Juvenile myelomonocytic leukemia with CBL mutation: two cases report and literature review

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Abstract

Juvenile myelomonocytic leukemia (JMML) is an aggressive clonal hematopoietic disorder of infancy and early childhood. About 15% of these patients have CBL mutation, which is usually a germline mutation with a high incidence of CBL syndrome. Conventional chemotherapy would be little benefit to these children, but epigenetic therapy with the DNA-hypomethylating agents can make a great difference in disease control. Here we report two infants diagnosed as JMML with CBL mutation. One case was treated with 6-mercaptopurine intermittently, but she was often hospitalized for pneumonia since the disease was not well controlled. The other one was treated with decitabine. He achieved clinical complete remission (CR) after three cycles of decitabine (20mg/m2/d×5 days, repeated every 4 weeks). Unfortunately, the patient's symptoms were recurrent two months later. Thus, JMML patient with CBL mutation has a good clinical response to decitabine, while how and how long it could be used remain to be further explored.

Background

Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disorder of childhood characterized by an excessive proliferation of cells of monocytic and granulocytic lineages with an incidence rate of 1.2 per million per year.[1]The main clinical manifestations of JMML were splenomegaly, thrombocytopenia, peripheral monocyte proliferation, elevated hemoglobin F , and sensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF).[2-4] Almost 90% of patients carry at least one genetic mutation of PTPN11, KRAS, NRAS, CBL, or NF1. According to the diagnostic criteria of the WHO in 2016 and the latest relevant reports, nearly 15% of JMML patients carry CBL mutation. [1, 5, 6]

In recent years, it is considered aberrant methylation of specific genes leads to abnormal signal pathways in patients with JMML, and the degree of methylation is closely related to the prognosis and complications of patients. Demethylation therapy may relieve patients' symptoms and prolong survival time.[7],[8]Here we report two patients were diagnosed with JMML with CBL mutation. One patient was treated with 6-mercaptopurine and the other one was treated with decitabine.

Patient 1

An eleven months old girl was suspected to JMML because of abnormal blood routine, lymphadenopathy, eczema-like dermatitis of the right leg, abdominal distention, the spleen were palpable 5 cm and the liver was 3 cm below the costal margin. Complete blood cell count showed leukocytosis (52.01×10^9/L), thrombocytopenia (68×10^9/L), anemia (99g/L), and striking monocytosis (11.35×10^9/L). The bone marrow (BM) aspirate revealed no blast cells and karyotype on BM cells was 46, XX. No BCR/ABL fusion transcript was found. The diagnosis of JMML was confirmed by the detection of a germline CBL (Y371H) mutation. Her

mother has the same mutation, but her condition was relieved spontaneously. Since the diagnosis, the patient was treated with 6-mercaptopurine intermittently according to the white blood cell count. Her white blood cell count is controlled at 5-15$\times10^9$/L, while platelet count fluctuates between 20$\times10^9$/L and 150$\times10^9$/L. She still has splenomegaly and is often hospitalized for pneumonia and gastroenteritis.

Patient 2

A six months old boy was diagnosed with the JMML because of the whole body hemorrhagic spot, cough, pulmonary infection. The spleen was palpable 2 cm below the costal margin. Blood routine showed leukocytosis (53.4$\times10^9$/L), thrombocytopenia (77$\times10^9$/L), and striking monocytosis (15.49$\times10^9$/L). The bone marrow (BM) revealed with 3% blast cells. No BCR/ABL fusion transcript and karyotype on BM cells was 46, XY. A genetic test showed that he had a germline CBL(C401Y) mutation, while this mutation was also detected in his father and sister with no clinical symptoms. Then we treated him with a DNA-hypomethylating agent which takes a course for every 4 weeks with 5 days of continuous decitabine (20mg/m^2/d). After three courses of treatment, the patient's spleen was significantly shrinking, with no obvious granulocytopenia and no other inflammation. He achieved complete remission. However, shortly after drug withdrawal, he developed splenomegaly again, accompanied by enteritis, axillary abscess, striking monocytosis, thrombocytopenia. Further vascular events occurred six months later that he developed inflammatory optical neuropathy in both two eyes, resulting in the loss of visual acuity. We treated him with glucocorticoid and gamma globulin in order to restore his vision but failed.

Discussion

JMML is characterized by excessive proliferation of monocytes and granulocytes. The leukemia cells can infiltrate the lung, liver, and spleen, leading to early respiratory insufficiency, hepatosplenomegaly, bleeding tendency, and even the possibility of transformation into acute myeloid leukemia (AML), which may lead to death in severe cases.[9],[10]JMML patients with CBL mutation generally have a higher survival rate and spontaneous remission rate.[11]*Locatelli, F and Tasian, S. K* suggested that these patients could adopt the "wait and watch" method, or the use of 6-mercaptopurine(6-MP) to prevent complications caused by tumor cell invasion.[5, 12, 13]In our center, one patient was treated with 6-MP, but the patient was often hospitalized because of thrombocytopenia, axillary lymphadenitis, enteritis, broncahopneumonia and other infiltrative reactions.

The existence of epigenetic alterations in JMML was certified by pilot studies of DNA hypermethylation at candidate gene loci.[14-16] Based on the degree of DNA methylation, patients in JMML are divided into three risk groups, of which CBL mutation is classified as the low methylation (LM) group.[17],[18]Through genetic testing, we found that there are healthy individuals carry CBL mutation without any clinical manifestations in these two families. This situation was also reported by *Kazuyuki Matsuda and Chiaki Taira* .[19]This may be related to the phenotypic differences caused by gene modification in epigenetics[8]. For those with clinical symptoms, germline mutations will persist even if complete remission is achieved after treatment,[20] and vasculitis is more likely to occur at a later stage of the disease.

By comparing other treatment options, JMML patients can be treated individually by judging the level of DNA methylation.[4]Decitabine is a potent DNA methylation-specific inhibitor, through S phase in cell proliferation irreversible inhibition of DNA methyltransferase. Decitabine can inhibit tumor cell proliferation as well as prevent drug resistance. Some clinical data show that decitabine can effectively improve the response rate and increase the median overall survival.[6],[10]In our case, we put forward for the first time in decitabine for low-dose and short-term treatment of CBL mutation in JMML. Our patient does have obvious clinical effects after the application of decitabine. After three courses, he reached complete remission(CR) according to the evaluation criteria.[20] A few months after he stopped using DNA-hypomethylating agents, his symptoms were recurrent and developed into irreversible binocular blindness caused by vasculitis. Animal experiments demonstrated that CBL mutation could lead to the corresponding protein deficiency in mice, accompanied by severe vasculitis and other pathological changes.[5, 21, 22]The reason is that CBL gene can regulate the stability and signal amplitude / duration of the JAK2. By transducing inflammatory signals downstream of

cytokines to regulate the development and function of immune cells.[23, 24],[25]Aberrant methylation of the target gene CpG island leads to abnormal activation of signal pathways, make massive abnormal T and B cells infiltrate blood vessels and organs, resulting in inflammation.[5, 6, 14]

The treatment experience of these two cases confirmed the positive demethylation effect of decitabine for children with JMML in CBL mutation, but the recurrence after withdrawal suggests that how to use it to control the disease as well as to prevent the occurrence of side effects. It remains to be further explored Whether DNA-hypomethylating agents can reduce the vasculitis in patients with CBL mutation.

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REFERENCES

[1]D. A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M. J. Borowitz, M. M. Le Beau, C. D. Bloomfield, M. Cazzola and J. W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood, 127, (2016), 2391-2405

[2]C. M. Niemeyer and C. P. Kratz, Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options, Br J Haematol, 140, (2008), 610-24

[3]T. I. Mughal, N. C. Cross, E. Padron, R. V. Tiu, M. Savona, L. Malcovati, R. Tibes, R. S. Komrokji, J. J. Kiladjian, G. Garcia-Manero, A. Orazi, R. Mesa, J. P. Maciejewski, P. Fenaux, R. Itzykson, G. Mufti, E. Solary and A. F. List, An International MDS/MPN Working Group's perspective and recommendations on molecular pathogenesis, diagnosis and clinical characterization of myelodysplastic/myeloproliferative neoplasms, Haematologica, 100, (2015), 1117-30

[4]M. Strullu, A. Caye, J. Lachenaud, B. Cassinat, S. Gazal, O. Fenneteau, N. Pouvreau, S. Pereira, C. Baumann, A. Contet, N. Sirvent, F. Mechinaud, I. Guellec, D. Adjaoud, C. Paillard, C. Alberti, M. Zenker, C. Chomienne, Y. Bertrand, A. Baruchel, A. Verloes and H. Cave, Juvenile myelomonocytic leukaemia and Noonan syndrome, J Med Genet, 51, (2014), 689-97

[5]F. Locatelli and C. M. Niemeyer, How I treat juvenile myelomonocytic leukemia, Blood, 125, (2015), 1083-90

[6]K. Sakashita, K. Matsuda and K. Koike, Diagnosis and treatment of juvenile myelomonocytic leukemia, Pediatr Int, 58, (2016), 681-90

[7]Y. Jiang, A. Dunbar, L. P. Gondek, S. Mohan, M. Rataul, C. O'Keefe, M. Sekeres, Y. Saunthararajah and J. P. Maciejewski, Aberrant DNA methylation is a dominant mechanism in MDS progression to AML, Blood, 113, (2009), 1315-25

[8]S. McPherson, M. F. McMullin and K. Mills, Epigenetics in Myeloproliferative Neoplasms, J Cell Mol Med, 21, (2017), 1660-1667

[9]J. Melo-Cardenas, Y. Xu, J. Wei, C. Tan, S. Kong, B. Gao, E. Montauti, G. Kirsammer, J. D. Licht, J. Yu, P. Ji, J. D. Crispino and D. Fang, USP22 deficiency leads to myeloid leukemia upon oncogenic Kras activation through a PU.1-dependent mechanism, Blood, 132, (2018), 423-434

[10]C. Flotho, S. Sommer and M. Lubbert, DNA-hypomethylating agents as epigenetic therapy before and after allogeneic hematopoietic stem cell transplantation in myelodysplastic syndromes and juvenile myelomonocytic leukemia, Semin Cancer Biol, 51, (2018), 68-79

[11]M. L. Loh, D. S. Sakai, C. Flotho, M. Kang, M. Fliegauf, S. Archambeault, C. G. Mullighan, L. Chen, E. Bergstraesser, C. E. Bueso-Ramos, P. D. Emanuel, H. Hasle, J. P. Issa, M. M. van den Heuvel-Eibrink,

F. Locatelli, J. Stary, M. Trebo, M. Wlodarski, M. Zecca, K. M. Shannon and C. M. Niemeyer, Mutations in CBL occur frequently in juvenile myelomonocytic leukemia, Blood, 114, (2009), 1859-63

[12]S. K. Tasian, J. A. Casas, D. Posocco, S. Gandre-Babbe, A. L. Gagne, G. Liang, M. L. Loh, M. J. Weiss, D. L. French and S. T. Chou, Mutation-specific signaling profiles and kinase inhibitor sensitivities of juvenile myelomonocytic leukemia revealed by induced pluripotent stem cells, Leukemia, 33, (2019), 181-190

[13]F. Locatelli, M. Algeri, P. Merli and L. Strocchio, Novel approaches to diagnosis and treatment of Juvenile Myelomonocytic Leukemia, Expert Rev Hematol, 11, (2018), 129-143

[14]C. Batz, I. Sandrock, C. M. Niemeyer and C. Flotho, Methylation of the PTEN gene CpG island is infrequent in juvenile myelomonocytic leukemia: Comments on "PTEN deficiency is a common defect in juvenile myelomonocytic leukemia" [Leuk. Res. 2009;33:671-677 (Epub 2008 November 17)], Leuk Res, 33, (2009), 1578-9; author reply 1580

[15]Y. L. Liu, R. P. Castleberry and P. D. Emanuel, PTEN deficiency is a common defect in juvenile myelomonocytic leukemia, Leuk Res, 33, (2009), 671-7

[16]C. Flotho, A. Paulun, C. Batz and C. M. Niemeyer, AKAP12, a gene with tumour suppressor properties, is a target of promoter DNA methylation in childhood myeloid malignancies, Br J Haematol, 138, (2007), 644-50

[17]D. B. Lipka, T. Witte, R. Toth, J. Yang, M. Wiesenfarth, P. Nollke, A. Fischer, D. Brocks, Z. Gu, J. Park, B. Strahm, M. Wlodarski, A. Yoshimi, R. Claus, M. Lubbert, H. Busch, M. Boerries, M. Hartmann, M. Schonung, U. Kilik, J. Langstein, J. A. Wierzbinska, C. Pabst, S. Garg, A. Catala, B. De Moerloose, M. Dworzak, H. Hasle, F. Locatelli, R. Masetti, M. Schmugge, O. Smith, J. Stary, M. Ussowicz, M. M. van den Heuvel-Eibrink, Y. Assenov, M. Schlesner, C. Niemeyer, C. Flotho and C. Plass, RAS-pathway mutation patterns define epigenetic subclasses in juvenile myelomonocytic leukemia, Nat Commun, 8, (2017), 2126

[18]H. Gill, A. Y. Leung and Y. L. Kwong, Molecular and Cellular Mechanisms of Myelodysplastic Syndrome: Implications on Targeted Therapy, Int J Mol Sci, 17, (2016), 440

[19]K. Matsuda, C. Taira, K. Sakashita, S. Saito, M. Tanaka-Yanagisawa, R. Yanagisawa, Y. Nakazawa, M. Shiohara, K. Fukushima, M. Oda, T. Honda, T. Nakahata and K. Koike, Long-term survival after nonintensive chemotherapy in some juvenile myelomonocytic leukemia patients with CBL mutations, and the possible presence of healthy persons with the mutations, Blood, 115, (2010), 5429-31

[20]C. M. Niemeyer, M. L. Loh, A. Cseh, T. Cooper, C. C. Dvorak, R. Chan, B. Xicoy, U. Germing, S. Kojima, A. Manabe, M. Dworzak, B. De Moerloose, J. Stary, O. P. Smith, R. Masetti, A. Catala, E. Bergstraesser, M. Ussowicz, O. Fabri, A. Baruchel, H. Cave, M. Zwaan, F. Locatelli, H. Hasle, M. M. van den Heuvel-Eibrink, C. Flotho and A. Yoshimi, Criteria for evaluating response and outcome in clinical trials for children with juvenile myelomonocytic leukemia, Haematologica, 100, (2015), 17-22

[21]R. R. Coe, M. L. McKinnon, M. Tarailo-Graovac, C. J. Ross, W. W. Wasserman, J. M. Friedman, P. C. Rogers and C. D. M. van Karnebeek, A case of splenomegaly in CBL syndrome, Eur J Med Genet, 60, (2017), 374-379

[22]M. Naramura, I. K. Jang, H. Kole, F. Huang, D. Haines and H. Gu, c-Cbl and Cbl-b regulate T cell responsiveness by promoting ligand-induced TCR down-modulation, Nat Immunol, 3, (2002), 1192-9

[23]K. Lv, J. Jiang, R. Donaghy, C. R. Riling, Y. Cheng, V. Chandra, K. Rozenova, W. An, B. C. Mohapatra, B. T. Goetz, V. Pillai, X. Han, E. A. Todd, G. R. Jeschke, W. Y. Langdon, S. Kumar, E. O. Hexner, H. Band and W. Tong, CBL family E3 ubiquitin ligases control JAK2 ubiquitination and stability in hematopoietic stem cells and myeloid malignancies, Genes Dev, 31, (2017), 1007-1023

[24]M. A. Schroeder, J. Choi, K. Staser and J. F. DiPersio, The Role of Janus Kinase Signaling in Graft-Versus-Host Disease and Graft Versus Leukemia, Biol Blood Marrow Transplant, 24, (2018), 1125-1134

[25]K. Matsuda, A. Shimada, N. Yoshida, A. Ogawa, A. Watanabe, S. Yajima, S. Iizuka, K. Koike, F. Yanai, K. Kawasaki, M. Yanagimachi, A. Kikuchi, Y. Ohtsuka, E. Hidaka, K. Yamauchi, M. Tanaka, R. Yanagisawa, Y. Nakazawa, M. Shiohara, A. Manabe, S. Kojima and K. Koike, Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations, Blood, 109, (2007), 5477-80