Clinical Epigenetics

EDITORIAL

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Clinical promise and applications of epigenetic biomarkers

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Epigenetic dysregulation is involved in a wide spectrum of diseases, and many studies have reported differential molecular epigenetic signatures between diseased tissues/cells and healthy control samples [1, 2]. Such epigenetic signals may point to promising therapeutic targets, but may also have great potential as biomarkers. Indeed, increasing evidence shows that epigenetic biomarkers have potential for prediction [3], early diagnosis [4–6] or prognosis [7] of disease, archiving a life course of environmental exposures [8, 9], as well as stratifying for and monitoring of therapy [3]; some have linked epigenetic variability to predictions of mortality [10–12]. Although in the field of oncology, various commercially available kits already exist [1, 13], clinical applications are lagging behind the discoveries of new biomarkers in other common diseases. In this thematic series of *Clinical Epigenetics*, we define the current progress in the development of epigenetic biomarkers for clinical use in cancer as well as in other common complex diseases and aging and include a variety of contributions ranging from original research to opinion pieces [14–17] and reviews [18, 19]. In assembling this collection, we also highlight critical issues related to the Ethical, Legal and Social Implications (ELSI) of epigenetics research [14, 15]. This includes the growing responsibility for us as scientists to investigate and uncover the mechanisms relating social determinants to human health [16] as technology advances and social inequities persist. Although epigenetic research and progress has accelerated dramatically in the past decade, the interest in applications of epigenetic age analysis in the realm of life insurance, direct to consumer testing and confirmation of juvenile-age for asylum (immigration) [14], highlights the obligation of engaged scientists to promote research standards and provide balanced interpretations of epigenetics observations.

In this *Clinical Epigenetics* series on epigenetic biomarkers, the range of topics (Fig. 1) supports the growing clinical translational relevance of epigenetic research, especially research on DNA methylation variability. Although best developed for cancer, the emergence of high-throughput assays has supported the development of epigenetic epidemiological research of (fetal) exposures and other common diseases.

The current collection of articles addresses fetal origins of complex diseases [20], lifestyle associations with the epigenome [8, 9, 21, 22], as well as predictive marks of heart disease [9, 21, 23–27] and cancer [5–7,

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Fig. 1 Word cloud showing the range of topics in the Clinical Epigenetics series on Epigenetic Biomarkers

22, 28–32]. Epigenetic marks of cancers have advanced beyond complex disease, and this special collection highlights several innovations including the application of cell-free DNA methylomes providing insights to ovarian cancer [5], a custom sequencing panel for acute myeloid leukemia [7], as well as advances with promise of clinical translation for lung [32], breast [22], renal [31], colorectal [29] or oropharyngeal cancers [28]. This compendium on cancer also includes a three gene test for cervical intraepithelial neoplasia and cervical cancer [6]. As the consideration of a clinical translational role for epigenetic biomarkers matures, we have also observed the potential relevance of fetal programming of adult diseases through the evaluation of placental methylomes [20], and advancements of insights to capture impacts of both social determinants [16] and lifestyle exposures [8, 9] on the epigenome. For clinical applications to nonneoplastic disease, multiple studies [21, 23, 24] have addressed the relevance of peripheral methylomes to cardiovascular disease, including the calculation of epigenetic clocks [21]. Methylation biomarkers in neurologic diseases and syndromes using leukocyte DNA holds particular promise as evidenced through the prediction of stroke risk [26], characterization of stroke outcomes [25], as well as the prediction of multiple sclerosis severity through the development of epigenetic risk scores [33]. Applications to ulcerative colitis [34] highlight opportunities for pharmaco-epigenetic targeting in nonneoplastic disease, here immune cell targets, and considerations for organ transplantation [19] may transform the clinical landscape related to posttransplant complications.

DNA methylation biomarkers

Although histone modifications and DNA hydroxymethylation might prove useful epi-markers in the future, so far DNA methylation has been exploited most for epigenetic biomarker development mainly due to relatively high stability of this epigenetic modification. A variety of methods are available to measure differences in DNA methylation. Most assays make use of bisulfite conversion before the methylation analysis, although emerging technologies avoid this potentially harsh DNA treatment in favor of bisulfite-free approaches [35–37]. For single gene analysis, the most common assays are (quantitative) methylation-specific PCR ((Q)MSP), bisulfite pyrosequencing, combined bisulfite restriction analysis (COBRA), targeted bisulfite sequencing and methylation-sensitive high-resolution melting (MS-HRM) [38, 39]. For diagnostic methylation assays of specific genes, QMSP is the most commonly used technique followed by bisulfite pyrosequencing [38–41] and can be regarded as established techniques applicable for biomarker development and clinical implementation [39].QMSP is a specific and sensitive method that allows accurate quantification, high-throughput testing and only requires a minimal amount of input DNA [40, 41]. The advantage of bisulfite pyrosequencing is that it provides an absolute level of methylation by determining the ratio of methylated and unmethylated cytosine residues separately [39, 42]. Disadvantages of the bisulfite pyrosequencing are that it is less sensitive to quantify methylated CpGs from dysplastic cells in an environment of many normal cells as found in multiple body materials, is only possible when sufficient CpG-free flanking sequences are available for primer design and is relatively expensive [42]. The analytical sensitivity of the methylation detection assay provided by PCR-based methods appears to be critical for diagnostic applications of epimutation testing. For example, using the same samples, MS-HRM was able to detect *BRCA1* epimutations, whereas the Illumina BeadChip microarray was not [43].

Analysis on the genome-wide level can be achieved by methylation (micro)arrays preceded by bisulfite conversion, such as EPIC arrays, immunoprecipitation of methylated DNA combined with next-generation sequencing and genome-wide bisulfite sequencing. Since the introduction of standard arrays allowing genome-wide interrogation of methylation over 10 years ago, epigenome-wide association studies (EWAS) have become a popular approach to identify biomarkers for both environmental exposures and disease outcomes [44, 45]. Case–control or prospective (longitudinal) cohort studies are the most widely used designs for the discovery of epigenetic biomarkers in EWAS [45]. To ensure the quality of reporting of such studies, general reporting guidelines on observational studies in epidemiology as specified in the STROBE (STrengthening the REporting of OBservational Studies in Epidemiology) statement should be used (see also: https://www.equator-network.org/reporting-guidelines/). However, reporting guidelines for observational studies such as case–control or cohort designs as specified in the STROBE checklist [46] are very general and guidelines specific for EWAS do not exist.

Similar to genome-wide association studies (GWAS), the early years of EWAS were characterized by a lack of consensus on data preparation and quality control, statistical analysis and reporting standards, including issues related to replication, multiple comparisons and generalizability. In essence, both GWAS and EWAS are observational studies investigating the association of either genetic (single-nucleotide polymorphisms [SNPs]) or methylation markers (CpG sites) with outcome traits. For GWAS, reporting guidelines were drawn up by the STrengthening the REporting of Genetic Association Studies (STREGA) panel [47] by extending the STROBE checklist with a number of items particularly relevant to genetic association studies, such as laboratory methods related to genotyping, genotyping accuracy, population stratification and adjustment for multiple testing. Unfortunately, such reporting guidelines and standards are lacking for EWAS making it more difficult to ensure standardized reporting and quality of such studies. In general, the study design and statistical analyses of biomarker discovery studies such as EWAS need to minimize sources of bias and optimize the chances of reporting true findings. For the latter, similar to GWAS, sufficient sample size (i.e., power), proper adjustment for multiple testing and replication of the findings in an independent sample are key. Unlike GWAS, epigenetic profiles are tissue specific, and lack of proper adjustment for cell-type heterogeneity is now generally recognized as one of the most important sources of confounding in EWAS [17].

A recent review concluded that the recent rise in EWAS has aided discovery of epigenetic biomarkers of disease outcomes, but translation of these findings into clinically useful applications such as prognostic biomarkers and therapeutic targets in the epigenome has so far been limited by inappropriate or inadequate statistical analyses, insufficiently powered studies, non-validated findings and an inability to establish causality [45].

Implementation of epigenetic biomarkers in health care

For the implementation of a new biomarker in health care, it is recommended to adhere to a five-phase framework as described by Pepe et al. [48]. The 5 phases are: (1) preclinical exploratory studies, (2) assessment in

noninvasive samples, (3) retrospective longitudinal studies, (4) prospective screening studies and (5) prospective intervention studies. Most importantly, for all phases, but especially for phases 4 and 5, blinding and randomization are essential to robustly validate biomarkers [48]. Most studies investigating DNA methylation marks as diagnostic tests are in phase 1 and 2. Only a few studies analyzed the application of methylation markers in prospective studies [49–54]. So far, only the promoter hypermethylation biomarkers *GSTP1* and *MGMT* have been implemented in health care, with *GSTP1* as a powerful diagnostic tool for prostate cancer [55, 56], and *MGMT* predicting a better response to treatment with alkylating agents chemotherapy in glioblastomas [3, 57].

Journal guidelines

Given the lack of specific reporting guidelines for epigenetic biomarker studies such as EWAS and in order to take epigenetic biomarker research to a higher level *Clinical Epigenetics* has formulated a number of key requirements. For a biomarker study, to be considered for publication in *Clinical Epigenetics*, the manuscript should: (i) contain a discovery and an independent validation samples, i.e., biological (rather than technical) replication. Validation data might also be obtained from publicly available repositories. Potential exceptions include rare diseases or findings yielding strong scientifically novel insights with convincingly described clinical relevance; (ii) provide access to raw data (according to the Findable Accessible Interoperable Reusable [FAIR] principles). This makes replication and verification of research easier, and the data can be used to study new research questions; (iii) have sufficient sample size (i.e., power) to detect realistic effect sizes and apply proper adjustment for multiple testing; (iv) when only using pre-existing datasets, include functional validations or present a solid discussion on functional implications.

Conclusions

It is important to have quality standards for (reporting on) epigenetic biomarker studies, as this ensures realistic expectations regarding the promise of new (classes of) biomarkers that are still at the research stage and/or in preclinical development. In tandem, this collection has highlighted some promising innovations and technical advances beyond data analytics, including advances related to the interrogation of cell-free DNA for cancer (ovarian) [5] and non-cancer (vascular) applications [27]. The growing potential of single cell and spatial technologies [58, 59] will likely further improve the translational success rate of these biomarkers into the clinic, establishing a clear role for epigenetics in precision medicine.

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