Pharmacology & Therapeutics 150 (2015) 1–22

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Teicher

Targeting histone lysine methylation in cancer

John McGrath, Patrick Trojer *

Constellation Pharmaceuticals, 215 1st Street Suite 200, Cambridge, MA, 02142, USA

ARTICLE INFO

Available online 9 January 2015

Keywords:
Methyltransferase
Demethylase
Chromatin
Epigenetic therapy
Histone methylation
Methyltransferase inhibitor

ABSTRACT

Within the vast landscape of histone modifications lysine methylation has gained increasing attention because of its profound regulatory potential. The methylation of lysine residues on histone proteins modulates chromatin structure and thereby contributes to the regulation of DNA-based nuclear processes such as transcription, replication and repair. Protein families with opposing catalytic activities, lysine methyltransferases (KMTs) and demethylases (KDMs), dynamically control levels of histone lysine methylation and individual enzymes within these families have become candidate oncology targets in recent years. A number of high quality small molecule inhibitors of these enzymes have been identified. Several of these compounds elicit selective cancer cell killing in vitro and robust efficacy in vivo, suggesting that targeting 'histone lysine methylation pathways' may be a relevant, emerging cancer therapeutic strategy. Here, we discuss individual histone lysine methylation pathway targets, the properties of currently available small molecule inhibitors and their application in the context of cancer.

Contents

1. Introduction

Chromatin is a complex assemblage of DNA and histone proteins (Wolffe, 1998) that allows large amounts of DNA to be accommodated in the confined space of a eukaryotic cell's nucleus. Dynamic regulation of chromatin structure is necessary to allow for the execution of processes that require access to DNA in response to physiological and environmental stimuli. Modulation of chromatin structure is accomplished by a number of mechanisms, including the alteration of nucleosome composition through replacement by histone variants (Biterge & Schneider, 2014; Weber & Henikoff, 2014), covalent histone modifications (Tan et al., 2011; Zentner & Henikoff, 2013; Rothbart & Strahl, 2014; Swygert & Peterson, 2014), ATP-dependent repositioning or removal of histones (Hargreaves & Crabtree, 2011; Swygert & Peterson, 2014), DNA modifications (Koh & Rao, 2013; Kohli & Zhang, 2013; Pastor et al., 2013; Delatte et al., 2014) and higher order chromatin

Abbreviations: 2-OG, 2-Oxoglutarate; AML, Acute Myeloid Leukemia; CML, Chronic Myelogenous Leukemia; DNMT1, DNA Methyltransferase 1; DLBCL, Diffuse Large B-cell Lymphoma; DOT1L, Disruptor of Telomeric Silencing 1-Like; EED (Eed), Embryonic Ectoderm Development; EHMT, Euchromatic histone-lysine N-methyltransferase; ESC, Embryonic Stem Cell; EZH2 (Ezh2), Enhancer of Zeste Homolog 2; FAD, Flavin Adenine Dinucleotide; GCB-DLBCL, Germinal Center B-cell Like Diffuse Large B-cell Lymphoma; HDAC, Histone Deacetylase; HTS, High Throughput Screening; IDH, Isocitrate Dehydrogenase; KDM, Lysine Demethylase; KMT, Lysine Methyltransferase; LOF, Loss of Function; MAO, Monoamine Oxidase; NHL, Non-Hodgkin Lymphoma; PDAC, Pancreatic Ductal Adenocarcinoma; RB, Retinoblastoma; RNAi, RNA interference; SAM, S-Adenosyl-L-Methionine; SAH, S-Adenosyl-L-Homocysteine; SCLC, Small Cell Lung Cancer; SSC, Squamous cell carcinoma; SUZ12 (Suz12), Suppressor of Zeste 12; TCA, Tricarboxylic Acid; TCP, Trans-2-phenylcyclopropan-1-amine; Wnt, Wingless-related integration site

* Corresponding author.
E-mail address: patrick.trojer@constellationpharma.com (P. Trojer).

http://dx.doi.org/10.1016/j.pharmthera.2015.01.002
0163-7258/

conformational changes (Woodcock & Ghosh, 2010). Posttranslational modifications of histones such as lysine acetylation can impact the charge distribution between histones and DNA, directly promoting chromatin opening or compaction, or they can influence the binding of chromatin-associated proteins, and thus promote or restrict their chromatin residency. Histone lysine methylation contributes to chromatin organization through the latter mechanism since it does not directly alter the charge on lysine.

The methylation of histone lysine residues was discovered in the 1960s (Allfrey & Mirsky, 1964; Murray, 1964) and was regarded for decades as an irreversible posttranslational modification. The discovery of a lysine demethylase in 2004 (Shi et al., 2004) provided the first evidence that the methylation of histone lysine residues is instead dynamic. The global turnover rates of individual histone lysine methylation marks are variable and may be either coupled or uncoupled from cellular replication and cell cycle progression (Zee et al., 2010, 2012). Also, histone methylation marks that appear to be slowly turned over on a global scale can show remarkable dynamics in a spatially and temporally defined context. Individual methylation marks show significant differences in overall abundance and the abundance of a given methylation mark may vary with respect to cell type (Jaffe et al., 2013).

Key lysine residues on core and linker histones have now been cataloged as methylation sites and the responsible enzymes that catalyze the addition and the removal of methyl groups have largely been identified (Greer & Shi, 2012). Despite the subtle physicochemical nature of lysine methylation, the regulatory potential of this 'histone lysine methylation network' is tremendous. The number of targetable histone lysine residues and the degree of methylation on each methylation site give rise to a highly complex repertoire of potential functional outputs, with further layers of complexity introduced by the fact that histone methylation marks do not function in isolation but rather cooperatively with other types of histone modification (Strahl & Allis, 2000). Individual methylated lysine residues are recognized by specific methyl-lysine recognition modules. Dozens of proteins belonging to various families according to the type of 'reader domain' have been identified (Taverna et al., 2007), while the characterization of binding specificities and the determination of functional consequences upon binding are still under investigation (Wilkinson & Gozani, 2014). Although we have only begun to unravel the mechanistic complexity of the histone lysine methylation network, its critical impact on governing important cellular processes including transcription, cell identity and genome stability is undisputed (Black et al., 2012; Greer & Shi, 2012; Lyons & Lomvardas, 2014; Rivera et al., 2014; Wozniak & Strahl, 2014). Aberrations in histone lysine methylation-controlled regulatory cues are frequently observed in cancer. Identification of key alterations in the histone lysine methylation network upon which cancer cells have become dependent may provide novel intervention points for human cancer therapy. Here, we discuss relevant oncology targets in this space as well as recently identified small molecule inhibitors of those targets, some of which are currently in (pre-)clinical development.

2. Dynamic histone lysine methylation

Two enzyme super-families, lysine methyltransferases (KMTs) and demethylases (KDMs), control histone lysine methylation states (Fig. 1).

KMTs utilize SAM as the methyl-group donor to transfer one, two or three methyl-groups to histone lysine residues. Most KMTs are highly selective with regard to the histone lysine residue upon which they operate as well as to the degree of methylation that they catalyze. It is now appreciated that KMTs also target non-histone substrates (for review see Clarke, 2013; Moore & Gozani, 2014). However, before the enzymatic activity of the first KMT was described (Rea et al., 2000) this family of proteins was long recognized as having a role in transcriptional regulation. Since KMTs are often multi-domain proteins and frequently participate in chromatin-bound, multi-subunit-containing protein complexes, it is likely that the catalytic activity contributes to but does not solely define KMT function. KMTs are categorized into two protein families based on catalytic domain sequence similarity and structural organization. The catalytic domain of KMTs is called the SET (*S*u(var)3-9, *E*nhancer of Zeste, *T*rithorax) domain, named after the *Drosophila* Polycomb proteins in which this domain was originally identified. (for a recent review and detailed domain organization of KMTs see Herz et al., 2013). The only exception is DOT1L, whose catalytic domain is structurally related to the catalytic domains of protein arginine methyltransferases (Nguyen & Zhang, 2011).

Enzymatic demethylation of histone lysines was experimentally confirmed in 2004 when a nuclear homolog of monoamine oxidases was shown to demethylate histone H3 at lysine 4 (Shi et al., 2004). KDMs are currently categorized into two protein families based on the organization of their catalytic domains and the type of oxidative mechanism that underlies the demethylation reaction (Fig. 1). The Jumonji (JmjC) domain-containing KDM family members utilize 2-oxoglutarate (2-OG; α-ketoglutarate) as a co-factor while KDM1A (LSD1, BHC110, AOF2) and KDM1B (LSD2) require FAD (Thinnes et al., 2014). Similar to the case of KMTs, KDMs are usually selective for a given lysine residue and individual KDMs specifically catalyze the removal of methyl groups from tri-, di- and mono-methylated lysines. In addition to their catalytic domains, KDMs frequently harbor chromatin-, DNA- and protein–protein interaction domains and often function as part of multi-subunit protein complexes. Over the past decade a wealth of data has been generated implicating KDMs in the regulation of nuclear processes including transcription (Kooistra & Helin, 2012).

Enzymatic modulation of histone lysine methylation is inherently linked to cellular metabolism since the universally required co-factors for both KMT and KDM reactions are key metabolites of the TCA cycle. Therefore, changes in intranuclear levels of these metabolites may impact their activity. The effect of changes in cellular metabolites on chromatin methylation is illustrated by the case of IDH1 and IDH2, metabolic enzymes that catalyze the oxidative decarboxylation of isocitrate to 2-OG. Mutations in these enzymes are observed in AML and glioblastoma (Dang et al., 2010), and result in an altered enzymatic activity. Mutated IDH1 and IDH2 utilize 2-OG as a substrate and convert it to 2-hydroxyglutarate (2-HG), a product inhibitor of 2-OG-utilizing enzymes. Thus, in the context of low 2-OG (and/or high 2-HG), KDMs and DNA hydroxylases are partially inhibited, causing a 'hypermethylator' phenotype that is often associated with cancer progression. Another example highlighting the connection between histone lysine methylation and cell physiology is provided by a study in which the metabolism of the amino acid threonine was investigated in embryonic stem cells. Threonine was demonstrated to contribute significantly to cellular glycine and acetyl coenzyme A levels required for SAM synthesis. Depletion of threonine reduced cellular SAM levels and selectively affected global histone H3 lysine 4 di- and tri-methylation levels (Shyh-Chang et al., 2013). The general observation that both KMTs and KDMs are dependent on intermediates of the TCA cycle strongly supports the concept that histone lysine methylation states can be dynamically regulated, especially at specific genomic locations where chromatin structure is actively re-organized in response to environmental stimuli.

3. Dysregulation of histone lysine methylation pathways in cancer

Large genomic sequencing campaigns of primary human tumor samples have provided evidence for recurrent mutations, translocations and somatic copy number gains or losses in KMTs, KDMs and other chromatin-associated protein-encoding genes (Table 1, Fig. 2; for instance see (Gonzalez-Perez et al., 2013; Kandoth et al., 2013; Zack et al., 2013; Lawrence et al., 2014)). Moreover, several KMTs and KDMs are specifically overexpressed in cancer and recent studies document aberrant global histone lysine methylation levels in a number of