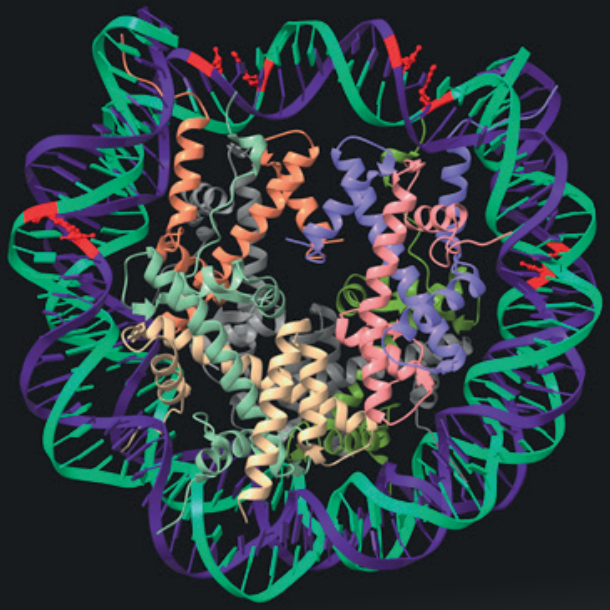


Epigenetic diagnostics and preventive medicine

Human nucleosome with CpG methylated (red) DNA Source: Shutterstock.com

Although we have a reasonable understanding of a number of mechanisms of epigenetic regulation, we are still have a lot more to learn about the impact and influence of epigenetic regulation, particularly in relation to disease. CLI caught up with Professor Michael Skinner (Center for Reproductive Biology, Washington State University, USA) to find out more about the effect of environmental factors on epigenetics and how this influences disease susceptibility.

What is epigenetics?

Epigenetics is defined as "molecular factors and processes around DNA that regulates genome activity (i.e. what genes are on or off), independent of DNA sequence, and are mitotically stable (i.e. when cells replicate)". The five main types of epigenetics are DNA methylation of CpG sites, histone modifications, non-coding ncRNA, chromatin structure, and RNA methylation. All gene expression requires corresponding epigenetic alterations to allow it to occur or shut it off.

What is an example of a 'normal' (i.e. non-disease state) role of epigenetics?

Epigenetics is required for all organisms and essential for all gene expression processes. There are no exceptions. Therefore, it is a required and normal molecular process. Specific positive or negative epigenetic changes are what allow a gene to be on and expressed or turned off. The first epigenetic process identified in the 1980s was X-chromosome inactivation via DNA methylation. The second was gene imprinting, which allows a specific paternal or maternal allele to be on or off and was described in the 1990s. The next was histone modifications in the 1990s, and then non-coding RNA around 2000.

The prevalence of many conditions and diseases has increased dramatically over the last 50 years. For many of these, traditional genome-wide association studies have identified genetic mutations linked to the pathology, but nevertheless these mutations only correlate with 1% of the disease population. How can epigenetic analysis help in our understanding of such conditions/diseases?

One of the primary functions of epigenetics is to respond to the environment and regulate gene expression. Therefore, environmental toxicants can alter the normal epigenetics directly to then increase later-life susceptibility for disease. In the last few decades, the number of environmental toxicants has increased dramatically, so we see a corresponding increase in most disease frequency. The primary source for abnormal physiology and disease is through environmental epigenetics, since the environment cannot generally cause genetic mutations in DNA sequence. This is why the frequency of autism has increased over tenfold in the last few decades. It is our environment, not our DNA sequence and genetics. Genetic mutations from genome-wide associations are always less than

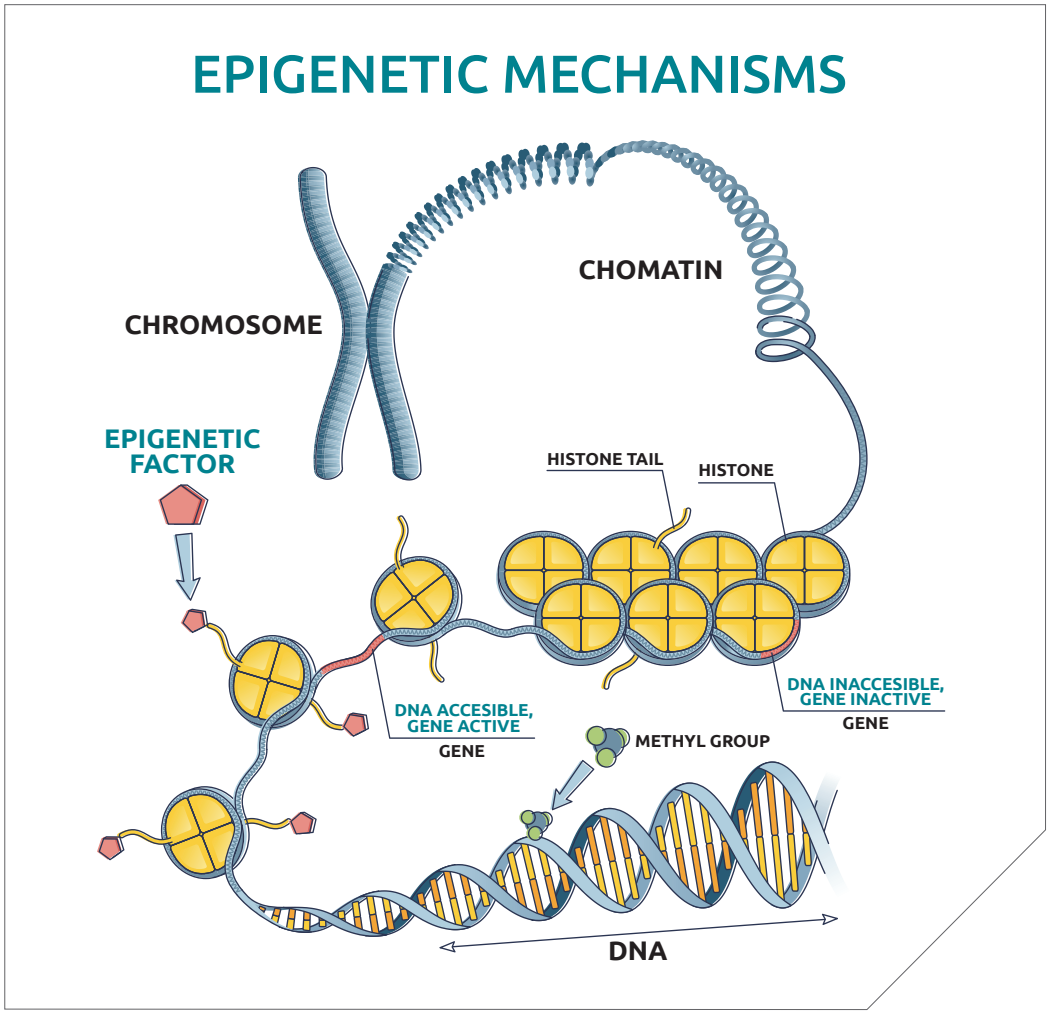
1% of the disease population. The frequency of chronic disease in the human population worldwide is now over 75%, and this is not from genetics but environmental epigenetics.

Because of the limited association of genetic mutations with some of these diseases that are becoming more and more prevalent, we understand that causation is complex and multifactorial and 'hand-wave' about 'environmental factors', such as exposure to agricultural chemicals and compounds in plastics. However, these days, with appropriate toxicology testing, why is this trend of increasing disease continuing?

This has two elements. The first is our current genetic determinism paradigm of genetic mutations being the cause of all disease and the only way evolution or biology works, is wrong. Environmental epigenetics has a far more prominent role in disease etiology, evolution, and biology, since it cannot change the genetics DNA sequence directly. The epigenetics is the intermediate for the environment to impact gene expression. Therefore, the new paradigm in disease etiology involves the integration of environmental epigenetics and genetics. Since our environment has degenerated with both nutrition and toxicant impacts, the result is increased disease frequency. The second factor is that we now know that environmental epigenetics can impact germline epigenetics and be transmitted to the next generation to promote an increase in disease susceptibility and frequency. Environmental factors such as glyphosate and atrazine do not affect the directly exposed generation, but has dramatic effects on subsequent generations. This is termed generational toxicology and it is not assessed by any regulatory agency today. However, if the exposure of this generation impacts your great-grandchildren, what we see with each new exposure is an increase of disease frequency in subsequent generations through this environmental epigenetics. An example is DDT [dichlorodiphenyltrichloroethane] exposure in the 1950s effecting all pregnant women and now three generations later we see a 50% obesity susceptibility trend, similar to what we found in our rodent models. Although altered diet and exercise promotes the disease, the susceptibility comes from environmental epigenetics.

So, often with epigenetic biomarkers, we diagnose disease risk or susceptibility. How can these findings be used to improve health?

As a result of the findings discussed above, it is to be expected that epigenetic biomarkers will be far more efficient at the diagnosis of disease susceptibility and can be used as a biomarker long before the



Epigenetic mechanisms Source: Shutterstock.com

disease develops. Since genetic mutations are such a low frequency event, the mutations have not been very efficient at preventive diagnostics, as few diseases have high frequency genetic alterations.

In contrast, altered epigenetics often is found in over 90% of the disease population and identified before disease onset. We initiated these types of studies in rodent models and found greater than 90% correlations in greater than 90% of the individuals with a specific disease using epigenetic biomarkers. In humans, we have identified epigenetic biomarkers for male infertility and paternal transmitted autism susceptibility, both with 90% accuracy in small sample data sets. One of the key advantages is the high frequency of consistent epigenetic modifications.

One of the major experimental flaws with epigenetic analysis is the use of mixed cell populations, such as blood. Although each cell type in the body has the same DNA sequence, such that mixed cell populations can be used for genetic studies, each cell type has a distinct epigenome that gives the cell its cell type specificity. So, in blood you have more than 20 cell types and if you do epigenetics, you can find changes, but they are often due to changes in cell population numbers, not direct epigenetic changes. Therefore, for epigenetics you need to use purified cell populations.

Once you get an epigenetic biomarker for disease susceptibility, several clinical options are possible. The first is better clinical management of the disease. The second is it allows preventive therapeutics to be considered and used. For example, an epigenetic biomarker for breast cancer early in life, long before the disease onset, could be used to allow a preventive treatment (such as tamoxifen) to be used in your 30s or 40s to delay the onset or prevent its development later in life.

What needs to happen to turn these findings into the development of clinically available tests?

Once an epigenetic biomarker for disease susceptibility is identified with a small clinical data set, such as our recent sperm biomarker for paternal transmission of autism susceptibility to his offspring, the next steps are an expanded clinical trail and development of an effective genome-wide high throughput assay.

The key development aspects are the use of a purified cell population that is easily collected, such as buccal cells. The second is a genome-wide analysis procedure to allow the optimal statistics and accuracy, all the epigenetic alterations should be assessed. Therefore, a genome-wide sequencing approach is generally needed, as small subsets of epimutations are not efficient in larger patient sample trials.

The expert

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Further resources

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