Apparent microRNA-target-specific histone modification in mammalian spermatogenesis

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Abstract: We identified several miRNAs' target genes that are significantly associated with histone modification during mammalian spermatogenesis. MiRNAs that target genes associated with the most significant histone modification are expressed before or during spermatogenesis, thus the results were convincing.

1. Introduction

Epigenetic regulation of mRNA expression is involved in a wide variety of biological events, e.g., disease progression, including cancers, development, and even evolution. Although epigenetics were recognized as important events several years ago, our present understanding of their mechanism remains incomplete. One reason for this deficiency is our lack of knowledge about cooperative mechanisms between distinct epigenetic factors. For example, although both microRNAs (miRNAs) and promoter methylation are related to the suppression of mRNA expression, whether these two factors regulate mRNA expression cooperatively is not known. Alternatively, although histone modification can both suppress and trigger mRNA expression, it is unknown what will result from simultaneous suppressive and expressive histone modification. Without this kind of knowledge, our understanding of how epigenetic factor regulate target genes will remain incomplete. Recently, Su et al. [1] found that miRNAs more frequently target genes with less methylated promoters. More recently, the author used bioinformatics to identify apparent reciprocal regulation of target genes between promoter methylation and miRNAs [2], [3], which has some support from the evolutionary point of views[4]. Thus, seeking more examples of apparent miRNA regulation of other epigenetic factors will deepen our understanding of the epigenetic regulation of mRNAs.

Histone modification is believed to affect mRNA expression through chromatin remodeling. For example, not only promoter methylation, but also methylation of histone is generally supposed to suppress transcription of genes. As both are equally methylated, it is possible that miRNA regulation of both processes exists. Indeed, a relationship between promoter methylation and histone modification has been suggested [5], [6].

In this paper, we analyzed publically available histone modification data obtained during mammalian spermatogenesis [7]. We targeted spermatogenesis because histone modification is believed to play critical roles in this process[8] and miRNAs are also thought to be generally important for differentiation[9]. Thus, cooperative regulation of mRNAs between miRNAs and histone modification likely takes place during spermatogenesis.

Various histone modification of multiple development stages in spermatogenesis were investigated with for targeting by microRNAs. We have found that the targeted genes of the majority miRNAs were significantly upregulated or suppressed during spermatogenesis. The frequent observation of apparent miRNA regulation of histone modification reflected the apparent dependence of histone modification on the frequency of being targeted by an individual miRNA. In addition, miRNAs whose target genes are most significantly associated with histone modification around the transcription start site (TSS) were expressed before or during spermatogenesis. This supported our analysis and experimental verification of these results are awaited.

2. Results and Discussion

2.1 Mouse miRNAs whose target genes are associated with differential histone modification during spermatogenesis around the TSS

Firstly, we used the MiRaGE method [10], [11] to investigate if histone modifications around the TSSs of miRNA target genes were enhanced or repressed during mouse spermatogenesis. Although MiRaGE was originally invented to infer miRNA regulation of target gene expression from target mRNA expression, it has also been used successfully to infer miRNA regulation of promoter methylation [2], [3]. Briefly, MiRaGE first computes the amount of differential mRNA expression/promoter methylation/histone modification of the ith gene at the jth sample, Δx_{ij}. It then computes the differential values to group the genes into two sets: genes targeted by the considered miRNA or genes not targeted by the considered miRNA but targeted by any other miRNAs. The exclusion from the analysis of genes not targeted by any miRNAs is because of the interrelations between genes targeted by any miRNAs. Thus, genes targeted by any miRNAs should be considered separately from genes targeted

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by no miRNAs. For example, each miRNA must compete with protein machinery, such as AGO proteins, that mediate its function. If the amounts of some miRNAs increased, these miRNAs might occupy protein machineries that otherwise could be used by other miRNAs[12]. This would affect the expression of mRNAs not targeted by these miRNAs, but targeted by any other miRNAs. However, this does not affect genes targeted by no miRNAs. Conversely, if some mRNAs' expressions increased, they would absorb miRNAs that otherwise could bind to other mRNAs[13]. Thus, the amount of genes targeted by any miRNAs affects the expression of other genes targeted by any miRNAs, but does not affect that of genes not targeted by any other miRNAs. Thus, mRNAs targeted by no miRNAs were excluded from the analysis. Then, *P*-values computed by the three statistical tests; i.e., the t-test, Wilcoxon rank sum test, and KolmogorovSmirnov test, were attributed to individual miRNAs. Despite the simplicity of this methodology, it has worked well for various applications[14], [15], [16], [17]. The obtained *P*-values were adjusted using the BenjaminiHochberg criterion[18]. Table 1 summarizes the number of mouse miRNAs whose target genes are associated with significant differential histone modification (significance means that the adjusted *P*-values are less than 0.05. The full list of mice miRNAs together with *P*-values and adjusted *P*-values is provided as additional file 1). Although the numbers vary depending on statistical test employed, they are almost consistent with each other. Thus, miRNA-target-specific histone modification seems to be observable during spermatogenesis.

The results shown in Table 1 did not qualitatively change depending upon which statistical test was used; therefore, we used the *P*-values computed by the *t* test for further analysis.

Table 1 The number of mouse miRNAs whose target genes are associated with significantly more or less modified histones, as inferred by the MiRaGE method. Statistical tests employed are KolmogorovSmirnov (KS) test, t test (t), and Wilcoxon rank sum test (wilcox). Adjusted *P*-values less than 0.05 were considered significant.

	$G < G'$			$G > G'$		
	KS	t	Wilcox	KS	t	Wilcox
Spermatocytes(SC)						
H3K4me1	120	84	90	48	4	4
H3K27me3	14	0	0	118	91	98
H3K4me3	129	112	72	19	0	1
H3K27ac	73	42	33	37	12	17
Spermatogonia(SG)						
H3K27me3	3	0	0	108	99	104
H3K4me3	124	43	78	2	0	0
H3K27ac	20	19	28	39	29	30
Spermatids(ST)						
H2AZ	97	24	60	69	59	33
H3K9ac	87	38	62	43	39	36
H3K4me1	86	78	77	28	2	3
H3K4me3	63	0	0	87	15	15
H3K27ac	35	23	29	23	23	14

3. Conclusions

In this paper, we used bioinformatics to identify apparent miRNA regulation of histone modification in mouse and human spermatogenesis. The apparent miRNA regulation of histone modification was biologically informative and feasible, and was unlikely to be caused by an artifact generated by the algorithm used. The apparent miRNA regulation of histone modification reflected the dependence of histone modification on the frequency of being targeted by an individual miRNA; therefore, it is likely to be a real biological effect. However, the mechanism of miRNA regulation of histone modification is not known and requires further experimentation and analysis.

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