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# Establishing a human adrenocortical carcinoma (ACC)-specific gene mutation signature

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**Abstract**

Adrenocortical carcinoma (ACC) is a rare and aggressive tumor whose molecular signaling pathways are not fully understood. Using an *in-silico* clinical data analysis approach we retrieved human gene mutation data from the highly reputed Cancer Genome Atlas (TCGA). ACC-specific gene mutations were correlated with proliferation marker FAM72 expression and Mutsig along with the algorithmic implementation of the 20/20 rule were used to validate their oncogenic potential. The newly identified oncogenic driver gene set (ZFPM1, LRIG1, CRIPAK, ZNF517, GARS and DGKZ), specifically and most repeatedly mutated in ACC, is involved in tumor suppression and cellular proliferation and thus could be useful for the prognosis and development of therapeutic approaches for the treatment of ACC.

**Keywords** Adrenal gland, CRIPAK, DGKZ, LRIG1, Oncogene, ZFPM1, Zinc finger.


## Introduction

Adrenocortical carcinoma (ACC) is a rare cancer affecting the adrenal cortex. ACC is a malignancy of the steroid hormone (i.e., mineralocorticoids, glucocorticoids, and androgens)-producing layer of the adrenal gland. ACC occurs mostly in women and has an incidence rate of 0.7–2 cases per million people per year [1]. ACC occurs primarily between 40 and 50 years of age and is often aggressive [2,3]. ACC has a high risk of metastatization even after early diagnosis and surgery [4]. Metastasis is the ability of tumor cells to leave the primary site and migrate to other tissues, leading to the spread of cancer throughout the body [5]. The molecular mechanisms of metastasis have been well-studied in carcinomas, in which epithelial to mesenchymal transition (EMT) is considered a critical process [5,6]. Invasion could either occur through the lymphatic system, through the venous system or through the extra-adrenal tissues [7]. Tissue biopsy is unhelpful and tumor protein p53 (TP53) expression appeared to be inadequate as prognostic marker in ACC; instead, quantification of cell proliferation marker MKI67 is critical for prognosis also in ACC [1,5,8,9]. Several genes have been proposed as cancer drivers in ACC, amongst them: TP53 and catenin $\beta$ 1 (CTNNB1) [10–12]. TP53 mutations have been observed in more than 50% of child patients and 4% of adult patients of ACC [11,12]. Alterations in the components of the WNT/$\beta$-catenin pathway are a prominent marker in ACC. CTNNB1 accumulation has been reported from cases of ACC [13,14] and somatic mutations in CTNNB1 have been observed in large cohort studies [13,15,16]. Activating mutations in CTNNB1 have been observed in approximately 25% of adrenocortical cancers [14]. CTNNB1 and TP53 mutations have been reported to be mutually exclusive in aggressive adrenal cancers [17]. CTNNB1 knockdown was reported to inhibit EMT, however, that may not apply to adrenal cells due to their mesodermal origins [18]. Other genes associated with malignant ACC include insulin growth factor 2 (IGF2) and splicing factor 1 (SF1). IGF2 overexpression is observed in more than 80% cases of ACC and was among the earliest abnormalities described in ACC [19]. SF1 overexpression is associated with poor prognosis in childhood and adult sporadic ACC [20].

Despite the importance of the IGF- and WNT/$\beta$-catenin signaling pathways, the pivotal driving factors behind ACC

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are still unknown and the treatment of ACC remains an unresolved challenge [10,13,19,21,22]. Menin 1 (MEN1), protein kinase cAMP dependent type-I regulatory subunit alpha (PRKAR1A), ribosomal protein L22 (RPL22), telomeric repeat binding factor 2 (TERF2), cyclin E1 (CCNE1) and neurofibromin 1 (NF1) were recently identified as possible cancer drivers in ACC [16]. However, it is highly likely that other genes are involved in ACC tumorigenesis, which have not been reported yet [16].

In our study, we conducted a complete analysis of the mutational data of ACC using the comprehensive public cBioPortal human cancer study database in order to determine new genes involved in ACC oncogenesis. We identified a new ACC-specific gene mutation signature, comprised of the six genes ZFPM1, LRIG1, CRIPAK, GARS, ZNF517 and DGKZ. We validated our findings using the Mutsig (for "Mutation Significance") [23] data as well as the 20/20 rule proposed by Vogelstein et al. [24]. Our new ACC-specific gene signature ties in with the WNT/$\beta$-catenin and MAPK signaling pathways and offers new targets for the effective treatment of ACC.

## Materials and methods

### Human cancer patient data sources

A publicly available human ACC study (http://www.cbioportal.org/) [25,26] from The Cancer Genome Atlas (TCGA) was analyzed for mutations and mRNA expression data (provisional data set). cBioPortal is a human cancer genomics database that contains 169 studies with 40,408 samples (as of January 2018)) covering 29 different tissues. cBioPortal combines data from TCGA (http://cancergenome.nih.gov/), the International Cancer Genome Consortium (ICGC; https://icgc.org/), the Wellcome Trust Sanger Institute's (WTSI) Cancer Genome Project (http://www.sanger.ac.uk/research/projects/cancergenome/) and the Cancer Genomics Hub (CGHub; https://cghub.ucsc.edu/). TCGA is a collaborative effort between the National Cancer Institute (NCI; http://www.cancer.gov/) and the National Human Genome Research Institute (NHGRI; https://www.genome.gov/). The TCGA ACC dataset on cBioPortal consisted of mutation data from 90 patients. mRNA expression data was available from 79 of those 90 patients. The mutation data consisted of somatic mutations in 6946 genes.

### ACC-specific gene signature identification: ACC-specific gene mutation – FAM72 (A-D) paralogs mRNA expression correlation analysis

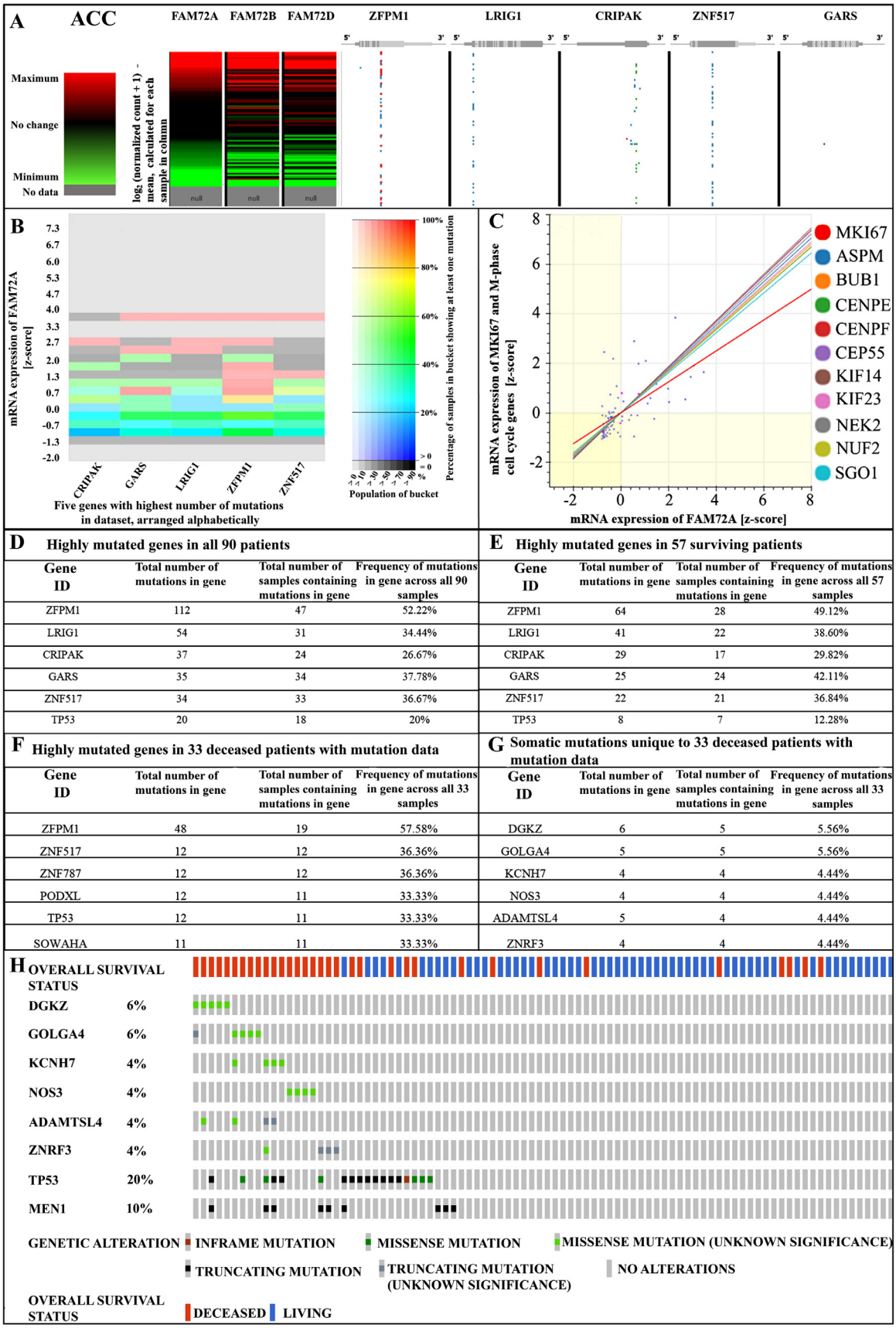
Complete mutation data for all genes was retrieved from the ACC TCGA study. The data was sorted by the frequency of mutations in each gene across the ACC study. The five genes demonstrating highest number of mutations were selected for display. Mutations in non-oncogenic genes, as described by Lawrence et al. and Greenman et al. [27,28], were not considered for the analysis. The mutations in the five genes demonstrating highest number of mutations in the study were compared to the mRNA expression of the proliferation marker FAM72 (A-D) paralogs in the ACC study and visualized with the Xena Functional Genomics Explorer [29]. FAM72 (A-D) is a set of four human-specific paralogs associated with neural stem cells [30–32] and is involved with cellular proliferation in cancerous cells where FAM72 expression is triggered by oncogenic mutations and thus they can be useful as cell proliferation markers [33]. Clinical data from the TCGA ACC study was retrieved from cBioPortal for patient-gene-specific analyses.

### ACC-specific mRNA expression analysis of the proliferation marker FAM72 (A-D) paralogs

mRNA expression *z*-scores (RNA sequencing (RNASeq V1/V2) from the 79 patients in the TCGA ACC study were locally computed on the foundation of raw expression data available on cBioPortal. A *z*-score is a statistical measurement indicating how many standard deviations the element is from the mean. The formula is $z = (X - m)/\sigma$, where $z$ is the *z*-score, $X$ is the value of the element, $m$ is the numerical mean of the population, and $\sigma$ is the standard deviation [34]. The *z*-score was normalized for all samples so that they sum to zero. Linear regression was determined between the FAM72A, the proliferation marker MKI67 [35] and genes expressed in the M-phase of the cell cycle, for all available samples across the TCGA ACC study. The regression curve analysis was visualized with the Python-based Bokeh online visualization tool [36].

### ACC-specific gene mutation – FAM72 (A-D) paralogs mRNA expression correlation analysis visualized by the bucket method

The mRNA expression *z*-scores for FAM72 (A-D) paralogs were separately grouped in buckets with a bucket size of 0.7 *z*-score units and consequently correlated with genes showing high numbers of ACC-tissue-specific gene mutations. The *Y*-axis denotes the *z*-score buckets for the selected FAM72 gene. The genes whose mutation numbers are to be visualized, lie on the X-axis. The data was then visualized with the Python-based Bokeh interactive visualization tool [36]. Number of mutations in a gene in the samples within a bucket are denoted by a color code. The color intensity of the buckets is directly proportional to the number of samples, while the colors visualize the relation of samples with a mutation to the total number of samples. Brighter colors indicate more samples in the bucket while paler colors indicate fewer samples in the bucket. Colors tending to the red side of the spectrum indicate increasing number of samples with a mutation in relation to the total number of samples in the bucket. Colors tending to the blue side of the spectrum indicate decreasing number of samples with a mutation in the bucket. Black bands denote absence of mutations or lack of expression data in the gene, while bright grey bands indicate absence of samples within the group. Bright pink boxes indicate that only one sample is present in the bucket that contains one mutation in the gene of interest.




**D** Highly mutated genes in all 90 patients

| Gene ID | Total number of mutations in gene | Total number of samples containing mutations in gene | Frequency of mutations in gene across all 90 samples |
|---|---|---|---|
| ZFPM1 | 112 | 47 | 52.22% |
| LRIG1 | 54 | 31 | 34.44% |
| CRIPAK | 37 | 24 | 26.67% |
| GARS | 35 | 34 | 37.78% |
| ZNF517 | 34 | 33 | 36.67% |
| TP53 | 20 | 18 | 20% |

**E** Highly mutated genes in 57 surviving patients

| Gene ID | Total number of mutations in gene | Total number of samples containing mutations in gene | Frequency of mutations in gene across all 57 samples |
|---|---|---|---|
| ZFPM1 | 64 | 28 | 49.12% |
| LRIG1 | 41 | 22 | 38.60% |
| CRIPAK | 29 | 17 | 29.82% |
| GARS | 25 | 24 | 42.11% |
| ZNF517 | 22 | 21 | 36.84% |
| TP53 | 8 | 7 | 12.28% |

**F** Highly mutated genes in 33 deceased patients with mutation data

| Gene ID | Total number of mutations in gene | Total number of samples containing mutations in gene | Frequency of mutations in gene across all 33 samples |
|---|---|---|---|
| ZFPM1 | 48 | 19 | 57.58% |
| ZNF517 | 12 | 12 | 36.36% |
| ZNF787 | 12 | 12 | 36.36% |
| PODXL | 12 | 11 | 33.33% |
| TP53 | 12 | 11 | 33.33% |
| SOWAHA | 11 | 11 | 33.33% |

**G** Somatic mutations unique to 33 deceased patients with mutation data

| Gene ID | Total number of mutations in gene | Total number of samples containing mutations in gene | Frequency of mutations in gene across all 33 samples |
|---|---|---|---|
| DGKZ | 6 | 5 | 5.56% |
| GOLGA4 | 5 | 5 | 5.56% |
| KCNH7 | 4 | 4 | 4.44% |
| NOS3 | 4 | 4 | 4.44% |
| ADAMTSL4 | 5 | 4 | 4.44% |
| ZNRF3 | 4 | 4 | 4.44% |




**Fig. 1**

### Gene-specific survival analysis

The prognostic significance of selected genes from ACC was analyzed using available Kaplan–Meier curves from the cBioPortal database and compared by the log-rank test [37].

### Application of the '20/20' rule and Mutsig to validate the oncogenic potential of selected genes in ACC

We applied the 20/20 rule as described by Vogelstein et al. [24] to our findings from the mutation data. In brief, the 20/20 rule classifies genes into oncogenes (ONG) or tumor suppressors (TSG). If the frequency of gain of function mutations in the gene is greater than 20%, then the gene is an ONG. If the frequency of recurrent loss of function mutations in a gene is greater than 20% and the frequency of gain is function mutations in the gene is less than 20%, then the gene is a TSG [24]. We used this approach on the somatic mutation data from the ACC study on cBioPortal and checked whether selected genes were sorted as ONG or TSG. Mutsig data information for each of the highest mutated genes was also retrieved from cBioPortal. Mutsig is a package of tools for analyzing mutation data. It operates on a cohort of patients and identifies mutations, genes, and other genomic elements predicted to be driver candidates [27]. The Mutsig package develops a background mutation model of the tumor and then analyzes mutations in each gene to identify genes that have been mutated at a higher frequency than background. Consequently, Mutsig assigns a Q-value to each mutated gene, based on a false discovery rate of mutations, and genes are then sorted based on their Q-value. Lower Q-value indicates a lower false discovery rate of mutational drivers, therefore the lower the Q-value, the higher the significance of the mutation in that gene. Here, our identified genes were validated as potential oncogenes by combining these two ('20/20' rule and Mutsig) methods. CTNNB1, PRKR1A, RB1, MEN1 and NF1 were used as positive controls, as they had been described as confirmed oncogenes in ACC from previous studies [16].

## Results

### Human gene mutation analysis of the TCGA ACC study

We found five genes prominently mutated in ACC: the leucine-rich repeats and immunoglobulin like domains 1 (LRIG1), the cysteine-rich PAK1 inhibitor (CRIPAK), the zinc finger protein 517 (ZNF517), the zinc finger protein FOG family member 1 (ZFPM1) and the glycyl-tRNA synthetase (GARS) (Fig. 1(A), Supplementary File 1). Although mucin 5B (MUC5B) had a higher number of mutations than CRIPAK, gene mutations in mucins have been reported to be passengers and not oncogenic [27]. We thus disregarded the mucins and focused on the remaining top five most mutated genes, namely ZFPM1, LRIG1, CRIPAK, GARS, and ZNF517.

Highest number of mutations were noted for the gene ZFPM1, followed by LRIG1 and GARS. The mRNA expression level of FAM72 paralogs is not linearly correlated with these mutations (Fig. 1(A)). Fig. 1(B) shows that mutations in ZFPM1, LRIG1 and CRIPAK are spread through the samples and no single driver oncogene could probably cause cellular proliferation, but an accumulation of mutations across a set of genes may be responsible for the cause of ACC. The linear MKI67-FAM72 mRNA expression correlation graph clearly demonstrates that FAM72 is highly expressed in proliferat-

Fig. 1 ACC-specific gene-mutation signature. Correlation between mRNA expression of human proliferation marker FAM72 (A-D) paralogs and proto-oncogenes / tumor suppressor genes frequently mutated in the TCGA ACC study comprised of 79 samples that contained both mRNA expression and mutation data. FAM72 paralogs were used as a comparison as they are known to be expressed in proliferative cells [38]. Comparison between the tumor samples sorted by sample in descending order of FAM72A expression (on the left hand), and the ACC-specific gene-mutation signature represented by the five genes demonstrating highest number of mutations (ZFPM1, LRIG1, CRIPAK, ZNF517 and GARS) in the same ACC samples sorted by number of mutations (on the right hand; sorted from left to right) (A). Red bands indicate increased expression, green bands indicate decreased expression and black bands indicate no change in expression. Blue dots (A, right hand) represent missense or in-frame mutations in the indicated gene in a sample, while red dots represent nonsense or frameshift indel mutations in that gene in the sample. ZFPM1 was the most frequently mutated gene in ACC and mutations occurred predominantly at the E444/L446 region. LRIG1 showed the second highest mutation frequency mutated at the same site L24V in its protein sequences. Bucket-wise distribution of mutations in the top five genes demonstrating highest number of mutations in ACC, sorted by FAM72A expression (B). The grey area in the heat maps and bucketed diagrams indicate lack of data. High gene expression correlation between FAM72A and the proliferative marker MKI67 as well as other M-phase-specific cell cycle genes indicates that FAM72A is highly expressed in proliferating ACC cells (C). The sample size was 79, standard error was 0.09, slope was 0.62, and two sided p-value was 0.0 for the linear regression plot. Mutations in selected genes across all 90 patients in the ACC study (D). Mutations in selected genes across 57 survivors (E). Frequency of mutations in ZFPM1 was slightly lower in survivors than overall patients, but mutation frequency in TP53 was 8% lower in samples from survivors. Most frequently mutated genes in 33 deceased patients with mutation data (F). Somatic mutations observed uniquely in deceased patients highlight a novel ACC-specific gene-mutation signature (DGKZ, GOLGA4, KCNH7, NOS3, ADAMTSL4, and ZNRF3) (G). OncoPrint data from the ACC study on cBioPortal for visualization of the relationship between somatic mutations in genes from (G) and survival of patient (H). Mutations in the genes stated in (D), (E), and (F) are well-established oncogenic drivers. However, the genes mentioned in (G) are – thus far – not reported to be oncogenic drivers, but may assist in metastasis. OncoPrint data in (H) clearly shows that mutations occurred in separate patients, all of whom are deceased, and did not overlap, implying that all genes from this novel gene-mutation signature from (G) could have each played a pivotal lethal role as primary driver oncogene in the oncogenic pathway of ACC or in metastasis in conjunction with other driver oncogenes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

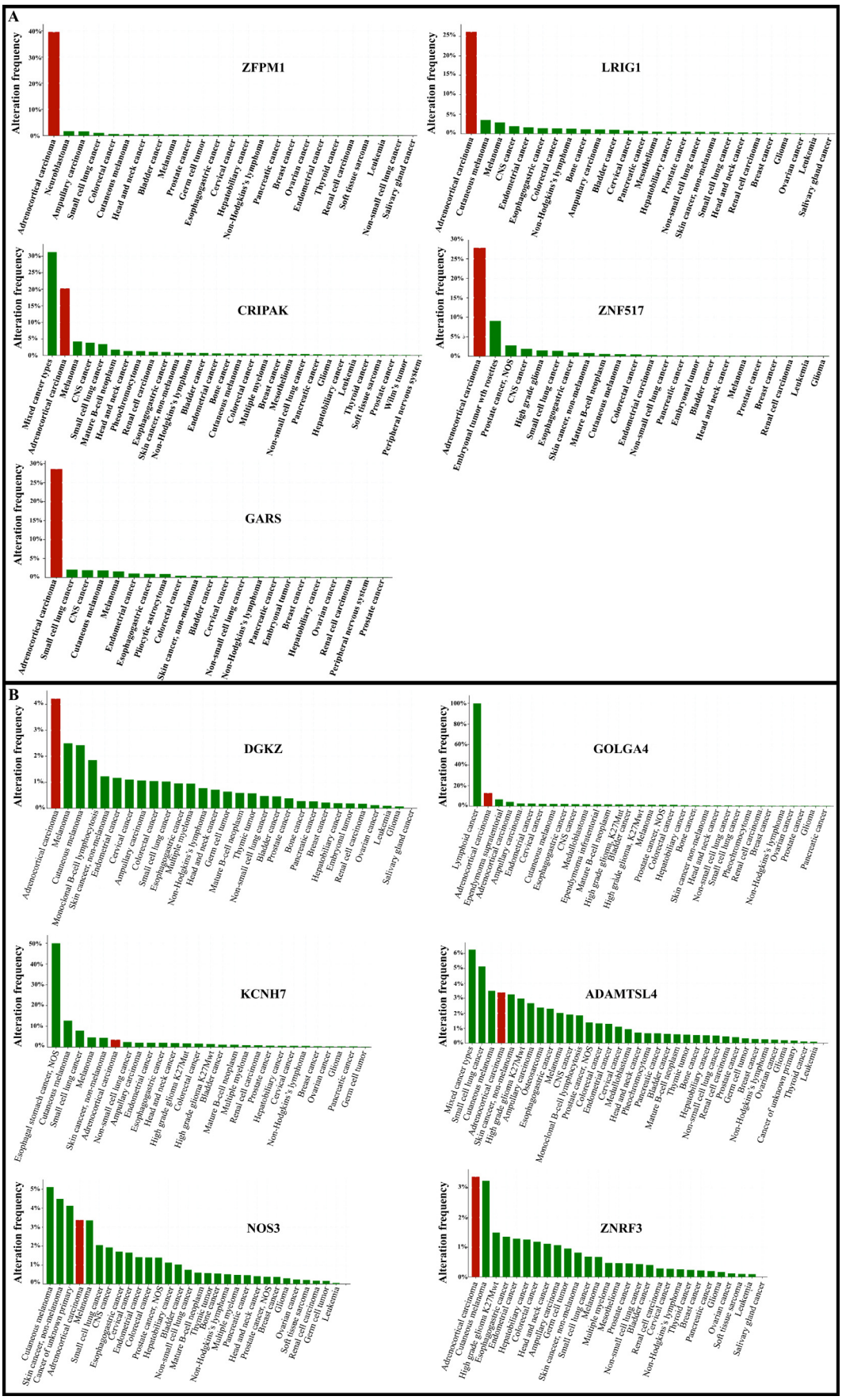

A
ZFPM1
LRIG1
CRIPAK
ZNF517
GARS
B
DGKZ
GOLGA4
KCNH7
ADAMTSL4
NOS3
ZNRF3
Alteration frequency

**Fig. 2**

ing ACC cells (Fig. 1(C)). Frequency of mutations in LRIG1, CRIPAK and GARS were slightly higher in survivors as compared to total number of patients, while the frequency of mutations in ZNF517 remained similar both in total number of patients as well as survivors. However, the frequency of mutations in TP53 was lower in survivors than in overall patients (Fig. 1(D)–(E)). We compared the list of frequently mutated genes overall with genes which were frequently mutated only in deceased patients. We found six genes, diacylglycerol kinase zeta (DGKZ), golgin A4 (GOLGA4), potassium voltage-gated channel subfamily H member 7 (KCNH7), ADAMTS like 4 (ADAMTSL4), nitric oxide synthase 3 (NOS3), and zinc and ring finger 3 (ZNRF3), which showed a significant number of mutations and adding to the novel ACC-specific gene mutation signature (Fig. 1(G)). We also observed a potential gender effect: mutations in DGKZ, GOLGA4 and NOS3 were observed mainly in women, with female to male occurrence ratios ranging from 3:1 to 4:0 (Supplementary File 1). This hints at a gender-specific role of these genes in ACC. Patient data and list of genes mutated solely in deceased patients is provided in Supplementary File 1.

We analyzed whether mutations in these top-five mutated genes, as well as genes observed only in deceased patients, were specific to ACC by checking alteration frequency of these genes across all human cancer tissue types available on the cBioPortal database. We found that alteration frequency of the ZFPM1, LRIG1, CRIPAK, GARS, and ZNF517 was significantly higher in ACC as compared to all other cancer types. This indicates that these five genes were specifically altered in ACC but not in other cancer tissues (Fig. 2). Moreover, the mutations in DGKZ and ZNRF3 were most frequently observed in ACC too, while GOLGA4, KCNH7, ADAMTSL4, and NOS3 did not show increased ACC-specific alteration frequencies (Fig. 2).

Moreover, we found that mutations in the five highest mutated genes were localized only to one or two positions in their amino acid (AA) sequences – particularly observed in ZFPM1, LRIG1, GARS and ZNF517; however, this AA location specificity was not observed in CRIPAK. These locations were specific to ACC and were not observed in any other cancer tissue (Fig. 3). Recurrent mutations at the same site provided further evidence of the ACC-specific oncogenic potential of these mutations for this specific gene set. The deceased patient-specific gene set did not show such a site-specificity for mutations (Supplementary File 1).

### Gene-specific survival analysis in ACC

Kaplan Meier survival curves for ZFPM1, LRIG1, CRIPAK, GARS, and ZNF517 show no change between patients with and without alterations in ACC (Supplementary File 1). However, survival curves for DGKZ, GOLGA4, KCNH7, ADAMTSL4, NOS3 and ZNRF3 show extensive differences between patients with and without mutations in them (Fig. 4). The low number of samples containing mutations in selected genes could be one reason for the large differences between curves.

### Application of the '20/20' rule to validate the oncogenic potential of selected genes

Applying the '20/20' test to the mutation data from ACC also confirmed our hypothesis that the genes of interest are potential oncogenes. This was additionally verified by the Mutsig Q-value data from cBioPortal (Supplementary File 1). We used genes reported previously as potential drivers in ACC [14,16] as positive controls and compared their 20/20 results and Mutsig Q-values with our gene set of interest. We found that ZFPM1, LRIG1, GARS, and ZNF517 show lower Q-values than the reported oncogenes. DGKZ qualifies as a potential oncogene by the 20/20 test, however, Q-values for DGKZ were unavailable. CRIPAK had a higher Q-value, even though it fits the parameters for an oncogene by the 20/20 test, while GOLGA4, KCNH7, ADAMTSL4, NOS3 and ZNRF3 were neither oncogenes nor tumor suppressors nor had any Q-value data. This could be due to the low number of mutations in the genes. Low Q-values for the genes of interest indicate that the genes are significantly mutated in ACC, lending credence to their status as potential ACC-specific oncogenes.

## Discussion

Although our understanding of the molecular mechanism driving ACC has advanced, the oncogenic mutations leading to ACC are poorly understood [1]. Thus, we aimed at unravelling novel ACC-specific targets and determined a novel gene set of six genes that were significantly and specifically mutated in ACC.

Kaplan–Meier curves for the gene set from deceased patients show dramatic differences between patients with and without mutations. This indicates that mutations in DGKZ, GOLGA4, KCNH7, ADAMTSL4, NOS3 or ZNRF3 dramatically worsen prognosis in ACC and are thus genes of special interest. Although the number of samples containing mutations in these genes is very low and could be a possible reason for differences in survival curves, the data correlate very well with the control gene TP53. In addition, GOLGA4, KCNH7, ADAMTSL4, NOS3 or ZNRF3 are not specifically mutated in ACC, nor do they fit 20/20 and Mutsig parameters. Hence we included DGKZ, but not the others, as part of our ACC-specific gene mutation signature. The specific integrated role of this

Fig. 2 Mutation frequency of the selected genes in ACC compared across all available (at cBioPortal) human cancer tissue types (adapted from cBioPortal). Cancer types lacking mutations in the specified genes have not been shown. Bars in red color indicate ACC. Mutation frequency in the five genes with highest number of mutations in ACC (A). Almost 40% of samples in ACC show mutations in ZFPM1, which is significantly higher than in any other cancer type. Similarly, mutations in LRIG1, CRIPAK, ZNF517 and GARS are significantly higher in ACC as compared to all other human cancer tissue types. This indicates that alterations in this particular five-gene set may be important for ACC oncogenesis. Mutation frequency in the top six genes observed only in deceased ACC patients (B). Mutation frequencies of these genes is relatively low in ACC (compared with genes shown in (A)), however, DGKZ and ZNRF3 are altered more in ACC as compared to other cancer types. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

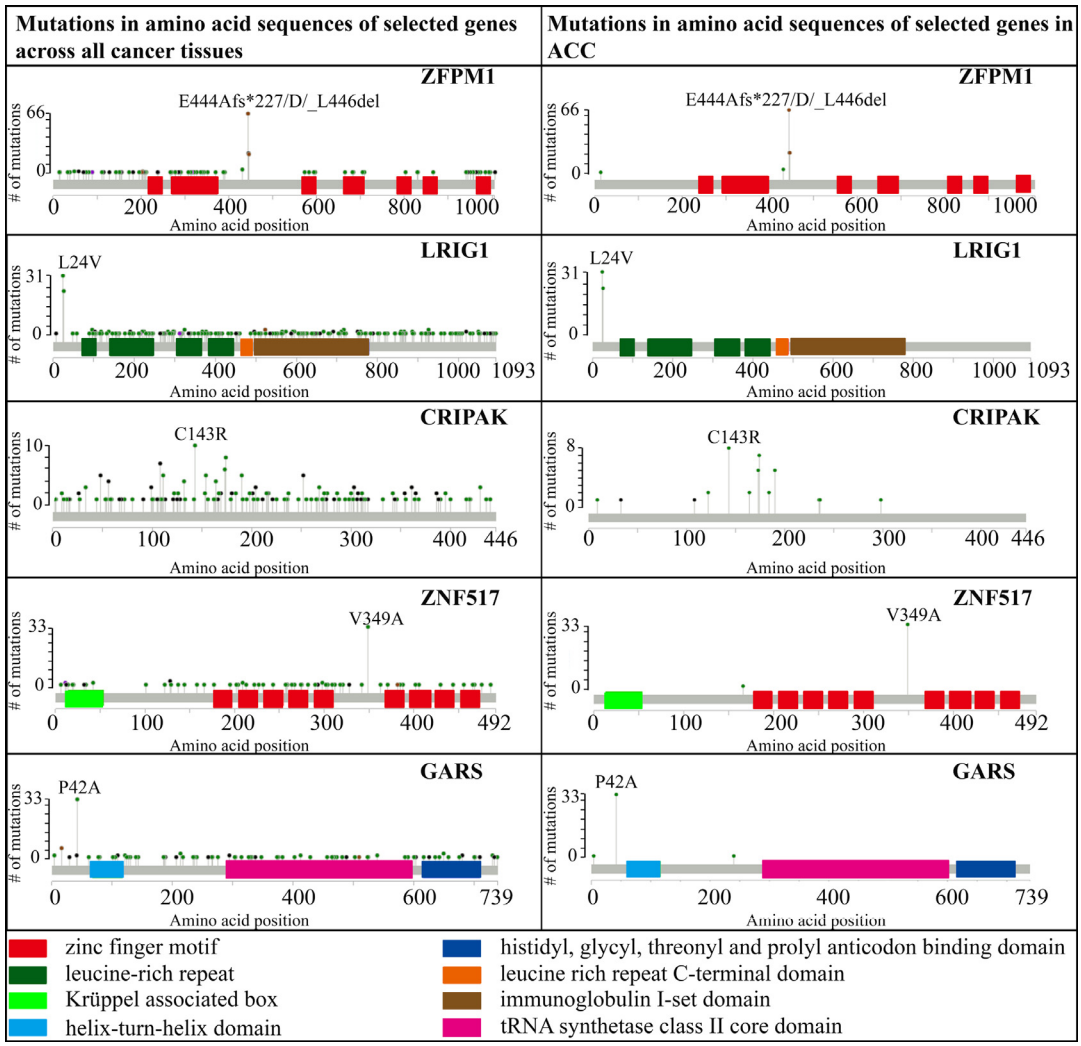



**Fig. 3** Comparative gene mutation analysis between site-specific AA mutations in ACC and all other available (at cBioPortal) human cancer tissue types. The top five genes with the highest number of gene mutations in ACC are shown. Mutations in ZFPM1 occur primarily at the E444 position in ACC, whereas in other cancer tissues the mutations are not localized to a specific site. Similarly, in ACC, mutations in LRIGI, ZNF517 and GARS occur mainly at the L24, V349 and P42 positions, respectively. Mutations in ZFPM1, LRIG1, ZNF517 and GARS occur at the same site in ACC, while occurring at various sites in all other cancer tissue types. Recurrent mutations at the same site in a gene imply that the location is a mutation hotspot in ACC, and thus could be an oncogenic trigger. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

ACC-specific gene mutation signature in a convergent ACC cancer cell signaling mechanism is as yet unclear. The signaling pathways involving our identified gene set as well as those from Zheng et al. [16] can be interlinked (Fig. 5).

Downstream signaling by tyrosine kinase receptors like EGFR activate multiple pathways including the mitogen-activated protein kinase (MAPK), phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) and WNT signaling pathways, and there is extensive cross-regulation between these pathways [48]. PAK1 belongs to the p21-activated serine/threonine kinase family and plays a critical role in linking multiple signaling pathways, including cell polarity, actin cytoskeleton reorganization and cellular proliferation [49,50]. PAK1 interacts directly with Rac family small GTPase 1 (RAC1) and PI3K, effecting downstream signaling by phosphorylating other kinases [51]. Increase in PAK1 expression has been observed in various cancers [49,51]. PAK1 is a promising target for therapy with both ATP-competitive as well as allosteric inhibitors being screened for treatment [52]. The functional role of CRIPAK is inhibition of the PAK1 kinase [43] and disruption in the function of CRIPAK via mutations would lead to a dysregulation of PAK1, thereby enhancing cellular proliferation (Fig. 5). Activation of the PI3K pathway leads to suppression of TP53 via MDM2 activation. AKT also phosphorylates and inhibits glycogen synthase kinase 3 (GSK3), thereby stabilizing downstream targets of GSK3 such as the G1 cyclins and TFs such as MYC proto-oncogene (MYC) and Jun proto-oncogene (JUN) [53]. AKT also phosphorylates CDKN1B, inactivating its cell-cycle prevention effect [53]. GSK3 phosphorylates CTNNB1, marking it for degradation. Mutation in CTNNB1 makes degradation impossible; thereby cause constitutive activation of target genes

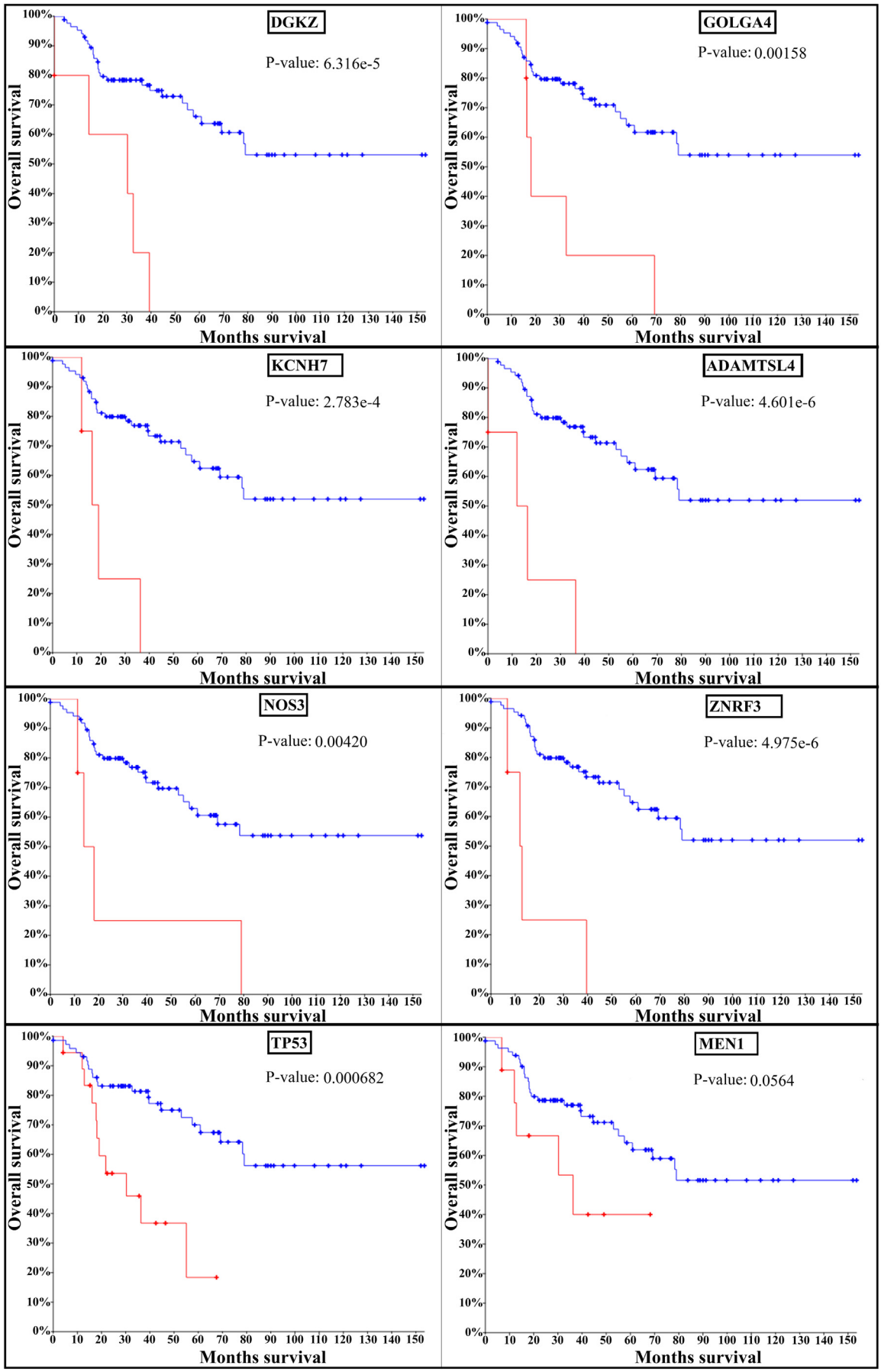

DGKZ
P-value: 6.316e-5
GOLGA4
P-value: 0.00158
KCNH7
P-value: 2.783e-4
ADAMTSL4
P-value: 4.601e-6
NOS3
P-value: 0.00420
ZNRF3
P-value: 4.975e-6
TP53
P-value: 0.000682
MEN1
P-value: 0.0564
Overall survival
Months survival

**Fig. 4**

such as MYC and cyclin D1 (CCND1). RPL22 is a 60S ribosomal subunit protein and activates TP53 expression by binding and inactivating MDM2, thereby suppressing cancer cell survival [46]. Inactivation of RPL22 and mutation in TP53 thus acts as a 'double whammy' in enhancing proliferation of cancer cells. The role of TP53 in oncogenesis is well established. TP53 can induce both cell cycle arrest and apoptosis, and mutations in TP53 are the most common ones observed in tumors [42]. PRKAR1A is the regulatory subunit of cAMP dependent protein kinase A (PKA) and mutations in PRKAR1A have been known to cause an autosomal dominant neoplasia syndrome called Carney complex [41]. The downstream signaling of EGFR also regulates CTNNB1, via the mucin-1 (MUC1) protein. Decrease in MUC1 expression suppresses EGFR-dependent tumorigenesis and CCND1 protein expression [54]. The menin (MEN1) protein has both tumor suppressive and proliferative functions. Mutations in MEN1 lead to multiple endocrine neoplasia, which causes tumors in endocrine glands, inactivating mutations in MEN1 are linked to inhibition of CDKN1B, and MEN1 has been reported to suppress AKT kinase activity in murine cells [55].

LRIG1 is a transmembrane protein which inhibits EGFR kinase receptor signaling by binding to it via its ectodomain [56]. LRIG1 functions as a tumor suppressor in multiple cancers [57–59] and increased expression of the (non-mutated) proto-oncogene LRIG1 is associated with good prognosis in breast, bladder, lung cancer, melanoma and glioma [60]. Inactivating mutations in LRIG1 lead to a 'switch on' of the EGFR kinase-signaling pathway, resulting in high cellular proliferation. LRIG1 has already been tested as a therapeutic for glioma. LRIG1 acts as a sensitizing agent for chemotherapeutics and injection of soluble LRIG1 has been reported to inhibit glioblastoma [61–64]. Therefore, LRIG1 could be a highly promising target for immunotherapy for ACC, given that it acts as an inhibitor of EGFR kinase. Ex-vivo sensitized dendritic cells to mutant LRIG1 could be targeted specifically to ACC cells expressing mutant LRIG1 to mediate an endogenous immune response to fight ACC. Antibodies targeting mutant LRIG1 could be generated, thereby killing the ACC cells via a cell-mediated cytotoxic processes. Another approach would be to target other genes on combination with LRIG1. FAM72 is a novel proliferation marker, which is overexpressed in non-neural cancer tissues [65,66]. Singling out FAM72 would mediate mitotic damage in all growing cells, however, targeting mutant LRIG1 and FAM72 together will ensure that only cancerous cells expressing mutated LRIG1 and overexpressing FAM72 will be targeted while leaving the other non-cancerous cells unharmed.

The zinc finger protein ZFPM1, also known as FOG1, acts as a cofactor with the transcription factor GATA1 and regulates differentiation and proliferation of blood cells at various stages [67,68]. ZFPM1 has been reported to be upregulated in myeloid leukemia [69] but no evidence of mutational effects have been reported. Both GATA1 and ZFPM1 interact with the nucleosome remodeling and deacetylase (NuRD) complex, which is comprised of retinoblastoma binding protein 4 (RBBP4), ATPases and histone deacetylases. This protein complex is critical in transcriptional repression of ZFPM1-GATA1 target genes [44,70]. Loss of function mutations in ZFPM1 could prevent differentiation, thereby activating proliferative mechanisms in the adrenocortical cells. RBBP4, in turn, forms a complex with RB1 and regulates the function of E2F1 [71]. This directly affects the cell cycle, as E2F1 is a transcriptional activator for G1 and S-phase genes.

GARS mutations are typically associated with the neuropathic Charcot-Marie-Tooth disorder [72]. However, there is some evidence to suggest that it also plays a role in the adaptive immune system by suppressing MAPK signaling in tumor cells [73]. GARS dysregulation has also been reported in a variety of cancers [74,75] though oncogenic mutations have not yet been reported. Recent evidence suggests that GARS acts as a chaperone for the proteins directly involved in the neddylation pathway [45]. Neddylation is the conjugation of the NEDD8 protein, via GARS, to its targets and is critical in cell cycle regulation: the NEDD8-cullin complex activates cullin-RING ubiquitin ligases, which degrade the p27 cell-cycle inhibitor and continues cell cycle progression [76]. Inhibition of GARS thus leads to reduction of metastasis [45]. This is of high interest as gain of function mutations in GARS would lead to higher levels of neddylation, thereby increasing cellular proliferation. This makes GARS a very attractive target for therapeutic applications.

There is little evidence about the function of the other zinc finger protein ZNF517. It forms part of the interactome of heat shock 90 kDa proteins (HSP90) [77] so it may be involved in protein folding or intracellular transport, but there is no other report of its cellular function. ZNF517 is a zinc-finger protein, which are the largest family of transcription factors. ZNFs work as transcriptional activators or repressors and play significant roles in cellular growth, maturation and metabolism [78]. ZNF517 contains Kruppel associated box (KRAB) domains, indicating its potential function as a transcriptional repressor. The KRAB domain binds to other corepressors via direct protein interactions leading to transcriptional repression of genes [79]. KRAB-ZNFs play important roles in cellular differentiation, apoptosis and cancer by acting as tumor suppressors and promoters of apoptosis [80]. Mutations in ZNF517 could lead to constitutive expression of downstream signaling pathways leading to proliferation. DGKZ is a lipid kinase involved in phosphatidic acid synthesis. DGKZ knockdown has been reported to inhibit proliferation in glioma cells [81]. DGKZ inhibits RAS guanyl-releasing protein (RASGRP1), thereby modulating RAS activity [47]. Inactivating mutations in DGKZ could lead to increased phosphorylation of RAS, thereby causing continuous activation of the MAPK or PI3K signaling pathways, ending in cellular proliferation. Mutations in these genes drastically worsen the prognosis, and therefore must act in

Fig. 4 Survival plots showing the prognosis of ACC patients with somatic mutations in DGKZ, GOLGA4, KCNH7, NOS3, ADAMTSL4 and ZNRF3, respectively (adapted from cBioPortal). DGKZ, GOLGA4, KCNH7, NOS3, ADAMTSL4 and ZNRF3 are the genes with highest number of somatic mutations observed only in deceased ACC patients. All patients with mutations in these genes show worse prognosis compared to all other ACC patients. MEN1 and TP53 survival plots are shown as control comparison. Red line: cases with alterations in query gene, blue line: cases without alterations in query gene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

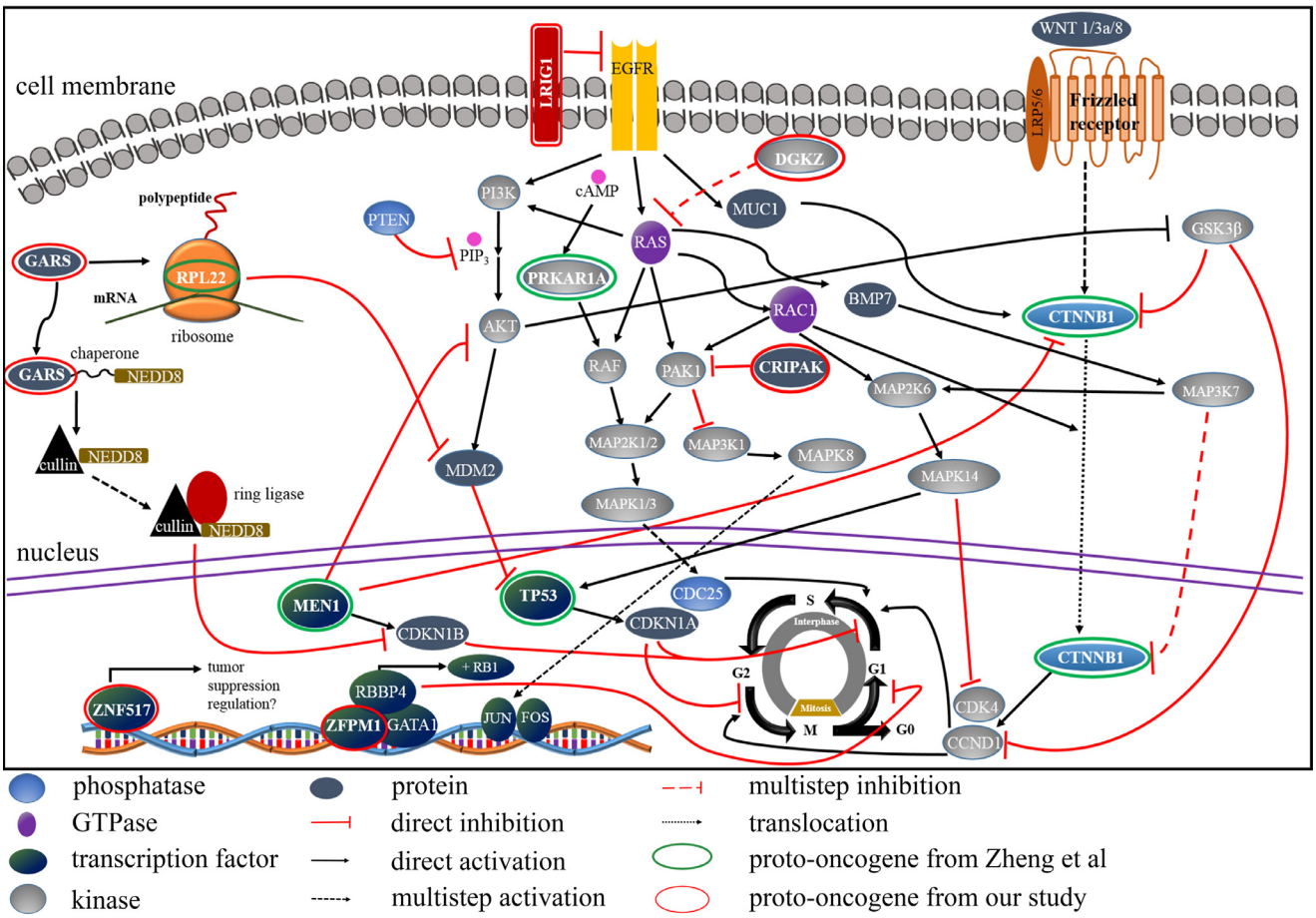



**Fig. 5** ACC-specific gene-mutation signature-activated cell signaling pathways with highlighted proto-oncogenes involved in tumorigenesis. Key to illustration is provided below the figure. Genes mentioned in Zheng et al. [16] have also been illustrated for comparison. LRIG1 binds to EGFR via its ectodomain, leading to suppression of EGFR-mediated signaling [39,40]. Loss-of function mutations in LRIG1 may lead to activation of the EGFR kinase-signaling pathway, thereby leading to constitutive expression of the PI3K and MAPK pathways. PI3K downstream signaling also reduces TP53 activity via MDM2 binding and PAK1 activation leads to cellular proliferation via MAPK signaling. Mutations in PRKR1A lead to an adrenal neoplasia called Carney complex [41]. Mutations in TP53 lead to inhibition of its tumor suppressor and pro-apoptotic role, thereby converting it into an oncogene [42]. Inactivating mutations in CRIPAK may lead to dysregulation of PAK1 [43] resulting in continuous downstream signaling of the MAPK and PI3K pathways. MEN1 mutations will activate AKT and CTNNB1, while also inactivating cyclin-dependent kinase inhibitor 1B (CDKN1B), also known as p27. CTNNB1 mutations lead to transcriptional activation of cell cycle activator like cyclin D. ZFPM1 forms a complex with RBBP4 and GATA1, and regulates cellular differentiation [44]. Mutations in ZFPM1 may dysregulate differentiation and cause abnormal growth of cells. GARS plays a critical role in neddylation and thereby regulates cellular proliferation [45]. The function and location of ZNF517 has not yet been described but it may act as a tumor suppressor. RPL22 inactivates MDM2, leading to TP53 expression [46]. DKGZ modulates cell cycle via DAG metabolization and RASGRP1 inhibition [47]. G0, quiescent or differentiated stage; G1, Gap1 phase; G2, Gap2 phase; M, Mitotic phase; S, Synthesis phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

conjunction with our novel gene set as well as other proto oncogenes such as MEN1, CTNNB1 and TP53.

EGFR signaling is the primary node through which the proliferative pathways can be initiated and most of the proto-oncogenes in ACC act downstream of EGFR. Inhibition of EGFR via LRIG1 is thus a key step in regulating, either partially or fully, the consequent signaling cascades. LRIG1 mutations would cause a continuous expression of the EGFR signaling cascade, thereby causing cellular proliferation. Similarly, mutations in GARS also serve to increase proliferation via a cascade that is independent of the PI3K/MAPK/WNT signaling pathways. Mutations in our selected genes thus appear to be more influential in ACC tumorigenesis than those described in earlier studies are.

Our study thus sheds new light on potential driver genes in this rare ACC cancer. Our findings describe a novel ACC-specific gene signature providing a gene set (ZFPM1, LRIG1, CRIPAK, GARS, ZNF517, and DGKZ) and validate its oncogenic potential, which could serve as a powerful therapeutic target.

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## Competing interests

The authors declare no conflicts of interest

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.10.005.

## References

[1] Fassnacht M, Kroiss M, Allolio B. Update in adrenocortical carcinoma. J Clin Endocrinol Metab 2013;98(12):4551–64.
[2] Kebebew E, et al. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? World J Surg 2006;30(5):872–8.
[3] Kerkhofs TM, et al. Adrenocortical carcinoma: a population-based study on incidence and survival in the Netherlands since 1993. Eur J Cancer 2013;49(11):2579–86.
[4] Creemers SG, et al. Identification of mutations in cell-free circulating tumor DNA in adrenocortical carcinoma: a case series. J Clin Endocrinol Metab 2017;102(10):3611–15.
[5] Lalli E, Luconi M. The next step: mechanisms driving adrenocortical carcinoma metastasis. Endocr Relat Cancer 2018;25(2):R31–48.
[6] Lambert AW, Pattabiraman DR, Weinberg RA. Emerging biological principles of metastasis. Cell 2017;168(4):670–91.
[7] Else T, et al. Adrenocortical carcinoma. Endocr Rev 2013;35(2):282–326.
[8] Beuschlein F, et al. Major prognostic role of Ki67 in localized adrenocortical carcinoma after complete resection. J Clin Endocrinol Metab 2015;100(3):841–9.
[9] Pereira SS, et al. Mechanisms of endocrinology: cell cycle regulation in adrenocortical carcinoma. Eur J Endocrinol 2018;179(2):R95–R110.
[10] Berthon A, et al. Constitutive beta-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development. Hum Mol Genet 2010;19(8):1561–76.
[11] Herrmann LJ, et al. TP53 germline mutations in adult patients with adrenocortical carcinoma. J Clin Endocrinol Metab 2012;97(3):E476–85.
[12] Raymond VM, et al. Prevalence of germline TP53 mutations in a prospective series of unselected patients with adrenocortical carcinoma. J Clin Endocrinol Metab 2013;98(1):E119–25.
[13] Gaujoux S, et al. beta-catenin activation is associated with specific clinical and pathologic characteristics and a poor outcome in adrenocortical carcinoma. Clin Cancer Res 2011;17(2):328–336.
[14] Tissier F, et al. Mutations of beta-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors. Cancer Res 2005;65(17):7622–7.
[15] Assie G, et al. Integrated genomic characterization of adrenocortical carcinoma. Nat Genet 2014;46(6):607–12.
[16] Zheng S, et al. Comprehensive pan-genomic characterization of adrenocortical carcinoma. Cancer Cell 2016;29(5):723–36.
[17] Ragazzon B, et al. Transcriptome analysis reveals that p53 and $\beta$-catenin alterations occur in a group of aggressive adrenocortical cancers. Cancer Res 2010;70(21):8276–81. doi:10.1158/0008-5472.CAN-10-2014.
[18] Salomon A, et al. Loss of beta-catenin in adrenocortical cancer cells causes growth inhibition and reversal of epithelial-to-mesenchymal transition. Oncotarget 2015;6(13):11421–33.
[19] Gicquel C, et al. Molecular markers and long-term recurrences in a large cohort of patients with sporadic adrenocortical tumors. Cancer Res 2001;61(18):6762–7.
[20] Sbiera S, et al. High diagnostic and prognostic value of steroidogenic factor-1 expression in adrenal tumors. J Clin Endocrinol Metab 2010;95(10):E161–71.
[21] de Fraipont F, et al. Gene expression profiling of human adrenocortical tumors using complementary deoxyribonucleic acid microarrays identifies several candidate genes as markers of malignancy. J Clin Endocrinol Metab 2005;90(3):1819–29.
[22] Giordano TJ, et al. Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. Am J Pathol 2003;162(2):521–31.
[23] Lawrence MS, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 2014;505(7484):495–501.
[24] Vogelstein B, et al. Cancer genome landscapes. Science 2013;339(6127):1546–58.
[25] Cerami E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2(5):401–4.
[26] Gao J, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):pl1.
[27] Lawrence MS, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013;499(7457):214–18.
[28] Greenman C, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446(7132):153–8.
[29] Goldman M, et al. Abstract 2584: The UCSC Xena system for cancer genomics data visualization and interpretation. Cancer Res 2017;77(13 Supplement):2584. doi:10.1158/1538-7445.AM2017-2584.
[30] Ho NT, et al. Cognitive functions: human vs. animal - 4:1 advantage |-FAM72-SRGAP2-|. J Mol Neurosci 2017;61(4):603–6.
[31] Ho NTT, Kutzner A, Heese K. Brain plasticity, cognitive functions and neural stem cells: a pivotal role for the brain-specific neural master gene |-SRGAP2-FAM72-|. Biol Chem 2017;399(1):55–61.
[32] Kutzner A, et al. All-or-(N)One - an epistemological characterization of the human tumorigenic neuronal paralogous FAM72 gene loci. Genomics 2015;106(5):278–85.
[33] Rahane CS, Kutzner A, Heese K. A cancer tissue-specific FAM72 expression profile defines a novel glioblastoma multiform (GBM) gene-mutation signature. J Neurooncol 2018. doi:10.1007/s11060-018-03029-3.
[34] Iverson GL. Z scores. In: Kreutzer JS, DeLuca J, Caplan B, editors. Encyclopedia of clinical neuropsychology. New York, NY: Springer New York; 2011. p. 2739–40.
[35] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182(3):311–22.
[36] Team BD. Bokeh: python library for interactive visualization. 2014. Available at: http://www.bokeh.pydata.org.
[37] Bland JM, Altman DG. The logrank test. BMJ 2004;328(7447):1073.
[38] Heese K. The protein p17 signaling pathways in cancer. Tumour Biol 2013;34(6):4081–7.

[39] Kou C, et al. LRIG1, a 3p tumor suppressor, represses EGFR signaling and is a novel epigenetic silenced gene in colorectal cancer. Biochem Biophys Res Commun 2015;464(2):519–25.
[40] Stutz MA, et al. LRIG1 negatively regulates the oncogenic EGF receptor mutant EGFRvIII. Oncogene 2008;27(43):5741–52.
[41] Kirschner LS, et al. Genetic heterogeneity and spectrum of mutations of the PRKAR1A gene in patients with the carney complex. Hum Mol Genet 2000;9(20):3037–46.
[42] Kandoth C, et al. Mutational landscape and significance across 12 major cancer types. Nature 2013;502(7471):333–9.
[43] Talukder AH, Meng Q, Kumar R. CRIPak, a novel endogenous Pak1 inhibitor. Oncogene 2006;25(9):1311–19.
[44] Miccio A, et al. NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development. EMBO J 2010;29(2):442–56.
[45] Mo Z, et al. Neddylation requires glycyl-tRNA synthetase to protect activated E2. Nat Struct Mol Biol 2016;23(8):730–7.
[46] Cao B, et al. Cancer-mutated ribosome protein L22 (RPL22/eL22) suppresses cancer cell survival by blocking p53-MDM2 circuit. Oncotarget 2017;8(53):90651–61.
[47] Topham MK, Epand RM. Mammalian diacylglycerol kinases: molecular interactions and biological functions of selected isoforms. Biochim Biophys Acta 2009;1790(6):416–24.
[48] Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci 2011;36(6):320–8.
[49] Ong CC, et al. p21-activated kinase 1: PAK'ed with potential. Oncotarget 2011;2(6):491–6.
[50] Guo D, et al. A Rac-cGMP signaling pathway. Cell 2007;128(2):341–55.
[51] Kumar R, Gururaj AE, Barnes CJ. p21-activated kinases in cancer. Nat Rev Cancer 2006;6(6):459–71.
[52] Semenova G, Chernoff J. Targeting PAK1. Biochem Soc Trans 2017;45(1):79–88.
[53] Manning BD, Toker A. AKT/PKB signaling: navigating the network. Cell 2017;169(3):381–405.
[54] Pochampalli MR, Bitler BG, Schroeder JA. Transforming growth factor alpha dependent cancer progression is modulated by Muc1. Cancer Res 2007;67(14):6591–8.
[55] Borsari S, et al. Loss of p27 expression is associated with MEN1 gene mutations in sporadic parathyroid adenomas. Endocrine 2017;55(2):386–97.
[56] Gur G, et al. LRIG1 restricts growth factor signaling by enhancing receptor ubiquitylation and degradation. EMBO J 2004;23(16):3270–81.
[57] Yokdang N, et al. LRIG1 opposes epithelial-to-mesenchymal transition and inhibits invasion of basal-like breast cancer cells. Oncogene 2016;35(22):2932–47.
[58] Neirinckx V, Hedman H, Niclou SP. Harnessing LRIG1-mediated inhibition of receptor tyrosine kinases for cancer therapy. Biochim Biophys Acta 2017;1868(1):109–16.
[59] Mao F, et al. Lrig1 is a haploinsufficient tumor suppressor gene in malignant glioma. Oncogenesis 2018;7(2):13.
[60] Rouam S, Moreau T, Broet P. Identifying common prognostic factors in genomic cancer studies: a novel index for censored outcomes. BMC Bioinf 2010;11(1):150.
[61] Johansson M, et al. The soluble form of the tumor suppressor Lrig1 potently inhibits in vivo glioma growth irrespective of EGF receptor status. Neuro Oncol 2013;15(9):1200–11.
[62] Qi XC, et al. LRIG1 dictates the chemo-sensitivity of temozolomide (TMZ) in U251 glioblastoma cells via down-regulation of EGFR/topoisomerase-2/Bcl-2. Biochem Biophys Res Commun 2013;437(4):565–72.
[63] Wang X, et al. LRIG1 enhances cisplatin sensitivity of glioma cell lines. Oncol Res 2012;20(5–6):205–11.
[64] Mao F, et al. LRIG proteins in glioma: functional roles, molecular mechanisms, and potential clinical implications. J Neurol Sci 2017;383:56–60.
[65] Guo C, et al. Ugene, a newly identified protein that is commonly overexpressed in cancer and binds uracil DNA glycosylase. Cancer Res 2008;68(15):6118–26.
[66] Nehar S, Mishra M, Heese K. Identification and characterisation of the novel amyloid-beta peptide-induced protein p17. FEBS Lett 2009;583(19):3247–53.
[67] Tanaka M, et al. Differentiation status dependent function of FOG-1. Genes Cells 2004;9(12):1213–26.
[68] Yang HY, et al. The suppression of zfpm-1 accelerates the erythropoietic differentiation of human CD34+ cells. Biochem Biophys Res Commun 2007;353(4):978–84.
[69] Marcucci G, et al. Prognostic significance of, and gene and microRNA expression signatures associated with, CEBPA mutations in cytogenetically normal acute myeloid leukemia with high-risk molecular features: a cancer and leukemia group b study. J Clin Oncol 2008;26(31):5078–87.
[70] Lejon S, et al. Insights into association of the NuRD complex with FOG-1 from the crystal structure of an RbAp48.FOG-1 complex. J Biol Chem 2011;286(2):1196–203.
[71] Li L, et al. Epigenetic modification of MiR-429 promotes liver tumour-initiating cell properties by targeting Rb binding protein 4. Gut 2015;64(1):156–67.
[72] He W, et al. CMT2D neuropathy is linked to the neomorphic binding activity of glycyl-tRNA synthetase. Nature 2015;526(7575):710–14.
[73] Park MC, et al. Secreted human glycyl-tRNA synthetase implicated in defense against ERK-activated tumorigenesis. Proc Natl Acad Sci USA 2012;109(11):E640–7.
[74] Kim S, You S, Hwang D. Aminoacyl-tRNA synthetases and tumorigenesis: more than housekeeping. Nat Rev Cancer 2011;11(708-18).
[75] Wasenius VM, et al. Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNA and tissue microarray study. Clin Cancer Res 2003;9(1):68–75.
[76] Pagano M, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science 1995;269(5224):682–5.
[77] Prince T, Williams H. 494 Heat shock protein 90 interactome is highly altered in adrenocortical carcinoma. European Urology Supplements 2016;15(3):e494.
[78] Jen J, Wang YC. Zinc finger proteins in cancer progression. J Biomed Sci 2016;23(1):53.
[79] Friedman JR, et al. KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev 1996;10(16):2067–78.
[80] Lupo A, et al. KRAB-zinc finger proteins: a repressor family displaying multiple biological functions. Curr Genomics 2013;14(4):268–78.
[81] Diao J, et al. Loss of diacylglycerol kinase-zeta inhibits cell proliferation and survival in human gliomas. Mol Neurobiol 2016;53(8):5425–35.