iMS2Net: A multiscale networking methodology to decipher metabolic synergy of organism

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Highlights

iMS2Net, a multiscale networking methodology to decipher iMS-spatial omics data

Elaboration of variation and covariation within/between organs to external stimuli

Understanding metabolic responses of organisms at cell and organ resolutions

A close association between lipid metabolism and inflammatory cytokine release

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iMS2Net: A multiscale networking methodology to decipher metabolic synergy of organism

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SUMMARY
The metabolic responses of organism to external stimuli are characterized by the multicellular- and multiorgan-based synergistic regulation. Network analysis is a powerful tool to investigate this multiscale interaction. The imaging mass spectrometry (iMS)-based spatial omics provides multidimensional and multiscale information, thus offering the possibility of network analysis to investigate metabolic response of organism to environmental stimuli. We present iMS datasetsourced multiscale network (iMS2Net) strategy to uncover prenatal environmental pollutant (PM2.5)-induced metabolic responses in the scales of cell and organ from metabolite abundances and metabolite-metabolite interaction using mouse fetal model, including metabolotypic similarity, metabolic vulnerability, metabolic co-variability and metabolic diversity within and between organs. Furthermore, network-based analysis results confirm close associations between lipid metabolites and inflammatory cytokine release. This networking methodology elicits particular advantages for modeling the dynamic and adaptive processes of organism under environmental stresses or pathophysiology and provides molecular mechanism to guide the occurrence and development of systemic diseases.

INTRODUCTION
Response to environmental challenges and maintenance of systemic homeostasis require the cooperative-ness of multiple organs. Organisms possess the inter-cellular and inter-organ communication system in order to respond to complex metabolic demands (Priest and Tontonoz, 2019; Castillo-Armengol et al., 2019; Ito and Adachi-Akahane, 2013). Elucidating the communications and regulations of biomolecules at inter-organs and inter-cells is a key to understand the interaction between organism and environment (Argilés et al., 2018; Huh and Veiga-Fernandes, 2020). Whereas the constant efforts to explore the bulk perturbation of metabolome, lipidome, and proteome based on individual organ using liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS) have been undertaken (Li et al., 2020, 2021; Xiao et al., 2021; Sowers et al., 2021), the collaborative response of multiple organs, such as covariation of metabolite-metabolite interactions within and between organs, is lacking.

Imaging mass spectrometry (iMS)-based spatial omics provides possibilities of elucidating complex regulatory mechanisms under the external environment or pathophysiological stimuli, because it allows the global profiling of lipids, metabolites, and proteins, as well as the spatial localization of target (sub-)organ in situ at the cell-specific resolution (Doerr, 2018; Sun et al., 2019; Zhao and Cai, 2020). More importantly, iMS provides the cell-resolved metabolic information to explain the cellular behaviors under environmental stress, such as metabolic heterogeneity, metabolic tolerance, metabolic diversity, etc (Zhao et al., 2021a; Chen et al., 2015; He et al., 2018; Zhang et al., 2020). Thus, some reports have supported the use of iMS-based omics to discover novel molecular mechanism in environmental-pollutant-exposed models, such as bisphenols-induced nephrotoxicity (Zhao et al., 2018b), hepatotoxicity (Zhao et al., 2018a), splenic injury (Zhao et al., 2020), and tumor proliferation and deterioration (Zhao et al., 2021c).

With the launch of omics, the network biology is developed by combining the omics-based system biology and network-based algorithms (Li et al., 2021; Xiao et al., 2021; Skinnider et al., 2021; Dai et al., 2020; Wang et al., 2022). Compared with the molecular-biology-based approaches and conventional multivariate methods, which are fractional investigated, network biology provides a landscape of metabolic response
in the organ or organism by describing the interaction between cells or organs comprehensively (Li et al., 2021; Xiao et al., 2021; Skinnider et al., 2021; Dai et al., 2020; Wang et al., 2022). Li et al. reports have demonstrated that the network analysis of LC-MS datasets can be used for the co-regulation of sterol lipids asso- ciated with gene intensity sub-regions from mouse brain (Li et al., 2021). However, the current network-based analysis mainly takes the molecule interaction from the whole organism into consideration, which is not favorable to the heterogeneity and interaction analysis from multispatial scale and in situ during the disease development. Therefore, we develop the multiscale networking methodology by using iMS-based lipidomics dataset.

Emerging experimental and epidemiological findings have demonstrated that prenatal exposure to aerodynamic diameter of 2.5 μm or less (PM2.5) is related to adverse health effects of offspring, such as the CNS disease, as well as multiorgan injuries including liver, kidney, brain, and gastrointestinal tract (Malley et al., 2017; van Rossem et al., 2015; Lavigne et al., 2017; Vrijheid et al., 2011). Generally, it is considered that PM2.5 exposure can either induce maternal and offspring inflammatory reactions or act on the offspring through placental barrier directly, which cause the developmental abnormalities and deleterious birth outcomes (Kannan et al., 2006; Zhao et al., 2021b). However, the elaborate mechanisms have not been fully elucidated, especially in the stage of fetal growth. In addition, it is well established that PM2.5 exposure induces the impairments of lung and heart by producing systematic or local inflammatory responses (Tian et al., 2021; Liu et al., 2021). Whereas, insufficient data exist to show a similar molecular mechanism for other organs. Comprehensive investigation to explore the susceptibility of multiple organs in PM2.5-exposed situation remains to be elucidated.

Herein, we establish a networking methodology by using the iMS-based lipidomics datasets and inflammatory responses. Our multiscale methodology, namely iMS2Net, which introduces five characteristic networks, combines the metabolite abundances and metabolite-metabolite interactions to reveal the relationship of prenatal PM2.5-exposed target and risk molecules of organ variation at cell-specific and organ-specific resolution. Furthermore, our findings are critical to screen the most sensitive (sub-) organs and to explain the occurrence and development of systemic diseases in response to toxic effects of compounds, which may assist in predicting the clinical relevance of adverse birth outcomes and environmental exposure.

RESULTS

We perform the iMS analysis in whole-body mouse fetus (embryonic day 18) exposed to PM2.5 during pregnancy in order to understand the potential PM2.5 toxicity in early stage. We select the widely used SCiLS Lab software to analyze the iMS data through a standard pipeline. Five components (Figure 1) and some
differential ion images (Figure S1) are achieved by this ion-abundance-based univariate or multivariate modeling. Although above conventional methodology has proved effective in iMS analysis, its application toward the discovery of metabolic cooperative response from inter-cells or inter-organs remains underexplored. Thus, iMS2Net is developed to explore the prenatal PM2.5-induced metabolic response in whole-body mouse fetus from the point of view of interaction.

Organ network of metabotypic similarity

Metabolic perception and response of organism to external stimuli mainly depend on cellular composition and cellular interaction that program the metabolite-metabolite interaction network and then shape the metabolic phenotype of an organ (Guijas et al., 2018; Nicholson et al., 2012). Thus, we need to first describe the metabolite-metabolite interactions between cells in order to investigate the metabolic response characteristic from organ level.

Here, we carry out cell-specific data analysis on control and PM2.5-exposed groups, respectively. Cell-specific differential correlation networks (DCNs) are constructed for each pixel (cell) to quantify the difference of metabolite-metabolite interactions between the pixel and the mean of a given reference organ. Then, relative distances between each two organs are calculated by Equation (11) and visualized by boxplots (Figure S2). We define and calculate the metabotypic similarities between organs using Equation (14), then construct the organ network of metabotypic similarity for both control and PM2.5-exposed groups (Figure 2). Some interesting results can be drawn from Figure 2 and S2 as follows.

Firstly, metabolic heterogeneity exists in each organ at the cell-specific level by analyzing the variation of relative distances between organs. For example, the edge numbers of cell-specific DCNs range from $2.3 \times 10^5$ to $1.1 \times 10^6$ in heart by referring to brain (Figure S2A). Secondly, organ-specific patterns exist in PM2.5 exposure-induced metabolic responses for different organs. Taking liver, heart, and intestine as the examples, the relative distances from liver to heart are much smaller in PM2.5-exposed group (Figure S2I) than that in control group (Figure S2B), whereas the relative distances from lung to intestine increase when exposed to PM2.5 (Figures S2D and S2K).

Thirdly, PM2.5 exposure increases the similarity of metabolite-metabolite interactions in all 7-organs, suggesting covariation patterns exist in metabolic response of organs to PM2.5 exposure. As the Figure S2 shows, most of the relative distances between organs in PM2.5-exposed group are much smaller (~50%) than that of in control group. It is worth noting that unlike other organs, metabotypic similarity of brain and hippocampal formation (Hpf) is highest in both control and PM2.5-exposed groups because of specific composition of brain structure. In addition, PM2.5 exposure greatly enhances the metabotypic similarity between heart and liver and heart and intestine, whereas PM2.5 exposure reduces the metabotypic similarity between liver and thymus (Figure 2).

So far, we reveal the impact of PM2.5 on organs metabolic response from a general perspective. Further investigation of metabolic perceiving and responding of intra- and inter-organs will be of utmost importance to clarify the unsolved molecular mechanisms of PM2.5 exposure.

Metabolic vulnerability network specific to PM2.5 exposure

With the exception of PM2.5-induced general metabolic variation from whole-body mouse fetus, we speculate that a new cellular metabolic homeostasis from inter-cells and inter-organs will be achieved to
respond to the environmental exposure of PM2.5 during pregnancy by metabolic reprogramming including the metabolite-metabolite interactions and the spatial distribution of metabolite abundances. To test this hypothesis, we develop the metabolic vulnerability network to decipher the cell-specific response to PM2.5 exposure in different organs from two perspectives of metabolite abundance and metabolite-metabolite interaction.

Cell-specific differential expressed ions

We apply Equation (7) to calculate the differential expressed ions (DEIs) for each pixel in an organ from PM2.5-exposed group by taking the reference as the corresponding organ from control group. Then we visualize the DEIs from different organs using heatmaps and violin plots (Figures 3A and 3B). The cell-specific DEIs tell us the inter-cellular heterogeneities of an organ when exposed to PM2.5 from the metabolites expression’s (i.e., abundances) point of view. The results also show that PM2.5 exposure brings different effects on the metabolite expression in each organ. Specifically, thymus is of the most DEIs (mean value 116.3), whereas intestine is of the fewest DEIs (mean value 15.3). Furthermore, the occurrence probability of DEIs is drastically different from each other even in a same organ because of the cell specificity. Hence, we define the vulnerability for an ion as the occurrence probability of the ion to be a cell-specific DEI and define the organ-vulnerable DEIs as the DEIs that appear in most of the pixels of the given organ. As shown in Figure S3, thymus has 59 vulnerable DEIs, whereas intestine has only 1 vulnerable DEI.

Cell-specific differential correlation network

Similarly, we can calculate differential correlation networks (DCNs) using Equations (8–9) for each pixel in PM2.5-exposed organs, then visualize the DCNs using heatmaps and violin plots for all organs (Figures 3C and 3D). Our results demonstrate that PM2.5 exposure induce the variation of metabolite-metabolite interactions in different degree, which is consistent with the analysis results of metabolite abundances (Figures 3A and 3B). The degrees of influence from high to low in proper order are thymus, liver, Hpf, lung, heart, and intestine. In addition, organ-vulnerable DCN is defined as the subnetwork shared by most of the cell-specific DCNs in the given organ. We achieve the vulnerable DCN for each organ (Figure S4), which is in agreement with results of cell-specific DCNs (Figures 3C and 3D), showing the size of organ-vulnerable DCNs in the order of highest to lowest, namely, thymus (125 nodes and 364 edges), liver (22 nodes and 44 edges), brain (57 nodes and 109 edges), Hpf (35 nodes and 73 edges), lung (44 nodes and 74 edges), heart (11 nodes and 15 edges), and intestine (10 nodes and 9 edges).
To comprehensively understand the co-metabolism between organs in response to PM2.5 exposure, organ covariation analysis is carried out in view of metabolite abundance and metabolite-metabolite interaction, respectively.

**Organ-covariant DEIs**

The covariant DEIs between control and PM2.5-exposed groups are screened using Equation (3) for each organ (Figure S5). Different from the aforementioned vulnerable DEIs, the covariant DEIs are the ions whose expressions are regulated synchronously in most of the cells in an organ. We can find that the ions number of covariant DEIs order from highest to lowest is thymus (82), brain (51), Hpf (43), lung (34), liver (32), heart (19), and intestine (12). In addition, the covariant DEIs that appear in each two organs can be calculated, and the top 3 between-organ covariant DEIs are brain-Hpf (39), brain-thymus (37), and Hpf-thymus (35). Using them as the base, the within-/between-organ covariant DEIs can be visualized together using an undirected network, denoted by organ network of metabolic covariation (Figure 4A). Among them, network node represents the covariant DEIs within an organ, and edge represents the covariant DEIs between two corresponding organs. To sum up, our results provide the PM2.5 exposure-associated perturbation patterns on mouse fetus. We also find that the organ-specific perturbance patterns (Figure S5) are similar with the cell-specific perturbance patterns (Figure S3) in view of metabolites abundance.

Considering the perturbance patterns comprehensively, there appear great differences between organs in response to PM2.5 exposure due to the inherent metabolic complexity of organs, the inhomogeneity of PM2.5 exposure, and the interaction between them as well. More specifically, organ-specific results show that thymus contains 82 of covariant DEIs (Figure 4A), which is equivalent to the cell-specific results (mean 116.3 of cell-specific DEIs, Figure 3B), indicating that PM2.5 exposure disturbs the thymus metabolism mainly through organ-specific pattern and secondarily through cell-specific pattern. In addition, PM2.5 exposure disturbs the liver metabolism mainly through cell-specific pattern (mean 110.3 of cell-specific DEIs, Figure 3A) and secondarily through organ-specific pattern (32 of covariant DEIs, Figure 4A).

**Organ-covariant DCN**

To explore the differences between organs in metabolic response to PM2.5 exposure, we apply Equations (4–5) to calculate the covariant DCN for each organ. We can find that the edge numbers of covariant DCNs order from highest to lowest is intestine ($19.2 \times 10^4$), liver ($14.7 \times 10^4$), heart ($11.9 \times 10^4$), brain ($19.9 \times 10^4$), lung ($9.2 \times 10^4$), Hpf ($0.7 \times 10^4$), and thymus ($0.2 \times 10^4$). The between-organ covariant DCN, i.e., the common sub-network of two covariant DCNs, is also calculated, and the first between-organ covariant DCN is heart-intestine ($7.17 \times 10^4$ edges). The covariant DCNs within and between organs are visualized in Figures 4B and S6. Among them, network node represents the covariant DCN of organ, and edge represents the between-organ covariant DCN of two corresponding organs. Contrary to the vulnerable DEIs results (Figure 4A), PM2.5 exposure induces significant alterations of metabolite-metabolite interactions in intestine, which has more universal differential correlations with most of organs, rather than thymus and Hpf. On this basis, the results demonstrate that metabolic responses of organs to PM2.5 exposure also exist in two important pathways, such as, thymus responds to PM2.5...
exposure through reprogramming in metabolite abundances, whereas intestine and heart respond to PM2.5 exposure through reprogramming in metabolite-metabolite interactions.

**Metabolic diversity of organ specific to PM2.5 exposure**

Metabolic diversity is considered as a determinant of multiple molecular processes, such as, tumor proliferation and metastasis, organ development, and environmental adaptation. Exploiting the metabolic diversity across the individual organ can show the interplay between metabolism dysregulation and disease (Vogel and Schulze, 2021; Murphy et al., 2020). However, metabolism of multi-/sub-organs can appear distinct diversity of metabolic profiling and multiple regulatory layers in response to the perturbations of external stimuli. Previous reports suggest that entropy approach provide cellular systemic behavior analysis rather than single elemental analysis (Menichetti et al., 2015). Here, we define two kinds of entropies, namely ionic entropy and spectral entropy, to quantify the diversities including the distribution of ions abundance and pixels spectra, respectively.

**Spectral entropy**

Spectral entropy is calculated using Equation (16) for each pixel to quantify the metabolic diversity of the pixel, then to investigate the overall alteration on metabolic profile of the pixel by PM2.5 exposure (Figure 5A). All organs increase significantly their spectral entropies in PM2.5-exposed group compared with control group. The results imply that all organs from mouse fetus exist in metabolic diversity to cope with PM2.5 exposure, which is the one of the great causes of PM2.5 exposure-induced systemic diseases. Ranks of organs with respect to spectral entropies are calculated by Student’s t test between the entropy sets of organs (Figure 5B). Thymus is the most significantly altered organ under PM2.5 exposure, followed by liver. The other five organs keep their ranks of spectral entropy unchanged in PM2.5-exposed group compared with control group, which implies that PM2.5 exposure might induce destruction, to a similar extend, of the metabolite-metabolite association of cells in all organs.

**Ionic entropy**

Ionic entropy is calculated using Equation (15) for each ion to quantify the metabolic diversity of the ion, then to investigate the overall alteration induced by PM2.5 exposure on the distributions of ion abundance in an organ (Figure 5C). All organs widen significantly their dynamic ranges of ions entropies, that is, some ions increase, whereas the others decrease their diversities of abundances after PM2.5 exposure. Most of

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**Figure 5. Metabotypic entropy in different organs**

(A) Violin plot of spectral entropy before (left) and after (right) PM2.5 exposure; (B) organ ranks of spectral entropy; (C) violin plot of ionic entropy before (left) and after (right) PM2.5 exposure; (D) heatmap of ionic entropy before (up) and after (down) PM2.5 exposure.
the organs except for heart and intestine increase their ion entropies in overall terms. For the decreased diversities of ion abundances in both heart and intestine after PM2.5 exposure, there are at least two reasons; in the first, PM2.5 exposure has less overall influence on heart and intestine metabolism compared with other organs (Figures 3B, 3D and 4A), and in the second, heart and intestine possess a higher degree of metabolic covariant (Figure 4B). More specifically, we plot all ionic entropies in each organ using heatmap in Figure 5D. Most of the ions decrease their diversities after PM2.5 exposure in both heart and intestine. Most of the ions whose m/z values lower than 900 Da increase their diversities after PM2.5 exposure in the other organs including brain, Hpf, lung, liver, and thymus.

Metabolic subnetwork signatures correlated with cytokines

To understand how the lipid metabolism correlates with cytokine release in mouse fetus exposed to PM2.5 during pregnancy, we perform both IMS-based lipidomics and cytokine profiling on 6 different organs and explore the variation of lipid regulation and immune response. Herein, we find that PM2.5 exposure alter the secretion of immune-related cytokines (Figure S7). For example, the secretion of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α) increase in brain, heart, intestine, liver, lung, and thymus, whereas anti-inflammatory cytokines IL-10 and TGF-β decrease in brain, heart, intestine, and lung in PM2.5-exposed group compared with control group. Subsequently, we investigate the IMS-based metabolic subnetworks significantly related to cytokine release from the metabolite abundances (DEIs) and metabolite-metabolite interactions (differential correlations).

Metabolic subnetwork based on cytokine-related DEIs

Fold changes of the mean abundance between control and PM2.5-exposed group are calculated for each organ. And the ion whose fold change is significantly correlated with the fold change of any cytokine across the six organs is defined as cytokine-related DEI. A total of 24 cytokine-related DEIs are found to be significantly correlated (Pearson correlation coefficient |r|>0.875) to at least one cytokine in view of the fold change. On this basis, we construct a subnetwork for each organ using the 24 ions and their interactions, as shown in Figure 6, in which the edges represent the interactions that are significantly different between control and PM2.5-exposed groups.

As shown in Figure 6, in heart and intestine exist the densest networks with 158 and 182 edges, implying the variation of metabolite abundances in response to cytokine release is consistent with metabolite-metabolite interaction patterns, which is a potential reason for decreased ionic entropy in both heart and intestine (Figure 5C). By contrast, there is an asynchronous characteristic between the variation of metabolite abundances in response to cytokine release and metabolite-metabolite interaction patterns in thymus with the loosest network (6 edges), which also clarifies to the potential reason for significant altered metabolism (Figure 3) and spectral entropy (Figure 5B) in thymus.

Metabolic subnetwork based on cytokine-related vulnerable correlations

Similarly, we screen out the metabolite-metabolite interactions whose vulnerabilities specific to PM2.5 exposure are significantly correlated with the fold change of cytokine. Then we construct a metabolic subnetwork for each organ and each cytokine using the cytokine-related vulnerable correlations, as shown in Figures S8–S12.

Taking IL-6 as an example, the results delivered by the vulnerable correlations suggest that fold change of IL-6 level is correlated with the variation of metabolite-metabolite interactions in networks (Figure S8). For example, PM2.5 exposure induces the IL-6 level variation of thymus with 6.82 fold changes compared with control group, whereas the vulnerabilities of most of the correlations between metabolites on the cytokine-related subnetwork of lung are the smallest in the 6 organs. Interestingly, variations of IL-6-related metabolic correlations in lung occur in an opposite manner (Figure S8). In addition, more cytokines-related metabolic subnetworks are detailed on Figure S9–S12. The m/z values of each cytokines-related metabolic subnetwork are labeled in Figure S13.

DISCUSSION

Despite the situation that PM2.5 concentrations decrease significantly with the implementation of emission reduction policies, the incidence of human diseases in PM2.5-exposed population is still expected to increase consecutively because of the adverse impact of prenatal PM2.5 exposure, at the next two decades (Turner et al., 2020). The results from toxicological analysis shows prenatal exposure to PM2.5 cause the systematic diseases of the fetus through several pathways, which is regulated by the cooperativeness of multiple organs or tissues.
In the current work, we introduce iMS2Net based on network analysis of iMS datasets that performs multiscale network calculation in prenatal PM2.5-exposed mouse fetus to decipher the metabolic response. iMS2Net is conducted by network modeling of the cell- and organ-specific response patterns to metabolic state, which allows for two analytical perspectives, both the metabolite abundances and metabolite-metabolite interaction. Metabolic responses of cells and organs to PM2.5 exposure generate multiple alterations on both metabolite abundance and metabolite-metabolite interaction. Most noteworthy, in these responses exist the great individual differences in different pixels from the same organ. Our results, which cannot be obtained by conventional iMS analysis, have richly validated that PM2.5 exposure induced the generation of new homeostasis by varying the intra-/inter-cellular/organic metabolic heterogeneity and patterns. Specifically, development and maturation of T cells in the thymus and its microenvironment plays an important role in the generation of a suitably functioning immune system. Some reports suggest that thymic function and its tolerance are critical factors in evaluating the susceptibility to CNS inflammation (Nunes-Alves et al., 2013; Alberti and Handel, 2021). Using iMS2Net, we discover the cooperativeness of multiorgans, such as brain-thymus, brain-Hpf, and Hpf-thymus.
Besides, we observe some susceptible organs contributing to the potential link between prenatal PM2.5 exposure and disease processes, such as thymus, brain, and intestine. Above all, our results provide some important evidences using the network analysis for PM2.5 toxicity from the perspective of multiorgan cooperativeness. Using this innovative networking methodology, the results show that multiorgans, not just brain, have proved susceptible to PM2.5 exposure during pregnancy. Given the complex cooperativeness and diversity of multiorgans, these likely contribute to PM2.5 trans-generational toxicity from mother to fetus.

As a class of metabolites, lipids play a fundamental role in various biological processes, such as membrane structure maintenance, signal transmission, and organ functional regulation. Metabolic homeostasis and interaction of lipids get involved in various systematic functions and communications of multiple organs in organisms (Oliveira et al., 2016; Hannun and Obeid, 2008). Inflammatory signaling and lipid regulation can influence mutually in disease development and response to external stimuli. Notably, inflammatory responses can modulate the lipid metabolism and pathway variation. Reversely, lipids also regulate the expression and secretion of pro- or anti-inflammatory cytokines (Glass and Olefsky, 2012; Wculek et al., 2022). Although distinctive superiority exists on IMS-based spectrum profiles, the accurate delineation and regulatory analysis of lipid metabolism and immune response landscape under external stimuli is still a major challenge. Our metabolic subnetwork analysis shows the close associations between lipid metabolites and inflammatory cytokines, implying a potential regulatory crosstalk between lipid metabolism and inflammation to cope with prenatal PM2.5 exposure.

These results highlight the combination of IMS-spatial omics and network analysis in investigating the biological communications between cells and organs in whole-body context that better profile molecular spatial interactions to cope with the external stimuli. This adequate network procedure has the potential to be used to rapidly discover the targeted molecule or (sub)-organ during the disease progression. We expect that iMS2Net and related work can be applied directly in professional data processing software to expand its applications.

Limitations of the study

Herein, a general limitation of this study includes the relatively simple interaction mode and application model used. It is preferred to develop higher order interaction, e.g., mutual information, not just the linear correlation based on iMS2Net. Control group and PM2.5-treated group with single exposure concentration and exposure time are used to perform the network analysis, but more treated animals at different exposure concentrations, times, or growth states would investigate the potential dose/time-dependent metabolic alterations, increase the statistical validity of results, and enlarge the application range of iMS2Net. Despite iMS2Net can be applied for the metabolic response between two single cells in which metabolites are dynamically fluctuated under external stimuli, we do not perform this application because there are too many cells to describe the metabolism profiling. By using other tools to detect cellular morphology and state, such as mass cytometry, complementary results that are important to investigate how the spatiotemporal changes between multi-biomolecules can be achieved.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS
J.D., Q.P., and L.D. performed the experiments of network analysis. C.Z., J.L., W.H., and X.Z. provided PM2.5 toxicological analysis and animal experimental results. C.Z. and Z.C. provided the iMS data. J.D., Q.P., and L.D. performed the experiments of network analysis. C.Z., J.L., W.H., and X.Z. provided the iMS data. J.D. and C.Z. co-wrote the manuscript and analyzed the data. J.D., C.Z., and Z.C. supervised and directed the study.

DECLARATION OF INTERESTS
The authors declare that there is no conflict of interest regarding the publication of this article.

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STAR★METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Critical commercial assays |        |            |
| Cytokine secretion analysis (IL-6) | Thermo Fisher (eBioscience) | KMC0061 |
| Cytokine secretion analysis (IL-1β) | Thermo Fisher (eBioscience) | BMS6002 |
| Cytokine secretion analysis (TNF-α) | Thermo Fisher (eBioscience) | BMS607-3 |
| Cytokine secretion analysis (IL-10) | Thermo Fisher (eBioscience) | BMS614 |
| Cytokine secretion analysis (TGF-β) | Thermo Fisher (eBioscience) | BMS608-4 |
| Chemicals, peptides, and recombinant proteins |        |            |
| N-(1-Naphthyl)-ethylenediamine dihydrochloride | Sigma Aldrich | 222488 |
| Experimental models: organisms/strains |        |            |
| Mouse: C57BL/6 | Animal Resources Center | C57BL/6JArc |
| Software and algorithms |        |            |
| FlexControl | Bruker Daltonics | Version 4.0 |
| FlexImaging | Bruker Daltonics | Version 4.1 |
| SCiLS Lab | Bruker Daltonics | SCiLS Lab MVS Version 2019b Premium 3D software |
| MATLAB | MathWorks | R2022a |

RESOURCE AVAILABILITY

Lead contact
Further information and request for resources and protocols should be directed to the lead contact, Prof. Zongwei Cai (zwcai@hkbu.edu.hk).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- MSI data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Prenatal PM2.5 exposed animal model

Collection and preparation of PM2.5 sample
The relevant procedures are described in our previous work (Zhao et al., 2019b). Shortly, PM2.5 samples are collected from Shanxi University in Taiyuan, China. Ambient PM2.5 concentration range is from 70 to 300 µm/(m³ d) during the period of sample collection. We transfer the PM2.5 samples from 90 mm diameter quartz microfiber filters (Whatman, QM-A, UK) into sterile Milli-Q water by sonication. Subsequently, PM2.5 suspensions are lyophilized by vacuum freeze dryer (SCIENTZ-30ND, Scientz Biotechnology Co., Ltd., China) and stored at –20°C.
Model construction

The procedures of animal experiments are approved by the Institutional Animal Care and Use Committee at Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences (Shenzhen, China) (IACUC number: SIAT-IACUC-200330-YGS-ZC-A1228). All of mice are treated humanely with the consideration of alleviating suffering. Six-week-old C57BL/6 male and female mice are housed under specific pathogen free condition with controlled temperature, humidity and 12 h dark: light cycle. One male and two females are bred and observed by a vaginal plug. And then, females are placed in a separate cage after successful mating. We collect the whole-body mouse fetus at embryonic day 18 for iMS and cytokine analysis including control and PM2.5-exposed group (n = 5). According to our previous work (Zhao et al., 2021b), PM2.5 sample features and daily mean concentration in Taiyuan, we select the 0.3 mg/(kg body d) as the exposure concentration of PM2.5 using intratracheal instillation for once per 2 days enduring for embryonic day 0 to embryonic day 18.

METHOD DETAILS

iMS analysis for lipid profiling

The procedures of matrix-assisted laser desorption ionization (MALDI)-iMS are described in our previous work (Zhao et al., 2018b, 2021b). In short, the mouse fetuses from control and PM2.5-exposed groups are sectioned at a 14 μm-thickness from their maximum cross-section by using CryoStar Nx79 cryostat (Thermo Fisher Scientific, Germany). Then, sections are thaw-mounted onto ITO slides for MALDI-iMS analysis. iMS experiments are carried out on the RapifleX MALDI Tissuetyper (Bruker Daltonics, Germany) with N-(1-Naphthyl)-ethylenediamine dihydrochloride matrix in negative reflector ion mode at 30% laser power. The lipid profiling from control and PM2.5-exposed groups are acquired at a mass range of \( m/z = 200-1,000 \) with spatial resolution at 50 μm.

Cytokine secretion analysis

The relevant procedures are described in our previous work (Zhao et al., 2019a). Briefly, cytokine concentrations are quantified by ELISA Ready Set Go Kit (eBioscience, CA, USA) according to the manufacture’s instructions. The absorbance in each well from control or PM2.5-exposed group is detected using a microplate reader (Perkin-Elmer Cetus, CA, USA) at 450 nm.

iMS data acquisition

The iMS data is pre-processed according to Rafols’ work (Rafols et al., 2018) including peak correction, peak picking, peak binning and total-ion-chromatogram (TIC) normalization. We obtain a 3D data matrix from selected slice of mouse fetus, which is located at the maximum cross-section. Then, pixels of 7 organs including brain, heart, Hpf, intestine, liver, lung and thymus are selected for further analysis. Pixels numbers for each organ in control and PM2.5-exposed groups are listed in Table S1. There are \( M = 995 \) ions in each pixel.

QUANTIFICATION AND STATISTICAL ANALYSIS

Organ-specific data analysis

Organ-specific differential expressed ions (DEIs). Let \((X)_{n_X \times M}, (Y)_{n_Y \times M}\) be the organ from control group with \(n_X\) pixels and \(M\) ions, \((Y)_{n_Y \times M}\) be the organ from PM2.5-exposed group with \(n_Y\) pixels and \(M\) ions. We use the effect size of Hedges’s \(g\) to quantify how much \(X\) differs from \(Y\) in ion \(j (j = 1, 2, \ldots, M)\) as (Lakens, 2013),

\[
Hedges's \ g(X, Y) = \frac{\mu(X) - \mu(Y)}{\sigma_pooled} \times \left(1 - \frac{3}{4(n_X + n_Y) - 9}\right)
\]

(Equation 1)

where \(\sigma_pooled\) is the pooled and weighted SD

\[
\sigma_pooled = \sqrt{\frac{(n_X - 1) \cdot \sigma^2(X) + (n_Y - 1) \cdot \sigma^2(Y)}{n_X + n_Y - 2}}
\]

(Equation 2)

The organ-specific DEIs are defined as the ions which are significantly different between two organs, and can be calculated by,

\[
DEI(X, Y) = \{ j \mid |Hedges's \ g(X, Y)| > g_r \}
\]

(Equation 3)
where \( g_r \) is the reference value of large effect size, \( g_r = 1.96 \) in our work.

**Organ-specific differential correlation network (DCN):** Let \( \text{PCC}(X) \) and \( \text{PCC}(Y) \) be the correlation matrices of an organ from control group \( X \) and PM2.5-exposed group \( Y \), it had been proved that the statistics of \( \Delta \text{PCC} = \text{PCC}(Y) - \text{PCC}(X) \) is normal distribution with Z score as (Fisher, 1921),

\[
Z(X,Y) = \frac{\left( 1 + \frac{1}{n_X} \cdot \text{PCC}(Y) - 1 \right) - \left( 1 + \frac{1}{n_Y} \cdot \text{PCC}(X) - 1 \right)}{\sqrt{\frac{1}{n_X - 3} + \frac{1}{n_Y - 3}}}
\]  
(Equation 4)

where \( n_X \) and \( n_Y \) be the sample sizes of \( X \) and \( Y \). Based on this conclusion, we define the organ-specific differential correlation network \( \text{DCN}(X,Y) \) whose adjacent matrix is calculated by,

\[
(e_{ij})_{M \times M} = \begin{cases} 
1 & \text{if } q_{ij}(X,Y) < \alpha \\
0 & \text{otherwise} 
\end{cases}
\]  
(Equation 5)

There is an edge between nodes \((i,j)\) only if \( e_{ij} = 1 \). And \( q_{ij}(X,Y) \) is the adjusted p value of \( e_{ij} \) using the Benjamini-Hochberg (Benjamini and Hochberg, 1995) procedure to control the false discovery rate (FDR), where p value can be obtained from z-score in Equation (4). The significant level \( \alpha = 0.05 \) is used in our work. Note that \( \text{DCN}(X,Y) \) is an undirected acyclic graph since \( e_{ij} = e_{ji} \) for \( \forall i, j = 1, 2, \ldots, N \).

**CELL-SPECIFIC DATA ANALYSIS**

Let \( X_r \) be a reference organ of \( n \) pixels and \( M \) ions. We introduce several important terms as follows.

**Cell-specific DEIs:** For a given pixel \( x = (x_j)_{1 \times M} \), assume that the abundances of ions are normal distribution in \( X \), then Z score of \( x_j \) would be,

\[
z(x_j|X') = \frac{x_j - \mu_j(X')}{\sigma_j(X')}
\]  
(Equation 6)

where, \( \mu_j(X') \) and \( \sigma_j(X') \) are the mean and the SD of metabolite \( j \) in \( X' \) respectively. The ion which is significantly different from the reference data \( X' \) with the significant level \( \alpha \) is the cell-specific DEI as follows,

\[
\text{DEI}(x|X') = \left\{ j \mid |q_j(x_j|X')| < \alpha, \forall x_j \in x \right\}
\]  
(Equation 7)

Where \( q_j(x_j|X') \) is the adjusted p value of sample \( x \) using the Benjamini-Hochberg procedure to control the false discovery rate, in which the p value of \( x \) can be obtained from the z-score in Equation (6). The significant level \( \alpha = 0.05 \) is used in our work.

**Cell-specific DCN:** Inspired by the sample-specific network from Liu et al.’s work (Liu et al., 2016) and the cell-resolved features derived from iMS, we define cell-specific differential correlation network (DCN) for a given pixel \( x \) to represent the differential metabolite-metabolite interactions in \( x \) with respect to a given reference \( X \), which is calculated as follows,

1. Calculate the Pearson correlation matrix of \( X' \), denoted as \( \text{PCC}_n \);
2. Combine \( X' \) with \( x \) to obtain a new dataset \( \begin{bmatrix} X' \\ x \end{bmatrix} \), then calculate the Pearson correlation matrix for the new dataset, denoted as \( \text{PCC}_{n+1} \);
3. Calculate z-score matrix for the difference of \( \Delta \text{PCC}_n = \text{PCC}_{n+1} - \text{PCC}_n \) as follows,

\[
(z_j)_{M \times M} = \frac{\Delta \text{PCC}_n \cdot (n - 1)}{1 - \text{PCC}_n}
\]  
(Equation 8)

4. Calculate the significance of correlation difference for each variable pair as,
Relative distance based on cell-specific DCN: Let \( X' \) be the reference organ, we define the relative distance between a given sample \( x \) and \( X' \) as the edges number in \( \text{DCN}(x|X') \),

\[
\text{rd}(x|X') = |\text{DCN}(x|X')| \quad \text{(Equation 10)}
\]

Similarly, we can define the relative distances set between two organs \( X^k \) and \( X' \) as,

\[
\text{RD}(X^k|X') = \{|\text{rd}(x|X')|, \forall x \in X^k\} \quad \text{(Equation 11)}
\]

Note that \( \text{RD}(X^k|X') \) is a set of scalars, and \( \text{RD}(X^k|X') \neq \text{RD}(X'^k|X') \) in most of cases.

Given two arbitrary organs \( X^k \) and \( X'^k \), we say that \( X^k \) and \( X'^k \) are statistically non-difference with respect to \( X' \) if there is no statistical difference between \( \text{RD}(X^k|X') \) and \( \text{RD}(X'^k|X') \).

Metabolic similarity between organs based on DCN: Let \( X = \{X^k, k = 1, 2, \ldots, K\} \) be the iMS data of \( K \) observation organs, \( X^k \in X \) be the reference organ, and \( \text{RD}(X^k|X') \) and \( \text{RD}(X'|X^k) \) be the relative distances of two given organs \( X^k, X' \in X \) with respect to \( X' \). Then we define the metabolic similarity between the two given organs \( X^k, X' \) as follows.

\[
\text{sim}(X^k|X', X) = \frac{1}{K} \sum_{j = 1, j \neq r}^{K} B(\text{RD}(X^j|X'), \text{RD}(X'|X')) \quad \text{(Equation 12)}
\]

where \( B(S1, S2) \) is a boolean function to test if median of \( S1 \) is significantly larger than that of \( S2 \) as follows,

\[
B(S1, S2) = \begin{cases} 
1 & \text{if } S1 \text{ is significantly larger than } S2 \\
0 & \text{otherwise}
\end{cases} \quad \text{(Equation 13)}
\]

Where \( S1 \) and \( S2 \) are medians of the two sets \( S1 \) and \( S2 \), respectively. In this work, we use the MATLAB function \( \text{ranksum(S1, S2, 'alpha', \alpha, 'tail', 'right')} \) to implement \( B(S1, S2) \) with the significant level \( \alpha = 0.05 \).

Since the similarity metric defined by \textit{Equation (12)} do not satisfy with the property of symmetry, \textit{i.e.,} \( \text{sim}(X^k|X') \neq \text{sim}(X'|X^k) \) in most of cases, we re-define the similarity of two organs as,

\[
\text{sim}(X^k, X') = \frac{1}{2} \left( \text{sim}(X^k|X') + \text{sim}(X'|X^k) \right) \quad \text{(Equation 14)}
\]

**METABOTYPIC ENTROPY**

Let \( \text{Prob}_j(i|X) \), \( p = 1, 2, \ldots, P \) be the abundance distribution of ion \( j \) across all pixels in a given organ \( X \), then the ion entropy of ion \( j \) can be defined and calculated by,

\[
\text{Ent}(j|X) = - \sum_{p=1}^{P} \text{Prob}_j(i|X) \cdot \log_2 \text{Prob}_j(i|X) \quad \text{(Equation 15)}
\]

Where \( P \) is the number of points in the distribution.

Regarding the abundances of ions from a pixel as the possible values of a random variable, we can calculate a probability distribution for each pixel, and define the spectral entropy as follows,
Ent(x) = - \sum_{p=1}^{P} \text{Prob}_p(x) \cdot \log_2 \text{Prob}_p(x) \quad \text{(Equation 16)}

where \text{Prob}_p(x), p = 1, 2, \cdots, P is the probability distribution for pixel x.

In this paper, we use the MATLAB function \textit{ksdensity} to fit the probability distribution of \text{Prob}(x) and \text{Prob}(j|X) with \( P = 50 \).