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Epigenetic editing: towards realization of the curable genome concept

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Abstract

Recent developments in biotechnology have enabled scientists to modulate DNA sequences in a precise way. These genome engineering technologies also open new possibilities to alter the epigenetic composition of the genome at any given genomic location thereby changing gene expression patterns, while leaving the primary DNA sequence intact. This new approach, so-called *epigenetic editing*, holds great promise to permanently reprogram cell identity. As reprogramming the epigenetic composition, and hence gene expression patterns is now technically feasible, the society needs to consider to what extent interference at the epigenetic level can be accepted. In this review, we discuss the potential epigenetic editing holds for research and therapy, and also touch upon societal implications of this rapidly growing research field.

Epigenetic editing

Epigenetics concerns the study of heritable, yet reversible, changes in gene expression that are affected by other mechanisms than changes in the primary DNA sequence. Epigenetic gene regulation is crucial for cell type specific gene expression patterns in higher eukaryotes, conferring stability of the cellular phenotype. On the other hand, epigenetics allows changes in expression in response to environmental or developmental cues. Derangements in epigenetic gene regulation have severe effects on cell behaviour and contribute to the initiation and maintenance of a diseased state. Nowadays, it is well accepted that many diseases are indeed associated with an altered epigenetic landscape [1, 2], examples range from behavioural disorders to metabolic diseases [3, 4]. Intriguingly, epigenetic enzymes driving the epigenetic composition are largely influenced by environmental conditions and therefore can enable a change in the epigenetic state. This explains the observations that nutrition or other lifestyle choices are associated with alterations in the composition of the epigenetic landscape. Moreover, since the epigenetic composition is reversible, it opens opportunities for therapeutic intervention at the epigenetic level. Many efforts are indeed ongoing to design inhibitors of epigenetic enzymes [5]. FDA-approved epigenetic drugs (small molecule inhibitors of epigenetic enzymes) have shown clinical effectiveness, mainly for the treatment of haematological malignancies. The recent review series 'Epigenetic drugs—from chemistry via biology to medicine and back' gives insights into the development and current status of epigenetic drugs [5]. Despite such promises, by inhibiting genome-wide acting enzymes, epigenetic drugs bring along genome-wide effects and also influence unwanted non-chromatin targets, preventing their wide-spread clinical application. To circumvent the genome-wide effects, gene-specific epigenetic alterations can be induced by targeting epigenetic enzymes, i.e. so-called epigenetic writers or erasers, to a given genomic location using programmable DNA binding platforms. This novel technology, referred to as epigenetic/epigenome editing, is receiving increasing attention the last few years as also exemplified by many recent reviews [6–14].

The molecular tools that are needed to perform epigenetic editing consist of at least two components: a DNA binding platform component and an epigenetic effector domain component (figure 1). The DNA-binding platform is engineered to bind a given stretch of base pairs in the desired gene. This DNA-binding platform serves as a GPS to find its desired genomic destination. The effector domain minimally consists of the catalytic domain of an epigenetic writer or an eraser that is able to alter defined epigenetic marks. The writer or eraser

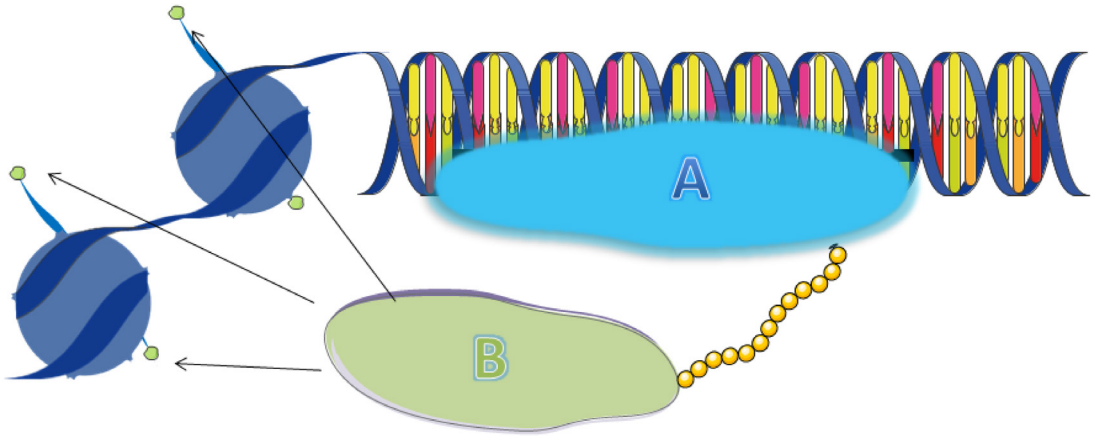

Figure 1. Epigenetic editing is used to actively rewrite epigenetic signatures at a genomic location of interest. The molecular tools used for this purpose consist of (A) a DNA binding platform to recognize the target sequence, and (B) an epigenetic modulator (or a catalytic domain thereof) which exerts its activating or repressive function by rewriting the epigenetic signature at a desired location.

rewrites the epigenetic marks at the given location with the ultimate goal to turn off or on the expression of the targeted gene. Epigenetic editing thus enables the reprogramming of epigenetic composition and can stably alter genome functioning without affecting the DNA-sequence itself. Since the vast majority of epigenetic marks are erased before/during the process of fertilization, the effects of this technique are less likely to be inherited by the next generation. Epigenetic editing might thus provide a less radical approach than traditional genetic editing.

The most frequently used DNA binding platforms for epigenetic editing are self-engineered zinc finger (ZF) proteins, transcription activator like effectors (TALEs) or the popular clustered regulatory interspaced palindromic repeats (CRISPRs) system. Shortly, ZF are naturally occurring transcription factors consisting of 30 amino acids per finger, which recognize 3 base pairs in the DNA target sequence. Several ZFs can be coupled together to increase efficiency and specificity. Major advantages of this tool are their relatively small size and low immunogenicity. TALEs are derived from plant pathogenic bacteria, they consist of 33–34 amino acids per moiety, each of which can recognize 1 nucleotide with their intervariable region [15]. Both platforms have been outcompeted by a bacterial immune system, called CRISPR-Cas, described in 2012 by Jennifer Doudna and Emmanuelle Charpentier [16]. This prokaryotic immune system is directed against foreign, invasive genetic elements (e.g. from viruses or phages): When bacterial cells are faced with an invasive pathogen, copies of the exogenous DNA are made and a small sequence of the foreign DNA, known as a spacer, is integrated into the CRISPR locus of the bacterial genome. Spacers are transcribed into a set of small RNA guides (sgRNA). When the bacterial cell encounters the same pathogen, the foreign DNA will be detected by these small RNAs. The small RNA binds to the invading DNA and directs its cleavage by Cas9 nucleases. The Cas9 enzyme introduces DNA double strand breaks at its binding site destroying the invading DNA. The system can be repurposed to target any given DNA: the double strand breaks are then repaired by the cell in an inaccurate way often resulting in gene inactivation. Importantly, when in addition DNA fragments that are homologous to the recognized sequence are introduced into the cell, the cell can exchange the damaged DNA by the introduced DNA via homologous recombination. Researchers have shown that this CRISPR-Cas system can be used to correct genetic mutations or introduce completely new pieces of DNA by designing the proper sgRNA and homologous DNA sequences. The CRISPR-Cas approach has revolutionized biomedical sciences and a patent war is currently ongoing between the inventors of the approach.

Applications of epigenetic editing

Upon fusion of self-engineered ZFs, TALEs or the catalytically dead dCas9 mutants (in combination with sgRNAs) with epigenetic writers or erasers, powerful tools are obtained to selectively change the epigenetic environment of a target genomic region. By actively changing the epigenetic landscape we can investigate the causal relationship between chromatin state, gene regulation and cell phenotype. As epigenetic editing holds great promise to permanently reprogram cell identity, and since most diseases nowadays are known to have dysregulated epigenetic components underlying the disease state, epigenetic editing might provide breakthroughs for various diseases, including those for which currently no cure or even treatment is available [17].

One of the many examples of the potential impact of gene-specific epigenetic interference technologies is cancer: in tumor cells, tumor suppressor genes are often genetically mutated, which makes them unable to perform their function to suppress tumor growth. Even more frequently though, these tumor suppressor genes are epigenetically altered such that they are no

longer transcribed. Conversely, in cancer cells so-called oncogenes that ensure continuous tumor cell division are permanently switched on. Correcting the epigenetic mechanisms underlying such cancer gene expression patterns might provide an alternative therapeutic approach or might contribute to reprogramming cancer into a chronic rather than a lethal disease state.

Alternatively, we might design tools to actively interfere with the development of therapy resistance: most women with early ER positive breast cancer, for example, are treated with oral adjuvant endocrine therapy. In pre- and postmenopausal women, different clinical strategies are employed to prevent endocrine signalling and cell proliferation. Selective ER modulators and downregulators (SERMs and SERDS) are the treatments of choice for premenopausal women and aromatase inhibitors (AIs) for postmenopausal women [18]. Often, these medications are effective in preventing disease relapse and death from the primary tumor, however in 30–40% of the initially responsive patients, relapse and a progression to metastatic disease can occur, resulting in a poor prognosis. Acquired resistance reflects tumour cell adaptation involving molecular changes that allow continued cell proliferation providing cells with a selective advantage. Acquired resistance to endocrine therapies is a long-term process in which genetic alterations act synergistically with epigenetic changes. The acquired resistance is the result of a complex interplay of factors being involved in various signalling pathways [19].

Epigenetic reprogramming, more specifically the epigenetic composition of regulatory elements (e.g. enhancers), is an integral component of cellular differentiation that facilitates lineage-specific transcriptional programs [20]. Recently it has been demonstrated that genome-wide epigenetic reprogramming (DNA methylation, posttranslational histone modifications and chromatin compaction) induces changes in gene regulatory networks and underlies long-term endocrine treatment resistance development [21–23]. One example of this is published in 2015 by Magnani *et al* where it is shown that endocrine therapy resistant cells are capable of activating endogenous cholesterol pathways through alterations in epigenetic histone modifications involving large topological domains and the activation of superenhancers, both *in vivo* and *in vitro* [24]. The overall effect of increased cholesterol concentration was to activate the estrogen receptor (ER), circumventing the cells reliance on estrogen. Other, epigenetic mechanisms of achieving this include DNA hypermethylation of *ESR1* [25] and overexpression of HDAC1 [26], both of which silence the expression of the ER, and allow other growth pathways to become dominant.

Resistance to endocrine therapy is an urgent medical problem. To proceed in this field, we need to identify and target cancer-specific epigenetic changes in individual patients during the course of resistance development and for this we need diagnostic tools (e.g. tissue biopsies or serum) to monitor, evaluate and predict the epigenetic component of treatment outcome.

From 2015 on, Dr Verschure at the University of Amsterdam (UvA) started as coordinator of an international research consortium (an Innovative Training Network (ITN) funded by EU H2020 MSCA-ITN-2014) to focus on uncovering the role of epigenetic regulation in resistance development for endocrine therapy in ER positive breast cancer. The EU research consortium entitled 'Epigenetic regulation of endocrine therapy resistance in breast cancer: a systems medicine approach to predict treatment outcome' (acronym: EpiPredict) consists of 15 parties, academic institutes and private companies, from 8 different countries, training a multidisciplinary group of 12 PhD students. The PhD students perform their research at 8 different laboratories, i.e. UvA, Imperial College London, the Deutsches Krebs-forschungs Zentrum, the University of Milano-Bicocca, the UMCG, the Hungarian Academy of Sciences, Epiontis GmbH, the Eidgenössische Technische Hochschule Basel.

Within EpiPredict, a systems medicine approach is employed to obtain mechanistic, detailed insights in to how changes of a patient's epigenome can affect gene expression, pathway activation and metabolic rewiring through a defined set of resistance involved pathways. We combine multidisciplinary research strategies and next generation technologies (epigenetic, gene expression, protein pathway activation, metabolic pathway profiling, gene-specific epigenetic interference technologies and computational approaches). The complex dynamic interactions that determine treatment resistance are virtually impossible to understand from only genome-wide experiments and bioinformatics analysis. Therefore, we establish mechanistic models [27, 28] from research/clinical data enabling re-iterative *in silico* experimentation. These models predict (dynamic) phenotypic responses upon changes in biological parameters (e.g. availability of ligands) thereby generating new hypotheses and wet-lab experiments to be tested with epigenetic interference technologies to further refine the model. The scientific mission of EpiPredict is to utilize mechanistic understanding of the involved epigenetic regulation and cell type switching underlying endocrine therapy resistance development to explore (i) robust diagnostic/prognostic tools to stratify patients for their likelihood of developing endocrine resistance and (ii) prediction measures for effectiveness of additional drugs counteracting resistance an important step towards tailored treatment-monitoring schedules. We will determine cellular heterogeneity and sub-cell type epigenetic state switching [29] and use CRISPR/dCas-based Epigenetic editing [30] to locally overwrite epigenetic signatures and verify the impact of defined alterations in epigenetic regulation on the ability of cells to end-up in an endocrine resistant state due to a concrete phenotypic switch. Epigenetic diagnostic tools to predict and monitor treatment outcome

will open-up an unexplored field of research with great potential for personalized medicine [31].

Dogmas

The gene targeted rewriting of epigenetic marks, at first introduced as epigenetic editing by us [32], was treated with quite some skepticism in its initial phase making it inherently difficult to overcome existing dogmas. In general, four major objections were raised against the concept: first of all, it was considered to be impossible to re-express epigenetically silenced genes, since these genes were supposed to be inaccessible. Silenced genes were believed to be located in compacted, epigenetically silenced genomic regions, and the transcription machinery was considered unable to access these tightly packed chromatin regions. Secondly, it was unclear whether epigenetic marks directly instruct gene expression or simply result as an indirect effect of an established gene expression pattern. Thirdly, at that time it was still inconceivable to consider a way to enable true gene-specific intervention. Recently, the CRISPR-Cas system provides clear examples of gene-specific modulation of gene expression patterns. In the end, even when the first three considerations would be proven surmountable, it was thought to be impossible to actually tip the gene expression balance from a silenced to an actively expressed state, or vice versa, in a long lasting manner.

Various labs, including ours, refuted existing dogmas, showing that silent genes are accessible and can be re-expressed [33–40]. Importantly, it was demonstrated that the local removal of DNA methylated CpG sites is sufficient to re-express epigenetically inactivated genes [36, 41, 42]. Moreover, it was shown that genes can be repressed by writing repressive epigenetic marks on, for example, the actively transcribed oncogene *her2/neu* [43–45]. Such studies challenged the dogma that epigenetic marks are not instructive in determining gene expression levels. Altogether, at this moment the concept of epigenetic editing is reaching wide spread acceptance, partially as a result of the introduction of the easy and cheap CRISPR-dCas approach.

Indeed, several publications demonstrate that writing methyl groups on cytosines of a gene induces downregulation of that gene [46]. The pending question is: can we accomplish a permanent reprogramming using a 'one and done' approach. The ambition would be to treat cells one-time, possibly with a combination of targeted epigenetic writers and erasers, allowing rewritten epigenetic marks to be memorized by the cell in a permanent manner. So far, the outcome of such studies focusing on epigenetic editing to achieve sustained gene repression are controversial [10, 47], which likely reflects the influence on the chromatin environment in allowing sustainability of the effect. In this respect, we recently demonstrated that writing methylation on lysine 4 of histone 3 was sufficient to induce re-expression of epigenetically silenced genes, although maintenance of the effect was again highly context-dependent [30].

Currently, many diseases have no clear clinical treatment or cure, however for several diseases we do have in depth knowledge relating to changes in gene expression profiles of disease-associated target genes and downstream genes. In principle epigenetic editing would enable us to reprogram the epigenetic profile of such genes, potentially reversing the diseased phenotype. Of course, it is crucial to understand the network wiring of involved genes which is often not straightforward to determine due to the complex behaviour of gene regulatory networks.

Pros and cons

Scientific breakthroughs, as exemplified here by genome engineering or epigenetic editing, will always be associated with ethical dilemmas: how to optimally exploit *The Good* (envisioned clinical cures of currently incurable diseases), while preventing *The Bad* (e.g. bioterrorism) and balancing *The Ugly* (like 'designer babies'). Communication within society is of utmost importance. The public needs to be well informed about new technologies to avoid a situation where new technologies are restricted or abandoned due to fear, as was the case when gene therapy was first introduced for disease treatment. The first clinical trials using gene therapy focused on compensating for a genetic mutation that caused the phenotype of severe combined immunodeficiency (SCID), so-called 'bubble boys'. SCID is an extremely rare genetic disorder characterized by disturbed development of functional T and B cells, resulting in an ineffective immune system. Disease victims (males) are extremely vulnerable to infections and permanently live in quarantine (a plastic house: 'bubble'). By the end of the last century, twenty SCID boys were treated with viral transduced gene therapy transferring a copy of the healthy gene to the immune cells. Nineteen of the treated SCID boys were eventually cured [48]. Although this by itself was a great success (*The Good*), the trial is certainly not recognized as such. As a matter of fact, of the twenty SCID boys that were treated, the gene therapeutic viruses caused leukaemia in five patients (*The Bad*). For society, this proved that gene therapy is very dangerous. Four out of five SCID boys with leukaemia were eventually cured of the condition, but unfortunately one deceased. Despite the tragic death of this one patient, the rest of the participants of the first gene therapy trial enjoy a normal life, which without the gene therapy had not been possible. Unfortunately, it seemed that funding agencies and companies back in the early 2000s wanted to stay far away from viral gene therapy, also because of biological warfare and ethical objections ('playing God'), which articulates *The Bad* and *The Ugly* side of gene therapy.

Also the CRISPR-Cas approach, as well as other novel biotechnological applications to interfere with

the (epi)genetic composition, promises a lot of *'The Good'* aspects. *'The Ugly'* (like non-professionals who order CRISPR-Cas reagents for their uncontrolled garage experiments: so-called biohackers) and *'The Bad'* (bioterrorism) sides of such approaches require solid policies. Obviously, we need to control adverse applications, but without destroying investments to strengthen *'The Good'* side. Reprogramming of genes to restore protein expression networks to a healthy situation would be a breakthrough for many diseases for which no therapy is available at the moment. In other words, 'the curable genome shines on the horizon'.

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