Spatially Resolved Lipidomic Profiling of Ovarian Cancer Using Ultrahigh Resolution Mass Spectrometry Imaging

Xin Ma,[1] Andro Botros,[2] Sylvia R. Yun,[2] Eun Young Park,[2] Olga Kim,[2] Ruihong Chen,[3] Murugesan Palaniappan,[3] Jaeyeon Kim,[2] Facundo M. Fernández[1]
[1]Georgia Institute of Technology, Department of Chemistry and Biochemistry, Atlanta, GA, United States
[2]Indiana University School of Medicine, Indianapolis, IN, United States
[3]Baylor College of Medicine, Houston, TX, United States

Introduction

Ovarian cancer (OC) is one of the deadliest cancers among women as no effective screening tools are available at its early stage.[1] Furthermore, the detailed mechanism of OC progression and metastasis remains unclear. We conducted spatially resolved lipidomic profiling of ovarian cancer tissues collected from a double-knockout (DKO) and a triple-mutant (TKO) mouse models. To investigate lipid distributions and alterations during OC development and progression, we performed MALDI imaging experiments on tissue sections in an ultrahigh resolution FTICR mass spectrometer and compared the profiles between DKO and TKO tissues using PCA and other multivariate techniques. Comparisons against control animals were also conducted with the aim to identify altered lipidomic pathways and better understand OC progression.

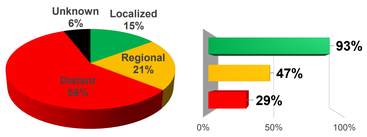


Figure 1. Overall percentage of the patients diagnosed with OC at different stages (left) and their corresponding five-year survival rates (right)

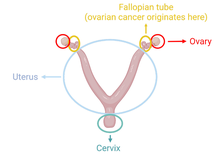
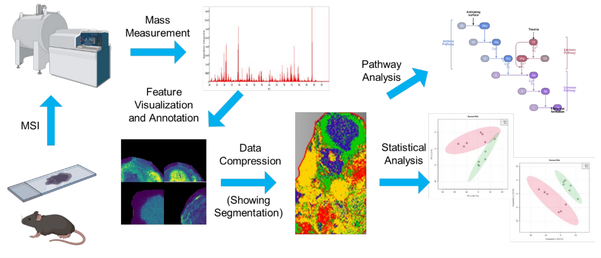


Figure 2. A schematic of the mouse reproductive system

Materials and Methods

- A double knockout (DKO) and a triple mutant (TKO) mouse model were studied. DKO: *Dicer1*$^{flox/flox}$*Pten*$^{flox/flox}$*Amhr2*$^{cre/+}$ double-knockout mice; TKO: *p53*$^{LSL\text{-}R172H/+}$*Dicer1*$^{flox/flox}$*Pten*$^{flox/flox}$*Amhr2*$^{cre/+}$ triple mutant mice.[2]
- Spatially resolved lipidomic studies were carried out in a 12 T ultrahigh resolution FTICR mass spectrometer using MALDI operated in negative ion mode, 1,5-diaminonaphthalene was used as the matrix.
- Images of four biological replicates from each animal model were collected and analyzed.
- Lipid features were putatively annotated with METASPACE[3] using Lipid Maps and HMDB databases.
- Lipidomic pathways related to ovarian cancer progression were identified to investigate the cancer pathogenesis.
- PLS-DA and oPLS-DA models were established for the differentiation of the high-grade serous carcinoma of the DKO and TKO tissues from the healthy control tissues.
- ROC curves were generated for the selection of potential cancer biomarkers.



Results

Spatial Segmentations

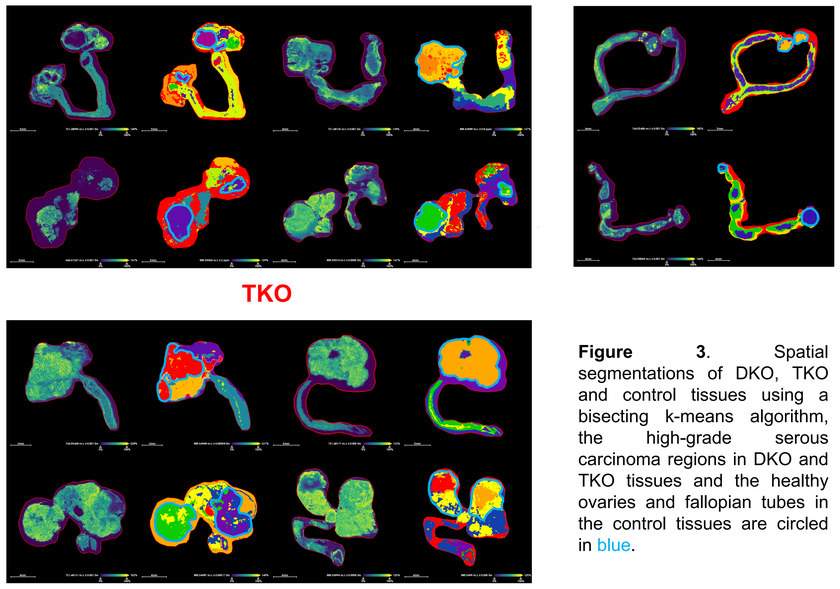
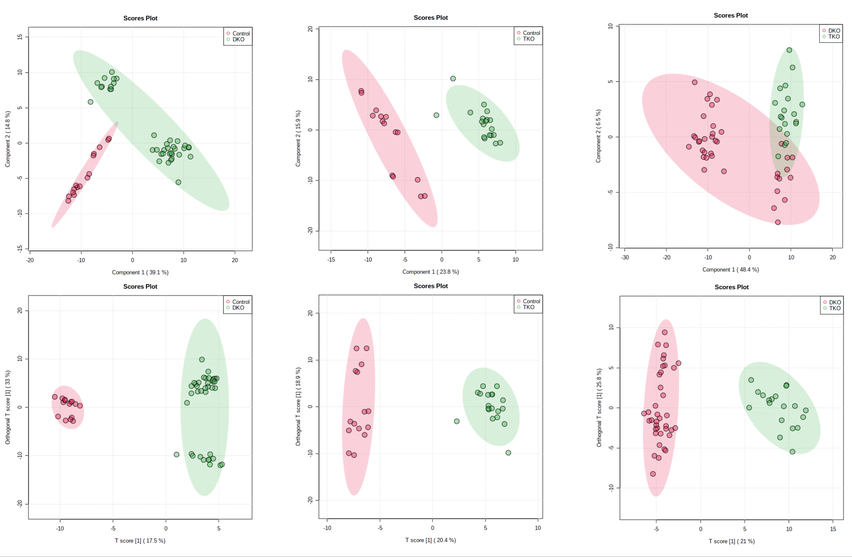
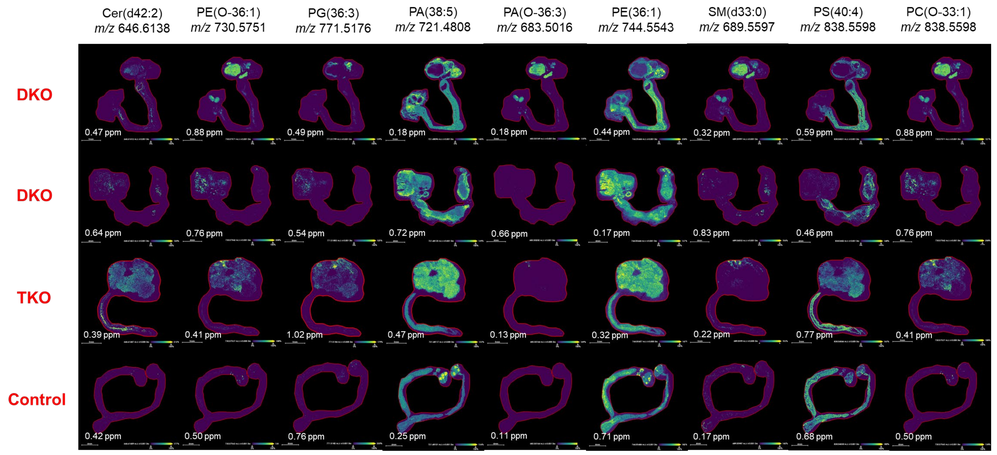


Figure 3. Spatial segmentations of DKO, TKO and control tissues using a bisecting k-means algorithm, the high-grade serous carcinoma regions in DKO and TKO tissues and the healthy ovaries and fallopian tubes in the control tissues are circled in blue.

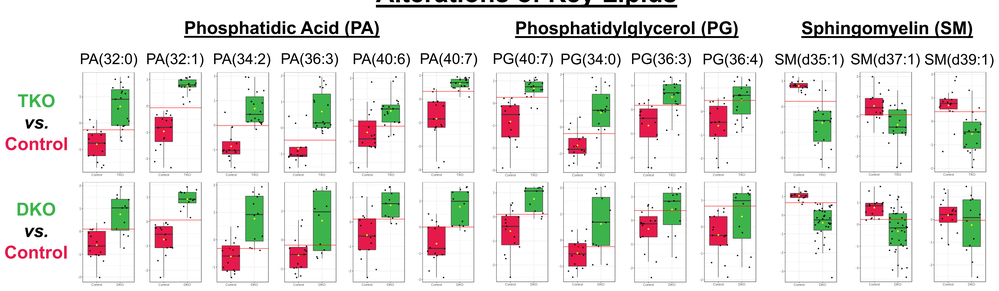
PLS-DA (Top Row) and oPLS-DA (Bottom Row) Models



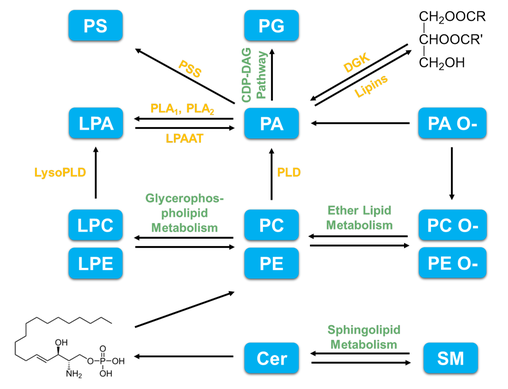
Images of Some Lipid Features



Alterations of Key Lipids



Major Lipidomic Pathways



- PA: central precursors for all glycerophospholipids, major lipid class for regulating cell proliferation.[4]
- LPAAT: lysophosphatidic acid acyltransferase, mainly involved in *de novo* membrane phospholipid biosynthesis.[5]
- DGK: diacylglycerol kinase; (Lyso) PLD: (Lyso) phospholipase D, key enzymes that are activated in response to cell growth and stress.[5]
- CDP-DAG: major pathway for *de novo* biosynthesis of complex glycerolipids.[6]
- Sphingolipids (Cer and SM) mainly involve in cell proliferation, differentiation, and apoptosis.[7]

PS: phosphatidylserine; PG: Phosphatidylglycerol; PC: phosphotidylcholine; PE: phosphotidylethanolamine; Cer: ceramide; SM: sphingomyelin.

*Blue box: lipids; Yellow: key enzymes; Green: key lipidomic pathways.

Conclusions

- Spatial metabolomics using mass spectrometry imaging enables direct investigation of lipidome distributions in the tissues.
- More accurate putative feature annotations with minimal mass errors are achieved by ultrahigh resolution mass spectrometry, thus allowing more accurate metabolic pathway analysis.
- Several major lipidomic pathways in the tissues were identified and revealed *in situ* lipidome alterations in the tissue, where PA is the major precursor for biosynthesis of other key glycerolipids and in response to cancer cell growth and proliferation.
- Statistical models including PLS-DA and oPLS-DA based on 202 annotated lipids were established, different animal models were differentiated.

References

1. American Cancer Society. *Cancer Facts & Figures 2021*. Atlanta, GA.
2. Kim, J.; Coffey, D. M. *et al. Endocrinology* **2015,** *156*, 1975–1981.
3. Palmer, A.; Phapale, P.; Chernyavsky, I. *et al. Nat. Methods* **2017**, *14*, 57–60.
4. Dória, M. L.; McKenzie, J. S.; Takats, Z. *et al. Sci. Rep.* **2016**, *6*, 39219.
5. Foster, D. A., Salloum, D. *et al. J. Biol. Chem.* **2014**, *289*, 22583–22588.
6. Koundouros, N.; Poulogiannis, G. *Br. J. Cancer* **2020**, *122*, 4–22.
7. Ogretmen, B. *Nat. Rev. Cancer* **2018**,*18*, 33–50.

Acknowledgements

We acknowledge support by NIH 1R01CA218664-01, R01NS101909-01, and NSF MRI CHE-1726528.