A multi-tissue atlas of spatial gene expression adaptations in late pregnancy

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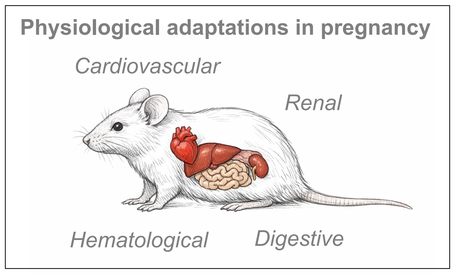
ABSTRACT

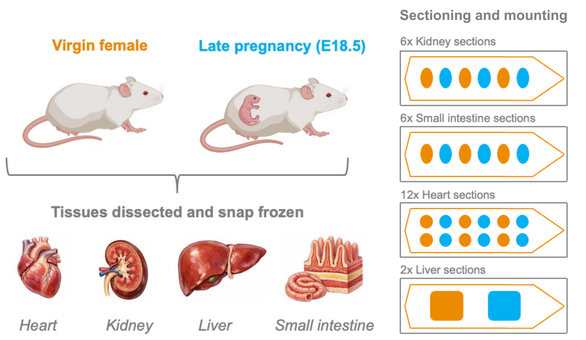
Pregnancy in mammals is associated with a range of physiological changes in multiple organ systems. In addition to reproductive organs, adaptations supporting pregnancy occur in the nervous, cardiovascular, renal, digestive and hematological systems, among others. Disruptions of these critical changes can have serious impacts on maternal and fetal health and ultimately the viability of the pregnancy.



To provide a comprehensive view of spatially defined gene expression changes in pregnancy, we used Illumina spatial technology with fresh frozen sections of liver, heart, kidney and small intestine from virgin and late pregnancy (E18.5) mice.

We were able to measure spatially localized gene expression changes directly related to known physiological adaptations that are critical to a viable pregnancy.

OVERVIEW



Sections from **virgin** and **pregnant** mice mounted on 4 spatial substrates

- **Each tissue type mounted on a separate Illumina spatial substrate**
- **Sections from the virgin and the pregnant mouse were alternated**
- **2-12 sections were fit on each substrate, depending on individual section size**

MATERIALS & METHODS

Samples were processed on Illumina spatial substrates and H&E images were captured on a Keyence BZ-X800 automated microscope. After cDNA barcoding and library preparation the samples were sequenced on 25B flow cells on a Novaseq X instrument. Data analysis was performed in Illumina Connected Analysis (ICA) and Illumina Connected Multiomics (ICM) as well as using third party tools. For cell typing all samples belonging to the same tissue type were aggregated together, basic QC steps were done, and the top 3000 most variable genes were chosen to calculate PCA. Harmony integration was performed to correct any batch effects and harmony embedding was used for leiden clustering. Biological cell types were annotated on leiden clusters based on marker genes and spatial locations. For differential expression gene analysis, DEGs (differentially expressed genes) were determined by Wilcoxon test between pregnant and virgin within the same cell type with adjusted p-value < 0.05 and log2FoldChange > 0.5 or < -0.5. All pathway enrichment analyses were done using the KEGG_2019_Mouse database.

SPATIAL ASSAY PERFORMANCE

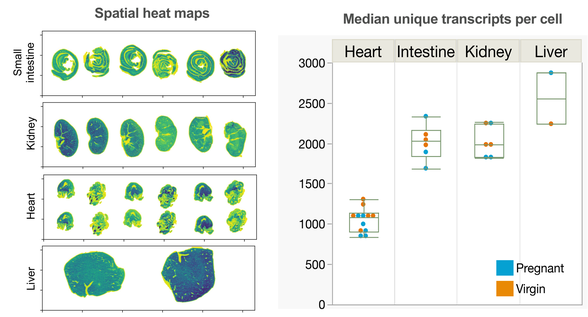


Figure 1. Spatial heatmaps for each substrate and median unique transcripts per cell. Sequencing depth for the box plots = 10k reads per cell

Cell segmentation and unsupervised clustering

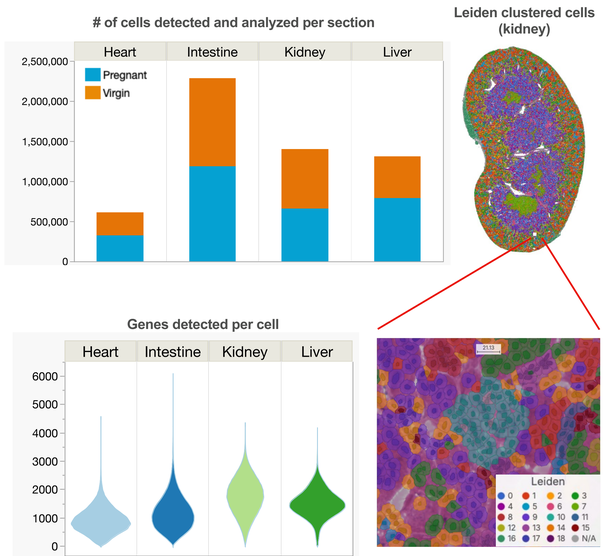
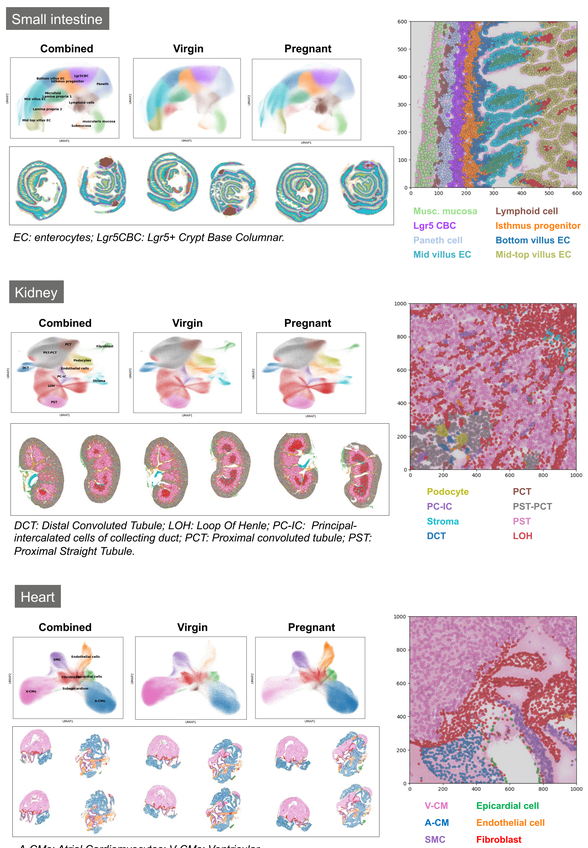


Figure 2. In total, 600k to 2.3M cells per spatial substrate were detected and analyzed in this experiment. Illumina Connected Multiomics (ICM) performed unsupervised Leiden clustering on all sections, exemplified here by clustered cells of a kidney section. Magnification shows individual cells colored by Leiden classification and superimposed on the original H&E image.

RESULTS

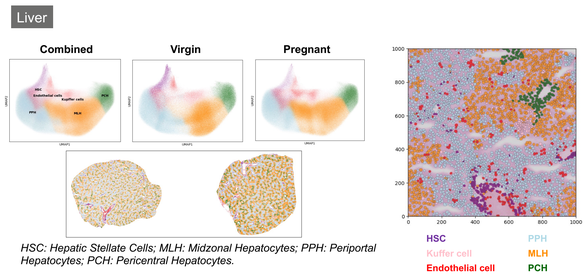
Consistent clustering and cell-typing across conditions within the same tissue type



EC: enterocytes; Lgr5CBC: Lgr5+ Crypt Base Columnar.

DCT: Distal Convoluted Tubule; LOH: Loop Of Henle; PC-IC: Principal-intercalated cells of collecting duct; PCT: Proximal convoluted tubule; PST: Proximal Straight Tubule.

A-CMs: Atrial Cardiomyocytes; V-CMs: Ventricular Cardiomyocytes; SMC: Smooth Muscle cells



HSC: Hepatic Stellate Cells; MLH: Midzonal Hepatocytes; PPH: Periportal Hepatocytes; PCH: Pericentral Hepatocytes.

Figure 3. UMAPs are shown for all samples combined after harmony integration as well as individually for virgin and pregnant samples. Cell types were annotated on leiden clusters based on marker genes and spatial locations. Zoom-in views are cell type colored cell contours on top of H&E image.

Lymphoid tissue expansion and pregnancy-specific gene regulation in small intestine

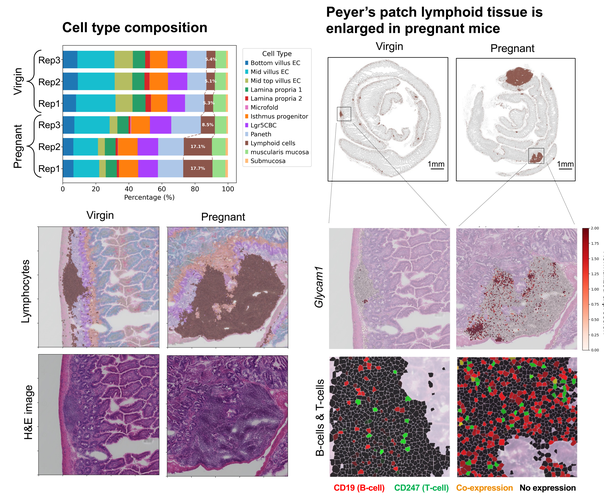


Figure 4. Proportion of annotated cell types in small intestine are summarized in the bar plot. Lymphoid cells organized in Peyer's patches are significantly increased in the pregnant sample. Peyer's patches in the pregnant sample have elevated numbers of activated B-cells (CD19) and T-cells (CD247) with very limited co-expression of these markers detected. *Glycam1*, a gene involved in immune cell trafficking, is also significantly upregulated.

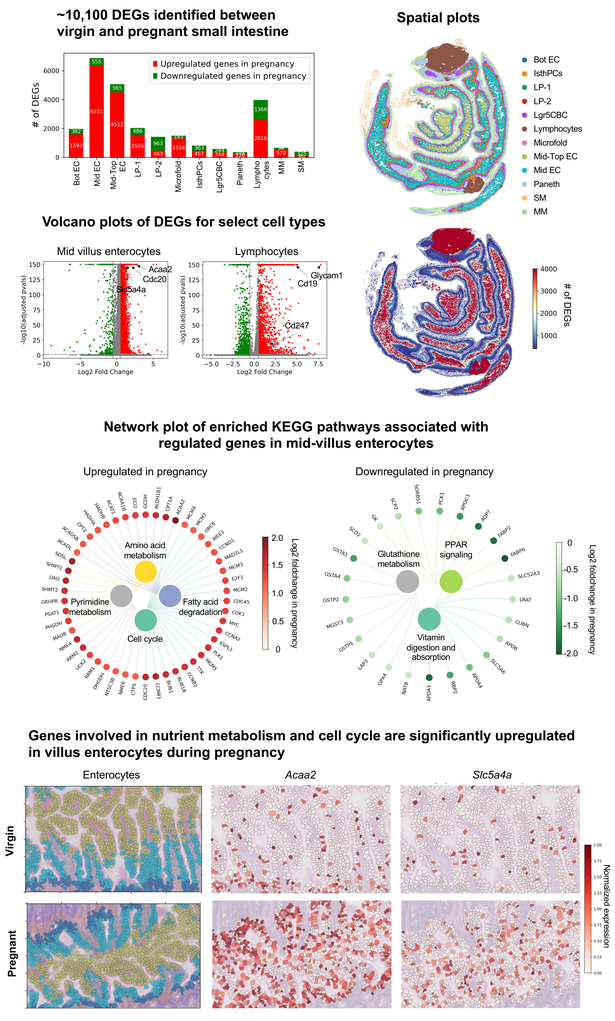


Figure 5. The bar graph shows the >10,000 differentially expressed genes in all identified cell types. Spatial plots of cell types and DEGs in an example pregnant section. KEGG analysis identified multiple regulated pathways in specific cell types. *Acaa2* is involved in fatty acid metabolism, *Slc5a4a* is sodium- and proton-sensitive transporter that plays a role in sustaining isthmus progenitor expansion and villus growth. Cdc20 is cell cycle protein which is essential for cell proliferation.

Metabolism-related gene regulation in midzonal hepatocytes during pregnancy

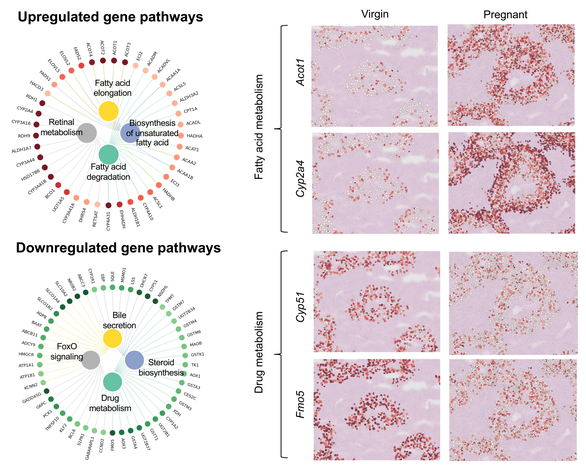


Figure 6. All cell types in the liver had significant gene regulation in late pregnancy and midzonal hepatocytes (MLH) were found to be a hotspot. Multiple KEGG pathways were identified based on up- and downregulated genes. The spatial maps show 4 examples of regulated genes in MLHs related to fatty acid and drug metabolism pathways.

Most gene regulation in the pregnant kidney occurs in cortical areas

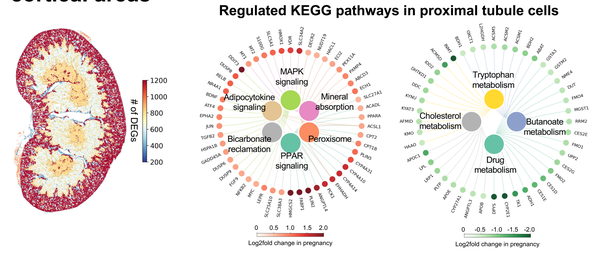


Figure 7. Spatial map of differential gene expression in late pregnancy. Gene expression regulation is most prominent in proximal convoluted tubule cells located in cortical areas. KEGG analysis shows pathway associated with up- and downregulated genes in PST-PCT cells

Pregnancy-related gene expression changes in heart

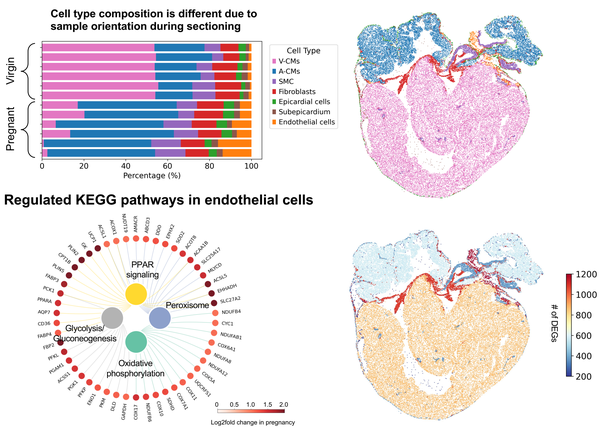


Figure 8. Differences in sample orientation (virgin vs pregnant) resulted in significant differences in cell type composition. A hotspot for gene regulation in late pregnancy was cardiac endothelial cells. KEGG pathway analysis for upregulated genes in endothelial cells shown.

CONCLUSIONS

1. Four different tissues known to undergo physiological adaptations in late pregnancy were successfully analyzed using Illumina spatial technology. Depending on tissue type and number of mounted sections, we detected **610,000** to **2,200,000** cells, and **35,000** to **40,000** genes per spatial substrate.
2. The high resolution of this spatial technology enabled accurate and detailed cell typing for each tissue type, and hot spots for pregnancy-related differential gene expression in defined cell types were identified. The total number of regulated genes ranged from **3,000** in kidney to **10,000** in small intestine.
3. KEGG pathway analysis identified regulated pathways for both up- and downregulated genes in all cell types investigated
4. This large dataset can be used to expand our understanding of physiological changes in late pregnancy.

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