Editorial

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Epigenomics Editorial

CpG-specific methylation at rheumatoid arthritis diagnosis as a marker of treatment response

"In rheumatoid arthritis, there are now a wide array of biological agents that appear effective at population level; however, at the individual level, only some respond and even less achieve clinical remission."

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Epigenetic mechanisms in rheumatoid arthritis pathogenesis

It is now well established that aberrant epigenetic profiles significantly contribute to the pathogenesis of a vast number of disease states including rheumatoid arthritis (RA). RA is an autoimmune disease that results in immune activation, autoantibody and inflammatory cytokine production, and the destruction of cartilage leading to deformity and disability. This is a common disease with a prevalence of around 1%.

In synovial fibroblasts, both global and gene-specific hypomethylation have been reported in LINE-1 [1] and *CXCL12* [2] respectively. In addition, Nakano *et al.* [3] have shown that an abundance of differentially methylated regions exists across the genome of RA patients. Even differential methylation of a single CpG site in the *IL-6* gene may contribute to the inflammatory state associated with RA [4]. Another study has shown that loss of DNMT1 activity leading to aberrant CpG methylation at the *MMP13* gene causes increased *MMP13* expression and subsequently collagen degradation in the cartilage [5]. While it is certainly of interest to understand and map the epigenetic changes that are causative of diseases such as RA, the use of contemporary DNA methylation-profiling technologies may also be utilized to influence patient treatment.

Treatment response based on epigenetic biomarkers

In the context of cancer treatment, methylation status of a number of genes has been linked with drug sensitivity in a vast number of cancer/tumor types [6]. Furthermore, it has also recently been hypothesized that DNA methylation may alter an individual's responsiveness to psychiatric drugs, possibly due to variations in serotonin synthesis genes [7]. Therefore, investigation into the effects of differential methylation on the efficacy of treatments in other common diseases appears worthwhile. In RA, there are now a wide array of biological agents that appear effective at population level; however, at the individual level, only some respond and even less achieve clinical remission. The trial and error approach of current drug treatment is both expensive and cumbersome. In the rheumatology field, therefore, a lot can be learned from how cancer researchers are utilizing epigenetic biomarkers.

With this in mind, Glossop *et al.* [8] recently reported some unique and interesting findings; 46 patients with suspected RA, who were not receiving disease-modifying antirheumatic drug treatment, were recruited. Upon diagnosis, one or more disease-modifying antirheumatic drugs (methotrexate, hydroxychloroquine or sulphasalazine) were prescribed, and disease activity quanti-

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fied 3 and 6 months later. Genome-wide methylation profiling of T-lymphocyte DNA at baseline revealed that 21 CpG sites exhibited differences in methylation between treatment responders and nonresponders, four of which remained statistically significant following Bonferonni adjustment. Most notably, two CpG sites in the *ADAMTSL2* and *BTN3A2* genes were strongly associated with treatment response; at baseline, 28 of 29 of responders possessed this specific methylation pattern at these two sites. These data are particularly powerful given that long-term outcome is associated with the response to first treatment [9]. Furthermore, a more immediate dose adjustment based on likely treatment responsiveness would not only reduce costs and drug wastage, but potentially attenuate the time frame by which the patient would experience optimal treatment and thereby potentially prevent irreversible joint damage, as we know there is a 'window of opportunity' in RA after which treatment appears ineffective.

> "Various therapeutic miRs are now in preclinical and clinical trials, and are therefore, likely to represent important treatment options for a number of conditions in future."

Use of the extensive HumanMethylation450k beadchip, validation of candidates using sodium bisulfite pyrosequencing, in addition to robust statistical measures, ensured that a large portion of the genome was covered using reliable techniques with low probability of type 1 errors. While the cost and coverage of the HumanMethylation450k beadchip should not be trivialized, further research of this nature should incorporate the very latest in microarray technology. In particular, the recently developed HumanMethylationEPIC beadchip has been expanded to include over 850,000 methylation sites. Again, we are aware of the difficulties surrounding participant recruitment and research concordance; however, larger sample sizes would enhance the robustness of the findings and could possibly identify further CpG sites associated with treatment response. It must be noted that the authors themselves acknowledge this. Additionally, while the authors state that short-term stimuli minimally affect DNA methylation, studies have shown that promoter methylation of a number of genes can be altered by a single bout of exercise [10,11], albeit within skeletal muscle and not lymphocytes. We propose, therefore, that a more extensive panel of baseline variables including habitual methyl-donor consumption through the diet and exercise, would provide even more robust data in larger populations. Overall, we congratulate the authors of this paper on highlighting a promising line of investigation which could significantly aid in the effectiveness and personalization of RA treatment.

Potential systemic sclerosis biomarkers & treatment

Given these data and the relatively well-established role of differentially methylated drug metabolism enzyme genes in modification of the responsiveness to drug treatment, it is plausible that DNA methylation may also serve as a biomarker of treatment response in other rheumatic conditions associated with aberrant epigenetic profiles such as systemic sclerosis (SSc) [12,13].

The aforementioned differential methylation of a CpG site in the *ADAMTSL2* gene, which is involved in TGF-β regulation, could be postulated to also influence SSc treatment responses due to the central role of aberrant TGF-β expression in SSc pathogenesis [14]. Furthermore, methotrexate, in its role as an immunosuppressant, is also used to treat SSc, which provides support to the possibility that SSc patients may also benefit from personalized treatment based on methylation-based predictions. Of course, prospective cohort studies would need to be conducted in order to confirm or refute such a supposition.

Gras *et al.* [15] reported that TGF-β may be linked with fibrosis via miRNA (miR)-dependent mechanisms; elevated miR-145 attenuates KLF4 which in turn augments α-SMA. The miR-29 family has also been implicated in SSc [16,17], while miR-155 is thought to be a promising therapeutic target for arthritis [18]. Various therapeutic miRs are now in preclinical and clinical trials, and are therefore, likely to represent important treatment options for a number of conditions in future. AntagomiRs, for example, are able to suppress aberrantly expressed miRs; however, no therapies for rheumatic conditions have currently advanced from *in vitro* validation stages. While this line of investigation does not necessarily relate specifically to methylation profiling to predict treatment sensitivity, it demonstrates that other epigenetic mechanisms can be manipulated by treatment in order to manage a variety of diseases.

Future perspective

Studies which highlight epigenetic modifications associated with response to treatment not only possess enormous clinical utility in terms of optimizing patient care, but may also aid in reducing the economic burden associated with unnecessary drug prescriptions. As suggested, future research should strive to expand upon studies such as those conducted by Glossop *et al.*; collaboration between institutions could significantly assist in expanding study numbers, thereby improving statistical robustness of data. Also, similar studies which potentially highlight the influence of DNA methylation on treatment response in other conditions could have a positive impact on a much larger number of patients. Finally, once studies such as this are vali-

dated in larger cohorts, it is essential that standardized procedures for measuring markers across different genomic sites are implemented.

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