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Key regulators of syncytiotrophoblast cell lineage development in human placentation

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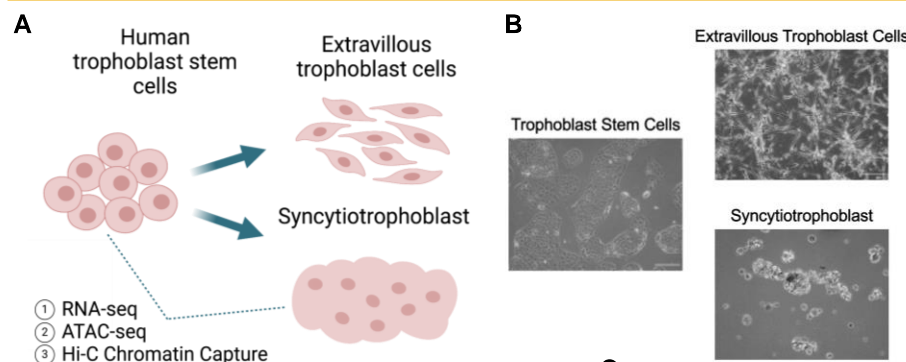
Abstract

Background. The placenta is a transient organ that forms during pregnancy to support fetal development and modulate exposure to environmental cues that impact chronic disease risk. The placenta supports fetal development in many ways including facilitating nutrient and oxygen exchange, removing harmful waste products, producing critical hormones (e.g., human chorionic gonadotropin), and conferring immune protection. These functions are largely carried out by terminally differentiated trophoblast referred to as syncytiotrophoblast and extravillous trophoblast cells. Despite the importance of syncytiotrophoblast and extravillous trophoblast cells, it is still unclear how they become specialized to support optimal fetal development. **Objective.** To identify transcriptional regulators of syncytiotrophoblast cell lineage development using a loss of function approach. **Methods.** Candidate transcription factors (TBX3, VGLL3, and ATF3) were knocked down in cytotrophoblast-derived human trophoblast stem cells using lentiviral-mediated delivery of short hairpin RNA (shTBX3, shVGLL3, or shATF3). A non-specific shRNA (shControl) was used as a control. Following transduction, cells were selected using puromycin and knockdown efficiency was confirmed at the transcript and protein level by RT-qPCR and western blotting, respectively. The impact of transcription factor knockdown on trophoblast stem cell differentiation into syncytiotrophoblast was evaluated through functional and transcriptomic assessments. **Results.** Transductions with both shTBX3 and shVGLL3 resulted in morphological abnormalities following syncytiotrophoblast cell differentiation compared to cells transduced with shControl. **Conclusions.** The critical contributions of candidate transcriptional regulators to syncytiotrophoblast cell lineage development can be evaluated using a loss of function approach in trophoblast stem cells. **Future Directions.** Preliminary results suggest that TBX3 and VGLL3 are critical for establishing the syncytiotrophoblast cell lineage. However, more in-depth characterization is needed to identify the molecular mechanism(s) through which TBX3 and VGLL3 regulate syncytiotrophoblast development. Future studies will include completing the shRNA knockdown of the remaining candidate transcription factor, ATF3, genome-wide assessments (e.g., ATAC-seq), and additional functional outputs such as human chorionic gonadotropin production, for all shRNA transductions.

Human Trophoblast Stem Cells



C

Stem vs. STB (TS[CT])

Figure 1. In vitro modeling of human trophoblast stem (TS) cells. A) Human TS cells (Okae et al. 2018, PMID: 29249463) give rise to extravillous trophoblast (EVT) and syncytiotrophoblast (STB) cell lineages. Regulatory networks controlling EVT and STB differentiation are not yet known. However, application of functional genomics approaches can help to identify candidate regulators. **B)** EVT or STB differentiation is associated with distinct morphological changes. **C)** Volcano plot displaying the significantly up- and down-regulated genes following STB differentiation identified with RNA-seq (n=3 per group; absolute log2fold change >1, adjusted p<0.05).

Objective: To identify transcriptional regulators of syncytiotrophoblast cell lineage development using a loss-of-function approach.

Experimental Approach

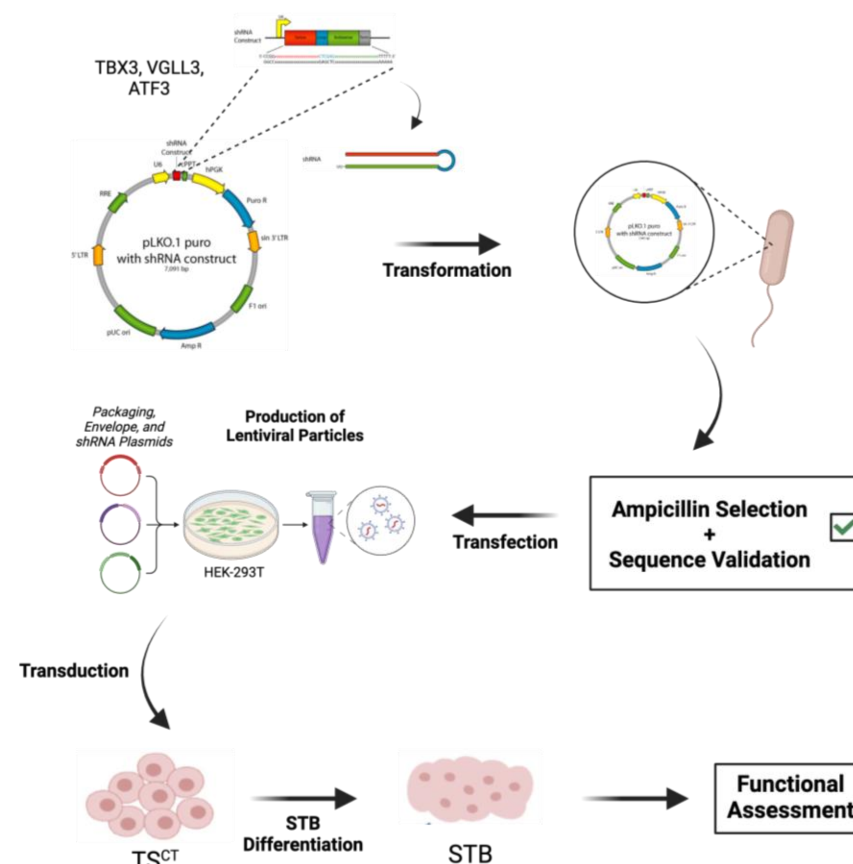


Figure 2. Experimental design for shRNA knockdown of candidate transcription factors TBX3, VGLL3, and ATF3. After cloning shRNA oligos into a pLKO.1 puromycin backbone designed for mammalian RNA interference, bacteria were transformed with plasmids for each of the target genes, as well as a scrambled shRNA control. Colonies expressing ampicillin resistance were selected and sent for sequencing to validate that they were correct. After validation, HEK-293T cells were transfected with packaging, envelope, and shRNA plasmids to produce lentiviral particles. These lentiviral particles were then used to transduce trophoblast stem cells prior to STB differentiation. STBs lacking each of the targets were then functionally assessed.

Results

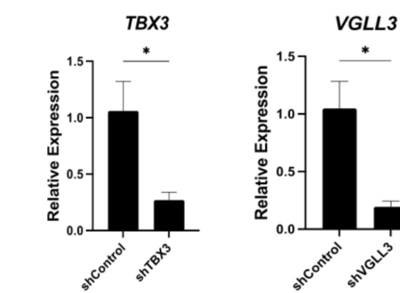


Figure 3. Validation of *TBX3* and *VGLL3* depletion through RT-qPCR. *TBX3* and *VGLL3* transcript levels in TS cells transduced with lentiviral particles containing shControl, shTBX3 (left), or shVGLL3 (right) shRNA (n=3 biological replicates per group; *p<0.05).

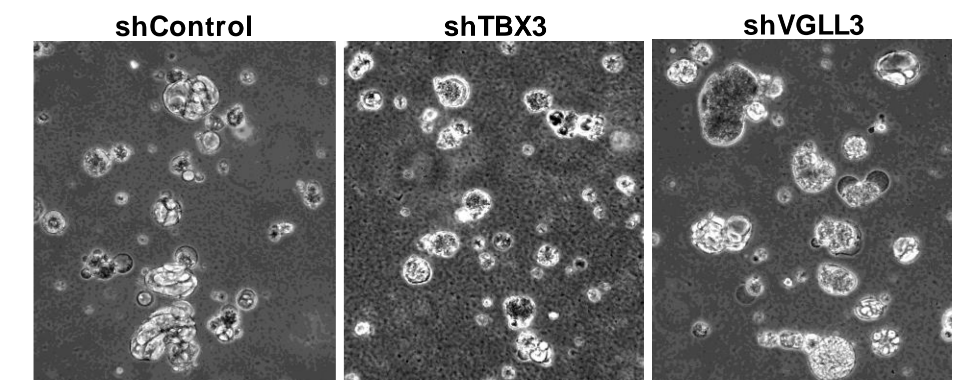


Figure 4. Assessing the effect of *TBX3* or *VGLL3* depletion on STB formation. Representative phase contrast images of STB-differentiated cells on day 6 of differentiation following transduction of cells in the stem state with lentiviral particles containing shTBX3, shVGLL3, or shControl shRNA sequences.

Conclusions and Future Directions

Transductions with both shTBX3 and shVGLL3 resulted in morphological abnormalities following STB cell differentiation compared to cells transduced with shControl. This suggests that a loss-of-function approach in human trophoblast cells using shRNA knockdown is an effective means to evaluate the potential contribution of candidate transcriptional regulators in driving trophoblast differentiation. While the preliminary data does implicate TBX3 and VGLL3 as important to establishment of the STB lineage, further experimentation is required to elucidate the molecular mechanism(s) through which TBX3 and VGLL3 regulate STB development. Future studies include:

- Completing the shRNA knockdown of the remaining candidate transcription factor, ATF3.
- Examining additional functional outputs such as chorionic gonadotropin production for all shRNA transductions.
- Exploring other models to study placentation, such as placental organoids or derivation of trophoblast stem cells from other tissue sources.

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