Utilizing Emerging Technology to Identify Non-coding Regulatory Elements in Human Myometrial Tissues

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Abstract

Purpose:
The myometrium is the muscular compartment of the uterus made of smooth muscle that maintains the structural integrity before and generates force at parturition. Dysregulated myometrial actions may lead to pregnancy complications such as preterm birth and dystocia. Homeostasis of the myometrium is governed by genetic networks, in part, though non-coding regulatory elements. The present study aims to identify cis-acting elements in the myometrial biopsies of healthy term pregnant participants.

Methods:
Three human myometrial specimens were obtained from lower segment uteri at term pregnancy prior to the onset of labor, followed by RNAseq and H3K4me1, H3K27ac, CTCF and PGR (progesterone receptor) ChIP-seq to profile transcriptome, enhancers, potential DNA looping anchors and PGR occupancy. Parturition association genome variants from literature were to establish their association with findings in the present study.

Results:
The 3 human subjects share 13090 active and 540 super enhancers. Approximately one third of active and 40% super enhancers are located nearby high-level expressing genes. Myometrial active enhancers exhibit over-representation of binding motifs of transcription factors known for myometrial homeostasis, hormone signaling mediators and smooth muscle gene regulation, including AP-1, PGR and SRF. Enriched functional annotations on cell-cell adhesion junction and steroid hormone receptor activities are found among the 355 active genes that are in close proximity of super enhancers. Notably, over 70% of super enhancers show the PGR occupancy, in accordance with the critical role of progesterone signaling in maintaining uterine quiescence before parturition. Parturition association genome variants in the non-coding genome have also been found in the myometrial enhancers, with some in a lustered pattern.

Conclusion:
We have successfully mapped and cataloged cis-acting elements in the myometrial genome. Our work identifies regulatory elements that may function to control expression of myometrial active genes, partly through interacting with progesterone signaling during pregnancy. The findings also implicate an impact of gestational duration-associated single nucleotide polymorphisms (SNPs) on myometrial gene expression and the genome topology.

Background

1. The myometrium maintains uterine integrity during pregnancy and provides contractile force at parturition.
2. The myometrium undergoes structural and functional remodeling transforming from a synthetic to a contractile state to meet the physiological demand of pregnancy.
3. Genetic and epigenetic regulatory mechanisms behind functional dynamics of the myometrium over pregnancy are largely unknown.
4. We hypothesize that unique sets of cis-acting elements attributes to distinct transcriptomic profiles underlying the stage-specific functional characters of the myometrium.
5. The goal of this project is to map the myometrial enhancers, identify transcription regulators for myometrial gene expression, and establish links between parturition associated genome variants and myometrial enhancers.

A Distinct Molecular Profile for the Myometrium

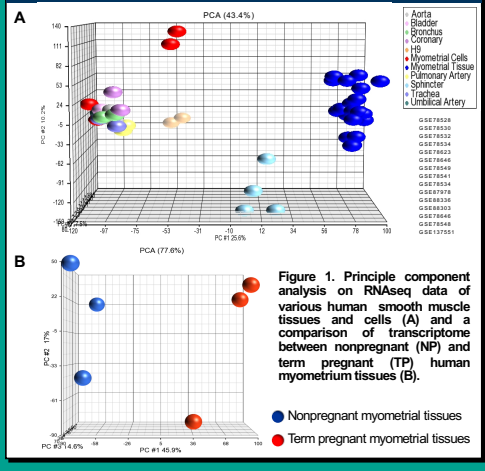


Figure 1. Principle component analysis on RNAseq data of various human smooth muscle tissues and cells (A) and a comparison of transcriptome between nonpregnant (NP) and term pregnant (TP) human myometrium tissues (B).

Regulatory Elements in the Human Myometrium

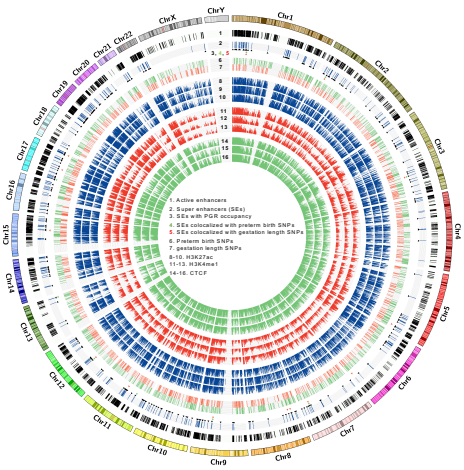
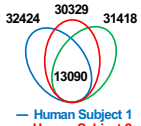


Figure 2. H3K27ac and H3K4me1 marks and CTCF occupancy in the term pregnant human myometrium. ChIP-seq assays were performed on myometrial tissues collected from the lower segment of the uterus of 3 human subjects greater than 37 weeks pregnancy by elected C-section. Active enhancers were annotated at the H3K4me1 and H3K27ac double positive regions (Calo and Wysocka, 2013, PMID: 23473601). Super enhancers are marked based on Whyte et al., 2013 (PMID: 23582322). Super enhancers that have PGR occupancy and parturition-associated genome variants (Zhang et al., 2017, PMID: 28877031) colocalization are also labeled.

Myometrial Active Enhancers

A

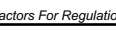


B

Total number of intervals	13090
Intervals associated with top third active genes (FPKM >= 15)	33.7 %
Intervals associated with middle third active genes (15 > FPKM >=5)	19.8 %
Intervals associated with bottom third active genes (5 > FPKM >=1)	13.3 %

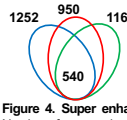
Figure 3. Active enhancers in term pregnant human myometrial tissues. (A) Number of active enhancers mapped in tissues of each individual human subject. (B) Association of commonly shared active enhancers with active genes in human myometrium. Active genes are defined by average FPKM ≥ 1 of RNAseq data of myometrial tissues among the three human subjects. The association between an interval and an active gene is defined by locating within 100 kb vicinity of each other.

Table 1. Over-Represented transcription factor binding motifs in active enhancers. Enrichment of motifs in the H3K4me1/H3K27ac marked active enhancer regions was determined by Hypergeometric Optimization of Motif EnRichment (HOMER) v4.10 (Heinz et al. 2010, PMID: 20513432). A subset of enriched motifs shown in denoted categories is displayed in this table.

Motif	Consensus Sequence	-Log *p*-Value
Known Myometrium Associated Transcription Factors		
AP-1		279
STAT5		34
NFkB		34
Steroid Hormone Receptors		
PGR		18
GRE		28
Transcription Factors For Regulation of Smooth Muscle Genes		
CArG (SRF)		22
ELK1		27

Myometrial Super Enhancers

A



B

Total number of intervals	540
Intervals associated with top third active genes (FPKM >= 15)	40.3 %
Intervals associated with middle third active genes (15 > FPKM >=5)	20.0 %
Intervals associated with bottom third active genes (5 > FPKM >=1)	7.3 %

Figure 4. Super enhancers in term pregnant human myometrial tissues. (A) Number of super enhancers mapped in tissues of each individual human subject. (B) Association of commonly shared active enhancers with active genes in human myometrium.

Table 2. Enriched gene ontology terms in the 355 active genes that are associated with super enhancers.

Gene Ontology Term	Enrichment *p*
Cell-cell adhesion junction	6.78×10^{-8}
Integrin Signaling Pathway	3.00×10^{-2}
Response to wounding	1.69×10^{-4}
Steroid hormone receptor activity	2.09×10^{-2}
Regulation of mRNA stability	1.28×10^{-2}

Enhancers with Parturition-Associated SNPs

Table 3. Numbers of myometrial enhancers in the same regions with parturition-associated genome variants. Top 10,000 gestational length and top 10,000 preterm birth associated SNPs reported previously (PMID: 28877031) are used to identify myometrial enhancers potentially linked to the parturition process.

	Preterm Birth SNPs Located in	Gestational Length SNPs Located in
# of Active Enhancers	61	61
# of Super Enhancers	10	14

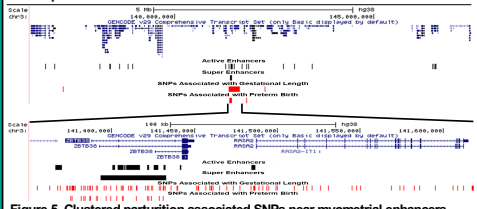


Figure 5. Clustered parturition associated SNPs near myometrial enhancers. Track view of the location of a super enhancer that has multiple parturition associated SNPs at and near the enhancer site.

PGR Occupied Myometrial Enhancers

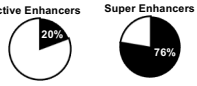


Figure 6. PGR occupancy in myometrial enhancers. 10845 PGR occupying sites in the human term pregnant myometrial tissue were identified by PGR ChIP-seq (GSE137550). Percentage of active and super enhancers that also show PGR occupancy are displayed.

Table 4. Selected enriched motifs in PGR occupied active enhancers.

Category	Motifs
Known myometrium associated TF	AP-1, STAT5, NFkB
Steroid hormone receptors	PGR, NR3C1, AR
Smooth muscle gene regulators	CArG, MyoD, Myf5, ELK1
Pregnancy Associated TF	CEBP, FOXO1

Summary

- Myometrial active enhancers are enriched with motifs of functionally known myometrial transcription factors, hormone nuclear receptors and smooth muscle regulators.
- Most myometrial super enhancers have active genes expressed in relatively high levels nearby and are occupied by the progesterone receptor, which is known to mediate progesterone signaling for maintaining myometrial quiescence before parturition.
- A subset of parturition associated genome variants in the non-coding genome is mapped to the myometrial enhancers, implicating potential functional significance of these enhancers.

Acknowledgement

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