SciForschen
Open HUB for Scientific Research

ISSN 2381-3318

International Journal of Cancer Research and Molecular Mechanisms

Case Report | Volume: 3.2 | Open Access

An Example of using Precision Medicine in Cancer Care: Dynamic Change of *TET2* Mutation in a Patient with Therapy-related Myelodysplastic Syndrome/ Acute Myeloid Leukemia

Peixian Chen[1], Yeong C Kim[2], Mojitaba Akhtari[1,3*] and San Ming Wang[2,4*]

[1]Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska, USA
[2]Department of Genetics, Cell Biology, and Anatomy, University of Nebraska Medical Center, Omaha, Nebraska, USA
[3]Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA
[4]Faculty of Health Sciences, University of Macau, Macau, China

***Corresponding authors:**

Mojitaba Akhtari, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA, **E-mail:** makhtari@usc.edu

San Ming Wang, Faculty of Health Sciences, University of Macau, Macau, China, **E-mail:** sanmingwang@umac.mo

Received date: 22 May 2017; **Accepted date:** 26 Jun 2017; **Published date:** 30 Jun 2017.

Citation: Chen P, Kim YC, Akhtari M, Wang SM (2017) An Example of using Precision Medicine in Cancer Care: Dynamic Change of *TET2* Mutation in a Patient with Therapy-related Myelodysplastic Syndrome/ Acute Myeloid Leukemia. Int J Cancer Res Mol Mech 3(2): doi http://dx.doi.org/10.16966/2381-3318.134

Abstract

Background: Therapy-related Myelodysplastic Syndrome/Acute Myeloid Leukemia (t-MDS/AML) is a serious complication for cancer patients undergoing chemotherapy or radiation treatment. Determining the mechanisms behind disease progression will help to design targeted treatment strategies. The objective of this study was to investigate the genetic basis for t-MDS/AML development.

Case: Exome sequencing analysis was performed for a t-MDS/AML patient to investigate the somatic mutation in coding genes at diagnosis, remission, allo-transplantation, and relapse.

Results: The study observed dynamic changes of somatic mutation during disease development. A TGTG insertion mutation was indentified at exon 3 in *Tet methylcytosine dioxygenase 2 (TET2)* at diagnosis. This mutation caused a frameshift, generating a pre-matured stop codon downstream, and leading to loss-of-function of *TET2*. At post-allotransplantation and relapse, this mutation decreased to basal levels.

Conclusions: The results suggest that the *TET2*-mutated clone might play more important roles in initiation than in relapse of t-MDS/AML, in which a new clone(s) with wild-type *TET2* may became dominant.

Keywords: Exome sequencing; Somatic mutation; tMDS/AML; *TET2*

Introduction

Chemotherapy and radiation in cancer patients can causea severe complication of therapy-related myelodysplastic syndrome/Acute myeloid leukemia (t-MDS/AML), a subgroup of MDS/AML disease [1,2]. Currently, successful preventative measures for this disease remain a challenge. The most effective treatment option is bone marrow allotransplantation with limited success [3,4]. Elucidating the genetic mechanism behind the development of t-MDS/AML is expected for developing new strategies for prevention and treatment of this disease.

Genetic factors are considered as the major cause for developing *de novo* MDS/AML, a more broad disease group compared to t-MDS/AML. Studies have extensively tested for genetic abnormalities, which led to the identification of mutations in multiple functionally important genes, including *TET2, TP53, DNMT3A, ASXL1, IDH1,* and *EZH2* [5,6]. Taking *TET2* as an example: *TET2* regulates DNA methylation though converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [5]. Mutated *TET2* fails to complete this conversion, can cause cancer-affecting particularly the myeloid lineage [6,7]. Multiple studies revealed that somatic mutations in *TET2* occur in *de novo* MDS/AML at high frequency [8-12]. A study in t-MDS/AML detected *TET2* mutations in four out of 38 (10.5%) t-MDS/AML cases [13]. However, evidence is lacking to determine if the same genetic defficiencies in *de novo* MDS/AML are responsible for t-MDS/AML. Further, why allotransplantation is not as effective in curing t-MDS/AML as in curing *de novo* MDS/AML remains unknown.

In the present study, we used exome sequencing [14] to analyze somatic mutations in coding genes in a t-MDS/AML patient at the disease stages of diagnosis, 69-days post-allotransplantation, 90-days post-allotransplantation, and relapse. Comparison of the mutations identified during the different disease stages revealed their dynamic changes during disease development. Among the mutations included a TGTG-insertion mutation in *TET2,* a gene well-known for its roles in *de novo* MDS/AML. We observed its highly presence at diagnosis but diminished at post-allotransplantation and relapse. This suggests that the *TET2*-mutated clone played important roles in initiating but not in relapse of the disease.

Case

Ethics statement

The subject involved in the study was informed using a questionaire addressing the following topics: reason behind study, purpose, study methods, risks, benefits to patient as well as others, alternatives to the study, renumeration, access to care at all times, protection of information, subject rights, and electing not to participate. The subject agreed to each of the questions, and signed in the consent form to participate in the study. The study was approved by the Institutional Review Board of University of Nebraska Medical Center under the protocol (488-11-FB).

Samples collection and DNA preparation

Samples were collected from the patient through the following: FFPE (Formalin Fixed Paraffin Embedded) fixed bone marrow cells at the diagnosis of t-MDS/AML, peripheral blood cells at pre-transplant (used only in Sanger sequencing validation), bone marrow samples at 69-days post allotransplantation, and bone marrow samples at 90-days post allotransplantation. A skin biopsy sample from the patient was collected before transplantation. A sample from peripheral blood cells of the bone marrow donor was also collected. Mononuclear cells from bone marrow and pripheral vein blood post allotransplantations were isolated by using Ficoll solution, and DNA was extracted from mononuclear cells using FlexiGene DNA kit (QiaGen, Coourtaboeuf, France). DNA from FFPE fixed cells at diagnosis and from skin biopsy were extracted using QIAamp kit following the manufacturer's protocol.

Exome sequencing and mapping

Exome sequencing followed the standard Illumina exome procedure (Illumina, San Diego, CA, USA). The process included DNA fragmentation, adapter ligation, and exome fragment capturing using the TruSeq Exome Enrichment Kit v2 (Illumina). Sequences were collected in a Illumina HiSeq2000 sequencer using the paired-end mode (2X100). The following steps were used to process the exome sequences: (a) sequences were mapped to the human genome reference sequence human genome 19 (hg19) using Bowtie 2 sofware [15]; (b) resulting SAM files were converted to BAM files; (c) duplicates were removed using Picard (http://picard.sourceforge.net); (d) GATK-RealignerTargetCreator was used to complete local realignment [16]; (e) VarScan 2 was used to call variants [17]; (f) somatic single nucleotide polymorphisms (SNP) and indel variants were identified through filtering public databases including the Single Nucleotide Polymorphism Database 137 (dbSNP137), 1000 Genomes, Exome Variant Server 6500 (http://evs.gs.washington.edu/EVS/) (MAF>0.01). The variants from the patient samples were filtered with those from skin biopsy and donor cells to identify the somatic mutations present in cancer samples only. Mutations causing nonsynonymous, splicing change, and stop gain/loss were identified. Those causing damage effects were then predicted using ANNOVAR [18], which predicts deleterious effects by at least one of the six prediction programs of Sorting Intolerant From Tolerant (SIFT) [19], PolyPhen2 [20], LRT [21], MutationTaster [22], PhyloP [23], and GERP++ [24]. Sanger sequencing was used to validate the *TET2* insert mutations.

Results

The patient analyzed in the study was a male Caucasian who at age 59 developed folicular lymphoma. Upon combinational chemotherapy with fludarabine, mitoxantrone, and dexamethasone, the patient achieved remission. He relapsed three years later and remissed upon treatment with rituximab. He relapsed again three years later and remissed again upon treatment with rituximab. Three year's later, the patient developed thrombocytopenia, and had no response to prednisone. Bone marrow examination revealed hypercellular bone marrow (50%) with megakarryocytic hyperplasia and dyspoiesis, along with an increased number of blast cells (20%). Cytogenetic analysis revealed the presence of two karyotype lines of 46, XY in 17 out of 21 metaphases and complex karyotype of 42-44, add(X)(q13),del(2)(p21),add(4)(q31),del(5)(q13q35),-6,-7,add(10)(q26),-18+1-2mar[8]/46,XY[12] in 4 out of 21 metaphases. The patient was diagnosed as t-MDS/AML. Upon two-cycle treatment of decitabibe, myeloblast cells in bone marrow decreased to 2%. Subsequently, the patient received bone marrow allotransplantation following standard care from the Stem Cell Transplant Center at the University of Nebraska Medical Center. Further, the patient received combinational treatment of fludarabine and busulfan. Bone marrow examination at 90-days post allotransplantation showed 5% blasts. Flowcytometry with 15-CD markers showed residual myelodysplasia. Cytogenetic analysis showed normal karyotype of 46 XY. MDS-specific FISH analysis [del5q31, KMT2A (MLL; 11q23) locus rearrangement, trisomy 8, monosomy 7, deletions of 7q31, 20q12 and 20q13] showed no sign of MDS-related abnormalities. The patient died three months later, at age 69.

DNA was extracted from bone marrow cells of the patient at the time of t-MDS/AML diagnosis, 69-days post-allotransplantation, and 90-days post-allotransplantation. DNA was also extracted from skin of the patient and from the peripheral blood cells of the allotransplant donor. DNA samples were used for exome sequencing. Genomic variants were called from each sample. Variants of normal polymorphisms were removed by filtering through dbSNP, 6500 Exomes, and 1000 Genome databases with a minor allele frequency (MAF)>0.01 as the cut-off. Further, the variants shared with those from patient's skin, and the variants in post-transplantation samples shared with donor's sample, were removed. From the remaining vaiants, nonsynonymous deleterious single nucleotide variation (SNV) and indels were identified at different disease stages: six mutations at diagnosis, 15 mutations at 69-days post-allotransplantation, and 20 mutations at 90-days post-allotransplantation (Tables 1 and 2). The mutations between the three stages were compared and showed that no mutations were shared between diagnosis and post-transplantation; eight mutations were shared between 69-days and 90-days post-transplantation (Figure 1).

Among the mutations detected, a four base insertion (TGTG) mutation at exon 3 of *TET2* was identified at diagnosis but not post-transplantation. Of the 2,002 amino acid residues of *TET2* (RefSeq NP_001120680.1), the insertion mutation at 990 caused a frameshift, generated a stop codon TAA at 1009, and led to the loss-of-function of *TET2* (Figure 2A). Comparing with the mutations identified in the t-MDS/AML study [13] showed that this mutation is a novel mutation in t-MDS/AML (Figure 2B).

Sanger sequencing was used to validate the *TET2* mutation at different stages of disease development. The mutation was confirmed to be a true somatic mutation. It was present at diagnosis, absent before transplantation, and reappeared post-transplantation at relapse, albeit at very low levels that was under the threshield of variant calling by the standard exome mapping conditions (Figure 3). To provide quantitative information for the *TET2* mutation, the ratio in Sanger sequence signals was compared between the wild-type GCCT and the insert TGTG at the same positions in the samples of diagnosis, 69-days post allotransplantation, and 90-days post allotransplantation. The results showed that the TGTG insert signal reached 35% of the wild-type GCCT signal at diagnosis, but the rates decreased to 9% at 69-days and 4% at 90-days post allotransplantation (Table 3). The number of wild-type GCCT sequences and insert TGTG sequences in the exome data were also counted, showing that the mutated sequences accounted for 14% (5/35) at diagnosis, 6% (4/64) at 69-days, and 6% (3/51) at 90-days post-allotransplantation. The two sets of quantitative results indicated that the mutated TET2 sequences were at much higher levels at diagnosis, but diminished at relapse and post allotransplantation.

Discussion

Precision medicine has been proposed to be a new model of medicine. By following each patient's disease process at genomic and molecular level, precision medicine provides hope to identify specific genetic markers for targeted treatment. Therefore, understanding the genetic changes during disease development is important to reach this goal. Instead of testing mutations once in a patient during disease, our study analyzed the somatic mutations in a t-MDS/AML patient across the entire disease development to trace the fate of somatic mutation during disease development. The results revealed dynamic changes of multiple somatic mutations as best

Table 1: Exome sequencing and mapping results.

Samples	Total reads	Total mapped reads	Total bases	Mapping rate	Coverage	Coverage at target regions*	Variants			
							Called variants		Somatic mutations	
							SNV	Indel	SNV	Indel
Diagnosis	49,472,270	48,961,020	4,996,699,270	99.00%	80	45	97,143	11,683	5	1
Post-transplant (69 days)	117,742,496	117,141,336	11,891,992,096	99.50%	192	103	131,837	14,869	13	1
Post-transplant (90 days)	123,512,958	122,895,392	12,474,808,758	99.50%	201	109	134,888	15,172	16	2
Skin	45,503,560	44,860,089	4,595,859,560	98.60%	74	39	71,589	6,832	NA	NA
Donor	95,402,302	95,070,506	9,635,632,502	99.70%	155	80	116,776	12,112	NA	NA

*Target regions: Illumina TruSeq Exome Enrichment Kit with 62,085,295 bp, only includes the bases within the targets were counted.

Table 2: Somatic mutations identified in the t-MDS/AML case.

Gene	Diagnosis	Relapse		Chr.	Position	Mutation type	RefSeq ID	Base change	Amino acid change	Damage*
		69 days	90 days							
CDC27	+	-	-	17	45214527	nonsynonymous SNV	NM_001114091	c.A1922G	p.Y641C	+
GOLGA8N	+	-	-	15	32895903	nonsynonymous SNV	NM_001282494	c.G1708A	p.A570T	+
LYZL2	+	-	-	10	30918597	nonsynonymous SNV	NM_183058	c.A38G	p.K13R	+
NBPF12	+	-	-	1	146412201	nonsynonymous SNV	NM_001278141	c.T2185G	p.S729A	+
TET2	**+**	**-**	**-**	**4**	**106158069**	**frameshift insertion**	**NM_001127208**	**exon3:c.2970_2971ins**	**p.H990fs**	**+**
TP53	+	-	-	17	7573996	nonsynonymous SNV	NM_001126115	c.T635C	p.L212P	+
AGAP8	-	+	+	10	51225970	nonsynonymous SNV	NM_001077686	c.A1012G	p.N338D	+
C2CD3	-	+	+	11	73796902	nonsynonymous SNV	NM_001286577	c.A3671G	p.Q1224R	+
CCDC57	-	+	+	17	80059734	nonsynonymous SNV	NM_198082	c.G2572A	p.A858T	+
KIR2DL3	-	+	+	19	55255357	nonsynonymous SNV	NM_015868	c.G485C	p.R162T	+
PLGRKT	-	+	+	9	5361143	nonsynonymous SNV	NM_018465	c.C257G	p.P86R	+
SLC2A3	-	+	+	12	8074055	nonsynonymous SNV	NM_006931	c.A1445G	p.E482G	+
SLC39A3	-	+	+	19	2733281	nonsynonymous SNV	NM_144564	c.T413C	p.M138T	+
SUPT20HL1	-	+	+	X	24382373-9	nonframeshift insertion	NM_001136234	exon1:c.1496_1497ins	p.I499delinsIAA	+
AHNAK2	-	+	-	14	105415655	nonsynonymous SNV	NM_138420	c.C6133G	p.L2045V	+
AHNAK2	-	+	-	14	105415666	nonsynonymous SNV	NM_138420	c.C6122T	p.P2041L	+
FAM86B2	-	+	-	8	12289662	nonsynonymous SNV	NM_001137610	c.C169T	p.H57Y	+
MALL	-	+	-	2	110845196	nonsynonymous SNV	NM_005434	c.G352C	p.E118Q	+
SLC25A5	-	+	-	X	118605001	stopgain SNV	NM_001152	c.G877T	p.E293X	+
TPSD1	-	+	-	16	1306817	nonsynonymous SNV	NM_012217	c.G274A	p.A92T	+
CYP2D6	-	-	+	22	42523636	nonsynonymous SNV	NM_001025161	c.G833T	p.R278L	+
FAM86B1	-	-	+	8	12047388	nonsynonymous SNV	NM_001083537	c.C169T	p.H57Y	+
GTF2I	-	-	+	7	74149837-40	frameshift deletion	NM_001518	exon15:c.1275_1278del	p.425_426del	+
KIR2DL1	-	-	+	19	55295215	nonsynonymous SNV	NM_014218	c.A997G	p.T333A	+
KMT2C	-	-	+	7	151945225	nonsynonymous SNV	NM_170606	c.A2294G	p.E765G	+
KRTAP9-9	-	-	+	17	39411670-85	nonframeshift insertion	NM_030975	exon1:c.33_34ins	p.P11delinsPTCCRT	+
SPATA31A4	-	-	+	9	41323271	nonsynonymous SNV	NM_001113541	c.C2033A	p.T678N	+
SPATA31A5	-	-	+	9	65504652	nonsynonymous SNV	NM_001113541	c.C2908T	p.L970F	+
TRIM49C	-	-	+	11	89774540	nonsynonymous SNV	NM_001195234	c.C1181A	p.T394N	+
USP17L11	-	-	+	4	9251645	nonsynonymous SNV	NM_001256854	c.G1290C	p.Q430H	+

*Predicted as damaging by at least one of the prediction programs of SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, RadialSVM and LR.

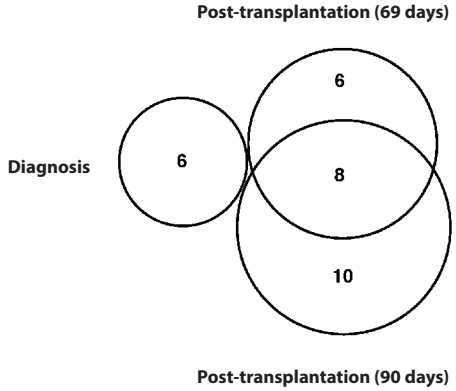

Figure 1: Dynamic changes in somatic mutations during disease development-Somatic mutations identified at diagnosis, relapse 69 days post-transplantation and 90 days post-transplantation were compared. No mutations were shared between diagnosis and post transplantations, and 8 mutations were shared between 69 days and 90 days post transplantation.

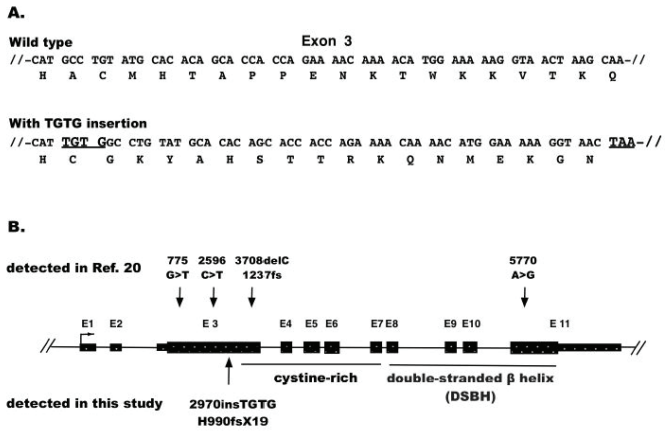

Figure 2: *TET2* mutation. A) TGTG-insertion mutation. It is located at exon 3, causing a coding frame shift and creating a pre-terminate TAA codon downstream. B) Positions of mutations in *TET2* in t-MDS/AML. The four mutations marked at the top level were identified by the study [20], the mutation marked at the bottom level was identified by this study.

examplified by the mutation in *TET2*, a gene known to play important roles in *de novo* MDS/AML [5-13].

As part of treatment for t-MDS/AML, successful allotransplantation is expected to replace the hematopoietic system of the patient with that of the bone marrow donor, allowing the patient to regain normal hematopoietic function. However, the relapse of the patient post transplantation implies that this did not happen, or happened transiently such that the hematopoietic system of the patient gained dominance and was responsible for the relapse. However, the clone(s) at the time of relapse was unlikely the same as the one at the time of diagnosis, as evidenced by (1) very different mutation pools at diagnosis and relapse (Figure 1), and (2) higher-level the mutated *TET2* at diagnosis but lower at the relapse (Figure 3 and Table 3), which was concurrent with lower blast rate at the relapse. It is possible that at the time of the last biopsy 90-days post transplantation, the *TET2*-mutated clone was still rare but became dominant afterwards. However, this possibility was not supported by the cytogenetic and FISH data at 90-days post-allotransplantation, which showed a normal karyotype and no sign for MDS-specific abnormalities.

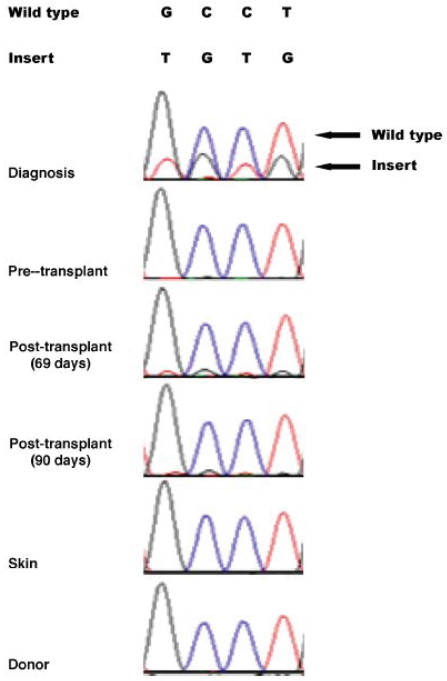

Figure 3: Dynamic presence of *TET2* TGTG insertion at different disease stages revealed by Sanger sequencing-The *TET2* TGTG insertion was highly present at diagnosis, absent pre-transplantation, and appeared again at post-transplantation and relapse but at basal levels. The same mutation was absent in patient's skin and donor's cells.

Table 3: Quantitative relationship between wild type and insert signals in TET2.

	Base	Relative signal height		
		Diagnosis	69 days post transplant	90 days post allotransplant
Wild type	G	5.5	6	6.2
	C	3.5	3.5	3.8
	C	3.5	3.5	3.9
	T	3.9	4	4.2
TGTG Insert	T	1.3	0.4	0.2
	G	1.6	0.5	0.3
	T	1.1	0.2	0.1
	G	1.6	0.4	0.1
Relative ratio	G/T	0.24	0.07	0.03
	C/G	0.46	0.14	0.08
	C/T	0.31	0.06	0.03
	T/G	0.41	0.10	0.02
Relative ratio/base		0.35	0.09	0.04

Our explanation is that the *TET2*-mutated clone contributed significantly in initiation but not in relapse of the t-MDS/AML patient.

Conclusions

The results from our study indicate that following dynamic change of somatic mutations in disease-specific genes allows more precise monitoring genetic basis of disease develoopment and will help selection of personized treatment for patients.

Data Deposition and Access

The exome data were deposited in European Nucleotide Archive (ENA) with accession ID: PRJEB18718

Conflict of Interest

Authors declare no competing interests in the study.

Author Contributions

PC performed the experiments, YK performed bioinformatics data analysis, MA identified and provided patient materials, MA, SMW conceived the study, SMW designed the study, and wrote the manuscript.

Acknowledgments

The study was supported by a grant from Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center (MA) and a pilot grant from Fred & Pamela Buffett Cancer Center, University of Nebraska Medical Center (SMW).

References

1. Rowley JD, Golomb HM, Vardiman JW (1981) Nonrandom chromosome abnormalities in acute leukemia and dysmyelopoietic syndromes in patients with previously treated malignant disease. Blood 58: 759-767.
2. Pedersen-Bjergaard J, Philip P, Larsen SO, Andersson M, Daugaard G, et al. (1993) Therapy-related myelodysplasia and acute myeloid leukemia. Cytogenetic characteristics of 115 consecutive cases and risk in seven cohorts of patients treated intensively for malignant diseases in the Copenhagen series. Leukemia 7: 1975-1986.
3. Witherspoon RP, Deeg HJ, Storer B, Anasetti C, Storb R, et al. (2001) Hematopoietic stem-cell transplantation for treatment-related leukemia or myelodysplasia. J Clin Oncol 19: 2134-2141.
4. Luger S, Sacks N (2002) Bone marrow transplantation for myelodysplastic syndrome--who? when? and which? Bone Marrow Transplant 30: 199-206.
5. Hu L, Li Z, Cheng J, Rao Q, Gong W, et al. (2013) Crystal structure of TET2-DNA complex: insight into TET-mediated 5mC oxidation. Cell 155: 1545-1555.
6. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, et al. (2010) Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 468: 839-843.
7. Muto T, Sashida G, Oshima M, Wendt GR, Mochizuki-Kashio M, et al. (2013) Concurrent loss of Ezh2 and Tet2 cooperates in the pathogenesis of myelodysplastic disorders. J Exp Med 210: 2627-2639.
8. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, et al. (2009) Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet 41: 838-842.
9. Kosmider O, Gelsi-Boyer V, Cheok M, Grabar S, Della-Valle V, et al. (2009) TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). Blood 114: 3285-3291.
10. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, et al. (2009) Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. Blood 113: 6403-6410.
11. Bacher U, Haferlach C, Schnittger S, Kohlmann A, Kern W, et al. (2010) Mutations of the TET2 and CBL genes: novel molecular markers in myeloid malignancies. Ann Hematol 89: 643-652.
12. Smith AE, Mohamedali AM, Kulasekararaj A, Lim Z, Gäken J, et al. (2010) Next-generation sequencing of the TET2 gene in 355 MDS and CMML patients reveals low-abundance mutant clones with early origins, but indicates no definite prognostic value. Blood 116: 3923-3932.
13. Shih AH, Chung SS, Dolezal EK, Zhang SJ, Abdel-Wahab OI, et al. (2013) Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia. Haematologica 98: 908-912.
14. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, et al. (2011) Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 12: 745-755.
15. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357-359.
16. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20: 1297-1303.
17. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, et al. (2012) VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 22: 568-576.
18. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38: e164.
19. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 4: 1073-1081.
20. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, et al. (2010) A method and server for predicting damaging missense mutations. Nat Methods 7: 248-249.
21. Chun S, Fay JC (2009) Identification of deleterious mutations within three human genomes. Genome Res 19: 1553-1561.
22. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D (2010) MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 7: 575-576.
23. Siepel A, Pollard KS, Haussler D (2006) New methods for detecting lineage-specific selection. Proceedings of the 10th International Conference on Research in Computational Molecular Biology (RECOMB 2006) 190–205.
24. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, et al. (2010) Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol 6: e1001025.