and a C-terminal *PDGFRB* domain (catalytic tyrosine kinase). The use of different primers combinations revealed the absence of the reciprocal 5' *PDGFRB/3'KIAA1509* fusion transcript. The FISH screening of 12 MPD patients with BAC clones specific for KIAA1509 and PDGFRB genes did not reveal the presence of other cases bearing the 5' KIAA1509/3' PDGFRB fusion gene. The patient with 5' KIAA1509/3' PDGFRB fusion transcript, was treated with imatinib and achieved hematological remission; the molecular response is still under evaluation. *Conclusions*. In this study we report the second MPD case with a t(5;14)(q32;q32) bearing a 5' KIAA1509/3' PDGFRB fusion gene. Our case differ from that previously reported in literature as KIAA1509 breakpoint was mapped within intron 11 instead of intron 9; any difference was observed in PDGFRB breakpoint location. As a consequence of this diversity, in our case a larger fusion protein was produced including an additional chromosome segregation ATPases domain. Treatment with imatinib resulted in hematologic response in both cases. Our data illustrate how molecular cytogenetic techniques may be useful to uncover recurrent chromosomal rearrangements in MPD patients.

1316

DETECTION AND MONITORING OF CYTOMEGALOVIRUS(CMV) IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS BY REAL-TIME PCR (RQ-PCR)

N. Obeidi, H. Ghaffari, A. Gharehbaghian, K. Alimoghaddam, M. Dehghan, A. Ghavamzadeh, A. Shamshiri

Bushehr Medical University, BUSHEHR, Iran, Hematology-Oncology & BMT Research Center, Tehran University of Medical Sciences, Iran Blood Transfusion Organization Research Center, Iran

CMV has been recognized as the most important viral pathogen in persons undergoing BMT. Monitoring of CMV reaction from latency is critical for these patients. We could detect CMV DNA in this patients by RQ-PCR For monitoring of CMV reaction. If copy number of CMV was increased, preemptive therapy will be initiated. 51 recipients of BMT (9-51 years) were monitored as weekly intervals until day 100 after transplantation. For amplification of the pp65 gene (UL83) RQ-PCR assay and pp65 Antigenemia method were preformed in parallel with 415 samples. By cloning of this region, we made standards for RQ-PCR. The results obtained by the two techniques were significantly correlated (p<0.01). We could detected 13x101-15x107copies/2x10^5 cells by RQ-PCR.76% of patients developed more than one episode of CMV replication. First positive result of RQ-PCR 13 days earlier than the Antigenemia. After preemptive therapy 16 days (7-21 days) needed to become negative result of RQ-PCR. There was no relationship between death and increase of CMV copy(p<0.419). There is no correlation between copy number of CMV virus and PP65 and WBC count (p<0.624,p<0.422). RQ-PCR was more sensitive than pp65 Antigenemia. After preemptive therapy, negative results of RQ-PCR were the best indicator for determining of successful treatment. Reaction of CMV in our patients mostly endogenous and depend on kind of immunosuppressive therapy. If copy number of CMV increased one log, CMV reaction developed 1.22 fold.

1317

MONITORING OF MINIMAL RESIDUAL DISEASE AND TREATMENT OF MOLECULAR RELAPSES IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

E.N. Shuravina, E.N. Parovitchnikova, I.A. Demidova, A.V. Misiurin, V.G. Savchenko

Russian National Center for Hematology, MOSCOW, Russian Federation

Backgrounds. Minimal residual disease monitoring of PML/RARA chimeric transcript is widely used method for detecting of molecular relapses (MR) in pts with APL during hematological remission. However, the necessity of therapy changing when MR is detected is still debated. *Aims.* We tried to find out whether the PML/RARA detection during hematological remission ultimately leads to relapse of APL and to develop the optimal treatment strategy of MR in APL pts. *Materials and Methods.* We investigated bone marrow samples by RT-PCR for PML/RARA chimeric transcript in 73 pts with newly diagnosed and morphologically proved APL. Primers synthesis for nested RT-PCR was performed using recommendations of BIOMED-1 Concerted Action (1999).). RT-PCR was performed on fresh marrow aspirates of all pts before treatment and periodically (2-3monthly) during all period of therapy (2 years after induction of remission). MR was defined as probable if chimeric transcript was detected once and was not find out by second investigation and as proved when *PML/RARA* was detected at least twice by consecutive investigations (in 2-4 weeks). *Results.* In 69 pts (94,5%) *PML/RARA* chimeric transcript was revealed during first investigation. 31 (45%) demonstrated bcr1 type of transcript, 38 (55%) - bcr3 type. In 4 pts (5,5%) *PML/RARA* was not found. During maintenance therapy in 19 of 52 pts (36,5%) MR was detected. In 5 patients from 6 with proved MR and in 3 pts from 13 with probable MR therapy was changed for Ara-C with idarubicine in early MR (12 months from remission induction) or ATRA + Interferon alfa in late onset of MR. No one of these pts developed hematological relapse. Maintenance was not changed in 11 pts (10 with probable MR, one - with proved MR) and 4 (36%) of them subsequently relapsed (one with proved MR). *Conclusions.* According to our data, detecting of *PML/RARA* in pts during maintenance therapy leads to high incidence of relapse in APL pts. Changing of therapy during MR significantly decreases the probability of hematological relapse [from 36% to 0% (p=0,001)].

1318

MONOCLONAL ANTIBODY TO CD34 INHIBITS PROLIFERATION AND INDUCES APOPTOSIS OF CD34+ STEM AND MYELOID CELL LINES

P.S.W. Stockbauer, K. Elknerov, J.S. Soucek, J.N. Nemcova, J. Schwarz

IHBT, PRAGUE, Czech Republic

Backgrounds. Monoclonal antibodies to epitopes of membrane differentiation antigens are widely used for therapeutical targeting of tumor cells. Aims. In some hematological malignancies, however, there is a need for more specific antibodies targeting the surface epitopes expressed on immature hematopoietic cells. *Methods.* We developed mouse monoclonal antibody of IgG1 class, clone 4H11, reactive with the class III (protein) epitope of human CD34 molecule. We detected the antiproliferative effect of CD34 antibody on human CD34+ stem and myeloid cell lines. Inhibition of proliferation was tested by uptake of tritiated thymidine and apoptosis was detected by Annexin-V-Fluorescence kit. *Results.* Anti-CD34 antibody 4H11 inhibited proliferation and induced apoptosis of CD34 positive cell lines at the concentration between 1-200 ug/ml after 12, 48 and 72 hours. The anti-CD34 antibody strongly inhibited proliferation and induced apoptosis of all CD34+ cell lines (MOLM-9, JURL, HEL, RPMI 8402) but not control CD34 negative cells. The antiproliferative effect was detected even at the antibody level of 2.5 ug/ml, and the antiproliferative effect was potentiated by simultaneous presence of differentiation inducing cytokines. The expression of CD34 antigen at the surface membrane of tested living cells was not modulated by 4H11 antibody. *Conclusions.* Based on the results obtained by the *ex vivo* model system of cultured leukemia cells we suggest that antigenic epitopes expressed on CD34 molecule should be considered as possible new molecular targets for the development of more effective targeted therapy of severe hematological malignancies, especially of immature myeloid lineage. (Supported by grant NR/8233-3 of the Internal grant agency of the Ministry of Health of the Czech Republic).

1319

ESTIMATION OF THE DIAGNOSTIC VALUE OF MYELOPEROXIDASE INDEX AND LDH IN MEGALOBLASTIC ANEMIA

J. Eivazi-Ziaei, S. Dastgiri

Tabriz Medical University, TABRIZ, Iran

Most cases of megaloblastic anemias corresponded to anemia with hyper-segmented neutrophils, macroovalocytosis and very high serum LDH level. Elevated neutrophil myeloperoxidase index (MPXI) may be indicative of a diagnosis of megaloblastic anemia. The aim of this study was to estimate the value of *MPXI* and *LDH* in the diagnosis of macrocytic anemia to facilitate the diagnostic algorithm prior to performing any bone marrow aspirate. *MPXI* and *LDH* were assessed using the first blood sample obtained prior to any transfusion or medical therapy, and after therapy in 29 patients diagnosed as megaloblastic anemia. MPXI was assessed using complete blood count (CBC), performed by Technicon H1 (Bayer) instrument. Mean value of MPXI significantly decreased after treatment (20.4, CI95%: 17-23 vs. -0.75, CI95%: -4-2.7, before and after treatment, respectively). The same significant pattern was also observed for LDH (4230, CI95%: 3096-5369 vs. 783, CI95%: 492-1075, before and after treatment, respectively). The proportional diagnostic value (%) was significantly higher when both MPXI and LDH (83 percent, p<0.001) were used together in the diagnosis of Megaloblastic Anemia while the same index was (71 percent, p<0.001) for MPXI and (48 percent, p<0.001) for LDH when they were used alone. MPXI and LDH values may have a diagnostic role on megaloblastic anemia. It might be used as a reliable screening tool before doing any other diagnostic procedure.