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Histone lysine methyltransferases and demethylases in *Plasmodium falciparum*

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Abstract

Dynamic histone lysine methylation, regulated by methyltransferases and demethylases, plays fundamental roles in chromatin structure and gene expression in a wide range of eukaryotic organisms. A large number of SET-domain-containing proteins make up the histone lysine methyltransferase (HKMT) family, which catalyses the methylation of different lysine residues with relatively high substrate specificities. Another large family of Jumonji C (JmjC)-domain-containing histone lysine demethylases (JHDMs) reverses histone lysine methylation with both lysine site and methyl-state specificities. Through bioinformatic analysis, at least nine SET-domain-containing genes were found in the malaria parasite *Plasmodium falciparum* and its sibling species. Phylogenetic analysis separated these putative HKMTs into five subfamilies with different putative substrate specificities. Consistent with the phylogenetic subdivision, methyl marks were found on K4, K9 and K36 of histone H3 and K20 of histone H4 by site-specific methyl-lysine antibodies. In addition, most SET-domain genes and histone methyl-lysine marks displayed dynamic changes during the parasite asexual erythrocytic cycle, suggesting that they constitute an important epigenetic mechanism of gene regulation in malaria parasites. Furthermore, the malaria parasite and other apicomplexan genomes also encode JmjC-domain-containing proteins that may serve as histone lysine demethylases. Whereas prokaryotic expression of putative active domains of four *P. falciparum* SET proteins did not yield detectable HKMT activity towards recombinant *P. falciparum* histones, two protein domains expressed in vitro in a eukaryotic system showed HKMT activities towards H3 and H4, respectively. With the discovery of these *Plasmodium* SET- and JmjC-domain genes in the malaria parasite genomes, future efforts will be directed towards elucidation of their substrate specificities and functions in various cellular processes of the parasites.

Keywords: *Plasmodium falciparum*; Histone; Methyltransferase; Demethylase; Phylogeny; Lysine methylation

1. Introduction

Among the diverse histone modifications occurring in eukaryotic cells, histone acetylation is largely linked to active genes, whereas histone lysine methylation is involved in both transcriptional activation and silencing (Li et al., 2007). Histone lysine methylation is regulated by the opposing actions of histone lysine methyltransferases (HKMTs) and histone lysine demethylases. HKMTs belong to two families of proteins: the SET [the *Drosophila* suppressor of variegation – Su(var), the Polycomb-group protein Enhancer of zeste – E(z) and Trithorax (TRX) group proteins] domain-containing protein family and non-SET-domain protein DOT1 (disruptor of telomeric silencing-1) (Martin and Zhang, 2005). To date, two families of histone lysine demethylases have also been identified: lysine-specific demethylases 1 (LSD1) and Jumonji C (JmjC)-domain-containing histone demethylases (JHDMs) (Klose et al., 2007). With the finding of a large number of HKMTs and histone demethylases, the significance of histone lysine methylation in regulating various biological processes and pathways in model organisms has begun to be fully appreciated. Recent studies in apicomplexan parasites have also demonstrated the importance of histone lysine methylation in parasite development and pathogene-

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sis (Chookajorn et al., 2007; Cui et al., 2007b; Lopez-Rubio et al., 2007; Sautel et al., 2007).

Plasmodium parasites have a complicated life cycle with two dramatically different hosts, a vertebrate and an invertebrate. In both hosts, the parasites display a tightly regulated transcription program (Bozdech et al., 2003; Le Roch et al., 2003). While basal transcription machinery such as proteins associated with RNA polymerase II is conserved (Callebaut et al., 2005), there is a paucity of recognisable specific transcription factors in the parasite genome (Aravind et al., 2003; Coulson et al., 2004). This notion of transcription factors in malaria parasites, together with the large number of chromatin-modifying proteins, has led to speculation that epigenetics plays a prominent role in transcription regulation in the parasite. Moreover, this notion can be extended to other apicomplexan parasites such as *Toxoplasma gondii,* suggesting that epigenetics is a conserved phenomenon in the apicomplexan lineage (Sullivan and Hakimi, 2006; Gissot et al., 2007). Of the many enzymes catalysing the reversible covalent histone modifications, the histone acetyltransferase (HAT) PfGCN5 is a key regulator of gene expression (Fan et al., 2004; Cui et al., 2007b), and represents a potential target for chemotherapy (Cui et al., 2007a). Furthermore, recent studies on antigenic switching in the parasite revealed the participation of the histone deacetylase (HDAC) Sir2 in regulating telomeric silencing and mono-allelic expression of the *var* genes (Duraisingh et al., 2005; Freitas-Junior et al., 2005). These studies underline the significance of epigenetic mechanisms in *Plasmodium* gene expression.

Like other eukaryotes, the malaria parasite chromosomes have a typical nucleosomal organisation involving both core and variant histones (Cary et al., 1994; Miao et al., 2006). In addition to histone acetylation, tandem mass spectrometry analysis of *P. falciparum* histones has revealed lysine and arginine methylation (Miao et al., 2006). Some of these methyl marks on histones have been shown to have evolutionarily conserved roles in transcription regulation. In malaria parasites, epigenetic markers have been investigated as a regulatory mechanism of antigenic switching. Di- and tri-methylation of H3K4 (an active gene marker) are found at the *var* promoter that is actively transcribed or poised for transcription (Lopez-Rubio et al., 2007). In contrast, tri-methylation of H3 at K9 (H3K9me3), a heterochromatin marker, has been shown to be associated with the silent *var* genes (Chookajorn et al., 2007; Lopez-Rubio et al., 2007), and is negatively correlated with global gene expression (Cui et al., 2007b). In this survey, we evaluated histone lysine methylation during *P. falciparum* development, performed in silico analysis of potential histone methyltransferases and demethylases in the parasite genomes, and speculated on their enzymatic activities within the context of phylogenetic groupings. These data will serve as the background for future enquiry into the roles of histone lysine methylation in regulating gene expression in malaria parasites.

2. Materials and methods

2.1. Identification of P. falciparum SET and JmjC-domain proteins

To identify SET and DOT1 family HKMTs and JHDMs in malaria parasites, the *Plasmodium* genomes (http://PlasmoDB.org) were searched with various BLAST algorithms using the consensus SET, DOT1 and JmjC motifs as the queries (Jenuwein et al., 1998; Janzen et al., 2006; Klose et al., 2007). The search was extended to cover other available Apicomplexa genomes (http://www.apidb.org). A more thorough survey and analysis of the protein architecture were performed using the Simple Modular Architecture Research Tool (SMART) (http://smart.embl.de), which uses a Hidden Markov Model (HMM) (Letunic et al., 2006). Alignment of individual proteins as well as signature domains identified by SMART was performed by using Clustal X with visual inspection. A cladogram was generated for *Plasmodium* and apicomplexan SET- and JmjC-domain proteins using the UPGMA method implemented in the MEGA 4 program with pairwise deletions (Tamura et al., 2007). The reliability of the tree topology was assessed by bootstrap analysis.

2.2. Parasite culture

Culture of the *P. falciparum* 3D7 clone and synchronisation by two rounds of 5% D-sorbitol treatment were performed as described previously (Trager and Jensen, 1976; Lambros and Vanderberg, 1979; Cui et al., 2007a,b). For time-course studies, parasites were taken at 12, 22, 32 and 42 h post-synchronisation to represent rings, early trophozoites, late trophozoites and schizonts as determined by microscopy. Infected erythrocytes were treated with 0.05% saponin to lyse the red blood cell membrane and the released parasites were collected by centrifugation and washed twice with cold PBS.

2.3. RNA extraction, cDNA synthesis,PCR and rapid amplification of cDNA ends (RACEs)

Total RNA was extracted from the parasites using Trizol Reagent (Invitrogen, Carlsbad, CA) and treated with RNase-free DNase I (Promega, Madison, WI) to remove contaminating genomic DNA (Cui et al., 2001). cDNA was synthesised from 1 μg of total parasite RNA using oligo-dT primer and Superscript III reverse transcriptase (RT) in a 20 μl reaction. Each cDNA reaction was diluted to 100 μl and 1 μl was used for PCR. To verify the prediction of introns in the SET-domain-containing open reading frames (ORFs), primers spanning the SET-domain-containing genes were designed to amplify cDNA for sequencing.

The 5′ ends of the SET- and Jmjc-domain genes were determined using the FirstChoice RNA-ligase mediated (RLM)-RACE kit (Ambion, Austin, TX), and the 3′ ends