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Epigenetics: Moving Forward

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Both the focus on epigenetics and the simple use of the term "epigenetic" have significantly augmented since the 1940s, when Sir Conrad Waddington opened the ground to this field. Since then, the definition of epigenetics became more inclusive, often defined as "*stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence*" (2008 Cold Spring Harbor Epigenetics meeting). In more common words, the term epigenetics derives from επί-genetics, which literally means "on top of" genetics, referring to the modifications of chromatin that are able to switch genes "on" or "off" affecting the cell's "interpretation" of genes and consequently function, specialization, phenotype, and cell fate [1].

Recently, the importance of the epigenetics has become evident from the plethora of articles, conferences, and consortia on the topic over the past decade. All over the world, research was intensified more and more on basic as well as biomedical-oriented epigenetic-based methodologies, targets, and applications. Funders initiated concerted actions to promote standardization and collaboration of the worldwide efforts aiming to unveil the role of transcriptional and epigenetic mechanisms in specification of cell fates and functions, such as the "American Association for Cancer Research Human Epigenome Task Force" and the "European Union Network of Excellence." The "International Human Epigenome Consortium" (IHEC) was founded to coordinate and standardize the production of reference epigenomes with a focus on cell states relevant to health and diseases, thereby accelerating translation of new knowledge to improve therapy [2]. IHEC has also coordinated the international efforts by bringing together the European Commission that funded "Blueprint consortium" (http://www.blueprint-epigenome.eu/) with, as a mere example, the NIH that funded "Roadmap on Epigenomics" (http://www.roadmapepigenomics.org). In addition, IHEC introduced common bioinformatics standards, models, and tools to analyze and interpret epigenomic data in a uniform and interoperable manner [3].

Epigenetic Drug Discovery, First Edition. Edited by Wolfgang Sippl and Manfred Jung.

1.1 Why This Enormously Increased Interest?

One reason is the need to address fundamental questions to understand the way the genome and environment interact in development and aging and how the epigenome affects or is affected by health and disease.

In addition, there is an urgent need to develop new ways to "drug" the epigenome and to translate discoveries into improvements of human health. Despite being quite stable and hereditable, epigenome modifications can be easily changed within the cell, affecting cell fate and functions. This epigenome plasticity opens the way to the pharmacological exploitation and to the identification and characterization of chromatin-targeting drugs. The identification of increasing numbers of new players acting as "*writers, erasers, or readers*" of the epigenome suggests that an intricate and very well-defined epi-modulated setting is responsible for maintaining the plasticity potential, ultimately guarantying cell identity and cell heterogeneity of otherwise similar tissues. Given that new modifications/new players are being uncovered, additional complexity arises, and a better understanding and frequent revisiting of the mechanism(s) of chromatin regulation and plasticity – ultimately at the single-cell level – are needed. The potential of this emerging knowledge toward its translation into biomedical applications is breathtaking. For example, a huge number of studies (many of which using high-throughput approaches) have unveiled the significance of certain histone marks, epi-enzymes, and chromatin-regulating factors in different human pathologies such as cancer, neurological disorders, diabetes, immunological pathologies, etc. [4]. Translating this basic knowledge to bedside practice has triggered investments in the identification and development of new drugs able to re-equilibrate deregulated epigenome areas acting by inhibiting or (currently more rarely) activating chromatin enzymes and/or by interfering the function of chromatin readers.

In addition to the rapidly accumulating knowledge on the mechanisms of action of chromatin-targeting "(epi)drugs," we have only beginning to unravel the different substrates of the epi-enzymes. "Epi-drugs" are designed to inhibit (or activate) histone-modifying enzymes or DNA methyltransferases or to interfere with readers of the resulting chromatin modifications. However, these chromatin modifiers (and the respective "epi-drugs") affect various substrates, including proteins in signal transduction pathways and cell structure. Such insights will turn out to be crucial to develop a better rational design of drugs treatment (and combination thereof), further exploiting and expanding the promise of epigenetically acting drugs.

It is still debated whether selective or broad chromatin modulators will be more effective [5]. As has been demonstrated in some cancer types harboring mutated enzymes, a selective "epi-drug" approach (active exclusively or preferentially on the mutant) may be preferred. On the other hand, a broad modulator might become more useful when concomitant alterations of different epi-targets are playing a role. This might also include hybrid molecules acting contextually on one epi-target and one non-epi-target.

Among the best studied chromatin-targeting drugs, HDAC inhibitors [6] and DNA-demethylating agents [7] have entered the clinic for anticancer treatment

and prevention. Despite that HDAC inhibitors mostly induce hyperacetylation, this cannot be considered as a parameter of response. This issue highlights the need for a detailed understanding and development of markers of treatment response along with (epi)drug development. This will become a challenging task considering that epigenetic-based approaches have been proposed for very different diseases. In cancer patients, the altered expression of epi-players (overexpression or silencing) or a qualitative deregulation such as the mutation in one of the epi-enzymes has been one of the parameters of choice although patient's stratification on the basis of HDAC expression levels appears not always predictive of a better response. The presence of a well-characterized target mutation may instead prove to be more useful for patient stratification. Small molecules able to selectively modulate the mutated enzymes/targets may display tumor-specific action.

Interestingly, different groups of enzymes display diverse ways of deregulation; for example, HDACs are generally quantitatively overexpressed in cancer [8] (with the exception of HDAC2 mutations [9], for example, in colon tumorigenesis), whereas HATs appears more frequently mutated [10, 11]. Furthermore, the direct and indirect deregulation of methylation control through mutations in DNA methyltransferases and isocitrate dehydrogenases (IDH) genes appears to go along with abnormal histone and DNA methylation as a common feature of tumors with IDH1 and IDH2 mutations and altered stem cell differentiation and eventual tumorigenesis [12]. Description of inactivating mutations in TET2 suggests that cellular transformation is in part caused by the deregulation of 5-mC conversion. The TET enzymes have particular relevance in hematological cancers and solid tumors with mutations causing TET inactivation [13].

1.2 Looking Forward to New Avenues of Epigenetics

The constant flow of discoveries in the epigenetic field adds new layers of complexity and may lead to novel approaches for treatment. Novel chromatin marks are identified, and insight from mining of these targets (alone and within the context of others) may rapidly change our view. For example, hydroxymethyl cytosine and its modulation is at present a focus of discussions aimed at unraveling its mode of action and its potential role in cancer as well as other human diseases [14] [15]. The levels of 5hmC in the brain of patients with neurodegenerative disorders have been reported to be highly compromised, indicating a potential role of 5hmC in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Huntington's disease. It has yet to be established whether this is the cause or the consequence of the onset and progression of these diseases [16].

The burst in acquisition of scientific knowledge and in evolving new technologies will also pave the way to new concepts in the regulation and deregulation of the epigenome. Emerging single-cell epigenomic methods [17] are being developed with the exciting potential to transform our knowledge of gene regulation [18]. Until recently, our epigenetic modifications have been studied in bulk measurements in populations of cells.

The development of single-cell technologies is likely to cause a profound transformation of epigenome studies and their interpretation, in particular, in cases where (epi)genetic heterogeneity is overriding. In recent years, many of the high-throughput sequencing technologies hither to assaying population have been adapted and became assayable at the single-cell level. Combined single-cell methods such as simultaneous assessment of the transcriptome and DNA methylome may provide deeper insight in epigenetic–transcriptional correlations, allowing analyses on the causal relationships between phenome and the epigenome state. Furthermore, combined genome and epigenome analyses will likely open up new avenues to dissect the complex contribution of genomic and epigenomic heterogeneities [19].

A better integration of high-throughput data, bioinformatics interpretation, novel epi-marks, and chromatin players has the potential to bridge basic knowledge with the clinics both for epi-marks mining for diagnosis of disease treatment and outcome prediction and for disease prevention. Furthermore, many chromatin-targeting drugs have been identified and characterized in the past decade for their beneficial action against different human diseases. Even though the beneficial effect and link to the selective chromatin-regulating action has to be better corroborated and strengthened, their clinical potential is clear. In agreement, HDACi have been approved for the therapy of cutaneous T-cell lymphoma (CTCL) and recently for the treatment of multiple myeloma [20], as are DNA-demethylating agents for the treatment of myelodysplastic syndrome (MDS). In addition, the action of HDAC inhibitors against cancer might also be linked to the modulation on the immune system, potentially shedding a different light for their clinical use [21]. That histone methylation is also altered in cancer that led to the identification of lysine methyltransferases and demethylases as promising targets for new anticancer drugs. Inhibitors (targeting the histone methyltransferases DOT1L and EZH2 as well as the demethylase LSD1) have already reached the first stages of clinical trials in cancer therapy [22].

Also pharmacological inhibition of BET proteins shows therapeutic action in a variety of different pathologies, particularly in models of cancer and inflammation [23]. Such effects have been attributed to subsets of downstream targets. While it is clear that the therapeutic potential is huge, the current understanding of molecular mechanisms that underlie the therapeutic effects of pharmacological BET bromodomain inhibition still need better understanding [24].

Drug discovery efforts in the epigenetic field are not only focused on cancer but also on more chronic diseases opening the way to new opportunities for the epi-targeted treatments. For example, I-BET151 has been reported to effectively prevent type 1 diabetes in a mouse model for this disease [25, 26], suggesting that an epigenetic treatment of diabetes might be at our doorstep. Along these lines, different classes of "epi-drugs" that have been suggested to decrease obesity and clinical trials at different stages are ongoing, aiming to a better definition of their potential [27]. Recent studies have identified SIRT1 activators that may delay multiple diseases of aging and extend lifespan *in vivo* [28]. In theory, such molecules could act against diseases, potentially extending healthy years of life. Potential roles of SIRT1 and SIRT2 modulation in neurodegenerative diseases

have been proposed [29, 30] and an SIRT1 inhibitor (Selisistat) is in clinical trial against Huntington's disease [31].

These are only examples of the critical need to illuminate the drug discovery efforts in the identification and characterization of the novel epi-drugs [32]. Thus, in this volume an overview of state-of-the-art knowledge and development in drug design for epi-targets, their mechanisms of actions, and the increasing spectrum of applications is presented. Furthermore, current methodologies are discussed including the structural biology of epigenetic targets, computer-based technologies, mass spectrometry, peptide microarrays, chemical probe development, and epigenetic multi-targeting. In addition, the "epi-drug" classes such as HDAC, SirT, HAT, methyltransferase and demethylase modulators, DNA modifiers, bromodomain, and methyl-lysine reader proteins are examined. Finally this volume will also address challenges and promises of parasitic epigenetic targets. A new promising approach is chemically induced proteolysis by so-called PROTACs (proteolysis targeting chimeras), where a ligand to the target of interest is fused to a moiety that leads, e.g. to ubiquitinylation and subsequent proteolytic degradation. This will phenocopy knockdowns, resp. knockout studies, and is promising prolonged target inactivation and might become a new paradigm in drug discovery and hence also in epigenetics [33–35].

Acknowledgments

Blueprint (282510), EPIGEN (MIUR-CNR); MIUR (20152TE5PK), AIRC (17217); COST EPICHEMBIO CM1406.

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