LETTER

REPLY TO LIU ET AL.:
Decidualization defect in severe preeclampsia

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Addressing our first line of evidence of a decidualization defect in severe preeclampsia (sPE) (1), Liu et al. (2) describe an in vitro decidualization model by using an endometrial cell line from hTERT-immortalized fibroblasts of a woman with nonmalignant myomas (ATCC: CRL-4003) with cAMP and medroxyprogesterone acetate for 5 d. They present an unpublished RNA-seq analysis of their decidualization model without reporting the number of technical or biological replicates.

We studied primary human endometrial stromal cells (hESCs) established from endometrial biopsies of former sPE patients or control women with normal pregnancies ($n = 13$ per group) (1). Upon decidualization using the same protocol (3), the sPE hESCs failed to show morphological changes, which were observed in the controls. Quantification of PRL and IGFBP1 secretion suggested a significantly sPE-associated down-regulation.

Lui et al. (2) fault our microarray analyses of decidualization for not identifying *PRL* and *IGFBP1* as differentially expressed due to large variances among samples (common with primary human cells) and the small sample size (normal pregnancies, $n = 7$; sPE, $n = 5$). In hESCs from normal pregnancies, 74 differentially expressed genes (DEGs) were identified (nondecidualized vs. decidualized), including *PRL* ($P = 0.0003$; corrected $P = 0.0594$) and *IGFBP1* ($P = 0.002$; corrected $P = 0.1448$). The corrected P values [Benjamini–Hochberg (4)], which we used to construct the heat maps, rely on a higher statistical significance than the P values. Furthermore, we show that hESCs from former sPE patients were transcriptionally inert when they were decidualized in vitro. Comparison with their counterparts from normal pregnancies revealed 129 DEGs. The expression of *PRL* ($P = 0.008$, corrected $P = 0.2012$) and *IGFBP1* ($P = 0.00009$, corrected $P = 0.0345$) was significantly down-regulated. *IGFBP1* was the most highly down-regulated gene (1).

Liu et al. (2) also criticize our transcriptomic analysis of the decidua basalis (DB; uterine lining adjacent to placenta) and decidua parietalis (DP; uterine lining away from placenta). They compare our data with other studies (5–7), finding a low correlation. This was not unexpected, given significant differences between the studies. (*i*) The gestational ages and PE diagnoses were different. We compared age-matched decidual samples from severe PE (29 wk) and nPTB (spontaneous preterm birth with no signs of infection; 30 wk). The others compared decidual samples from PE and term decidual samples. (*ii*) The collection methods were different: laser-capture microdissection (ours) and vacuum aspiration of the placental bed (the others). (*iii*) The decidual sites sampled were different. We noted that the DB dataset identified few genes because laser-capture yielded samples that contained a mixture of cells, including placental cytotrophoblasts. In contrast, our DP data identified many DEGs in sPE because of the clear separation between these cells and cytotrophoblasts of the smooth chorion, which enabled capture of a purer population (8). Our results suggest substantial differences between DP and DB. (*iv*) The other studies used a less stringent false-discovery rate (FDR) ($P \leq 0.1$ vs. FDR $P < 0.05$). (*v*) The overlap among the three studies used for comparison was very low (Fig. 1).

In summary, we think that the lack of overlap among the datasets is attributable to the very significant differences in cell sources and experimental design.

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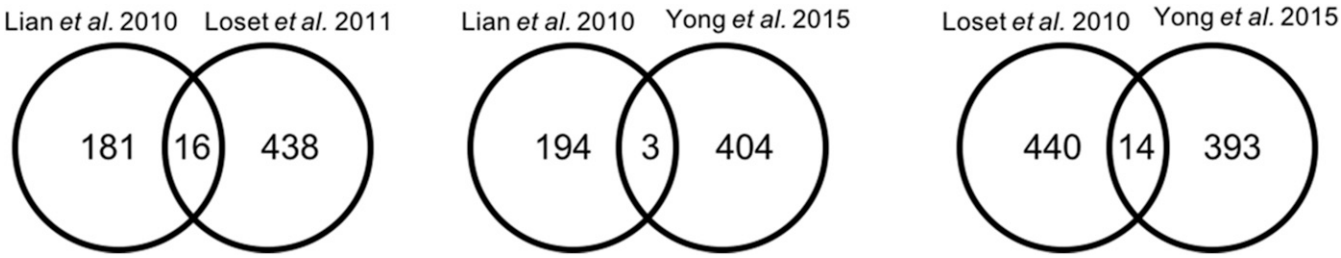

Fig. 1. Shared genes obtained after comparative analysis of the three studies used by Liu et al. (2, 4–6).

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