From Transcripts to Proteins

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From transcripts to proteins – new insight into aldosterone producing adenoma

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Running title: Proteome in aldosterone producing adenoma

Primary aldosteronism (PA) is the most frequent cause of secondary arterial hypertension, with an estimated prevalence of up to 10% in referred patients and 5% in primary care [1]. The disease results mainly from the presence of a unilateral aldosterone producing adenoma (APA) or bilateral adrenal hyperplasia. Mutations in genes coding for ion channels (*KCNJ5*, *CACNA1D*) and ATPases (*ATP1A1*, *ATP2B3*), have been identified in APA [1]. In addition, similar somatic mutations have been found in particular structures of the adrenal cortex, named the aldosterone producing cell clusters (APCC). Those structures are supposed to produce aldosterone in an autonomous fashion. Somatic mutations in APCC have been described in adrenal glands from normal subjects[2], from patients with lateralized PA without APA [3] and only very recently also in adrenals from patients with bilateral adrenal hyperplasia [4]. The frequency of somatic mutations in APA is around 50% [1]; however, a recent study applying next generation sequencing on aldosterone synthase expressing adrenal nodules suggest that somatic mutations in aldosterone-driving genes may be found in up to 88% of APA [5].

Despite the impressive advances of the last few years on how gene mutations lead to increased aldosterone production, still many questions remain unanswered concerning the pathophysiology of PA, including the mechanisms and dynamics of nodule formation, particularly in multinodular adrenals, and the role of APCC in APA development.

In an extremely original work published in this issue, Swierczynska et al have performed deep quantitative (phospho)proteomic analysis of six pairs of APA and matched nontumoral adrenal cortices. APA had diverse genetic origin, three harboured mutations in *KCNJ5*, one in *CACNA1D*, and two had no mutation. The authors identified, out of 5555 proteins common to all samples, 18 which were significantly down-regulated and 11 significantly up-regulated in all APA. Proteome analysis confirmed increased expression of CYP11B2 (aldosterone synthase) and HSD3B2 (3 beta-hydroxysteroid dehydrogenase), steroidogenic enzymes

involved in increased aldosterone output, consistently among all samples; CYP21A2 (steroid 21-hydroxylase) was increased in 4 APA. They also discovered new phosphorylation sites on HSD3B2 and CYP21A2, which were deregulated in some APA. In contrast to previous studies investigating mRNA expression [6], only minor changes in proteins regulating intracellular cholesterol supply were identified, with increased expression of lipolysis-stimulated lipoprotein receptor LSR being increased in four APA. Pathway analysis confirmed the increased expression of proteins belonging to amino acid metabolism and steroidogenesis, as well as to calcium signaling.

A particular interesting finding of this study is the discovery that APA show higher levels of proteins involved in N-glycosylation and enzymes involved in GABA degradation, as well as enhanced mTORC1 signalling. Remarkably, the GTPase RHOC, which was upregulated independently of the mutation status in all APA, significantly increased expression of *CYP11B2* at the transcriptional level in transfected adrenocortical H295R cells. APA were also characterized by disturbed extracellular matrix composition and actin cytoskeleton rearrangements, with reduction in proteins related to collagen and collagen fibril assembly, structural components of the extracellular matrix and enzymes involved in extracellular matrix turnover. Investigation of changes in phosphorylation identified mTORC1 signaling as significantly upregulated in APA. Different kinases were predicted to be deregulated in the phosphoproteome analysis; here again, the affected pathways were related to cytoskeleton remodelling and axonal guidance, suggesting that APA development may be accompanied or be related to changes in cytoskeleton rearrangements and possibly innervation. The latter is of particular interest given the many neuropeptides regulating aldosterone production in a paracrine fashion.

Concerning the mutation status, principal component analysis showed that APA with KCNJ5 mutations, but also their adjacent cortex, cluster separately from the other samples. 57

proteins were exclusively reduced and 89 exclusively increased in *KCNJ5* mutated samples compared to the other APA. *KCNJ5* mutated samples also showed lower levels of CYP11B1 and CYP17A1 and higher expression of CYP11B2. Data on gene or protein expression in *KCNJ5* mutated tumors vs other APA are inconsistent in the literature, and certainly require further studies. However, a previous transcriptome analysis performed on 102 samples did not show differential clustering between *KCNJ5* mutated and tumors not harbouring KCNJ5 mutations [7]. Furthermore, recent studies report higher CYP17A1 expression [8] and no difference in CYP11B2 expression [9] in *KCNJ5* mutated tumors compared to other groups by immunohistochemistry. Another important question to answer will be how non-tumoral tissue from patients carrying a *KCNJ5* mutations cluster differently from those of patients not carrying such a mutation in the APA. Besides paracrine factors secreted by the tumor, this fact, which requires to be replicated on a larger number of samples, raises the questions as to whether adrenal glands in patients with APA carrying *KCNJ5* mutation are different from the other categories of tumors in particular with respect to nodulation and number of APCC. An alternative explanation may be the sex difference, as *KCNJ5* mutation carriers were all females in this study.

Although performed on a small number of samples, results from this study provide a great resource for future research and highlight novel pathways possibly involved in APA development. Besides the question on how the different pathways differentially regulated in APA may act on aldosterone production and cell proliferation, an interesting issue is how protein expression correlates with transcriptional regulation. It is generally accepted that steady-state differences between protein levels are largely explained by variation of transcript concentrations, while post-transcriptional regulation processes are important during highly dynamic phases, such as cellular differentiation or stress response. The complex regulation of protein expression, including non-coding RNA, post-transcriptional modifications and

different mechanisms of protein degradation, as well as the dynamics of mRNA transcription vs protein expression, adds to the complexity of the interpretation. In this study, several regulated proteins follow patterns of transcriptional changes previously described in APA, including increased expression of CYP11B2 and HSD3B2, or VSNL1 [10]. Conversely, other changes were not confirmed at the protein level. Beyond discrepancies between mRNA and protein expression, results might be confounded by the small number of samples explored in this study and by the extreme heterogeneity of APA at the cellular, molecular and genetic level. Indeed, recent studies clearly showed that APA are composed of different type of cells, that they differentially express steroidogenic enzymes and in some instances also harbour different genetic abnormalities in different areas of the tumour [1]. Future studies will be key for replicating the findings of this study and expanding our knowledge on proteome changes in relation with well-defined pathological and genetic entities.

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Disclosures

The authors have nothing to disclose.

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