Published in final edited form as:

Fertil Steril. 2016 September 1; 106(3): 551. doi:10.1016/j.fertnstert.2016.06.008.

Differential DNA methylation analysis optimally requires purified cell populations

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Because each cell type has a unique epigenome to help provide cell specificity, the use of a mixed cell population in the analysis of DNA methylation is a reflection of all cell types present. Therefore, changes in cell population number or composition can suggest differential DNA methylation that is not due to alterations in DNA methylation. The analysis of differential DNA methylation optimally requires the use of purified cell populations.

There are over 200 cell types in the human body, and each tissue contains multiple cell types. Blood contains over 20 different cell types. Although all cell types contain the same DNA with a similar DNA sequence, each cell type has a very distinct epigenome to give the cell its cell specificity. Therefore, the difference between a neuron and a hepatocyte is in large part due to the distinct epigenomes of the two cell types. Because all cell types have the same DNA and sequence, genetic studies can easily use mixed cell populations as well as the whole organism, if required, to do genetic analysis. In contrast, epigenetic investigations, such as differential DNA methylation analysis, require consideration of mixed cell populations and purity of the cell types used.

One of the first types of studies to investigate environmental epigenetics in humans used twin studies with discordant disease and whole blood analysis (1). More recently the need for the use of purified cell types is appreciated in epigenome-wide association studies EWAS) with the use of purified cell types (2). Although interesting observations have been provided with blood analysis, due to the presence of over 20 cell types interpretation of the observations is difficult. Small changes of 10% within different cell populations can generate a change in DNA methylation that is not due to alterations in DNA methylation at the specific genomic sites. Subsequent analysis of purified cell types would be required to confirm the differential DNA methylation analysis.

There have been attempts to develop bioinformatics approaches to assess epigenetics in mixed cell populations (3), but without extensive information on different cell types epigenomes this type of approach is in its early days. When simply mapping epigenetic marks to the genome and not looking at differential DNA methylation regions (DMR), this issue of mixed cell populations is less problematic. There are also specific epigenetic sites that are consistent between cell populations, and a current example of this is imprinted

genes. Those imprinted DNA methylation regions are generally conserved between cell types, so a mixed cell population can be considered for such sites. However, imprinted genes are generally less responsive to environmental factors, so they do not generally change. Certainly the vast majority of DNA methylation regions do not have this property. Future improvements in bioinformatics and simulation analysis with more detailed epigenome maps for various cell types with in a mixture such as blood may improve mixed cell analysis, but these resources are not currently available.

A study in this issue of *Fertility and Sterility* by Estill et al. (4), "Assisted Reproductive Technologies Alter DNA Methylation Profiles in Bloodspots of Newborn Infants," provides the interesting observation that assisted reproductive technologies (ART) may impact the epigenetics of the child. A limitation in this study is that whole blood was used such that a mixed cell population complicates any data interpretation. The authors concluded that this was not an issue, but changes in cell population percentiles can impact the differential DNA methylation data observed. Future studies will need to confirm and validate the specific DMR observed using purified cell types. However, this study does provide the potential sites for further investigation and suggests that ART and these DMRs need further investigation.

In contrast with genetic analysis, epigenetic analysis optimally requires purified cell populations. Changes in mixed cell populations of 5% to 10% can suggest the presence of a DMR that is not due to epigenetic change at the DMR but rather to alterations in cell populations. This limitation of a mixed cell population analysis needs to be noted and confirmed with purified cell populations. Future technology, bioinformatics, and epigenome resources may facilitate mixed cell population studies and not require confirmation with purified cell types. Currently, epigenetic analyses such as differential DNA methylation region identification optimally require purified cell populations in the experimental design.

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