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Mini Review

Computer-aided Molecular Design of Compounds Targeting Histone Modifying Enzymes

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ABSTRACT

Growing evidences show that epigenetic mechanisms play crucial roles in the genesis and progression of many physiopathological processes. As a result, research in epigenetic grew at a fast pace in the last decade. In particular, the study of histone post-translational modifications encountered an extraordinary progression and many modifications have been characterized and associated to fundamental biological processes and pathological conditions. Histone modifications are the catalytic result of a large set of enzyme families that operate covalent modifications on specific residues at the histone tails. Taken together, these modifications elicit a complex and concerted processing that greatly contribute to the chromatin remodeling and may drive different pathological conditions, especially cancer. For this reason, several epigenetic targets are currently under validation for drug discovery purposes and different academic and industrial programs have been already launched to produce the first pre-clinical and clinical outcomes. In this scenario, computer-aided molecular design techniques are offering important tools, mainly as a consequence of the increasing structural information available for these targets. In this mini-review we will briefly discuss the most common types of known histone modifications and the corresponding operating enzymes by emphasizing the computer-aided molecular design approaches that can be of use to speed-up the efforts to generate new pharmaceutically relevant compounds.

Contents

1. Introduction

In the last decades, major efforts have been done in genetics though it is evident that unraveling complex biological mechanisms like cancer requires a larger framework of research that recently evolved toward the better understanding of the immune system regulation [1], the rediscovery of metabolism [2] and, importantly, the role of epigenetics [3]. The term epigenetics refers to the mechanisms of temporal and spatial control of gene activity that do not entail modification of the DNA sequence but influence the physiological and pathological development of an organism. The molecular mechanisms by which epigenetic changes occur are complex and cover a wide range of processes [4]. Epigenetic mechanisms can occur at biochemical level in three different ways:

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i) through histone post-translational modifications (PTMs), which will be the object of this mini-review, as well as the molecular recognition of non-catalytic readers of histones [5], ii) through the DNA methylation, i.e. the methylation of cytosines to 5-methylcytosines, which is the object of recent reviews [6–8], and iii) through regulation of gene expression by non-coding RNA (ncRNA), which is also an emerging topic of research, covered by recent reviews [9–11]. All these processes contribute to define the epigenetic mechanisms by which gene expression is activated or silenced [12–16].

Post-translational modifications of histones occur at the N-terminal tails of the protein chains and consist in covalent modifications that are catalyzed by different classes of enzymes [17,18]. The ensemble of these modifications is commonly referred as to be the *histone code* referring to the idea that all histone PTMs determine the activity state of an underlying gene [19]. One of the hallmarks of the histone code is that it can be positively or negatively correlated with specific transcriptional states or organization of chromatin [20–23]. This is accomplished through a fine regulation of histone PTMs controlled by an enzymatic machinery, which existence and function have been elucidated partly, but with an extraordinary progression in the last years [23–29]. Importantly, further understanding of epigenetic phenomena occurring on histone proteins is critical to shed light on biological processes that are progressively translating into the development of new medical options [29–31]. In this direction, different studies have highlighted how the histone alterations contribute to the onset and growth of a variety of cancers [7,23,24,27,32–41], among other pathologies. Consequently, enzymes operating PTMs on histones are constituting attractive therapeutic targets for the development of new therapies [13,31,42–44]. It should be noted that, while the resulting effects on chromatin collectively depend on the ensemble of histone PTMs, these are operated by precise variations of physicochemical properties that we recently reviewed [17]. For these reasons, large efforts from both academic and industrial settings have been dedicated in the last year to identify and evaluate new biologically active compounds against histone modifying enzymes. Fuelled by the increasing availability of structural information, several endeavors have been initiated and helped by the usage of computer-aided molecular design techniques. Thus, in this mini-review, we aim to highlight the aspects relating histone modifications in the light of the future applications of computational techniques to the research of new probe or lead-like epigenetic modulators.

2. Type of Histone Modifications and Their Biological and Clinical Relevance

To understand the relevance of computational techniques in histone-related epigenetic targets, it is important to highlight that these post-translational modifications are functionalizations/defunctionalizations of specific residues, which are lysine, arginine, serine, threonine, histidine, tyrosine, cysteine and glutamic acid, located at the N-terminal tails of each chain. Fig. 1 summarizes all the most common PTMs that can occur on histones. By far, lysine represents the residues with most chemical versatility, as it is capable to undergo several kinds and grades of modifications. Consequently, histone methyltransferases, demethylases, acetyltransferases and deacetylases have been recently ascribed an important role as new classes of biological targets for drug discovery [18,45–49]. Arginine represents also a residue that is modified by enzymes recognized for drug development, in particular histone methyltransferases. Differently to these previous cases, enzymes that modify histone serines, threonines, histidines, tyrosines, cysteines and glutamic acids have not been exploited yet for the discovery of new modulating compounds. Nevertheless, it is expected that further elucidation of their biological role and protein structure will spur such endeavors. It is worth to note that other kinds of modifications like propionylation, butyrylation, crotonylation, 2-hydroxyisobutyrylation have been reported [50].

Different studies elucidate the impact that PTMs have on chromatin and their relevance in human physiology and pathology [16,18,25,26,31,51–57]. Interestingly, their biological role greatly varies, depending on the kind of modification. Therefore, for instance, the acetylation appears to be the most promiscuous histone modification and is always associated to transcriptional activity. Conversely, histone methylation has a high degree of selectivity toward specific histone residues and can be associated with both repression and transcription [58,59]. In addition PTMs can "cross-talk", meaning that modifications can occur in a concerted or a subsequent manner [25,60–63].

In the last decade, epigenetic modifications of histones have been mainly studied in the context of cancer, particularly histone deacetylases of classes I, II and IV (HDACs) [64]. Indeed, abnormal activity of the enzymes responsible for deacetylation of histones, modification that alters the chromatin structure repressing transcription, has been shown to be implicated in several diseases [18,65]. Because of these compelling evidences, HDACs have been recognized as consolidated drug target, in particular for breast cancer, colorectal cancer, leukemia, lymphoma, ovarian and prostate cancer. In addition, another class of histone deacetylases named sirtuins (or class III deacetylases), which uses NAD^+ to catalyze the removal of an acetyl group, also came into the light as new therapeutic targets [66,67]. This family of enzymes, in fact, was found to be involved in relevant physiological and pathological processes, as well as in aging-related disorders, metabolic and inflammatory conditions and processes involving DNA regulation and integrity, including cancer [68–71]. Interestingly, also the correspondent families of enzymes that revert the catalytic activity of histone deacetylation, i.e. acetyltransferases (HAT), have also met a great deal of interest. Two classes of HAT exist and consist of enzymes able to acetylate multiple sites in the histone tails and additional sites on the globular histone core, for the first class, while the second class mostly consists of cytoplasmic enzymes able to acetylate newly synthesized histones prior to their deposition into chromatin. Abnormal regulation of HATs has been linked to leukemia and several studies connecting them to prostate and gastric cancers have been realized [72,73].

The second most studied histone modification is methylation. As anticipated above, protein methyltransferases (PMT) emerged recently as new important targets for cancer therapy, since they were found to be overexpressed or repressed in several types of cancer, precisely in breast cancer, leukemia, myeloma, ovarian, prostate and kidney cancers. Several recently published reviews describe mechanism and biological roles of PMTs [6,41,45,46,74–81]. Indeed, due to their importance in different pathological conditions, several drug discovery programs have been launched in order to design specific compounds able to modulate these targets [46]. Equally, the recent focus in understanding histone methylation led to the characterization of histone demethylases (HDM). The first described protein has been the lysine specific demethylase 1 (LSD1) [82]. Soon after, a new class of proteins having demethylase activity, the JMJC (Jumonji C) domain family, was discovered and characterized [83]. LSD and JMJC demethylases have been reported to be regulators of various cellular processes. Therefore, a special effort, as in the case of PMTs, is currently made, aimed to the discovery of small-molecule inhibitors with therapeutic potential [47,83].

Beside the above classes of enzymes, there is a mounting evidence that also other type of modifications can constitute important paradigm in epigenetics and may underlie new biological target for therapeutic purposes. For instance, some studies highlighted specific roles of ubiquitination [84–87], poly-ADP-ribosylation [88–96] and glycosylation [61,97–101] to the epigenetic code. However, the therapeutic potential of these modifications still needs to be validated for the therapeutic and drug discovery point of view. Equally, other modifications like histone phosphorylation, citrullination (deamination) [102], biotinylation [103,104], tail clipping and proline isomerization [25], are still poorly understood and their role in human pathologies remains largely unclear [25]. In the next paragraph, we aim to describe the state-of-the-art of these modifications, analyzing, from a chemical point of view, their role on the dynamics of histone proteins.