Primary aldosteronism (PA) is a secondary hypertensive disease caused by autonomous aldosterone production that often caused by aldosterone-producing adenoma (APA). Immunohistochemistry of aldosterone synthase (CYP11B2) shows the presence of aldosterone-producing cell clusters (APCCs) even in non-PA adult adrenal cortex (Ref. 1,2). An APCC-like portion also exists in possible APCC-to-APA transitional regions (pAATLs) in PA adrenals (Ref. 3). However, whether APCCs produce aldosterone or 18-oxocortisol (18-oxoF), a serum marker of APA, remains unknown due to lack of technology to visualize adrenocortico steroids on tissue sections.

In the present study, we utilized highly sensitive Fourier transform ion cyclotron resonance mass spectrometry to image various adrenocortico steroids, including 18-oxoF, in adrenal tissue sections from 8 PA patients with APCC (Cases 1-4), pAATL (Case 5), and APA (Cases 6-8). Further analyses by tandem mass spectrometry imaging allowed us to differentially visualize aldosterone from cortisone, which share identical m/z. These advanced imaging techniques revealed that aldosterone and 18-oxoF co-accumulated within CYP11B2-expressing regions. These imaging outcomes along with a growing body of adrenal research led us to build a progressive development hypothesis of an aldosterone-producing pathology in the adrenal glands.

**Abstract**

**Patients and methods**

MALDI-IMS: Matrix-Assisted Laser Desorption/Ionization-Imaging Mass Spectrometry

**Results**

Figure 1: Immunohistochemistry for CYP11B2 in human adrenalectomized samples. Panels (A-D), (E), and (F-H) show immunohistochemistry for samples of APCCs (arrowheads), pAATL, and APA, respectively. In (C-H), areas marked with red dotted lines indicate a tumor (T); outside of these areas constitutes the adjacent normal adrenal tissue (non-tumor portion: NT). Tumors in (C-D) and (E-H) are non-functional adenoma and APA, respectively. All panels are shown at the same magnification. Bars indicate 1 mm. Case numbers marked by blue, orange, and green indicate cases of APCC (cases 1–4), pAATL (case 5), and APA (cases 6–8), respectively. In (C–H), cases marked with red dotted lines indicate a tumor (T). In (C–H), areas marked with red dotted lines indicate a tumor (T); outside of these areas constitutes the adjacent normal adrenal tissue (non-tumor portion: NT). Tumors in (C-D) and (E-H) are non-functional adenoma and APA, respectively. All panels are shown at the same magnification. Bars indicate 1 mm. Case numbers marked by blue, orange, and green indicate cases of APCC (cases 1–4), pAATL (case 5), and APA (cases 6–8), respectively. In order to differentiate the aldosterone-specific signal from the cortisone-derived signal on adrenal sections, we further established a tandem-MS imaging method with a linear ion trap-type instrument. An initial attempt using the tandem MS method (MS^2) revealed that derivatized aldosterone and cortisone still showed the same ion transition, i.e., from m/z 474.3 to 415.2, representing a common derivatization reaction of the Gir-T molecule. However, one additional tandem MS (MS-MS-MS: MS^3) enabled the differentiation of distinct steroid structural and gave independent signals for aldosterone and cortisone, i.e., m/z 474.3 > 415.2 > 397.2 and m/z 474.2 > 415.2 > 385.2, respectively.

Figure 2: MALDI imaging using FT-ICR-MS of rat adrenal sections. Distributions of Gir-T-aldosterone, and Gir-T-cortisone on a rat adrenal section are shown in the left and right, respectively.

Figure 3: MALDI imaging of APCC, pAATL, and APA using FT-IMS-MS (a–p). Case numbers marked by blue, orange, and green indicate cases of APCC (cases 1–4), pAATL (case 5), and APA (cases 8), respectively. The signals of the derivatized steroids, Gir-T-aldosterone (left) and Gir-T-cortisone (right) are shown on a single section of each case.

Figure 4: Discrimination of aldosterone from cortisone, an isomer of aldosterone. In order to differentiate the aldosterone-specific signal from the cortisone-derived signal on adrenal sections, we further established a tandem-MS imaging method with a linear ion trap-type instrument. An initial attempt using the tandem MS method (MS^2) revealed that derivatized aldosterone and cortisone still showed the same ion transition, i.e., from m/z 474.3 to 415.2, representing a common derivatization reaction of the Gir-T molecule. However, one additional tandem MS (MS-MS-MS: MS^3) enabled the differentiation of distinct steroid structures and gave independent signals for aldosterone and cortisone, i.e., m/z 474.3 > 415.2 > 397.2 and m/z 474.2 > 415.2 > 385.2, respectively.

**Conclusions**

Steroid imaging by MALDI-IMS revealed heterogeneous steroid localization patterns provided insight into the spatio-temporal relationship between altered steroid hormone production and a cell lineage leading toward primary aldosteronism lesions.

**References**

