Comprehensive genomic characterization of three spatially and temporally distinct tumors from different organs in a single patient exhibiting both BRCA2 and VHL germline mutations

#2687W

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Patient Background — Tumors & Susceptibility Genes

Inherited genetic mutations have been strongly associated with heightened risk for a broad range of cancer subtypes. Over the last two decades, large efforts have focused on recognizing cancer driver genes and better understanding their role in cancer progression, resulting in identification and characterization of important drivers. In very rare cases, a single individual has germline mutations in more than one of these cancer driver genes.

To spatially and temporally profile tumor initiation in the setting of *de novo* germline tumor suppressor mutations, we applied next generation sequencing to analyze tumors arising from three distinct tissues in a female patient affected by Von-Hippel Lindau disease. Using the ACE Extended Cancer Panel, a targeted enrichment sequencing platform including over 1,300 cancer genes and 200 miRNAs, we sequenced each neoplastic site as well as adjacent normal tissues. Both the DNA and RNA were sequenced to high depth, and small variants, gene expression, copy number alterations, and gene fusions were assessed. Additionally, by sequencing both DNA and RNA, we evaluated allele specific expression and verified copy number changes affecting gene expression.

One Patient — Three Spatially and Temporally Distinct Tumors

Tumor Type	Age Of Onset	Tissue Type	Stage	Treatment	Status
Pheochromocytoma (PCC)	50	Adrenal	1	Surgery	Remission
Renal Cell Carcinoma (RCC)	51	Kidney	1 & Recur	Pazopanib	In Treatment
Basal Cell Carcinoma (BCC)	54	Skin	1	Surgery	Remission

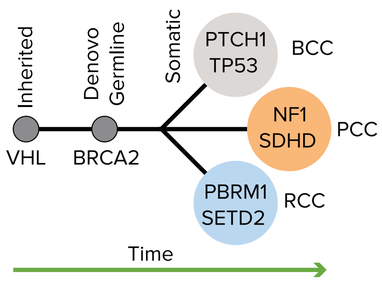
We performed deep sequencing on three tumors that arose in distinct tissues more than 1 year apart using our ACE Cancer Panel. The patient has been independently treated 3 times and is currently in remission for BCC and PCC and in continued treatment for RCC.

Two Germline Cancer Susceptibility Genes (BRCA2 & VHL)

		Germline Small Variant			
Gene	Variant Class	Mutation	Germline?	Allele Freq DNA	Allele Freq RNA
VHL	Missense	p.L157P	Yes	***50%***	***67%***
BRCA2	Frame Shift	p.S1982fs	Yes	***51%***	***50%***

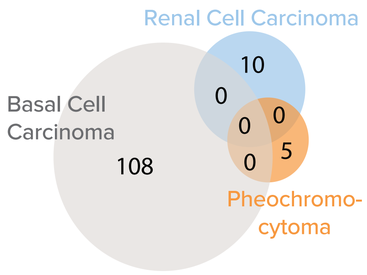
BRCA2 is known for its role in breast, ovarian, and skin cancers. VHL mutations are rare (1/36,000 individuals) and known for their role in several cancers, including kidney RCC, and PCC. To our knowledge, this is the first time extensive genetic profiling has been performed on a patient with both BRCA2 and VHL mutations.

Temporally and Spatially Separated Mutations Drive Tumors



Our patient had inherited their VHL missense mutation and obtained a *de novo* germline frameshift mutation in BRCA2. Both of these mutations were observed in each adjacent normal tissue , suggesting the BRCA2 frameshift was a very early mutation and is not mosaic. Additionally, we detected mutations in what are perhaps the most well characterized cancer driver genes for each our the three distinct tumor types, indicating each tumor progressed along characteristic paths regardless of their common germline susceptibility mutations.

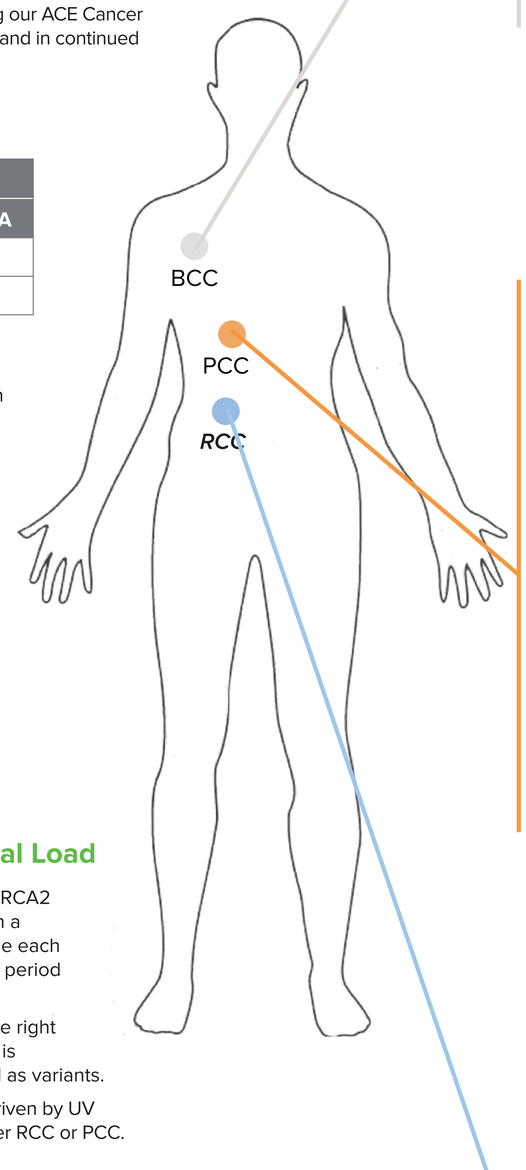
All Somatic Mutations Are Private and BCC Has a Higher Mutational Load



Not considering the germline susceptibility variants BRCA2 and VHL, each somatic mutation was only detected in a single tumor sample. This strongly suggests that, while each tumor occurred in succession within a relatively short period of years, each mutation has arisen independently.

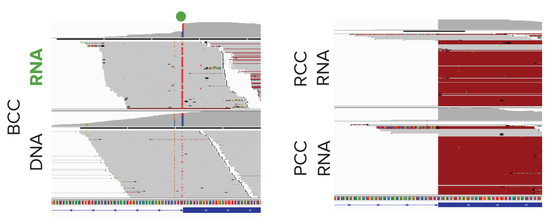
Furthermore, when analyzing the tumor profiles on the right (BCC, PCC, and RCC), it can be seen that each tumor is predominately driven by unique sets of genes as well as variants.

It is also important to note that BCC, which is largely driven by UV damage, contains significantly more variants than either RCC or PCC.



Tumor Analysis is Improved by Integrated RNA/DNA Analysis

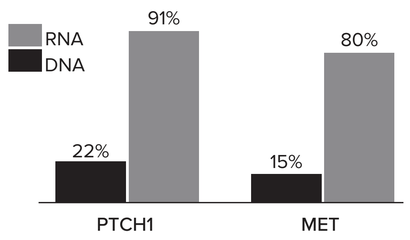
PTCH1 Splice Site Mutation in BCC Tumor Clearly Affecting RNA Processing



We identified a splice donor variant in the PTCH1 gene in our BCC tumor. This gene is the most well known driver gene in this tumor type. By inspecting this variant in both the RNA and DNA, we are able to observe both the concordant presence of this variant and more importantly, the effect this variant has on transcript splicing.

In the BCC RNA sample the reads are clearly continuing into the intron, as would occur if this variant is indeed damaging splicing at this site. In contrast, in both the RCC and PCC samples, which do not have this variant, display a sharp exon intron transcript boundary.

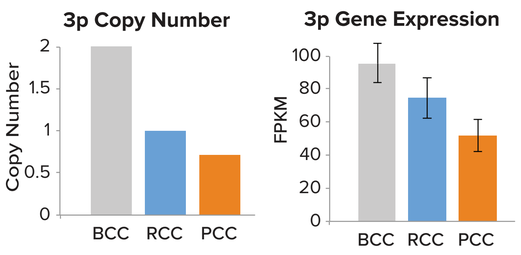
High Profile Cancer Driver Gene Variants Exhibiting Allele-Specific Expression



Alelle-specific expression is the preferential expression of a particular allele and can lead to drastic changes in variant expression.

We observe allele specific expression in multiple well known somatic cancer driver genes in our patient. For example, we observed that small variants in both PTCH1, which is described above, and MET are the minor alleles in DNA. However, when inspected in the RNA these variants are dominant, being expressed at >4 times higher levels than in the DNA. This event would not be detectable when analyzing DNA alone.

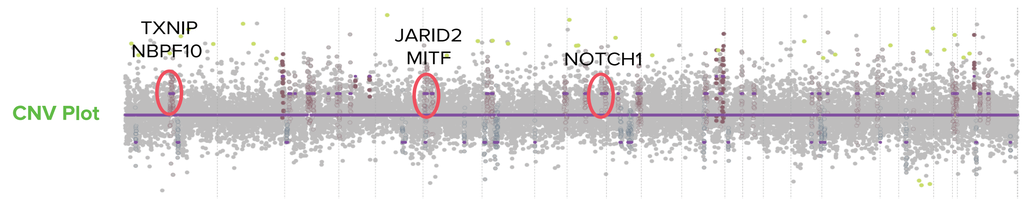
Loss of Chr 3p in RCC and PCC is Associated with Significantly Reduced Gene Expression



CNVs provide indications that genes in the affected region are either up or down regulated. However, without having expression data, it is not possible to be observe of these responses. By integrating RNA and DNA results we can clearly observe there is both a copy number loss in 3p of RCC and PCC as well as a reduction in gene expression in this region containing many important driver genes, including VHL, SETD2, and PBRM1.

BRCA2, PTCH1, and TP53 Are Driving Progression of Tumor 3 (BCC)

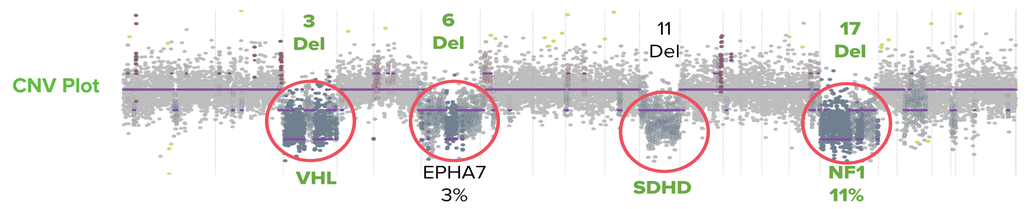
Driver Mutations		Small Variant				CNV	
Gene	Small Variant or CNV?	Mutation	Small Variant Germline?	Allele Freq DNA	Allele Freq RNA	Associated With CNV	Common In BCC?
BRCA2	Frame Shift	p.S1982fs	Yes	***46%***	***51%***	***No***	***<1%***
VHL	Missense	p.L157P	Yes	***52%***	***55%***	***No***	***<1%***
PTCH1	Splice Donor	c.2560+1G>A	No	22%	***91%***	***No***	***75%***
TP53	Stopgain	p.R337*	No	***1%***	***12%***	No	***66%***
MET	Missense	p.P574L	No	***15%***	***80%***	No	***<1%***



We detected 3 well known driver mutations in the patient's BCC sample (BRCA2, PTCH1, and TP53). BRCA2 is one of the most widely studied cancer susceptibility germline mutations and plays a role in BCC progression, among other cancers. Most importantly, we identified a splice donor mutation in PTCH1, which is very well known cancer driver of BCC, being mutated in 75% of patient samples. This gene is so important in BCC that mutations of PTCH1 alone are sufficient for tumor formation in mice. It is also worth noting that very interesting allele specific expression is occurring in this tumor, as PTCH1 and MET display significantly higher ratios of variant allele expression in RNA than DNA (22% → 91% AF and 15% → 80% AF, respectively). Further the high profile TP53 stopgain mutation was only detected through RNA analysis, as the mutant allele is expressed at a 12X higher ratio than was detected in DNA.

VHL, SDHD, and NF1 Are Driving Progression of Tumor 2 (PCC)

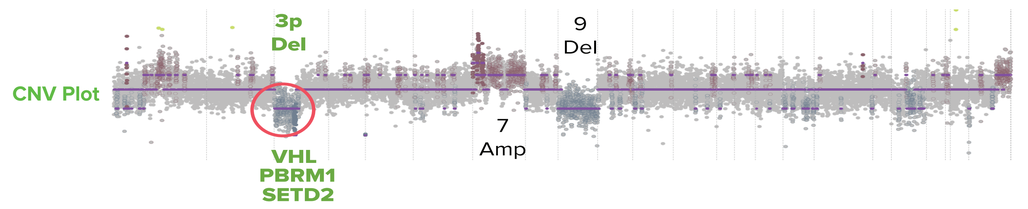
Driver Mutations		Small Variant				CNV	
Gene	Small Variant or CNV?	Mutation	Small Variant Germline?	Allele Freq DNA	Allele Freq RNA	Associated With CNV	Common In Pheo?
VHL	Missense & CNV	p.L157P	Yes	***71%***	***80%***	***1 Copy Del***	***13%***
BRCA2	Frame Shift	p.S1982fs	Yes	***51%***	***NA***	***No***	***<1%***
NF1	CNV	NA	No	NA	NA	***1 Copy Del***	***25%***
SDHD	CNV	NA	No	NA	NA	***1 Copy Del***	***5%***
EPHA7	CNV	NA	No	NA	NA	***1 Copy Del***	***3%***



In the PCC tumor, we identified a large number of whole chromosome deletions (Chr3, Chr6, Chr11, Chr17). Three of the four deleted chromosomes contain the most commonly mutated cancer driver genes in PCC (VHL, NF1, and SDHD). Small variant mutations were also detected on known PCC genes VHL (germline) and TXNIP, each of which were identified in both the tumor RNA and DNA at comparable frequencies.

VHL, PBRM1, and SETD2 Are Driving Progression of Tumor 1 (RCC)

Driver Mutations		Small Variant				CNV	
Gene	Small Variant or CNV?	Mutation	Small Variant Germline?	Allele Freq DNA	Allele Freq RNA	Associated With CNV	Common In RCC?
VHL	Missense & CNV	p.L157P	Yes	***50%***	***68%***	***1 Copy Del***	***52%***
BRCA2	Frame Shift	p.S1982fs	Yes	***49%***	***30%***	***No***	***<1%***
PBRM1	Missense & CNV	p.N698K	No	30%	***23%***	***1 Copy Del***	***33%***
SETD2	CNV	NA	NA	NA	NA	***1 Copy Del***	***12%***



We identified candidate cancer driver mutations in 5 genes (VHL, PBRM1, SETD2, AXIN1, GSK3B) which had been shown to be mutated in RCC (TCGA). Three of these are well known and likely constitute the most important RCC cancer driver genes (VHL, PBRM1, SETD2). Each of these small variant mutations was identified in both the tumor RNA and DNA at comparable frequencies. Consistent with data from the TCGA, we identified biallelic inactivation of VHL through 3p LOH and a germline VHL inactivating mutation.

Conclusions

This is the first study to investigate the progression of multiple tumors arising from dual BRCA2/VHL germline mutations in a single patient. In addition to identifying the impact of inherited BRCA2 and VHL mutations in each tumor, we extensively characterized driver mutations in all tumors, identifying private mutations in each. Interestingly, each tumor arose from entirely independent somatic mutations, with each tumor containing multiple well characterized driver mutations that are well known for driving each tumor type.

By applying both RNA and DNA sequencing to our analysis we were able to both increase confidence in concordant variant calls, detect as allele specific expression, or simply detect very low level genome variants which are become apparent through higher expression levels. By applying an integrated RNA/DNA approach with a cancer focused augmented enrichment panel, we were able to gain a greater understanding of our patient's rare and challenging tumor progression.

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