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Research Article

Genetic and immune profiling for potential therapeutic targets in adult human craniopharyngioma

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ABSTRACT

Craniopharyngioma is a rare tumor in adults. Although histologically benign, it can be locally aggressive and may require additional therapeutic modalities to surgical resection. Analyses including next generation sequencing, chromogenic and *in situ* hybridization, immunohistochemistry, and gene amplification were used to profile craniopharyngiomas (n=6) for frequently altered therapeutic targets. Four of six patients had the $BRAF^{V600E}$ missense mutation, frequent in the papillary craniopharyngioma subtype. One patient had a missense mutation in the *WNT* pathway, specifically *CTNNB1*, often associated with the adamantinomatous subtype. Craniopharyngiomas lacked microsatellite instability, had low tumor mutational burden, but did express PD-L1 protein, indicating potential therapeutic value for immune checkpoint inhibition. We identified mutations not previously described, including an *E318K* missense mutation in the *MITF* gene, an *R1407* frameshift in the *SETD2* gene of the PIK3CA pathway, *R462H* in the *NF2* gene, and a *I463V* mutation in *TSC2*. Two patients testing positive for *EGFR* expression were negative for the *EGFRvIII* variant. Herein, we identified several alterations such as those in $BRAF^{V600E}$ and PD-L1, which may be considered as targets for combination therapy of residual craniopharygiomas.

Introduction

Craniopharyngioma is a rare, benign but heterogeneous tumor of the pituitary stalk, comprising 1-3% of all brain tumors [1]. It is the most common childhood suprasellar tumor; however, it has a bimodal age distribution and may be observed in adults between age 50 and the late 70s, who are the focus of this manuscript [2]. Two theories have been debated regarding the etiology of craniopharyngioma. The first one proposes that craniopharyngiomas develop from the transformation of oral ectodermal embryologic remnants of the Rathke pouch, whereas the other hypothesis argues that this tumor originates from metaplasia of the primordial adenohypophysis cells [3, 4]. These tumors are typically treated with surgery; however, residual tumor and recurrence can pose a treatment quandary because little is known about the genetic landscape of these tumors beyond two defining mutations: *BRAF V600E* and *CTNNB1* [5, 6].

Papillary craniopharyngioma, primarily seen in adults, is associated with *BRAF V600E* mutation whereas the adamantinomatous type, which is more common in children, is linked to mutations in the ß-catenin gene or a mediator of the Wnt pathway *CTNNB1*; however, both subtypes have been described in adults. Craniopharyngiomas are not histologically malignant, but they often are locally aggressive and can thus cause debilitating visual, endocrine, and neurologic symptoms and a decrease in survival. There are two treatment options available, either

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attempting an aggressive complete resection, or performing a more conservative resection in preparation for adjuvant radiation therapy. Both options have potential complications, including cerebrovascular injury, neurocognitive decline, and metabolic alterations, including frequent panhypopituitarism [7-10]. Furthermore, the partial resection and radiation therapy combination leaves remnants of the tumor, which can lead to recurrence and repetitive surgical risks, exposes patients to a higher risk of radiation-induced secondary malignancy, and multiple recurrences are associated with malignant transformation [11-13]. Consequently, genetic profiling may provide insight into new therapeutic strategies and a better understanding of the etiology, development and progression of these tumors. As such, we hypothesized that sequencing for cancer hotspot mutations may reveal novel therapeutic targets that could be considered in scenarios where patients have sub totally resected or unresectable craniopharygioma.

Materials and methods

Study population

Multiplatform analysis covering the tumor mutational burden (TMB), microsatellite instability (MSI), high-throughput sequencing, *in situ* hybridization, and immunohistochemical study was performed on six craniopharyngioma tumors in adults and identified in the Caris Life Sciences database. The purpose of the database is to provide a genetic profiling record, but annotation of clinical data is limited. As such, the history, treatment, and survivorship outcomes of patients are not included. The histologic diagnosis is based on WHO guidelines (ICD10-2016).

Genetic analysis

Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor blocks using the QIAamp DNA FFPE DNA Extraction Kit (Qiagen Sciences, Germantown, MD 20874). Genes of interest, cited in Supplementary Table 1, were amplified using the Illumina TruSEQ amplicon cancer hotspot (47 genes; n=1)(Illumina, San Diego, CA) or the Agilent customized pan-cancer panel (592 genes; n=4)(Agilent Technologies, Santa Clara, CA) depending on the availability of both tissue and sequencing panels, with an overlap of the genes in both panels regardless of the size, and sequenced with the Illumina MiSEQ and Illumina NextSEQ platforms, respectively, out of a total of 1.4 megabases of DNA. The analysis focused on the TMB, MSI, and specific gene mutations and their transcriptional effect. TMB was measured by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations, the threshold used for TMB was 17 mutations/megabase based on concordance data with MSI in colorectal cancer. MSI was examined using over 7,000 target microsatellite loci and compared to the reference genome hg19 from the University of California, Santa Cruz (UCSC) Genome Browser database. The threshold to determine MSI by NGS was 46 or more loci with insertions or deletions to generate a sensitivity of > 95% and specificity of > 99%. Variants were detected with a >99% confidence interval based on the frequency of identified mutations and amplicon coverage, with an average coverage of > 500 and an analytic sensitivity of 5%.

Gene amplification and expression

Both fluorescent and chromogenic *in situ* hybridization were used to detect amplifications in *cMET, Her2* and *cMET* amplifications, respectively, as well as gene fusion of *ALK*. Analysis by immunohistochemistry (IHC) was performed on full FFPE sections to assess the expression of EGFR, Her2/Neu, cMET, PD-L1 and ALK chosen based on the relevance in cancer. Slides were stained using automated techniques, per the manufacturer's instructions, and were optimized and validated per Clinical Laboratory Improvement Amendments CLIA/CAO and international Organization for Standardization (ISO) requirements. Staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0-100%). Results were categorized as positive or negative by defined thresholds specific to each marker based on published clinical literature that associates biomarker status with patient responses to therapeutic agents. For PD-L1, the primary antibody used was SP142 (Spring Biosciences). The staining was regarded as positive if its intensity on the membrane of the tumor cells was >=2+ and the percentage of positively stained cells was >5%. A board-certified pathologist evaluated all IHC results independently. For gene fusion detection, anchored multiplex PCR was performed for targeted RNA sequencing using the ArcherDx fusion assay (Archer FusionPlex Solid Tumor panel). The formalin-fixed paraffin-embedded tumor samples were microdissected to enrich the sample to ≥20% tumor nuclei, and mRNA was isolated, and reverse transcribed into complementary DNA (cDNA). Unidirectional gene-specific primers were used to enrich for target regions, followed by Next-Generation sequencing (Illumina MiSeq platform). Targets included 52 genes, and the full list can be found at http://archerdx.com/fusionplex-assays/solid-tumor.

Table 1 Craniopharyngioma study demographics

Number of patients (n)	6
Age	
Median, years (range)	54.5(33-78)
Sex	
Male, n (%)	3 (50%)
Female, n (%)	3 (50%)
Primary, n (%)	4 (66.6%)
Recurrent, n (%)	1 (16.7%)
NOS, n (%)	1 (16.7%)
Craniopharyngioma subtype	
Papillary, n (%)	3 (50%)
Adamantinomatous, n (%)	1 (16.7%)
Undefined, n (%)	2 (33.3%)
Location	
Parasellar, n (%)	1 (16.7%)
Suprasellar, n (%)	2 (33.3%)
Rathke pouch, n (%)	1 (16.7%)
Frontal lobe, n (%)	1 (16.7%)
NOS, n (%)	1 (16.7%)

Results

Demographics

The study cohort included six adult patients who were diagnosed with

craniopharyngioma. The patients' ages ranged from 33 to 78 years, with the median age being 54.5 years. Four patients presented with a newly diagnosed craniopharyngioma, and the disease was metastatic in one patient. The mass was in the parasellar in one, in the suprasellar region in two, in the Rathke pouch in one, in the frontal lobe (recurrent) in one, and in an unspecified location in another. Based on histology, three of the tumors were papillary, one adamantinomatous, and two were undefined because of the distorted architecture that does not fall in any of the predefined subtypes implying a possibility of a mixed subtypes or a new distinct phenotype (Table 1).

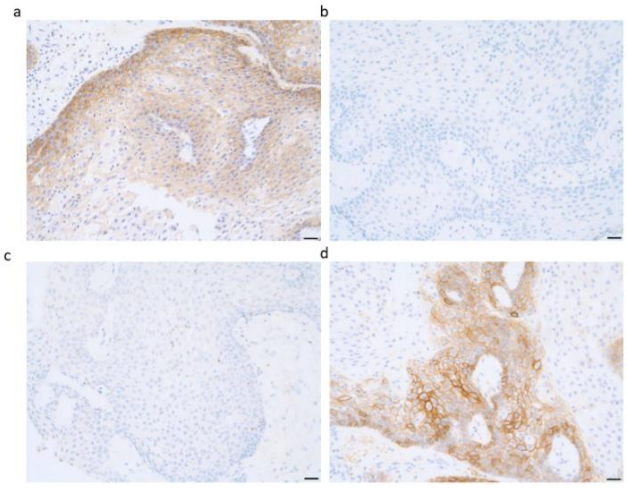

Figure 1: Representative immunohistochemical analysis of (a) the epidermal growth factor receptor (EGFR), (b) Her2, (c) ALK, (d) PD-L1 in tumor cells from patient #2. Staining was positive for expression of both the EGFR and PD-L1, but not for Her2 or ALK. (Magnification = 20X in a through d.)

Craniopharyngiomas are genomically stable but express PD-L1

To clarify whether craniopharygiomas expressed biomarkers associated with a potential response to immune checkpoint inhibitors, the tumors were assessed for both MSI and TMB. Of the patients tested (n=4), none showed MSI and all showed a relatively low TMB including the recurrent case (Table 2). No mutations in the DNA repair genes (MLH1, MSH2, MSH6, PMS2) were detected (data not shown). Tumors in four of the five patients profiled were positive for PD-L1 expression, as assayed by IHC at a cut point of 2+ staining intensity of at least 5% cells (Figure 1). All tumors demonstrated some PD-L1 staining.

Craniopharyngiomas express a variety of mutations with known pathogenic effects

Pathogenic mutations known for craniopharyngiomas are summarized in (Table 2). Four out of six patients had mutations in *BRAF*, specifically the *V600E* missense mutation known to be expressed in the papillary subtype of craniopharyngioma. One patient had a mutation in the WNT pathway, specifically a missense mutation in *CTNNB1* typically associated with adamantinomatous craniopharyngiomas. The same patient with mutation in *CTNNB1* also had a mutation in the *NF2* gene—specifically an *R462H* mutation of unknown significance that may act as a driver. Novel mutations not previously described included an *E318K* missense mutation in the *MITF* gene and an *R1407* frameshift in the *SETD2* gene. One patient had a kinase domain mutation in exon 20 (H1047R) in PIK3CA gene that's been reported to activate the PI3K/Akt/mTOR pathway.

Table 2 Patients with craniopharyngioma--mutational profiles

Patient	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**
Microsatellite instability	NS	NS	Stable	Stable	Stable	Stable
Tumor mutational burden (per Mb)	NS	NS	7	8	6	4
Mutations of known significance						
BRAF		V600E	V600E	V600E		V600E
CTNNB1					G34E	
MITF			E318K			
PIK3CA	H1047R					
SETD2					R1407fs	
Oncogenes						
ALK	NS	NS	WT	WT	WT	WT
BCL2	NS	NS	WT	WT	WT	WT
BRAF	NS	V600E	V600E	V600E	WT	V600E
KIT	NS	NS	WT	WT	WT	WT
MYCN	NS	NS	WT	WT	WT	WT
HER2	NS	NS	WT	WT	WT	WT
JAK2	NS	NS	WT	WT	WT	WT
KRAS	NS	NS	WT	WT	WT	WT
HRAS	NS	Ind	WT	WT	WT	WT
N-ras	NS	NS	WT	WT	WT	WT
Tumor suppressors						
APC	NS	NS	WT	WT	WT	WT
BRCA1	NS	NS	WT	WT	WT	WT

BRCA2	NS	NS	WT	WT	WT	WT
CDKN2A	NS	NS	WT	WT	WT	WT
SMAD4	NS	NS	WT	WT	WT	WT
Men1	NS	NS	WT	WT	WT	WT
NF1	NS	NS	WT	WT	WT	WT
NF2	NS	NS	WT	WT	R462H	WT
PTEN	NS	NS	WT	WT	WT	WT
Rb	NS	NS	WT	WT	WT	WT
TP53	NS	NS	WT	WT	WT	WT
TSC1	NS	NS	WT	WT	WT	WT
TSC2	NS	NS	WT	I463V	WT	WT
Targeted therapy status						
EGFR	Positive	Positive	NS	NS	NS	NS
PD-L1	NS	Positive	Positive	Negative	Positive	Positive

Craniopharyngiomas overexpress EGFR

Using fluorescent and chromogenic *in situ* hybridization, we evaluated for amplifications of cMET (n=2) and Her2 (n=3) and no amplifications were seen. ALK FISH was tested on one tumor and no gene fusion was detected. RNA sequencing was done on another two tumors and no gene fusion was detected of the 52 genes interrogated. Gene copy number alteration was also evaluated on 442 of the 592 genes sequenced on the four tumors and no amplification event was seen. Immunohistochemistry on EGFR was done in two tumors and showed overexpression on both (2/2).

Discussion

To date, there has not been comprehensive sequencing information or extensive immune profiling reported on craniopharyngiomas. Previous craniopharyngioma sequencing studies have only focused on either codon hotspot mutations in *BRAF* and *CTNNB1* or evaluations that were limited to 23- or 46-gene panels [5, 14-17]. Immune profiling is limited to few previous studies [18, 19]. Whole exome sequencing was previously performed on craniopharyngioma, however this does not detect hotspot genes that are directly implicated in cancer [5]. As such, we performed genetic sequencing of 592 genes, gene amplification assessments, and immune profiling analysis on craniopharyngiomas to study the TMB, MSI, and genetic alterations that could be further explored as therapeutic targets. Consistent with prior reports, our study found that the $BRAF^{V600E}$ mutation was the most common mutation in craniopharygiomas, and we also identified another tumor with a *CTNNB1* mutation with a *G34E* substitution [15]. These two unique mutations have been previously described to occur exclusively in the papillary ($BRAF^{V600E}$) and adamantinomatous (*CTNNB1*) subtypes, respectively, and were proposed to be driver mutations of their correspondent subtypes; however, their single driver oncogenic potential has been questioned [20, 21]. Despite the relatively low mutational burden seen in craniopharyngiomas, we found several unique mutations, including one in the melanocyte-inducing transcription factor (*MITF*) gene (*E318K*) and another in the *SET* Domain Containing 2 gene (*SETD2*) (*R1407* frameshift). These two mutations have not been previously described in craniopharyngiomas but are associated with other types of tumors. *MITF* (*E318K*) mutation has been associated with neural crest-derived tumors, melanomas, and renal cell carcinomas, whereas the *SETD2* frameshift mutation was previously described in gastrointestinal tumors [22-24]. Histone deacetylase (HDAC)-inhibitor drugs could be considered for treatment in the clinical scenario of upregulated MITF and SETD2 inhibitors are currently being investigated in the treatment of leukemia [25, 26].

The higher the tumor mutational burden is, the more the immune system recognizes the cell as non-self and attacks it. In our study, the levels of TMB and MSI (a condition known as genetic hypermutation) were low, there were no alterations in DNA repair genes, but we did observe expression of the PD-L1 in most samples regardless of the tumor subtype. The utility of a given biomarker such as TMB, MSI, or PD-L1 to correlate with therapeutic response to immune checkpoint inhibitors is lineage dependent and it is unknown if these types of agents would be efficacious for craniopharyngiomas. PD-L1 expression in the stromal fibrovascular core in the papillary subtypes of craniopharygiomas and on the cystic lining in the adamantinomatous subtypes has been previously described [19]. In an attempt to find treatment strategies, Coy et al., specifically looked at overlap between PD-L1 expression and genetic alterations such as *BRAF* papillary and *CTNNB1* mutations. With such substantial overlap between *BRAF* mutations and PD-L1 expression, our combined findings would support consideration of a clinical trial using BRAF/MEK inhibitors in combination with immune checkpoint inhibitors in craniopharyngioma patients with refractory or residual disease and in the neoadjuvant setting prior to radiation therapy. This combination is currently being evaluated for safety and efficacy in melanoma patients (NCT02130466).

Craniopharyngiomas could result from a loss-of-function mutation in a tumor suppressor gene or a gain of function in an oncogene. For loss-of-function mutations, both alleles of a tumor suppressor gene must be lost in order to induce a tumor, unlike the case in oncogenes in which only one allele needs to be mutated. In the current study, we found losses of the neurofibromatosis (*NF*) *type 2* (*R462H*) gene and the tuberous sclerosis type 2 (*I463V*) gene, which have not been previously described. *NF2* alterations have been previously shown to be associated with schwannoma, ependymoma, and meningioma, and tuberous sclerosis with ependymoma [27, 28]. It is unclear what role these two genes may play in the underlying development of craniopharyngioma, including in the rare instance of familial craniopharyngioma, but this is an area for future investigation [29, 30]. Our molecular profiling also showed that the phosphoinositide-3-kinase, catalytic, alpha polypeptide (*PIK3CA*) gene, which is involved in cellular proliferation and inhibition of

apoptosis, was mutated in one case. Somatic mutations of PIK3CA are common in a variety of primary tumors such as those of the colon, breast, and stomach [31]. Phosphatidylinositol 3-kinase (PIK3) is known to regulate the tuberous sclerosis (*TSC*) tumor suppressor gene [32]. Both the *PIK3CA* and the *TSC2* mutations were observed in two patients in our study, suggesting that the roles of *PIK3CA* and *TSC2* merit further investigation as to their contributions to the etiology of craniopharyngioma. mTOR inhibitors could be considered for those patients with TSC2 mutations [33]. The only FDA-approved pan-PIK3 inhibitor is Copanlisib, but it is nonspecific and may have unacceptable toxicity due to off-target effect [34]. Specific PIK3 inhibitors are being employed in clinical trials of advanced stage cancers, and the positive overall response rates and progression-free survival rates being observed for *PIK3CA*-mutant tumors may make this a useful therapeutic strategy for a subset of craniopharygiomas [35, 36].

The epidermal growth factor receptor (EGFR), but not the EGFRvIII variant, is expressed in craniopharygiomas as validated by the IHC, and EGFR upregulation is implicated in cell differentiation, proliferation, apoptosis, and migration of these tumors [37]. Furthermore, EGFR expression has been reported in craniopoharyngioma and EGFR phosphorylation has been shown to enhance adamantinomatous craniopharyngioma cell migration and has been proposed as an escape mechanism for radiation therapy [38, 39]. EGFR inhibitors such gefitinib, erlotinib, and lapatinib are now routine treatments in non-small cell lung cancer and breast cancer and could be considered for off-label use in craniopharygiomas. The response to BRAF inhibitors in papillary craniopharyngioma has shown promise, but the tumor recurs shortly after treatment interruption in most cases [40]. Subsequently, BRAF inhibition combined with the MEK inhibitor trametinib has shown a decrease in proliferation of tumor cells *in vitro* and in preclinical xenograft models and produced a dramatic response in a refractory papillary craniopharyngioma case [41, 42]. This is not entirely surprising because this is an established combination strategy for the treatment of melanoma [43]. However, it is unclear whether the genetic variability that underlies each subtype would uniformly demonstrate clinical benefit, but based on the aforementioned data, a clinical trial of this combination would be justified in the adult craniopharygioma patient population.

We would have liked to profile many more of these cases, as further exploration of several mutations in a larger population is warranted. This is likely to require multicenter efforts and commitment to increase the sample size and increase the power of such extensive sequencing. Another limitation of the current study is that the sequencing was done from FFPE blocks, resulting in low coverage for some of the genes in the panel sequenced, and thereby their exclusion. We also are unable to associate the genetic findings with prognosis nor to conclude whether their roles are as driver mutations. Moreover, we note that many studies currently focus on the adamantinomatous subtype, taking for granted the high frequency of the $BRAF^{V600E}$ mutation and the availability of BRAF and MEK inhibitors, which have demonstrated marked antitumor activity within the CNS [44]. As such, the current study provides additional justification for the triple combination of BRAF and MEK inhibitors plus immune checkpoint inhibitors.

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Contributions

CK and DZ performed computational and data analysis, wrote the manuscript and prepared for submission. AH wrote the manuscript, planned experimental design, provided oversight. ZG, JX, DS acquired patient samples and performed the technical experiments

Conflicts of interest

ABH serves on the Caris Life Sciences Scientific Advisory Board and is a stockholder in the company. ZG, JX, and DS are employees of Caris Life Sciences.

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Ethical approval

This study involved collection of existing data and publicly available diagnostic specimens, and the information gathering process precludes direct and indirect identification of subjects, which therefore exempts it from requiring institutional review board approval under HHS regulations at 45 CFR 46.101(b).

Data availability

The authors affirm that all data necessary for confirming the conclusions of this article are present within the article, figures, tables, and the database available through Caris Life Sciences.

Supplementary Table 1: List of genes sequenced

BRAF	FANCD2	BCL9	CDKN2C	EML4	FLT1	IKBKE	MDM4	NSD1	PRCC	SH3GL1	TNFAIP3
ABL1	GATA1	BCOR	CDX2	EP300	FLT4	IKZF1	MDS2	NT5C2	PRDM1	SLC34A2	TNFRSF 14
AKT1	MAML2	BCORL1	CHCHD7	EPHA3	FNBP1	IL2	MECOM	NTRK1	PRDM16	SLC45A3	TNFRSF 17
ALK	MRE11	BCR	CHEK1	EPHA5	FOXA1	IL21R	MED12	NTRK2	PRF1	SMAD2	TOP1
APC	MYH11	BIRC3	CHEK2	EPHB1	FOXL2	IL6ST	MEF2B	NTRK3	PRKAR1 A	SMARC A4	TPM3

ATM	PTPRC	BLM	CHIC2	EPS15	FOXO1	IL7R	MAP2K1	NUMA1	PRKDC	SMARCE1	TPM4
KIT	RNF213	BMPR1A	CHN1	ERBB3	FOXO3	INHBA	MAP2K2	NUP214	PRRX1	SNX29	TPR
CDH1	ZNF384	BRCA1	CIC	ERC1	FOXO4	IRF4	MEN1	NUP93	PSIP1	SOCS1	TRAF7
MET	MITF	BRCA2	CIITA	ERCC1	FOXP1	IRS2	MKL1	NUP98	PTCH1	SOX10	TRIM26
CSF1R	ABI1	BRD3	CLP1	ERCC2	FSTL3	ITK	MLF1	NUTM1	RABEP1	SOX2	TRIM27
CTNNB1	ABL2	BRD4	CLTC	ERCC3	FUBP1	JAK1	MLLT1	NUTM2B	RAC1	SPECC1	TRIM33
EGFR	ACKR3	BRIP1	CNBP	ERCC4	FUS	JAZF1	MLLT10	OLIG2	RAD21	SPEN	TRIP11
ERBB2	ACSL3	BTG1	CNOT3	ERCC5	GAS7	JUN	MLLT11	OMD	RAD50	SPOP	TRRAP
ERBB4	ACSL6	BTK	CNTRL	ERG	GATA2	KAT6A	MLLT3	P2RY8	RAD51	SRC	TSC1
FBXW7	AFF1	BUB1B	COL1A1	ESR1	GATA3	KAT6B	AFDN	PAFAH1B2	RAD51B	SRGAP3	TSC2
FGFR1	AFF4	EMSY	COPB1	ETV1	GID4	KCNJ5	MLLT6	PAK3	RAF1	SRSF2	TSHR
FGFR2	AKAP9	C15orf65	COX6C	ETV4	GMPS	KDM5A	MN1	PALB2	RALGDS	SRSF3	TTL
FLT3	AKT2	WDCP	CREB1	ETV5	GNA13	KDM5C	MNX1	PATZ1	RANBP17	SS18	U2AF1
GNA11	AKT3	CACNA1D	CREB3L1	ETV6	GOLGA5	KDM6A	MSH2	PAX3	RAP1GDS1	SS18L1	UBR5
GNAQ	ALDH2	CALR	CREB3L2	EWSR1	GOPC	KDSR	MSH6	PAX5	RARA	SSX1	USP6
GNAS	AMER1	CAMTA1	CREBBP	EXT1	GPC3	KEAP1	MSI2	PAX7	RBM15	STAG2	VEGFA
HNF1A	AR	CANT1	CRKL	EXT2	GPHN	KIAA1549	MSN	PAX8	RECQL4	STAT3	VEGFB
HRAS	ARAF	CARD11	CRLF2	EZH2	ADGRA2	KIF5B	MTCP1	PBRM1	REL	STAT4	VTI1A
IDH1	ARFRP1	CARS	CRTC1	EZR	GRIN2A	KLF4	MTOR	PBX1	RHOH	STAT5B	WAS
JAK2	ARHGAP26	KNL1	CRTC3	FAM46C	GSK3B	KLHL6	MUC1	PCM1	RICTOR	STIL	NSD2
JAK3	ARHGEF12	CASP8	CSF3R	FANCA	H3F3A	KLK2	MUTYH	PCSK7	RMI2	SUFU	NSD3
KDR	ARID1A	CBFA2T3	CTCF	FANCC	H3F3B	KMT2A	MYB	PDCD1	RNF43	SUZ12	WIF1
KRAS	ARNT	CBFB	CTLA4	FANCE	HERPUD1	KMT2C	MYC	PDCD1LG2	ROS1	SYK	WISP3
MLH1	ASPSCR1	CBL	CTNNA1	FANCF	HEY1	KMT2D	MYCL	PDE4DIP	RPL10	TAF15	WRN
MPL	ASXL1	CBLB	CYLD	FANCG	HGF	KTN1	MYCN	PDGFB	RPL22	TAL1	WT1
NOTCH1	ATF1	CBLC	CYP2D6	FANCL	HIP1	LASP1	MYD88	PDGFRB	RPL5	TAL2	WWTR1
NPM1	ATIC	CCDC6	DAXX	FAS	HIST1H3B	LCK	MYH9	PDK1	RPN1	TBL1XR1	XPA
NRAS	ATP1A1	CCNB1IP1	DDB2	FBXO11	HIST1H4I	LCP1	NACA	PER1	RPTOR	TCEA1	XPC
PDGFRA	ATP2B3	CCND1	DDIT3	FCRL4	HLF	LGR5	NBN	PHF6	RUNX1	TCF12	XPO1
PIK3CA	ATR	CCND2	DDR2	FEV	HMGA1	LHFPL6	NCKIPSD	PHOX2B	RUNx1T1	TCF3	YWHAE
PTEN	ATRX	CCND3	DDX10	FGF10	HMGA2	LIFR	NCOA1	PICALM	SBDS	TCF7L2	ZBTB16
PTPN11	AURKA	CCNE1	DDX5	FGF14	HMGN2P46	LMO1	NCOA2	PIK3CG	SDC4	TCL1A	ZMYM2
RB1	AURKB	CD274	DDX6	FGF19	HNRNPA2B1	LMO2	NCOA4	PIK3R1	SDHAF2	TERT	ZNF217
RET	AXL	CD74	DEK	FGF23	HOOK3	LPP	NDRG1	PIK3R2	SDHB	TET1	ZNF331
SMAD4	BAP1	CD79A	DICER1	FGF3	HOXA11	LRIG3	NF1	PIM1	SDHC	TET2	ZNF521
SMARCB1	BARD1	CD79B	DNM2	FGF4	HOXA13	LRP1B	NF2	PLAG1	SDHD	TFE3	ZNF703

SMO	BCL10	CDC73	DNMT3A	FGF6	HOXA9	LYL1	NFE2L2	PML	SEPT5	TFEB	ZRSR2
STK11	BCL11A	CDH11	DOT1L	FGFR1OP	HOXC11	MAF	NFIB	PMS1	SEPT6	TFG	MSI
TP53	BCL11B	CDK12	EBF1	FGFR3	HOXC13	MAFB	NFKB2	PMS2	SEPT9	TFPT	TMB
VHL	BCL2	CDK4	ECT2L	FGFR4	HOXD11	MALT1	NFKBIA	POLE	SET	TFRC	
AFF3	BCL2L11	CDK6	EIF4A2	FH	HOXD13	MAP2K4	NIN	POT1	SETBP1	TGFBR2	
ARID2	BCL2L2	CDK8	ELF4	FHIT	HSP90AA1	MAP3K1	NKX2-1	POU2AF1	SETD2	THRAP3	
AXIN1	BCL3	CDKN1B	ELK4	FIP1L1	HSP90AB1	MAX	NONO	POU5F1	SF3B1	TLX1	
CEBPA	BCL6	CDKN2A	ELL	FLCN	IDH2	MCL1	NOTCH2	PPARG	SFPQ	TLX3	
CLTCL1	BCL7A	CDKN2B	ELN	FLI1	IGF1R	MDM2	NR4A3	PPP2R1A	SH2B3	TMPRSS2	

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