Sequencing a super multiple synchronous lung cancer reveals a novel variant in driver gene *ARID1B*

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Venn diagram showing the distributions of validated mutations in the 5 lesions.

Central Message

With the use of target DNA region sequencing, the only shared mutation, a new somatic mutation in *ARID1B* and a germ-line mutation in *EGFR*, was identified in samples from a patient's lung with 166 nodules.

See Editorial Commentary page e193.

▶ Video clip is available online.

Multiple synchronous lung cancer presents a clinical dilemma. From 2012 to 2016, we treated a patient with 166 nodules in the lung. We selected 5 typical samples to target the DNA region sequencing. The results demonstrate that only 1 somatic mutation was shared by all 5 tumor samples. A large number of primary tumors appeared in the same organ. We found a new somatic mutation in *ARID1B* and a germ-line mutation in *EGFR*.

CASE REPORT

In November 2012, bilateral multiple lung nodules (maximum diameter 1.2 cm) and multiple thyroid nodules (0.3 to 0.5 cm in diameter) were found in a 52-year-old Chinese woman on routine examination. The patient underwent subtotal thyroidectomy for

FIGURE 1. Pedigree and cancer status of the patients. The III5 indicates the patient's sister, and the IV1 indicates the patient's niece.

VIDEO 1. A total of 166 nodules were identified, with maximum diameters of 2 to 17 mm. Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(18\)30059-X/fulltext](http://www.jtcvsonline.org/article/S0022-5223(18)30059-X/fulltext).

papillary cancer of the thyroid in November 2012, and no recurrence was detected. The patient had never smoked cigarettes and did not have a drug or alcohol addiction. The patient reported a sister and niece with lung cancer (Figure 1).

From November 2012 to April 2016, surveillance of computed tomography scan showed 166 nodules measuring 2 to 17 mm in diameter distributed across 5 lobes of the lung, including pure ground-glass nodules, partial-solid nodules, solid nodules, and cavitary nodules (Video 1). One of the largest lesions in the left upper lobe had become progressively solid.

Left-upper lobectomy by video-assisted thoracoscopic surgery was performed in May 2016. Three major lesions were found to be invasive adenocarcinoma, 4 lesions were found to be atypical adenomatous hyperplasia, and 13 lesions were found to be adenocarcinoma in situ by paraffin section pathology (Figure 2, B). The most typical samples were selected for immunohistochemistry of lung adenocarcinoma (Figure 2, C). Other lesions underwent paired box gene 8 and thyroglobulin analysis in this case to rule out papillary thyroid carcinoma metastasis. All lesions were at stage T1a or Tis. No lymph node metastasis was found.

We chose 5 obvious lesions for further examination. After assessment, 5 tumor samples were identified as 1 thyroid cancer, 1 invasive adenocarcinoma, 2 adenocarcinomas in situ, and 1 adenomatous hyperplasia. DNA from blood and paraffin section of 5 tumors were subjected to the Illumina HiSeq X Ten system (Illumina Inc, San Diego, Calif) after target region capture using

a cancer panel, of which the coding region of 483 cancer-related genes and selected introns from 19 genes were included (Table 1).

Somatic point mutations were detected for each of the 5 tumor samples and then filtered according to the criteria. In total, there were 106 filtered point mutations, among which only 1 mutation was shared by all the 5 tumor samples (Figure 3). This missense mutation was located in *ARID1B* p.A334G. In addition, sequence analysis revealed another missense mutation in exon 20 of the *EGFR* gene (c.G2327A, p.R776H). This mutation was present in normal lymphoid cells, indicating that it was a germline mutation.

DISCUSSION

We report a female patient who was a nonsmoker and had super multiple synchronous adenocarcinoma of the lung. We identified a novel somatic *ARID1B* mutation shared by 5 independent tumors from the same individual. *ARID1B* is reported in 40 of 922 specimens of adenocarcinoma of the lung in the COSMIC database. It is reported with a frequency of nonsynonymous mutations detected in 33 samples with a frequency of 0.17.¹ As a member of the human SWItch/sucrose nonfermentable chromatin remodeling complex, *ARID1B*, namely, the AT-rich interaction domain 1B, is closely related with cancer formation and has been identified as a cancer-driver gene.^{2,3} Vengoechea and colleagues⁴ also reported deletion of 6q25 including *ARID1B* in a patient with papillary thyroid cancer. As chromatin regulators, *ARID1B* mutations can reduce the stability of the lung cancer cell genome by affecting chromatin remodeling.^{5,6} Another study showed that a lack in the expression of *ARID1A* and *ARID1B* could weaken the cancer cell in DNA repair and even make it vulnerable to DNA damage.⁷ In our case, a novel mutation of *ARID1B* shared by the 5 tumor samples was identified. Therefore, we suspected that the mutation played a significant role in the patient's cancer formation. Furthermore, the patient also carried a germline missense mutation R776H of *EGFR*. According to the existing research, *EGFR* mutation induces cell proliferation and promotes tumor development.⁸

CONCLUSIONS

Further studies will be performed in the future to demonstrate how the downstream functional protein of *ARID1B* and *EGFR* mutation contributes to the normal tissue carcinogenesis. In addition, we will also explore the relationship between *ARID1B* and *EGFR* mutation and its role in the oncogene mutation network.

FIGURE 2. Growth and identification of the lesions. A, Computed tomography images show that 1 large nodule in the left upper lobe became progressively solid. B, Pathological images of invasive adenocarcinoma, adenocarcinoma in situ, and atypical adenomatous hyperplasia. C, Hematoxylin-eosin staining and ICH images confirm 2 smaller lesions to be primary adenocarcinoma. Scale bar = 10 μ m. HE, Hematoxylin-eosin; PAX8, paired box gene 8; TG, thyroglobulin; PD-L1, programmed death-ligand 1; ALK, anaplastic lymphoma kinase.

TABLE 1. Cancer panel coding region of 483 cancer-related genes and selected introns from 19 genes

A						
<i>ABCB1</i>	<i>ABCC1</i>	<i>ABCC2</i>	<i>ABCC4</i>	<i>ABCC6</i>	<i>ABCG2</i>	<i>ABL1</i>
<i>ACK1/TNK2</i>	<i>ACVR1B</i>	<i>AKT1</i>	<i>AKT2</i>	<i>AKT3</i>	<i>ALK*</i>	<i>AMER1</i>
<i>APC*</i>	<i>AR</i>	<i>ARAF</i>	<i>ARFRP1</i>	<i>ARID1A</i>	<i>ARID1B</i>	<i>ARID2</i>
<i>ASXL1</i>	<i>AT1C</i>	<i>ATM*</i>	<i>ATP7A</i>	<i>ATR</i>	<i>ATRX</i>	<i>AURKA</i>
<i>AURKB</i>	<i>AXIN1</i>	<i>AXL</i>				
B						
<i>B2M</i>	<i>BAIAP3</i>	<i>BAP1*</i>	<i>BARD1*</i>	<i>BCL2</i>	<i>BCL2L2</i>	<i>BCL6</i>
<i>BCOR</i>	<i>BCORL1</i>	<i>BCR</i>	<i>BIRC5</i>	<i>BLK</i>	<i>BLM*</i>	<i>BRAF</i>
<i>BRCA1*</i>	<i>BRCA2*</i>	<i>BRIP1*</i>	<i>BRK/PTK6</i>	<i>BSG/CD147</i>	<i>BTK</i>	
C						
<i>C11orf30</i>	<i>C18orf56</i>	<i>C8orf34</i>	<i>CAMK2G</i>	<i>CAMKK2</i>	<i>CARD11</i>	<i>CASP8</i>
<i>CBFB</i>	<i>CBL</i>	<i>CBR1</i>	<i>CBR3</i>	<i>CCND1</i>	<i>CCND2</i>	<i>CCND3</i>
<i>CCNE1</i>	<i>CCR4</i>	<i>CD19</i>	<i>CD22</i>	<i>CD274</i>	<i>CD33</i>	<i>CD38</i>
<i>CD3EAP</i>	<i>CD52</i>	<i>CD74</i>	<i>CD79A</i>	<i>CD79B</i>	<i>CDA</i>	<i>CDC73*</i>
<i>CDH1*</i>	<i>CDK1</i>	<i>CDK12</i>	<i>CDK2</i>	<i>CDK4*</i>	<i>CDK5</i>	<i>CDK6</i>
<i>CDK7</i>	<i>CDK8</i>	<i>CDK9</i>	<i>CDKN1B</i>	<i>CDKN2A*</i>	<i>CDKN2B</i>	<i>CDKN2C</i>
<i>CEBPA*</i>	<i>CHEK1</i>	<i>CHEK2*</i>	<i>CHST3</i>	<i>CIC</i>	<i>CSNK1A1</i>	<i>COMT</i>
<i>CREBBP</i>	<i>CRKL</i>	<i>CRLF2</i>	<i>CSF1R</i>	<i>CSK</i>	<i>CTCF</i>	<i>CTLA4</i>
<i>CTNNA1</i>	<i>CTNNB1</i>	<i>CYBA</i>	<i>CYLD*</i>	<i>CYP19A1</i>	<i>CYP1A1</i>	<i>CYP1A2</i>
<i>CYP1B1</i>	<i>CYP2A6</i>	<i>CYP2B6</i>	<i>CYP2C19</i>	<i>CYP2C8</i>	<i>CYP2C9</i>	<i>CYP2D6</i>
<i>CYP2E1</i>	<i>CYP3A4</i>	<i>CYP3A5</i>	<i>CYP4B1</i>			
D						
<i>DAXX</i>	<i>DDR1</i>	<i>DDR2</i>	<i>DNMT1</i>	<i>DNMT3A</i>	<i>DOT1L</i>	<i>DPYD</i>
<i>DSCAM</i>						
E						
<i>E2F1</i>	<i>EGF</i>	<i>EGFL7</i>	<i>EGFR*</i>	<i>EGR1</i>	<i>EMC8</i>	<i>EML4</i>
<i>ENOSF1</i>	<i>EP300</i>	<i>EPH/EPHA1</i>	<i>EPHA2</i>	<i>EPHA3</i>	<i>EPHA4</i>	<i>EPHA5</i>
<i>EPHA7</i>	<i>EPHA8</i>	<i>EPHB1</i>	<i>EPHB2</i>	<i>EPHB3</i>	<i>EPHX1</i>	<i>ERBB2/HER2</i>
<i>ERBB3</i>	<i>ERBB4</i>	<i>ERCC1</i>	<i>ERCC2*</i>	<i>ERG</i>	<i>ESR1/ER</i>	<i>ETV1</i>
<i>ETV4</i>	<i>ETV5</i>	<i>ETV6</i>	<i>EWSR1</i>	<i>EZH2*</i>		
F						
<i>FAM46C</i>	<i>FANCA*</i>	<i>FANCC</i>	<i>FANCD2*</i>	<i>FANCE*</i>	<i>FANCF*</i>	<i>FANCG*</i>
<i>FANCL*</i>	<i>FBXW7</i>	<i>FCGR3A</i>	<i>FGF10</i>	<i>FGF14</i>	<i>FGF19</i>	<i>FGF23</i>
<i>FGF3</i>	<i>FGF4</i>	<i>FGF6</i>	<i>FGFR1</i>	<i>FGFR2</i>	<i>FGFR3</i>	<i>FGFR4</i>
<i>FGR</i>	<i>FKBP1A</i>	<i>FLT1</i>	<i>FLT3</i>	<i>FLT4</i>	<i>FOXL2</i>	<i>FRK</i>
<i>FUBP1</i>	<i>FYN</i>	<i>FZD7</i>				
G						
<i>GSTT1</i>	<i>GALNT14</i>	<i>GATA1</i>	<i>GATA2*</i>	<i>GATA3</i>	<i>GCK</i>	<i>GID4</i>
<i>GINS2</i>	<i>GNA11</i>	<i>GNA13</i>	<i>GNAQ</i>	<i>GNAS</i>	<i>GPC3*</i>	<i>GPR124</i>
<i>GRIN2A</i>	<i>GSK3B</i>	<i>GSTM1</i>	<i>GSTM3</i>	<i>GSTP1</i>	<i>GSTT1</i>	
H						
<i>HCK</i>	<i>HGF</i>	<i>HIF-1/HIF1A</i>	<i>HIST1H3B</i>	<i>HNF1A*</i>	<i>HRAS*</i>	<i>HSP90AA1</i>
<i>H3F3A</i>						

(Continued)

TABLE 1. Continued

I						
IDH1	IDH2	IGF1	IGF1R/IGFR	IGF2	IGF2R	IKBKB
IKBKE	IKZF1	IL7R	INHBA	INSR/IR	IRF4	IRS2
ITK						
J						
JAK1	JAK2	JAK3	JUN			
K						
KAT6A	KDM5A	KDM5C	KDM6A	KDR/VEGFR	KEAP1	KIT*
KLC3	KLHL6	KMT2A/MLL	KMT2B/MLL4	KMT2C/MLL3	KMT2D/MLL2	KRAS
L						
LCK	LIMK1	LMO1	LRP1B	LRP2	LYN	
M						
MAP2K1	MAP2K2	MAP2K4	MAP3K1	MAP4K4	MAP4K5	MAPK1
MAPK10	MAPK14	MAPK8	MAPK9	MAPKAPK2	MARK1	MCL1
MDM2	MDM4	MED12	MEF2B	MEN1*	MERTK	MET*
MITF*	MKNK2	MLH1*	MPL	MRE11A*	MS4A1	MSH2*
MSH6*	MTDH	MTHFR	MTOR	MTRR	MUTYH*	MYC
MYCL1	MYCN	MYD88				
N						
NAT1	NAT2	NCAM1	NCF4	NCOA3	NCOR1	NEK11
NF1*	NF2*	NFE2L2	NFKBIA	NKX2-1	NOS3	NOTCH1
NOTCH2	NPM1	NQO1	NRAS	NTRK1	NTRK2	NTRK3
NUP93						
P						
PAK1	PAK3	PALB2*	PARP1	PARP2	PAX5	PTPRD
PBRM1	PDCD1	PDGFRA	PDGFRB	PDK1	PHF6	PHKA2
PIGF	PIK3CA	PIK3CB	PIK3CG	PIK3R1	PIK3R2	PKC/PRRT2
PKCγ/PRKCG	PKCε/PRKCE	PLK1	PPARD	PPP1R13L	PPP2R1A	PRDM1
PRDX4	PRKAA1	PRKARIA*	PRKCA	PRKCB	PRKDC	PTCH1*
PTEN*	PTK2	PTPN11				
R						
RAC2	RAD50*	RAD51	RAF1	RARA	RB1*	RET*
RICTOR	RMDN2	RNF43	ROCK1	RON/MST1R	ROS1	RPL13
RPS6KA1	RPS6KB1	RPTOR	RRM1	RUNX1		
S						
SCF/KITLG	SDHA*	SDHAF1	SDHAF2*	SDHB*	SULT1C4	SYK
SDHC*	SDHD*	SETD2*	SF3B1	SGK1	SHH	SIK1
SKP2	SLC10A2	SLC15A2	SLC22A1	SLC22A16	SLC22A2	SLC22A6
SLCO1B1	SLCO1B3	SMAD2	SMAD4*	SMARCA4	SMARCB1*	SMO
SOCS1	SOD2	SOX10	SOX2	SOX9	SPEN	SPG7
SPOP	SRC	SRD5A2	SRMS	STAG2	STAT1	STAT2
STAT3	STAT4	STAT5A	STAT5B	STAT6	STEAP1	STK11*
STK3	STK4	SUFU*	SULT1A1	SULT1A2		

(Continued)

TABLE 1. Continued

T						
TCF7L1	TCF7L2	TEK	TET2	TGFBR1	TGFBR2	TK1
TMPRSS2	TNF	TNFAIP3	TNFRSF14	TNFRSF8	TNFSF11	TNFSF13B
TOP1	TP53*	TPMT	TPX2	TRAIL-R1	TRAIL-R2	TSC1*
TSC2*	TSHR	TYMS/TS	TYRO3			
U						
U2AF1	UBE2I	UGT1A1	UGT1A9	UGT2B15	UGT2B17	UGT2B7
UMPS						
V						
VEGFA	VEGFB	VHL*				
W						
WEE1	WISP3	WNK3	WT1*			
X						
XPC*	XPO1	XRCC1	XRCC4			
Y						
YES1						
Z						
ZAP70	ZC3HAV1	ZNF217	ZNF703			

*Labeled 63 genetic risk for gene detection.

FIGURE 3. Venn diagram showing the distributions of validated mutations in the 5 lesions. TT, Thyroid tumor; LT, lung tumor.

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