Comprehensive Pan-Genomic Characterization of Adrenocortical Carcinoma

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In Brief

Adrenocortical carcinoma (ACC) is an uncommon endocrine cancer that is best treated surgically with R0 resection, if found at an early stage. Advanced stages of ACC respond poorly to multi-modal therapies and patient survival remains poor over the past decades. Comprehensive and integrative multi-omic studies of ACC can provide a better insight in tumor initiation and progression. The identification of oncogenic alterations may lead to development of novel therapies that can be personalized based on an individual's tumor profile to achieve better outcomes.

Zheng and colleagues analyzed 91 ACC tumors from four continents and included histology and clinicopathologic features in their cohort. Molecular data from their cohort was gleaned via whole exome sequencing, mRNA and miRNA sequencing, DNA copy number variation with SNP arrays, DNA methylation profiles from DNA methylation arrays, and targeted proteome analysis via reverse phase protein arrays.

Using exome and RNA sequencing significant mutated genes in ACC identified by the MutSigCV algorithm included 3 known genes involved in ACC (TP53, CTNNB1, MEN1) and 2 previously unknown (PRKAR1A, and RPL22). The protein kinase cAMP-dependent regulatory type I alpha gene (PRKAR1A) inactivating somatic mutations and homozygous deletion were found in 7 and 3 cases, respectively. While germline mutations of PRKAR1A cause Carney complex, somatic mutations can be found in benign adrenal tumors and rarely in ACC. 7% of ACC in this cohort harbored somatic alteration of RPL22. Additional mutated cancer genes (NF1 and MLL4) not selected by MutSigCV (due to Type II error) were noted when the mutated genes were compared to the Cancer Gene Census.

Somatic copy number alteration (SCNA) and loss of heterozygosity analysis with GISTIC 2.0 revealed amplification of genes involved in maintenance of telomeres (TERT, TERF2) and deletions of RB1, CDKN2A. Homozygous deletion of ZNRF3 was observed in 16% of their tumors. Arm level copy number variation was found to be common in 89 ACC cases and three groups were stratified based on arm level copy alterations. These included 1) chromosomal (61%) - highest number of whole chromosomal arms gains and losses, 2) noisy (30%) - high number of chromosomal breaks and frequent loss of 1p with 1q intact, and 3) quiet (9%) - few large copy number alterations observed. Kaplan-Meier survival analysis showed significant decreased survival in the noisy group compared to the other two groups suggesting an aggressive phenotype with these copy number alterations. The noisy group was validated in an independent cohort of 119 cases of ACC but the quiet group was not observed in this cohort.

Whole genome doubling (WGD), tumor purity, and ploidy were analyzed with the ABSOLUTE algorithm. The authors noted 68% WGD in the noisy group, 51% WGD in the chromosomal

group, and no WGD in the quiet group. Gene set enrichment analysis revealed upregulation of pathways involving DNA replication repair, telomere maintenance, and cell cycle regulation in WGD tumors. ACC with WGD had shorter telomeres and higher TERT expression than WGD tumors, suggesting a compensatory mechanism to maintain telomere length.

Transcriptomic and genomic data were examined with unsupervised clustering to derive molecular classes for each platform (mRNA: n=3, microRNA: n=6, DNA-methylation: n=3, copy number: n=3, and protein: n=3 groups). Integrated analysis across all platforms was performed. Select subsets of theses molecular classes were placed into a cluster of cluster (CoC) analysis and 3 unique CoC groups resulted. Disease progression rates of the 3 clusters CoCs was well separated with CoC 3 having highest rate of progression and shortest survival. When compared to previously defined aggressive C1A and indolent C1B ACC groups (1),(2), CoC 1 mostly stratified to C1B and CoC 2 and CoC 3 to C1A. Because DNA-methylation subtypes had high impact on CoC analysis, DNA- -methylation data was used to create methylation signature that robustly classified this ACC cohort into 3 survival groups with 92.4% accuracy. This simplified approach can be implemented in clinical practice as accurate methylation data can be obtained from formalin-fixed, paraffin-embedded tumor samples.

Additionally, the authors found that most genes(TP53, ZNRF3, CTNNB1, TERT, and PRKAR1A) were altered by somatic mutations or DNA copy-number alterationsexcept CDKN2A which was targeted by both deletion and promoter DNA methylation. Increased activity of the Wnt/beta-catenin pathway in 41% their cohort was due to alterations of ZNRF3, CTNNB1, APC, and MEN1. p53 apoptosis/Rb1 cell-cycle pathways were altered in 44.9% of ACC secondary to somatic mutations in TP53, CDKN2A, RB1, CDK4, and CCNE1. Interestingly, when combining somatic mutations, copy number alterations, and epigenetic changes the CoC 1 group had no driver mutations, however the proliferative marker (MKI67) was lower in CoC 1 group consistent with a more indolent phenotype.

Pan-cancer analyses revealed 6 mutational signatures in 85 ACCs and these data were compared to an independent cohort of 22 signatures (3). Signature 1 looked like age and DNA mismatch repair deficiency signature with C > T of CGs. Signature 1 compared similarly to most GI cancers. Signature 2 resembled the smoking signature; similar to adenocarcinoma and squamous cell carcinoma of the lung. Signature 5 resembled UV and APOBEC signature.

An ACC differentiation score (ADS) was derived using 25 genes highly expressed in the adult adrenal cortex. Functional ACCs had high ADS without correlation (p=0.41) to Weiss Score. ACC with high ADS did correlate to Wnt mutations (p=0.0091).

Critique

This bioinformatic analysis of a large international ACC cohort revealed several previously unknown driver genes for ACC. However, to designate these newly-identified genes as "driver" genes in ACC initiation and progression, functional studies of these genes should be performed. WGD in ACC is also suggested to be a marker for progression in this study. Better understanding of the mechanisms resulting in WGD can lead to identification of effective novel therapy in ACC. The authors were also able to confirm the two pathologic classes of ACC with distinct clinical outcomes first proposed by Weiss et al. in 1989 on a molecular basis and further stratified ACC into to three classes. Dysregulation of various

pathways in ACC was also elucidated, several were clinically actionable. Their pan genomic analysis of ACC reveals that this malignancy is a very complex process involving the mutation/alterations of several genes leading to the modification of multiple downstream molecular events which may require multi-targeted therapies to fight this cancer effectively. Using WES data, clinically actionable alterations with existing drugs were identified in 22 ACC samples. This approach implements the concept of precision medicine guided by tumor molecular profiles to maximize the anti-tumor effect.

Because this is largely a bioinformatic analysis of ACC one should consider the limitations of the heuristic algorithms used to obtain the computational results and ensuing conclusions. For instance, MutSigCV has the ability to find tumor suppressor driver genes well but is less capable at revealing driver oncogenes that may fall below the frequency threshold (1). GISTIC 2.0 is an algorithm used to obtain SCNA data. The assumptions used in the modeling to develop the GISTIC 2.0 software might explain why the quiet group had very few SCNAs (2). Tumor purity, ploidy, and WGD was determined with the ABSOLUTE computational software. A limitation of this algorithm arises when applied to tumors with undetected SCNAs (none seen in the majority of the quiet group) and an incorrect ploidy could be ascertained (3).

Future Directions

This landmark study may be used by investigators to launch a multitude of studies which might include examination of clinically actionable alterations, functional studies of newly-identified genes, mechanism of WGD, and subgene analysis (1) to find additional driver mutations. The insight in these alterations requires further studies to validate and to develop novel cancer therapeutics designed for the various molecular classes of ACC and applied to our patients' specific tumor profiles. The raw data is also available in the TCGA which promotes ease of access.

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Additional High Yield Reading:

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