Deep targeted and Exome sequencing Reveals High Allelic Frequencies of Tumor Mutations in the High Risk Airway Epithelium

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Background

- Insights into tumor mutational burden of the airway epithelium may play important role in stratifying individuals at different levels of risk for lung cancer.
- Comparison of the genetic profiles of normal airway epithelial cells to tumor mutations can yield valuable information regarding shared mutations as well as presence of neoantigens.
- CAPP-Seq is a method of next generation targeted sequencing that is sensitive enough to detect very low frequency mutations, originally developed to detect circulating tumor DNA for monitoring of molecular residual disease.
- In individuals who have smoked and are high risk for developing lung cancer, the lungs develop widespread somatic mutations in the field of cancerization.

Research question

Does field of cancerization harbor mutations of diagnostic value? Can these mutations be used for lung cancer risk assessment and early detection?

Hypothesis

We hypothesized that cytologically normal bronchial epithelium of patients with lung cancer and those at high risk for lung cancer harbor driver mutations

Methods

- Bronchial brushings were collected from consented patients at the time of bronchoscopy. Lung tumor tissues from matched individuals were also sequenced.
- Tammemagi risk model was used to calculate the risk of developing lung cancer.
- DNA was extracted from the bronchial epithelial cells from the bronchial brushings of individuals at-risk (low and high) for lung cancer and patients with lung cancer.
- Next generation sequence-based CAPP-Seq (CAncer Personalized Profiling by deep Sequencing) and whole exome sequencing (Exome-Seq) were performed using DNA from the same tube.

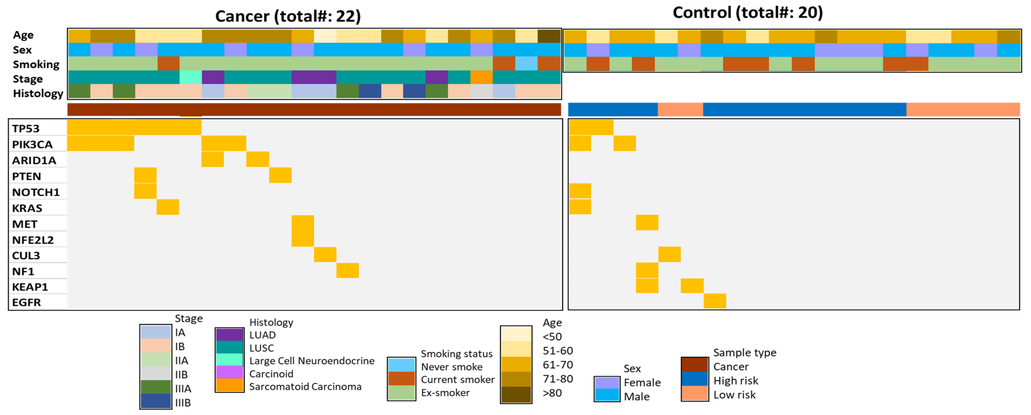
Samples used in CAPP-Seq and Exome-Seq analyses

	CAPP-Seq		Exome-Seq			
	Brushings	Tumor A	Brushings	Tumor A	Tumor B	Tumor C
low risk	10	n/a	9	n/a	n/a	n/a
high risk	10	n/a	8	n/a	n/a	n/a
lung cancer	22	21	17	19	18	17

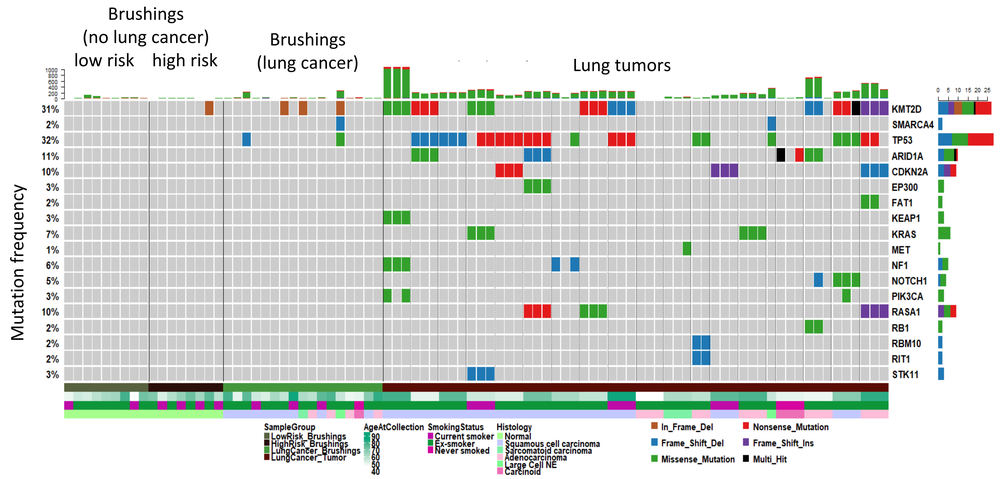
Mutations detected in patient groups

	CAPP-Seq			Exome-Seq		
	Brushings (no lung cancer)	Brushings (lung cancer)	Lung tumor	Brushings (no lung cancer)	Brushings (lung cancer)	Lung tumor
Patients (n)	20	22	21	17	17	19
Patients, mutations identified (n)	10	13	18	17	17	19
Total number of detected mutations	24	62	183	550	937	13785
Average allelic frequency (%)	0.48	1.4	20	2.84	3.68	21.57

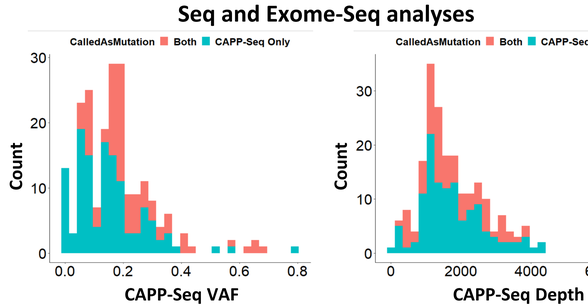
Driver mutations detected in the cytologically normal bronchial epithelium by CAPP-Seq



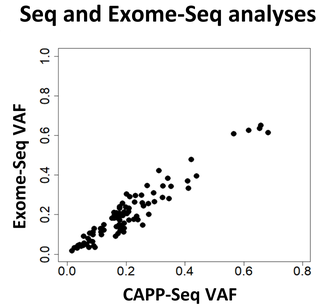
Driver mutations detected in the cytologically normal bronchial epithelium by exome-Seq



Improved VAF and depth with combined CAPP-Seq and Exome-Seq analyses



Similar VAF demonstrated by CAPP-Seq and Exome-Seq analyses



Driver mutations detected in bronchial epithelium

	Patients	Genes (number)
CAPP-Seq		
Brushings, (no lung cancer) high risk	5 out of 13	TP53, PIK3CA, NOTCH1, KRAS, MET,NF1, KEAP1 and EGFR (8)
Brushings,(no lung cancer) low risk	2 out of 7	CUL3, KEAP1 (2)
Brushings (lung cancer) and lung tumor	13 out of 22	TP53, PIK3CA, ARID1A, PTEN, NOTCH1, KRAS, MET, NFE2L2, CUL3, NF1 (10)
Driver tumor mutations in bronchial brushings, same patient	7 out of 21	TP53, PIK3CA, ARID1A, PTEN, NOTCH1, KRAS, MET, NFE2L2, CUL3, NF1, KEAP1, and EGFR (12)
Exome-Seq		
Brushings, (no lung cancer) high risk	1 out of 8	KMT2D (1)
Brushings (lung cancer)	4 out of 17	KMT2D, SMARCA4, TP53 (3)
Lung tumor	18 out of 19	KMT2D, SMARCA4, TP53, ARID1A, CDKN2A, EP300, FAT1, KEAP1, DRAS, MET, NF1, NOTCH1, PIK3CA, RASA1, RB1, RBM10, RIT1, STK11 (18)

CAPP-Seq

- At least one mutation was called in 18 out of 22 bronchial brushings of individuals with lung cancer.
- Most frequent mutations were in TP53 and PIK3CA.

Exome-Seq

- Using the same bronchial brushings, 90 mutations were identified that were also identified by CAPPSeq.
- Most of the mutations identified by CAPPSeq with >5% VAF were also identified by Exom-Seq.
- Out of 9 mutations in TP53 identified in the brushings by CAPPSeq, 4 were identified by Exom-Seq.
- Mutation in KMTD2, a histone methylation enzyme and a tumor suppressor, was identified by Exom-Seq in the brushings and the tumor tissues of patients with lung cancer as well as in the brushings of one patient (high risk) without lung cancer.

Improved VAF and depth

- Mutations with a higher depth in CAPP-Seq showed similar trend to be identified in Exom-Seq.
- Mutations with a higher VAF in CAPP-Seq were easier to be identified in Exom-Seq.
- Mutation VAF threshold limited Exom-Seq mutation identification.

Conclusions

- Targeted sequencing with CAPP-Seq provided a sensitive method for identifying low frequency mutations in the tumor and airways.
- We identified driver gene mutations in the cytologically normal bronchial epithelium as well as in the lung tumor tissues of the same patient.
- Individuals at-risk for lung cancer also harbored driver gene mutations.
- This field of cancerization effect may provide a tool for risk assessment for the early detection of lung cancer.

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