based assays will be progressively incorporated as cancer biomarkers of early detection and prognosis and as predictors of response to therapy.

In the context of personalized medicine, how will epigenomics interface with other molecular diagnostic developments?

David Sidransky: Epigenomics will be one of several commonly tested alterations in defining the best way to manage or treat a patient with cancer.

Peter W. Laird: Epigenomic profiles convey information that complements gene expression and mutation profiles. Some molecular subtypes, such as CpG island methylator phenotypes are more easily recognized by epigenomic profiling than by expression, copy number, or mutation profiling. Therefore, I anticipate that epigenomic analysis will be an indispensable tool in our diagnostic arsenal to select the most appropriate therapeutic strategies for each patient.

Paul Cairns: For cancer, for example, it is very likely that intrinsic and acquired chemoresistance will have an epigenetic component. Inactivation of *BRCA1* (breast cancer 1, early onset) by hypermethylation in sporadic breast or ovarian cancer should confer similar sensitivity to poly(ADP-ribose) polymerase inhibitors as do inactivating point mutations in the hereditary cancers. For other common human diseases it is possible, even likely, that epigenomic background will be as important as the genome in susceptibility.

Bharati Bapat: Detection of one or several epigenetic biomarkers will be integrated with other molecular diagnostics tests to determine the optimal strategies for cancer patient treatments.

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