Article

Mass Spectrometry Imaging (MSI) Delineates Thymus-Centric Metabolism In Vivo as an Effect of Systemic Administration of Dexamethasone

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Abstract: Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) is increasingly used in a broad range of research due to its ability to visualize the spatial distribution of metabolites in vivo. Here, we have developed a method, named thoracic Mass Spectrometry Imaging (tMSI), as a standard protocol of molecular imaging of whole-animal sectioning in various settings of mice in vivo. Further application of the strategy that involved the systemic administration of dexamethasone (DEX) in mice, enabled a dynamic shift in the energy status of multiple thoracic organs to be visualized, based on tMSI data of purine and pyrimidine metabolites. Furthermore, with the introduction of uniform manifold approximation and projection (UMAP) for tMSI data, metabolic profiles normally localized in the cortex and cortico-medullary junction (CMJ) of the thymus were drastically shifted as minor profiles into the medulla of DEX-treated thymus. As a massive apoptotic cell death in the thymic cortex was noticeable, a single molecule, which was upregulated in the cortex of the thymus, enabled us to predict ongoing immunosuppression by in vivo DEX-administration.

Keywords: MALDI-MSI; thymus; purine and pyrimidine; dexamethasone; UMAP; apoptosis

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Thymus is a multi-lobed organ, composed of cortical and medullary areas, which plays an important role in T cell differentiation. During intrathymic T-cell development, differentiating thymocytes migrate through distinct thymic compartments, interacting with the cortical and medullary microenvironments of the thymic lobules. Different signals guide the entrance of precursor cells into the thymus [1,2], the migration of developing thymocytes inside the organ [3], and the expression of mature thymocytes within the periphery of the immune system [4,5]. During this journey, thymocytes experience distinct types of interactions and receive appropriate signals for cell survival, proliferation, and differentiation that culminate in the generation of a T cell repertoire capable of responding to foreign antigens [6]. It is important to know how the T cells, both inside and outside of the thymus, obtained their metabolic phenotypes in accordance with their functional demands. Here, we seek definitions both inside and outside a thymic microenvironment, which triggers the necessary signals for the aforementioned process in terms of cellular metabolic phenotypes in vivo.

Recently, the application of a matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) has proved to be a powerful approach when conducting direct tissue analysis. The instrument is a combination of an optical microscope, which allows for the observation of high-resolution morphological images, with a mass spectrometer, which identifies and visualizes the distribution of specific molecules [7]. The accurate and high-resolution mass images of tissue sections obtained through the use of an imaging mass microscope will help us to consider what kind of molecules are concerned

with thymic structure and function to support T cell survival, proliferation, differentiation and migration [8].

Corticosteroids are often used for the treatment of myasthenia gravis (MG). The most common form of MG is due to anti-acetylcholine receptor (AChR) antibodies [9] and is frequently associated with thymic pathology, including thymoma, and follicular hyperplasia [10,11]. Several lines of study have shown that corticotherapy had striking effects on MG thymuses, i.e., the number and size of germinal centers (GC) are reduced in patients treated with corticosteroids [12]. Thus far, microarray experiment have clarified that the genes dysregulated in thymic hyperplasia, and normalized by corticosteroids, should play a role in the intra-thymic pathogenic mechanisms. However, problems remain mostly unsolved regarding what happened during the effector phase at the proteome and metabolome levels. Understanding how the pathology of the thymus reacts in steroid-treated patients must be of particular interest for many clinicians involved in the treatment of autoimmune disease. In this study, the challenge was to visualize the metabolic landscape of the thorax before and after dexamethasone (DEX) administration. DEX is a corticosteroid used in a wide range of conditions for its anti-inflammatory and immunosuppressant effects [13,14]. Here, we attempted to set up a standard protocol of MALDI-MSI, which targeted whole thoracic organs in combination with uniform manifold approximation and projection (UMAP), in order to discover what would happen to mice *in vivo*, under various clinical settings.

1. Experimental Section

All animal experiments were approved by the Doshisha University Research Ethics Committee and were performed in accordance with the relevant guidelines and regulations of the committee. Female ICR mice (four weeks of age) were purchased from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan). Four weeks is the age at which the thymic weight is at its maximum [15]. After 12 h of intraperitoneal administration of 5 mg/kg of DEX (MP Biomedicals, Paris, France) and saline as a control, the mice were sacrificed and analyzed under deep anesthetic status with isoflurane (Mylan, Canonsburg, PA, USA). Then, mice were snap-frozen in liquid nitrogen. This step was in accordance with the ARRIVE guidelines for *in vivo* animal study and the use of proper anesthetics [16].

2. Preparation of Tissue Sections

Fresh frozen chest organ blocks were sectioned at -20°C using a cryostat (CM 3050; Leica, Wetzlar Hesse, Germany) to a thickness of 12 μm . The thoracic portion of the mice were removed and fixed on the stage with glue materials, such as agar or optimal-cutting-temperature polymer, and care was taken to avoid cross-contamination as these materials would interfere with MSI [17]. The frozen sections were thaw-mounted on indium–tin–oxide (ITO) coated glass slides (Bruker Daltonics, Bremen, Germany) for MSI.

3. Histological Analysis

The tissue sections after MSI were stained for histological analysis with hematoxylin (Merck KGaA, Darmstadt, Germany) and eosin (Wako, Japan). The serial frozen sections were stained by TdT-mediated dUTP Nick End Labeling (TUNEL) [18]. The H&E staining after MSI was as follows: (1) fix in a 4% paraformaldehyde for 30 min, (2) wash in water for 5 min, (3) stain in hematoxylin for 5 min, (4) wash in water for 5 min, (5) dip in 1% HCl 70% ethanol for 2 s, (6) wash in water for 5 min, (7) counterstain in eosin Y for 20 s, (8) wash and dehydrate in 100% ethanol for 5 min, (9) wash and dehydrate again in 100% ethanol for 5 min, (10) dip in xylene for 5 min, and (11) dip in xylene again for 5 min. The sections were covered with marinol (Wako, Japan), followed by a glass cover slide, and dried at room temperature. TUNEL-stained sections were created using the TUNEL stain kit (Merck MILLIPORE, Darmstadt, Germany) as directed by the manufacturer's protocol. Histological sections were scanned with a NanoZoomer 2.0 HT slide scanner (Hamamatsu Photonics, Hamamatsu, Japan) at $40\times$ magnification and observed using the NDP.view2 software program (Hamamatsu Photonics). HE-stained images were converted to black and

white mode in 8-bit type grayscale by image J software (NIH, <http://imagej.nih.gov/ij/>, accessed on 14 October 2021).

4. Mass Spectrometry Imaging (MSI) Data Acquisition

The frozen sections were kept in plastic tubes with silica gel (Wako, Japan), which were subsequently dried in a vacuum dryer. As recommended in a previous report, we used 9-aminoacridine (9-AA; Merck KGaA, Darmstadt, Germany) as a matrix for negative ion modes to detect various metabolites such as nucleotides [19–25]. The sections were manually sprayed with 4 mg/mL of 9-AA in 70% methanol by a 0.2 mm nozzle caliber airbrush (Procon boy FWA Platinum; Mr. Hobby, Kyoto, Japan). MSI was performed using an atmospheric pressure MALDI-QIT-TOF-MS [26] (Mass Microscope as a prototype; Shimadzu, Kyoto, Japan) equipped with a 355-nm Nd: YAG laser.

5. MSI Data Processing

MALDI measurements were recorded in a mass range of m/z 250–900. The lateral resolution was set to 50 μm . Using the software, IMAGEREVEAL (Shimadzu, Kyoto, Japan), ion image reconstruction and statistical analysis were performed from MS data normalized to total ion current to eliminate fluctuations in ionization efficiency. For HE staining after MSI experiments, 9-AA was removed from the slides by dipping them in methanol for 2 min. The optical images of the HE stained sections were imported into IMAGEREVEAL. A nonlinear dimensionality-reduction technique, uniform manifold approximation and projection (UMAP), was recently developed for the analysis of high-dimensional data. Applying UMAP to MSI data has gained popularity as it can preserve local structures of high-dimensional data in a low map representation. The number of selected peaks for the statistical analysis was set to 3000. To annotate peaks in MSI data, we used the Human Metabolome Database [27] (<http://hmdb.ca>, accessed on 14 October 2021) and METLIN [28,29] (<https://metlin.scripps.edu>, accessed on 14 October 2021). UMAP [30,31] was clustered, fully reduced and spectral processing was conducted with a clustering algorithm.

6. Results

6.1. Whole Animal Sectioning of the Mice

In order to obtain specific and sensitive biomarkers that would reflect health and diseased status, we developed a direct thoracic sectioning method without taking the conventional approach and using Kawamoto's film method [32]. In a single slide, we can characterize histopathologic and metabolic microenvironments of the thymus as well as multiple organs within the thoracic cavity (Figure 1 and Supplementary Figures S1–S3). In a single murine thoracic tissue section, multiple organs such as bone marrow, thymus, lymph node, smooth muscle, esophagus, trachea, lung, spinal cord, adipose tissue, arteriovenous blood vessels and blood were included to be analyzed for both histology and MSI. For histology, whole-body staining was enabled by an original protocol and, consequently, lymphatic organs were stained with relatively strong contrast by hematoxylin and eosin (HE) staining. In order to obtain comparable tissue sections among DEX-treated and non-treated mice, the coronal section of the thorax, at the point between the arch of the aorta and the bifurcation of subclavian branch, was carefully selected. The current stage was suitable for a metabolomics study as the aorta and veins were lined in a relatively wide area just beneath the thymus (Figure 1).

Figure 1. Orientation and structural basis of the murine thymus. (A) Macroscopic image of ICR mouse. Mid-sagittal section of the murine body from head to upper thorax was shown. Four yellow dotted lines, as (i–iv) from the cervical to the caudal orientation, show the level of the coronal sections in the upper thorax. For those tissue sections, HE staining was done in (D). (B,C) Major arterial branches are depicted in the illustrations. (B) ventral view, (C) left lateral view in 3-D mode. * Brachiocephalic artery, ‡ left common carotid artery, § left subclavian artery, ± aortic arch, # blood vessel that branch from the internal thoracic artery into the thymus. (D) Four coronal sections of the murine thorax. From (i) to (iv), sections were directed from head to thorax orientation. Coronal section between (iii) and (iv) was used in the following experiments. 1. Bone marrow, 2. Thymus, 3. Lymph node, 4. Esophagus, 5. Trachea, 6. Lung, 7. Spinal cord.

6.2. Dexamethasone Induced Massive Apoptotic Cell Death in the Thymus

After 12 h of DEX administration, massive apoptotic cell death was observed, especially in the cortex of the thymus of DEX-treated mice. This was well reflected by TUNEL staining in Figure 2. Moreover, while converted visions of HE staining into binary mode clearly defined cortex and medulla in control thymus, cortico-medullary junction (CMJ) appeared as the third compartment in DEX treated thymus (Figure 2). By contrast, in control thymus, CMJ was hard to define using simple HE staining or binary version.

Figure 2. Microscopic image of the murine thorax before and after DEX administration. (A) HE-stained thoracic tissue sections after IMS. Left: Control; Right: DEX administered ICR mouse. 1. Bone marrow, 2. Thymus, 3. Lymph node, 4. Esophagus, 5. Trachea, 6. Lung, 7. Spinal cord. (B) HE (a,b) and TUNEL (e,f) staining of the thoracic sections. The binary images of HE staining were also shown in (i,j). Scale bar; 2.5 mm (a,e); 1 mm (b,f); 500 μ m (c,d,g,h). (c,d,g,h,k,l) were the enlarged figure of the squared area from (a,b,e,f,i,j), respectively. $\pm$ shows aortic arch.

6.3. Dexamethasone Alters Global Energy Status of the Mice In Vivo

For the serial section of HE staining mentioned above, an analysis of mass spectrometry imaging (MSI) was successfully completed with micro-mass microscopy (Figure 3). First, 9-aminoacridine (9-AA) was utilized as a matrix to visualize annotated metabolites such as purine and pyrimidine metabolites [23,25], as well as 2,3-bisphosphoglycerates (2,3-BPG), as shown in Figure 3B. While 2,3-BPG at m/z 264.94 marks intra-vascular structure due to its relationship with red blood cells, metabolites such as uridine diphosphate (UDP)-glucose at m/z 565.03 and UDP-N-acetylhexosamine (HexNAc) at m/z 606.05 were strongly detected in cortex of the thymus, bone marrow, and adipose tissues. One should consider that 2,3-BPG was colocalized with metabolites in circulation, which belonged to a humoral factor and UDP-glucose and UDP-HexNAc that represented the metabolic status of a mostly intracellular solid organ. While there seemed to be no significant difference

in the intensity of 2,3-BPG between DEX treated and non-treated mice, the distribution of 2,3-BPG shown by tMSI was dependent on its background vascular structure (Figure 3).

Figure 3. Thoracic Mass Spectrometry Imaging (tMSI). (A) Average mass spectrum of the normal murine thorax section obtained from MALDI-IT-TOF analyzer in the negative-ion mode. The signals were obtained ranging from $m/z = 250$ to 900. Annotated single peak with qualified metabolites in the current literature and by ourselves are shown. (B) MSI clarifies the localization of 2, 3-BPG (b,f), UDP-glucose (c,g), and UDP-HexNac (d,h) shown as a heat map mode. HE staining from control (CON) and DEX treated mice, were shown (as (a) and (e), respectively). Color scale for 2, 3-BPG (b,f) was 0 to 21.237%, for UDP-glucose (c,g) was 0 to 43.871%, and for UDP-HexNac (d,h) was 0 to 42.86%, respectively.

In Figure 4, we have shown nucleotide imaging from DEX treated and non-treated mice. As is often the case with MALDI-MSI, metabolites such as ATP will decay rapidly due to post-mortem (PM) changes within 1 min after death of the animal. A key issue to resolve is prevention of the PM enzymatic degradation of metabolites during the IMS sample preparation process. Our method of tMSI is superior whole-body imaging technology, which saves time for sample collection and, subsequently, freezing in comparison with conventional methods. As a result, adenosine triphosphate (ATP) at m/z 505.96 and uridine triphosphate (UTP) at m/z 482.94, were reported as find-me signals that were released in a regulated way during apoptosis [33], and distributed mainly in the medulla before DEX treatment. With the subsequent massive apoptotic cell death caused by DEX treatment, mostly in the thymic cortex, the signals from ATP and UTP remained in the medulla. Assuming that the intravascular ATP level stayed in the same range as shown in the signals within the aorta and veins, juxtaposed to thymus, the distribution of these nucleotides between the cortex and medulla were reverted by DEX treatment. ATP as well as adenosine diphosphate (ADP) at m/z 426.00 were the only two nucleotides detected inside the aorta and veins, possibly reflecting a released fraction from apoptotic cell death in circulation. In Figure 5, we have calculated and visualized the energy status of the thoracic organs according to the following formula: $[(ATP) + 0.5 (ADP)] / [(ATP) + (ADP) + (AMP)]$

with or without DEX treatment for the mice [34] where adenosine monophosphate (AMP) at m/z 346.04.

Figure 4. MSI derived from purine and pyrimidine metabolites. Panel (a–c,g–i) are from control mouse and (d–f,j–l) are from DEX-treated mouse. AMP: (g,j); ADP: (h,k); ATP: (i) and (l). UMP: (a) and (d); UDP: (b,e); UTP: (c,f). Color scale for adenine nucleotides are between 0 and 53.989% (g–l) and for uridine nucleotides are between 0 and 60.628% (a–f), respectively.

Figure 5. Visualization of energy charge (EC) status of multiple thoracic organs with or without DEX administration. EC images were calculated and shown by using the defined formula as $[(ATP) + 0.5 (ADP)] / [(ATP) + (ADP) + (AMP)]$. EC in the thymic cortex was significantly reduced after DEX administration. In the medulla of DEX-treated thymus, heterogeneous EC distribution was noticeable. Color scale for control and DEX treated mouse are the same as between 9.708 and 82.876%.

By contrast, all other nucleotides: uridine monophosphate (UMP) at m/z 323.01, uridine diphosphate (UDP) at m/z 402.97, and uridine triphosphate (UTP) at m/z 482.94,

(Figure 4); guanine monophosphate (GMP) at m/z 362.04, guanine diphosphate (GDP) at m/z 442.00, and guanine triphosphate (GTP) at m/z 521.96 (Supplementary Figure S4); deoxythymidine monophosphate (dTMP) at m/z 321.03, deoxythymidine diphosphate (dTDP) at m/z 401.00, and deoxythymidine triphosphate (dTTP) at m/z 480.96; cytidine monophosphate (CMP) at m/z 322.04, cytidine diphosphate (CDP) at m/z 402.00, and cytidine triphosphate (CTP) at m/z 481.98 (Supplementary Figure S5) were not detected inside the vasculatures. These observations may support the idea that apoptotic cells released certain metabolites as ‘good-bye’ signals to actively modulate outcomes in tissues which were originally reported by an untargeted secretome of apoptotic cells that used Jurkat cells [35]. ATP and ADP were good candidates for those humoral signals.

As well as visualizing adenine nucleotides, analysis of their degradation products might be useful in detecting alterations in energy status. In particular, inosine monophosphate (IMP), a deamination product of AMP, is of interest in this context. The distribution of IMP at m/z 347.03 in both control and DEX-treated mice is shown in Supplementary Figure S6. This was one of most prominent metabolic effects of systemic corticosteroid therapy, which were noticeable in multiple tissues, including aortic walls, esophagus and skeletal muscles of the thorax. Notably, this was also detected in the grey matter of the spinal cord (Supplementary Figure S6).

IMP levels were negatively related to the ATP/ADP ratio, which suggested an imbalance between ATP utilization and resynthesis. This was totally consistent with the current study as the shift in energy status of thoracic muscle shown in Figure 5 was inversely related to the shift in IMP distribution, as shown in Supplementary Figure S6, in DEX treated mice. The cause and consequences of these disturbances in muscle energy metabolism in COPD patients [36], as well as DEX treatment in the current study, need further exploration.

6.4. Imaging Global Metabolomics in Multi-Organ Platform of the Mice

Segmentation analysis was performed based on the acquired data using machine learning methods, and utilized IMAGEREVEAL software and Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) [30,31]. In Figure 5, a definitive metabolic compartment was clearly visualized in the thorax before and after DEX treatment in mice. Massive apoptosis found in the cortex of DEX-treated thymus were classified as a light green color in UMAP. Apoptosis-related metabolism in the thorax of the mice were prevalent on the cortex with DEX treatment. With advanced calculations using 82 fractions of UMAP technique, the spinal cord was clearly demarcated with white matter (pink) and grey matter (yellow) as a butterfly shape. Furthermore, the third compartment in grey matter was intermingled with blue colored compartments in Figure 6, and displayed a very similar pattern to the distribution of nucleus of interneurons in anterior and posterior horns of grey matter. With this calculation applied to the thymus, we have succeeded in dividing the metabolic compartment within the thymus as: (1) outer-most compartment consistent with thymus cortex (red), (2) outer medulla or junctional area often described as CMJ (blue), and (3) inner-most medulla (purple) in normal mice (Figure 7). Interestingly, there were several TUNEL-staining positive locations, mostly extra-thymic, which shared a consistent metabolic profile, expressed in green color. Most importantly, we have succeeded in categorizing CMJ from a metabolic point of view, in normal murine thymus.

Figure 6. UMAP analysis from tMSI data. (a) Segmentation images based on UMAP analysis were shown (a,b). Each segment calculated by UMAP was visualized in a three-dimensional space with graph layout algorithms (c). Color used in (c) is identical to (a,b). (a) control mouse, (b) DEX-treated mouse, (d–g) UMAP-profiled metabolic status of the spinal cord. Three metabolomic clusters were marked with blue (d), yellow (e), and pink (f). Yellow color is consistent with grey matter and pink color is consistent with white matter. Blue color looks like nucleus of various interneurons included in grey matter. Three colored images were merged on the HE staining of the spinal cord shown in (g).

(A)
Figure 7. Cont.

(B)

(C)

Figure 7. (A) UMAP-profiled metabolic status of the thymus. Four clusters were shown as green (b), red (c), blue (d), and purple (e). Four metabolomic profiles were merged with HE staining (f). In each panel, left-sided tissues were from control thymus and right-sided tissues were from DEX-treated mice. (B) Four UMAP profiles were merged against blank. (C) TUNEL staining of the squared areas in (B) were enlarged as (1,2) from control, (3,4) from DEX treated mouse. (1,2) from bone marrow and (3) from trachea and lymph node. (4) from cortex and medulla of the DEX-treated thymus.

Another point is that the metabolic heterogeneity of the thymic medulla was first visualized using the current technique, which was obviously influenced by DEX treatment. Notably, molecular profiles of the cortical thymus in normal mice had a unique molecular profile, shown in red color in Figure 6, which mostly disappeared in the DEX-treated thymus cortex and shifted toward juxtaposed posterior portion of the medulla. In normal mouse thymus medulla, metabolic heterogeneity was observed in a regular manner as two layers of CMJ and inner-most medulla. With DEX treatment, the metabolic compartment representing CMJ (blue) and the innermost medulla (purple) compartment in normal thymus was intermingled, as shown in Figure 6. These observations have a close relationship to the histological findings with TUNEL

positive cells (Figure 6) before and after DEX treatment, and the distribution of metabolites corresponding to the structure and distribution of each organ was visualized as a cluster map (Figure 6). Crucially, the aforementioned trends above were reproduced well by independent experiments, as shown in Supplementary Figure S7.

Here, we have shown a novel DEX-responsive molecule, mostly depicted in the cortex of the thymus (Figure 8). We have developed a multivariate analytical algorithm based on an IMS dataset, which enabled us to find metabolic biomarkers of systemic effect of DEX administration; however, these metabolites were not referred to in the current data base, i.e., METLIN except m/z 558.04 (ADP-ribose), as shown in Supplementary Table S1. Of note, m/z 625.16 was specifically upregulated in DEX-treated thymic cortex and bone marrow, as well as the lymph node. Although these molecules must be further analyzed for identification in future studies, these findings can be utilized as a biomarker for monitoring systemic effects of corticosteroid usage. In applying MSI into human thymus pathology in future research, these metabolites may be utilized to diagnose thymus pathology such as GC hyperplasia and thymoma to help with the surgical decision of MG regarding image-guided therapy (IGT) [37–40].

Figure 8. Differentially detected ionic images from mouse thorax by DEX administration. Images at m/z 558.04 (a,e), 579.03 (b,f), m/z 625.16 (c,d), and 640.12 (e,f) were shown. (a–d) are from control mouse and (e–h) from DEX-treated mouse. Ionic intensity was visualized with heat map mode. m/z 558.04 is annotated as ADP-ribose by MS/MS analysis (Supplementary Table S1). Color scale for each ion is between 0 and 28.543% (a,e), 0 to 24.954% (b,f), 0 to 20.334% (c,d), and 0 to 13.687% (e,f), respectively.

7. Conclusions

Whole-body imaging mass spectrometry was developed for the study of spatially resolved immunometabolism within a murine corticosteroid administration model. Metabolic effects of corticosteroid administration in mice were reflected in thymus-centric alterations in purine and pyrimidine metabolites.

Applying a machine learning method with dimensionality reduction technology, we demonstrated that the metabolic profile in thymus was consistent with histological compartments such as cortex, medulla and the corticomedullary junction of the control thymus.

Applying the aforementioned technology into corticosteroid induced thymus evidenced massive apoptotic changes in the thymic cortex, which was extracted as a common metabolic profile, as well as single specific biomarker metabolites.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/app112211038/s1>. Figure S1: HE staining of the serial sections of murine thymus, coronal sections. Upper panels are from left lobe and lower panels are from right lobe of the thymus. Cortex and medulla of the thymus can easily be defined by the current HE staining. Marked points are the arterial branches of the internal thoracic artery, which enter into the thymus. Blood vessels are marked as #, and juxtaposed with major organs and the heart. Scales are 2.5 mm; Figure S2: Control mouse. HE staining of the control murine thoracic transverse tissue section for tMSI. Multiple organs

in the thorax from normal mouse were shown. Each dotted square area was enlarged as numbered: (1) Bone marrow and thymus, (2) A branch from the internal thoracic artery enters the thymus, (3) Adipose tissues surrounding and juxtaposed to the thymus and aorta, (4) Lymph node; several follicle structures are apparently observed as a concentric circle, (5) Enlarged view of bone marrow; typical bone marrow hematopoietic cells such as megakaryocytes are shown. Figure S3: HE staining of the DEX-treated murine thoracic transverse tissue section for tMSI. Figure S4: Results of single mass spectrometry imaging, annotated as purine metabolites, are shown: (a,c,e) are from control mouse and (b,d,f) are from DEX treated mouse, (a,b) GMP at m/z 362.04, (c,d) GDP at m/z 442.00, (e,f) GTP at m/z 521.96. Color scale for each ion is the same as between 0 and 47.023%. Figure S5: Results of single mass spectrometry imaging annotated as pyrimidine metabolites are shown: (a,b,c) and (g,h,i) are from control mouse and (d,e,f) and (j,k,l) are from DEX treated mouse, (a,d) dTMP at m/z 321.03, (b,e) dTDP at m/z 401.00, (c,f) dTTP at m/z 480.96, (g,j) CMP at m/z 322.04, (h,k) CDP at m/z 402.00, (i,l) CTP at m/z 481.98. Color scale for deoxythymidine nucleotides is the same as between 0 and 47.023% and cytidine nucleotides is the same as between 0 and 39.715%, respectively. Figure S6: Ionic images of inosine mono phosphate (IMP), an intermediate metabolite in purine synthesis are shown. IMP as m/z 347.03. tMSI of normal (left) and DEX treated (right) mice. Color scale for IMP is the same as that between 0 and 30.22%. Figure S7: UMAP analysis from MSI data. Three independent experiments support a definite metabolic compartment shift induced by DEX treatment in mice. An intraperitoneal injection method is always accompanied with a certain level of variation; however, major findings of metabolic shift were consistent among three independent experiments. Table S1: A list of detected and identified nucleotides (purine and pyrimidine) metabolites by MS/MS analysis.

Author Contributions: Y.T. conducted experiments and wrote manuscript. S.Y. gathered statistics based on MALDI-MSI dataset. T.N. supported mass spectrometry imaging technology and M.I. was responsible for overall experiments, measurements, statistics and draft writing. All authors have read and agreed to the published version of the manuscript.

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