中文題目： 全轉錄體定序分析顯示新奇的融合基因變異在染色體第七對部分缺失的急性骨髓性白血病病人

英文題目： Whole transcriptome sequencing reveals novel gene fusions in acute myeloid leukemia patients with chromosome 7q partial deletion

作　　者： 林明恩[1], 陳建源[1*], 黃彥華[2], 邱賀晨[2], 徐瑞澤[2], 蔡孟勳[3], 楊永正[2], 田蕙芬[1], 鄭安理[1, 4]

服務單位：[1]台大醫院內科部, [2]陽明大學生物資訊所, [3]台大基因體中心, [4]台大醫院腫瘤部

Background:

Next generation sequencing is a novel tool to explore the delicate cancer lesions of human genome in recent years. Although around 20 oncogenes had been found to involve the mechanism of acute myeloid leukemia (AML), the pathogenesis of AML is still under investigated. Chromosome 7q deletion / monosomy 7 is a disease entity in acute myeloid leukemia, which usually is associated with complex cytogenetic changes and is clinically manifested with poor therapeutic response. We use the method of whole transcriptome sequencing to explore the genetic lesions in AML patients with chromosome 7q deletions.

Materials and Methods:

The leukemic marrow samples were collected after inform consent. The leukemic cells were separated with Ficoll-Hypaque solution and cryopreserved with trizol. The quality and quantity of total RNA were checked to fit the limit of whole transcriptome sequencing. Total RNA was negatively selected with ribosome RNA magnetic beads, and then the mRNA was created as the transcriptome library as the Illumina's and Applied Biosystem SOLiD system guide. The transcriptome library was pair-end with bilateral 75 base pairs (bps) and a 350bp insert.

The reads were trimmed according to the quality score. Then the reads were aligned to NCBI human genome hg19 (GRCh37) and transcriptome. The fusion analysis was performed by pipeline FusionDetector. The point mutation and single nucleotide polymorphism were performed by Polyphen-2, and Condel.

Result

1. Quality and quantity of whole transcriptome sequencing

Whole transcriptome sequencing were performed in 8 AML marrow samples. Seven patients were chromosome 7q partial deletion and one patient was inv(16) as the control to detect the fusion CBFB/MYH11. The mean reads in each transcriptome were 450,421,127 (ranged from 247,946,931 to 708,972,354). The alignment was ranged from 50-60% .

2. Methods to detect fusion genes in whole transcriptome sequencing

There were several bioinformatics methods to detect the fusion genes in current literature. Our bioinformatic group modified and created the pipeline fusiondetector to detect the fusion lesions.

Thousands of candidate fusion genes were calculated in each transcriptome. The control fusion gene CBFB/MYH11 could be detected in the pipeline fusion-detector. We find the candidate fusion gene and validated by PCR and sequencing.

3. Novel fusion genes in AML patients

There were no recurrent fusion genes detected in the AML with chromosome 7q partial deletion. NSD1/NUP98 fusion gene was found in a patient 46,XX,add(7)(q22)[2]/46,XX,del(3)(?q13q21), del(11)(?p13p15)[1]/46,XX[9]. TPM4/KLF2 fusion gene was found in a patient with 46,XY,del(7)(q22q36)[8]/46,XY[2]. TALDO1/DSN1 fusion gene was found in a patient with 43,XY,-5,add(7)(q22),add(11)(p15),add(12)(p11),-16,del(18)(p11),-19, add(20)(q13), dic(?;22)(?;p12)[11] / 46,XY[9]. DSN1 is a key component gene of kinetochore complex. Disrupt the DSN1 gene could cause abnormal function of mitosis. This fusion genes has been validated by PCR and sequencing. Further molecular cloning and functional study is ongoing.

4. Small indels (insertion, deletion, and inversion) and point mutations in transcriptome sequencing

Conclusion

AML patients with chromosome 7q deletion are a heterogeneous group with occult genetic change. Whole transcriptome sequencing and massive parallel analysis with bioinformatics could detect the occult fusion genes of human cancer genome. The point mutation, SNP and small indels such as insertion, inversion, and deletion also could be found by transcriptome sequencing. The leukemogenesis could be further explored with the technique of next generation sequencing.