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COMMENTARY

Histone methylation, alternative splicing and neuronal differentiation

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ABSTRACT

Alternative splicing, as well as chromatin structure, greatly contributes to specific transcriptional programs that promote neuronal differentiation. The activity of G9a, the enzyme responsible for mono- and di-methylation of lysine 9 on histone H3 (H3K9me1 and H3K9me2) in mammalian euchromatin, has been widely implicated in the differentiation of a variety of cell types and tissues. In a recent work from our group (Fiszbein et al., 2016) we have shown that alternative splicing of G9a regulates its nuclear localization and, therefore, the efficiency of H3K9 methylation, which promotes neuronal differentiation. We discuss here our results in the light of a report from other group (Laurent et al. 2015) demonstrating a key role for the alternative splicing of the histone demethylase LSD1 in controlling specific gene expression in neurons. All together, these results illustrate the importance of alternative splicing in the generation of a proper equilibrium between methylation and demethylation of histones for the regulation of neuron-specific transcriptional programs.

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Cell differentiation requires activation and inactivation of different transcriptional, mRNA processing and translational programs. Alternative splicing, as the most important source of diversity of mRNAs and proteins, greatly contributes to these tissue-specific expression patterns.[2,13,25] It is known that more than 60 percent of alternative splicing events in humans are differentially regulated among different tissues.[35] In addition to the roles of specific sets of transcription and splicing factors, it has become clear that epigenetic mechanisms are critical for setting of differentiation programs as well as to maintain distinct acquired phenotypes.[31]

Changes on chromatin structure[4,11,17] and alternative splicing[18,33] have been shown to be prevalent in the regulation of differentiation and functioning in the nervous system. For example, the balance between acetylation and deacetylation of histones is important for proper progression lineage during the differentiation of neuronal precursors, since Trichostatine A, a drug that inhibits histone deacetylation prevented the progression of progenitors into mature oligodendrocytes.[22] However, it has also been shown that another drug, valproic acid that is also an inhibitor of histone deacetylase activity, induced neuronal differentiation of adult hippocampal neural progenitors.[9]

Furthermore, genome-wide trasncriptomics analyses revealed that alternative splicing is particularly widespread in the vertebrate nervous system.[2,18,25]

Various neuronal pathologies associated with mutations or changes in the activity of regulators of both chromatin structure and alternative splicing have been reported, such as defects in long-term memory observed due to a generalized hypoacetylation by mutations in the gene encoding the CREB binding protein.[1] In this context, our recent work on the relevance of alternative splicing of the histone modifying enzyme G9a in the regulation of neuronal differentiation[8] illustrates the existing cross-talk between alternative splicing and chromatin structure in the nervous system.

In order to evaluate the significant role of alternative splicing in the modulation of the functional properties of chromatin-modifying enzymes, Lois and Colleagues[20] analyzed a set of 160 genes encoding

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Table 1. List of methyltranferase and demethylase enzymes codifying genes that show alternative splicing isoforms in human and/or mouse.

Enzyme name	Gene symbol	Function
euchromatic histone-lysine N-methyltransferase 2	G9a / EHMT2	methyltransferase
euchromatic histone-lysine N-methyltransferase 1	GLP / EHMT1	methyltransferase
DOT1-like, histone H3 methyltransferase	DOTIL	methyltransferase
enhancer of zeste homolog 2	EZH2	methyltransferase
protein arginine methyltransferase 1	PRMT1	methyltransferase
Protein arginine N-methyltransferase 5	PRMT5	methyltransferase
SET domain containing (lysine methyltransferase) 8	SETD8	methyltransferase
suppressor of variegation 3–9 homolog 1	SUV39H1	methyltransferase
suppressor of variegation 3–9 homolog 2	SUV39H2	methyltransferase
JmjC domain-containing histone demethylation protein 1A	JHDM1A	demethylase
JmjC domain-containing histone demethylation protein 1B	JHDM1B	demethylase
JmjC domain-containing histone demethylation protein 2B	JHDM2B	demethylase
JmjC domain-containing histone demethylation protein 3B	JHDM3B	demethylase
JmjC domain-containing histone demethylation protein 3C	JHDM3C	demethylase

chromatin modifying enzymes and focused on the changes affecting protein domains. The authors found that 49% of the genes present alternatively spliced isoforms and that 60% of these genes have isoforms with at least one affected characterized domain. Table 1 shows a list of methyltransferases and demethylases with multiple alternatively splicing isoforms in human and mouse.

G9a, also known as EHMT2 (euchromatic histone methyltransferase 2), is the enzyme responsible for mono- and di-methylation of lysine 9 on histone H3 (H3K9me1 and H3K9me2) in mammals. As a methyltransferase, G9a catalyzes not only the addition of methyl groups in H3 but also has numerous targets of its activity as CDYL1, WIZ, p53 and also G9a by automethylation. Mostly recognizing lysine- and arginine-rich regions,[27] its function and biochemistry have been thoroughly studied and various pharmacological inhibitors of its activity have been developed.[29]

G9a has been widely implicated in the differentiation of a variety of cell types and tissues. It is known that G9a is essential for the differentiation and growth of tenocytes, since in G9a-KO mice the expression of several tendon-specific transcription factors is suppressed.[34] Moreover, it was reported that G9a cooperates with Sharp1, a potent repressor of skeletal muscle differentiation[19] and that deficiency in G9a inhibits differentiation of monocytes[36] and T helper cells.[16] G9a is required for the formation of brown adipose tissue in detriment of the specification muscle lineage[26] and mice deficient in G9a and GLP showed severe heart defects due to decreased expression of specific cardiac genes.[10] In addition, G9a-KO animals showed upregulation of genes related with retinal progenitor cells[12] and a drastic loss of mature gametes.[32] Finally, G9a has been reported as a key regulator of pluripotency related genes promoting specific gene silencing through histone methylation during differentiation of embryonic stem cells.[3,6] G9a loss allows the restatement of the gene Oct3/4 pluripotency and reprogramming of differentiated cells[6,7] and at the same time increases the frequency of induced pluripotent stem cells.[21]

It has been shown that G9a has a key role in the nervous system through the control of cognition and adaptive responses due to the suppression of non-neuronal genes.[28] Its ortholog in *Drosophila* has been described as a regulator of peripheral dendrite growth, classical learning and expression of genes related to memory.[14] Moreover, it has been shown that G9a affects the specification of different neuronal subtypes[24] and regulates the neurodegeneration induced by ethanol.[30]

In our recent publication,[8] the role of G9a in cell differentiation is extended. We found that the methyltransferase activity of G9a is required for neuronal differentiation of the N2a cell line in culture and that the levels of H3K9me2 are upregulated in N2a mature neurons. Moreover, this change positively correlates with the inclusion of G9a alternative exon 10 (E10) in the mature mRNA. Although the existence of 2 alternatively spliced isoforms of G9a differing on the presence/absence of E10 was first described in 2001,[5] most of the literature refers to G9a without further consideration of the putative roles of its alternative splicing variants. We have demonstrated that, although E10 inclusion does not affect G9a intrinsic catalytic activity, it results in increased H3K9me2 global levels due to higher nuclear localization of the enzyme. In parallel, Mauger et al.[23] showed that levels of E10 inclusion vary greatly in different cell types, suggesting that

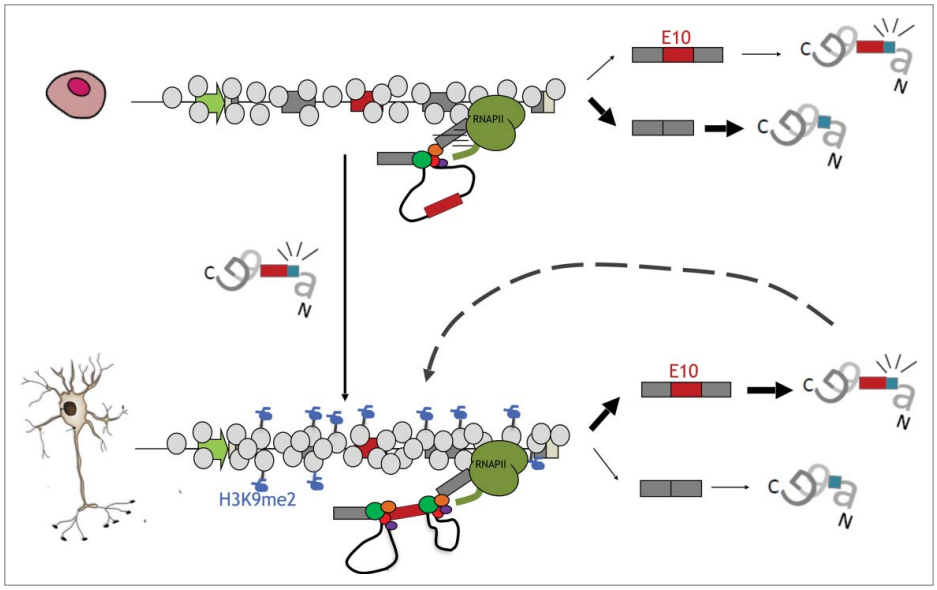

Figure 1. Schematic diagram of the implication of G9a alternative splicing in neuronal differentiation. In neuronal precursors, the prevalence of the G9a isoform lacking E10 correlates with low levels of intragenic H3K9 di-methylation. Upon differentiation, the alternative splicing pattern of G9a changes in favor of the E10 inclusion which, by promoting G9a nuclear localization increases H3K9me2 levels in the G9a gene. This model suggest a positive feedback loop in which G9a regulates its own alternative splicing by intragenic methylation of its histone marks during neuronal differentiation.

splicing might control some G9a enzymatic properties. Similarly to our results obtained *in vivo*, the authors found that inclusion of E10 does not change G9a activity measured *in vitro*. We analyzed the subcellular localization of G9a during neuronal differentiation of N2a cells by immunofluorescence and found that the pattern changes from a cell-wide to an almost only nuclear localization, and this observation correlates with the observed increase in E10 inclusion into G9a mRNA. To determine whether E10 inclusion just correlates with, or causes G9a nuclear localization, we transiently overexpressed G9a with and without E10 in different cell lines. These experiments led us to conclude that E10 inclusion promotes G9a nuclear localization. To explain our results, since the alternatively spliced exon does not encode a nuclear localization signal (NLS), we hypothesized that inclusion of E10 might enhance the exposure of the neighboring constitutive NLS due to its intrinsically unstructured nature. Moreover, we showed that G9a regulates its own alternative splicing and the isoform including amino acids encoded by E10 promotes more inclusion of E10 in the mature mRNA. Regarding the mechanism by which G9a regulates its own alternative splicing, we hypothesized that during neuronal differentiation, G9a methylates its own intragenic histone marks provoking a more compact chromatin structure that could, in turn, promote more inclusion of alternative exons (Fig. 1). However, the possibility of a direct or indirect mechanism of G9a regulating its own alternative splicing patterns remains open. Finally, we found that the regulation of G9a alternative splicing appears to be necessary for efficient neuronal differentiation.

G9a does not seem to be the only chromatin modifying enzyme whose alternative splicing regulates neuronal differentiation. It has been reported that a specific isoform of lysine-specific demethylase 1 (LSD1) has a key role through histone demethylation.[15] The authors found that the inclusion of an alternative exon in LSD1 promotes the adquisition of selective histone substrate specificities and regulates important transcriptional programs in neurons. LSD1 has been characterized as the enzyme responsible for H3K4me1/2 and H3K9me1/2 demethylation. This recent work emphasizes the role of an alternative exon inclusion in LSD1 to mediate H3K9me2 demethylation in collaboration with a new protein partner, supervillin, which is essential for neuronal maturation. The authors found that knockdown specifically the isoform that includes the alternative exon compromises neuronal differentiation of SH-SY5Y cells and increases H3K9me2 levels at its target promoters. Taken together, these results and our recent report, suggest that a correct equilibrium of methylation and demethylation of H3K9

is fundamental for the regulation of specific expression programs that promote neuronal differentiation. Furthermore, both the methyltransferase G9a and the demethylase LSD1 could possibly have different gene targets and the joint regulation of both enzymes could be key to repress non-neuronal genes and activate neuronal-genes at the same time.

Overall, the results on G9a suggest a positive feedback loop that reinforce cellular commitment to differentiation and bring alternative splicing into focus, highlighting the key role of alternative isoforms during neuronal differentiation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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