Analysis of DNA methylation profiles and single nuclei RNA-seq of pediatric adrenocortical tumors reveal subgroups of clinical relevance and distinct tumor biology

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Pediatric adrenocortical tumors (pACTs) represent a group of rare entities arising from the cortex of the adrenal gland. The mean patient age at diagnosis is 4,8 years. pACTs are divided into highly malignant pediatric adrenocortical carcinomas (pACCs) and the more benign pediatric adrenocortical adenomas (pACAs), although the exact identification of patients at high risk and the accurate pathological differentiation between pACCs and pACAs remain difficult. Most patients show signs of virilization, Cushing syndrome, or both, and germline variants of *TP53* are common. Complete tumor resection is required to achieve cure, which is particularly difficult in children with advanced disease. In our study, we analyzed DNA methylation data of pACTs from 149 patients and identified four distinct methylation subgroups of clinical relevance: One of which confered a significantly poorer prognosis than the other subgroups, with a 5-year overall survival (OS) of only 27%, while OS in the other subgroups ranged between 81%-95%. Importantly, the high-risk subgroup also contained tumors previously considered as pACA. In addition, we performed single nuclei RNA sequencing (snRNA-Seq) to reveal the biological heterogeneity underlying this disease. The methylation-derived subgroups differed in the composition of regulatory modules (so called meta-signatures), with the high-risk subgroup being enriched for proliferation-related programs (with an upregulation of e.g. *PLK1*, a pharmacologically targetable protein kinase, and *CCNE2*). Other regulatory modules which were enriched in the standard risk ACC groups displayed genes involved in steroidogenesis (such as *SULT2A1*), but also in the detoxification of xenobiotics (such as *CYP2E1*), which may contribute to chemotherapy resistance in these tumors. Performing trajectory analyses, we found that the single cell distribution of pediatric ACC recapitulates the centripetal differentiation of the adrenocortex from Zona glomerulosa to Zona reticularis - with the high-risk tumors displaying more glomerulosa-like transcriptional profiles. In accordance with this, we detected strongly upregulated WNT signaling (mainly through *WNT4*) in these high-risk tumors when performing receptor-ligand analyses. Overall, our study not only sheds light on the intertumoral and intratumoral heterogeneity of pACTs but provides means for improved risk stratification by establishing four distinct subgroups. Moreover, the single cell dissection of these tumors highlights novel drug targets, thus potentially opening new avenues for targeted therapy.