Cell Line Genomic DNAs for the Molecular Diagnosis of Cancer

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Abstract

Introduction: Large-scale cancer genome programs have generated a rich data set comprising genetic abnormalities observed in thousands of clinical patient tumors, which provides a major opportunity for the molecular detection of cancer. However, the lack of controls for molecular tests has been a challenge. Because of the reproducible nature of the cell lines, genomic DNAs of fully characterized and authenticated cell lines provide a solution.

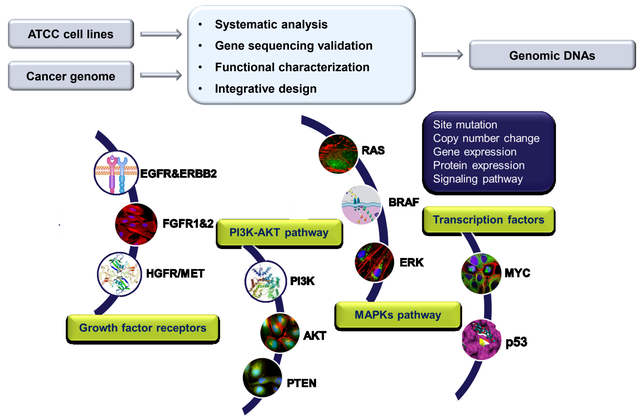
Methods: Genomic DNAs were extracted from over 70 commonly used human cancer cell lines derived from the breast, lung, colon, and pancreas, as well as hematopoietic and lymphoid tissue. Cancer gene mutations were identified by next-generation sequencing. Gene copy number changes were analyzed using the qBiomarker Copy Number PCR Assays kit (QIAGEN). Moreover, the selected cell lines were analyzed by quantitative polymerase chain reaction (qPCR), Western blot, and immunofluorescence (IF) staining to verify gene and protein expression mutation.

Results: Here, we present over 70 genomic DNAs isolated from authenticated cancer cell lines that contain desired biomarkers for oncological assay development. In addition to driver mutations such as BRAF V600, KRAS G12, PI3K E545 and EGFR T790, the gene copy number amplifications of AKT, FGFR, MET, and ERBB2, and the deletion of PTEN are presented in the cell lines from which the genomic DNAs were extracted. Our analysis shows the systematic molecular characterization and clustering of those human tumor cell lines, which represent the most common human cancer types found in the clinic, such as lung, breast, colon, pancreatic, and skin cancer. These cell lines were fully analyzed by next-generation sequencing to capture the driver gene mutations and allelic frequency. Gene DNA copy number variations were determined as well. Moreover, the gene expression, protein expression, and relevant cell signaling pathway activations have also been profiled. To be paired with mutations, a set of wild type controls derived from normal tissues were characterized in parallel.

Conclusions: Overall, genomic DNA from authenticated and well characterized cell lines provide suitable control materials to develop assays for genetic testing.

Introduction

ATCC tumor cell panels and related genomic DNAs



Results

Human cancer cell lines contain biomarkers – mutations and copy number variations

ATCC® No.	Cell line name	Gene	AKT1 copy number variation	Measured CNV of AKT1	AKT2 copy number variation	Measured CNV of AKT2	Tumor source
CRL-2321™	HCC1143	AKT1	Amplification	6.21	–	–	Breast
CRL-1469™	PANC-1	AKT2	–	–	Amplification	25.46	Pancreas
CRL-1622™	KLE	AKT2	–	–	Amplification	12.51	Endometrium
HTB-161™	NIH:OVCAR-3	AKT2	–	–	Amplification	9.78	Ovary
HTB-183™	NCI-H661	AKT2	–	–	Slight amplification	5.56	Lung

ATCC® No.	Cell line name	Gene	FGFR1 copy number variation	Measured CNV of FGFR1	FGFR2 copy number variation	Measured CNV of FGFR2	Tumor source
HTB-23™	MDA-MB-134-VI	FGFR1	Amplification	14.22	–	–	Breast
CRL-2066™	DMS 114	FGFR1	Amplification	7.17	–	–	Lung
CCL-235™	SW837	FGFR1	Slight amplification	3.9	–	–	Colon
CCL-246™	KG-1	FGFR1	Slight amplification	3.87	–	–	Leukemia
CRL-5974™	SNU-16	FGFR2	–	–	Amplification	451.16	Stomach
HTB-103™	KATO III	FGFR2	–	–	Amplification	138.62	Stomach

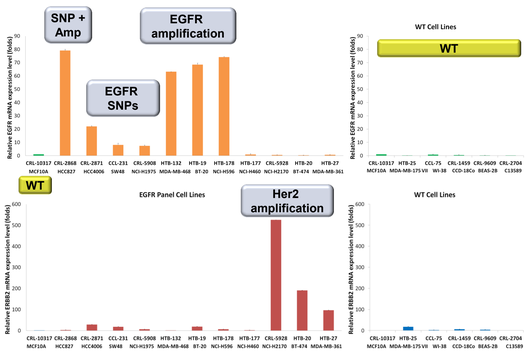
ATCC® No.	Cell line name	Gene	MYC copy number variation	Measured CNV of MYC	Tumor source
CRL-5974™	SNU-16	MYC	Amplification	50.48	Stomach
CRL-2081™	MSTO-211H	MYC	Amplification	38.92	Mesothelioma
HTB-175™	NCI-H82	MYC	Amplification	35.63	Lung
HTB-171™	NCI-H446	MYC	Amplification	19.06	Lung
CCL-240™	HL-60	MYC	Slight amplification	9.43	Leukemia

ATCC® No.	Cell line name	Gene	MET copy number variation	Measured CNV of MET	Tumor source
CRL-5973™	SNU-5	MET	Amplification	71.88	Stomach
HTB-135™	Hs 746T	MET	Amplification	23.96	Stomach
CRL-2351™	AU565	MET	Slight amplification	1.99	Breast

ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
CRL-2577™	RKO	MAPK3	p.R96R	c.288C>T	3346	C = 36.5, T = 40.5	Colon
		PIK3CA	p.H1047R	c.3140A>G	1359	A = 45.5, G = 54.4	
		BRAF	p.V600E	c.1799T>A	257	T = 29.6, A = 69.6	
HTB-9™	5637	MAPK1	p.R79K	c.236G>A	67777	G = 56.1, A = 43.8	Bladder
HTB-65™	MeWo	MAPK3	p.P246S	c.736C>T	9476	C = 41.4, T = 58.6	Skin
CRL-9446™	CHL-1	MAPK3	p.I228V	c.682A>G	9124	G = 99.8	Skin
HTB-2™	RT4	MAPK3	p.A109A	c.327G>A	14152	G = 62.6, A = 37.2	Bladder

ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-31™	C-33-A	PTEN	p.R233*	c.697C>T	65522	C = 51.9, T = 48.0	Cervix
HTB-111™	AN3 CA	PTEN	p.R130fs	c.389_389delG	14373	Deletion = 99.3	Endometrium
CRL-1718™	CCF-STTG1	PTEN	p.L112R	c.335T>G	20249	G = 99.6	Brain

Real time PCR analysis of mRNA levels



ATCC® No.	Cell line name	Gene	Amino acid Change	cDNA Change	NGS Coverage	% Zygosity	Tumor source
CCL-231™	SW48	EGFR	p.G719S	c.2155G>A	35993	G = 69.4, A = 30.3	Colon
CRL-5908™	NCI-H1975	EGFR	p.T790M	c.2369C>T	11704	C = 33.0, T = 66.9	Lung
			p.L858R	c.2573T>G	9441	T = 33.7, G = 66.2	

ATCC® No.	Cell line name	Gene	EGFR copy number variation	Measured CNV of EGFR	ERBB2 copy number variation	Measured CNV of ERBB2	Tumor source
CRL-2868™	HCC827	EGFR	Amplification	63.01	–	–	Lung
HTB-132™	MDA-MB-468	EGFR	Amplification	25.02	–	–	Breast
HTB-19™	BT-20	EGFR	Amplification	15.73	–	–	Breast
HTB-178™	NCI-H596	EGFR	Amplification	0.06	–	–	Lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	Lung
CRL-5928™	NCI-H2170	ERBB2	–	–	Amplification	128.89	Lung
HTB-20™	BT-474	ERBB2	–	–	Amplification	29.7	Breast
HTB-27™	MDA-MB-361	ERBB2	–	–	Amplification	16.85	Breast

ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
CRL-2177™	SW 1271	NRAS	p.Q61R	c.182A>G	26732	G = 99.8%	Lung
CRL-2273™	CHP-212	NRAS	p.Q61K	c.181C>A	49859	C = 50.7, A = 49.1	Brain
CRL-7585™	Hs 852.T	NRAS	p.G12V	c.35G>T	66411	G = 38.0, T = 61.8	Skin
CRL-9068™	NCI-H929	NRAS	p.G13D	c.38G>A	21896	A = 53.9, G = 45.9	Myeloma
TIB-202™	THP-1	NRAS	p.G12D	c.35G>A	60288	A = 70.1, G = 29.9	Leukemia
CRL-2547™	Panc 10.05	KRAS	p.G12D	c.35G>A	42708	G = 52.7, A = 47.3	Pancreas
CRL-2549™	Panc 03.27	KRAS	p.G12V	c.35G>T	58913	G = 47.0, T = 52.9	Pancreas
HTB-174™	NCI-H441	KRAS	p.G12V	c.35G>T	87521	G = 52.8, T = 47.1	Lung
CL-187™	LS 180	KRAS	p.G12D	c.35G>A	91234	G = 51.3, A = 48.6	Colon
CCL-225™	HCT-15	KRAS	p.G13D	c.38G>A	49764	G = 52.1, A = 47.8	Colon

ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-19™	BT-20	PIK3CA	p.H1047R	c.3140A>G	7062	A = 64.3, G = 35.6	Breast
HTB-131™	MDA-MB-453	PIK3CA	p.H1047R	c.3140A>G	10415	A = 35.6, G = 64.2	Breast
HTB-112™	HEC-1-A	PIK3CA	p.G1049R	c.3145G>C	6981	G = 38.8, C = 61.0	Endometrium
HTB-178™	NCI-H596	PIK3CA	p.E545K	c.1633G>A	2669	G = 68.5, A = 31.4	Lung
CRL-1739™	AGS	PIK3CA	p.E545A	c.1634A>C	9377	A = 23.6, C = 76.3	Stomach
CCL-237™	SW948	PIK3CA	p.E542K	c.1624G>A	13713	G = 52.7, A = 47.2	Colon
HTB-121™	BT-483	PIK3CA	p.E542K	c.1634A>C	11779	A = 49.8, C = 50.0	Breast
HTB-27™	MDA-MB-361	PIK3CA	p.E545K	c.1633G>A	4681	G = 79.7, A = 20.1	Breast
			p.K567R	c.1700A>G	916	A = 64.2, G = 35.8	

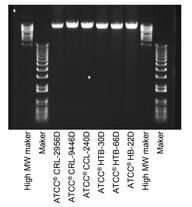
ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-66™	RPMI-7951	BRAF	p.V600E	c.1799T>A	1599	T = 62.5, A = 37.1	Skin
CCL-238™	SW1417	BRAF	p.V600E	c.1799T>A	3697	T = 58.6, A = 41.2	Colon
CRL-7898™	A101D	BRAF	p.V600E	c.1799T>A	5643	T = 43.8, A = 55.8	Skin
CCL-224™	COLO 201	BRAF	p.V600E	c.1799T>A	4122	T = 22.0, A = 77.8	Colon
CRL-1676™	WM-266-4	BRAF	p.V600D	c.1799_1800TG >AT	6776	T = 37.7, A = 62.1, G = 37.4, T = 62.5	Skin

ATCC® No.	Cell line name	Gene	Amino acid Change	DNA Change	NGS Coverage	% Zygosity	Tumor source
HTB-62™	P3HR-1	MYC	p.Y27S	c.80A>C	50463	A = 9.6, C = 89.9	Burkitt's lymphoma
			p.E54D	c.162G>A	66485	G = 10.8, A = 82.6	
			p.P72S	c.214C>T	68482	C = 17.6, T = 81.2	
			p.Q113H	c.339G>C	68395	G = 9.6, C = 89.2	
CRL-1648™	CA46	MYC	p.V20I	c.58G>A	22792	G = 12.1, A = 86.5	Burkitt's lymphoma
			p.P72S	c.214C>T	30456	C = 13.8, T = 85.3	
			p.P75H	c.224C>A	29467	C = 17.2, A = 80.3	
			p.L193V	c.577C>G	21112	C = 54.9, G = 44.7	
			p.Q321H	c.963G>C	30065	G = 50.1, C = 49.5	
CRL-1647™	ST486	MYC	p.Q51L	c.152A>T	50033	A = 8.1, T = 87.8	Burkitt's lymphoma
			p.P72T	c.214C>A	49383	C = 21.0, A = 77.7	
			p.T110P	c.328A>C	50765	A = 7.2, C = 92.2	
			p.A198V	c.593C>T	31630	C = 5.7, T = 93.0	

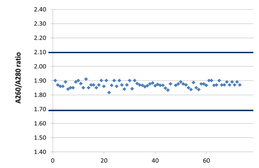
Genomic DNAs are produced by a validated automation system

Genomic DNA purity

Electrophoresis

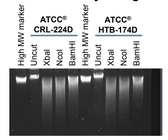


A_{260}/A_{280} ratios of 72 genomic DNAs

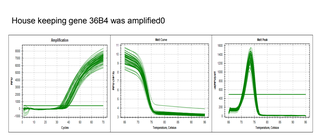


Genomic DNA functional tests

Restriction enzyme digestion



qPCR assay



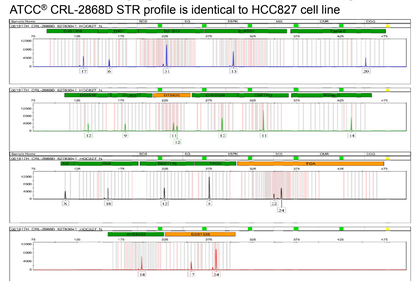
Next-generation sequencing – Illumina

Genomic DNAs extracted from different passages of cells were compared

Cell line	gDNA samples	Gene	AA Change	DNA Change	NGS Coverage	% Zygosity
RPMI-7951 (pl)	gDNA s1	BRAF	p.V600E	c.1799T>A	9244	T = 61.1, A = 38.8
RPMI-7951 (pm)	gDNA s2	BRAF	p.V600E	c.1799T>A	8787	T = 61.8, A = 38.1
RPMI-7951 (ph)	gDNA s3	BRAF	p.V600E	c.1799T>A	9644	T = 59.7, A = 40.2
Panc 10.05 (pl)	gDNA s4	KRAS	p.G12D	c.35G>A	56929	A = 53.5, G = 46.3
Panc 10.05 (pm)	gDNA s5	KRAS	p.G12D	c.35G>A	49775	A = 52.8, G = 47.0
Panc 10.05 (ph)	gDNA s6	KRAS	p.G12D	c.35G>A	49951	A = 52.7, G = 47.2

STR to verify genomic DNA identity

ATCC® CRL-2868D STR profile is identical to HCC827 cell line



Summary

Over 70 genomic DNAs isolated from authenticated human cancer lines contain the desired biomarkers for oncological analysis and assay development, which are useful tools in the nucleic acid-based detection for cancer.