Placenta xxx (2015) 1–11

Contents lists available at ScienceDirect

Placenta

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

# Changes in the enhancer landscape during early placental development uncover a trophoblast invasion gene-enhancer network

Geetu Tuteja [a,1], Tisha Chung [a], Gill Bejerano [a,b,c,*]

[a] Department of Developmental Biology, Stanford University, Stanford, CA 94305, USA
[b] Department of Computer Science, Stanford University, Stanford, CA 94305, USA
[c] Division of Medical Genetics, Department of Pediatrics, Stanford University, Stanford, CA 94305, USA

ARTICLE INFO

*Article history:*
Received 9 July 2015
Received in revised form
21 October 2015
Accepted 2 November 2015

*Keywords:*
Trophoblast invasion
Cis-regulation
Gene-enhancer network

ABSTRACT

*Introduction:* Trophoblast invasion establishes adequate blood flow between mother and fetus in early placental development. However, little is known about the cis-regulatory mechanisms underlying this important process. We aimed to identify enhancer elements that are active during trophoblast invasion, and build a trophoblast invasion gene-enhancer network.

*Methods:* We carried out ChIP-Seq for an enhancer-associated mark (H3k27Ac) at two time points during early placental development in mouse. One time point when invasion is at its peak (e7.5) and another time point shortly afterwards (e9.5). We use computational analysis to identify putative enhancers, as well as the transcription factor binding sites within them, that are specific to the time point of trophoblast invasion.

*Results:* We compared read profiles at e7.5 and e9.5 to identify 1,977 e7.5-specific enhancers. Within a subset of e7.5-specific enhancers, we discovered a cell migration associated regulatory code, consisting of three transcription factor motifs: AP1, Ets, and Tcfap2. To validate differential expression of the transcription factors that bind these motifs, we performed RNA-Seq in the same context. Finally, we integrated these data with publicly available protein–protein interaction data and constructed a trophoblast invasion gene-enhancer network.

*Discussion:* The data we generated and analysis we carried out improves our understanding of the regulatory mechanisms of trophoblast invasion, by suggesting a transcriptional code exists in the enhancers of cell migration genes. Furthermore, the network we constructed highlights novel candidate genes that may be critical for trophoblast invasion.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

In hemochorial placentation, trophoblast cells of the fetal placenta line the implantation site and invade the maternal decidua, enabling maternal spiral artery remodeling, and establishing adequate blood flow between mother and fetus [1–4]. Defects in this process of trophoblast invasion can lead to a number of disorders that have detrimental effects on both the mother and the fetus. Excessive trophoblast invasion may contribute to the pathogenesis of placenta creta, a severe pregnancy complication in which abnormally deep attachment of the placenta to the myometrium is observed [5–7]. Shallow invasion results in hypoxic trophoblast tissue, which can cause oxidative stress in the placenta and has been associated with pre-eclampsia and intrauterine growth restriction [8,9].

Yet the mechanisms underlying trophoblast invasion are poorly understood. Several genes and transcription factors (TFs) have been identified as important for regulating this process [2,10,11]. Nevertheless, little is known about how the genes are regulated, or about the enhancers to which the TFs bind to regulate gene expression. Enhancer regions (distal cis-regulatory regions that regulate spatiotemporal gene expression) are crucial to many processes, and often contribute to disease when disrupted [12,13]. Single nucleotide polymorphisms (SNPs) associated with placental disorders often reside in non-coding DNA, indicating the importance of non-coding enhancer elements in these disorders [14].

* Corresponding author. Beckman Center B-300, 279 Campus Drive West, Stanford, CA 94305, USA.
*E-mail address:* bejerano@stanford.edu (G. Bejerano).
[1] Present address: Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA.

http://dx.doi.org/10.1016/j.placenta.2015.11.001
0143-4004/© 2015 Elsevier Ltd. All rights reserved.

Next-generation sequencing of mouse [15,16] and human [17–19] placentas, as well as computational prediction [20], have been performed to identify putative placenta enhancer elements, but none of these has focused on the process of trophoblast invasion. Identifying enhancers genome-wide that contribute to the process of trophoblast invasion requires assaying placental tissue at an early time point, while invasion is taking place.

To identify enhancer elements that are important during trophoblast invasion from the fetal perspective, we carried out ChIP-Seq for H3k27Ac on mouse fetal placental tissue at e7.5 and e9.5. H3k27Ac is a histone modification that is associated with active enhancers [21]. E7.5 has been described as the time point for maximal trophoblast invasion in mice [22], while e9.5 is a late post-implantation stage that we used for comparison to isolate enhancers specific to e7.5 placentas and thus more likely to be crucial for the invasion process. While human and mouse placentation differ in certain respects, genetic manipulation of the mouse has been critical in providing insights into human placental development [4]. Furthermore, mouse and human placenta share homologous cell types and gene expression patterns [23]. For example, the outermost cell layer of the placenta is composed of trophoblast giant cells (TGCs) in mouse, and of extravillous cytotrophoblast cells in human, both of which display invasive behavior and express Matrix Metallopeptidase 9 (*Mmp9*) and Stimulated by Retinoic Acid 13 (*Stra13*) [23].

By comparing the enhancer landscape of the mouse placenta at e7.5 to that at e9.5, we were able to identify 1,977 enhancers with higher signal at e7.5. We then performed a motif scoring analysis and discovered three transcription factor motifs (for AP1, Ets and Tcfap2) enriched within these enhancers. The subset of enhancers containing these three TF motifs is frequently associated with genes that are important for the invasion process in mouse and human. We performed transcriptome profiling to identify the specific TF family members likely binding to these enhancers. Finally, we incorporated protein–protein interaction data to construct a gene-enhancer network, which provides additional evidence for co-regulation of genes with enhancers containing the AP1, Ets, and Tcfap2 motifs, and highlights novel candidate genes that may be important for trophoblast invasion. By performing genomic analysis at two time points that are close in development, we were able to better understand regulatory mechanisms of a process that is particularly active during a small window of time.

## 2. Material and methods

### 2.1. ChIP-Seq library construction and sequencing

Ectoplacental cone (EPC) tissue was dissected from embryos at e7.5, obtained from timed-pregnant CD-1 mice (Charles Rivers Labs). The EPC was separated from the maternal decidua, embryo, and other extraembryonic tissue as described in Ref. [24]. At e9.5, the fetal portion of the placenta was dissected. ChIP was carried out on two biological replicates per time point. For each biological replicate at e7.5, EPCs from 25 pregnant mice (~10 embryos per mouse) were combined prior to chromatin isolation. For each biological replicate at e9.5, placentas from ~10 pregnant mice were combined prior to chromatin isolation. Chromatin isolation and immunoprecipitation were carried out as previously described [25], but with 5 μg chromatin and 1.2 μg of H3k27ac antibody (Abcam ab4729, lot: GR24371-1) for the immunoprecipitation assay.

ChIP-Seq libraries were prepared as described previously [26] using 2 ng starting material for each biological replicate. For each biological replicate, 2 ng of input DNA was also prepared as a control. Libraries were sequenced by the Stanford Center for Genomics and Personalized Medicine (SCGPM) using the Illumina GAIIx, and single-end 36 base pair reads.

### 2.2. ChIP-Seq data processing and peak-calling

GAIIx output was also analyzed by the SCGPM, using the Genome Analyzer Pipeline. Sequence tags that aligned uniquely to the mouse genome build mm9 with 0,1, or 2 mismatches, according to the ELAND alignment algorithm, were retained for further analysis. The number of sequence tags for each sample can be found in Supplementary Table S1.

Sequence tags from biological replicates were combined prior to peak calling. Peak calling was performed using GLITR [26] with default parameters, a 5% false discovery rate cutoff, and a maximum peak height ≥20. Data were further filtered to remove peaks that were also identified in input control data, and because of our interest in distal cis-regulatory elements, to remove peaks that overlapped exons (using the UCSC mm9.knownGene table) or that were within 2.5 kb of a gene transcriptional start site.

### 2.3. Luciferase assays

Mouse TGCs were differentiated from trophoblast stem cells as described previously [20]. We did not select for a particular type of giant cell, so the TGCs likely represent a heterogeneous population as was found previously [27]. Putative enhancers were cloned into the pGL4.23 Ligation Independent Cloning vector, and transfection assays and luciferase assays were performed as described previously [20]. For cloning, peak coordinates were adjusted to respect conserved DNA boundaries based on Multiz alignments from Mouse to Rat and Human. Primers used to amplify genomic regions are listed in Supplementary Table S2.

### 2.4. Identification of enhancers with higher activity at e7.5 versus e9.5

In order to identify enhancers with the most differential activity between time points, we first combined the peak files of both time points, to obtain peaks that are active at e7.5 or e9.5. Overlapping peaks were merged. For each peak, a fold was calculated by normalizing the maximum number of overlapping tags within the peak by the number of tags that mapped to chromosome:

$$e7.5\ fold = \frac{\left(\frac{\max\ OverlappingTags(e7.5)}{chromosomeTags(e7.5)}\right)}{\left(\frac{\max\ OverlappingTags(e9.5)}{chromosomeTags(e9.5)}\right)}$$

From the set of merged peaks, we then obtained: (1) those peaks that have higher signal at e7.5, as the peaks with the highest 25% peak-height fold difference at e7.5 compared to e9.5; and (2) those peaks that have higher signal at e9.5, as the peaks with the highest 25% peak-height fold difference compared to e7.5. Peaks that were not in set (1) or (2) were considered to have strong activity at both time points.

### 2.5. Significance of overlap with mouse functional data sets

Mouse H3k27Ac data from 19 tissue types [15] were downloaded from the Ren Lab website: http://chromosome.sdsc.edu/mouse/download.html. The point coordinate in the H3k27Ac file was padded by ±500 bp, and regions overlapping exons or within 2.5 kb of a transcription start site in mm9 were removed.

To assess overlap significance of the 19 H3k27ac sets with the e7.5/e9.5 placenta peaks, we first created 10,000 shuffles of the peaks. For each shuffle, we counted the number of times

Please cite this article in press as: G. Tuteja, et al., Changes in the enhancer landscape during early placental development uncover a trophoblast invasion gene-enhancer network, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.11.001

Download English Version:

https://daneshyari.com/en/article/5894351

Download Persian Version:

https://daneshyari.com/article/5894351

Daneshyari.com