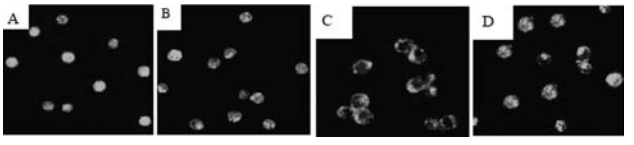
and 40 μmol/L quercertin respectively, there were marked down-regulation of MRP1 gene expression, as compared with mock-treated group (p<0.01). In HL-60 cell line, the DNR fluorescence was mainly distributed in the nucleus, cytoplasm and cell membrane, with nucleus intensely, cytoplasm uniformly and diffusely, membrane continuously staining pattern (Figure A). Compared with mock-treated group, the distributions of DNR fluorescence were not obviously changed after treated with different concentrations of quercertin (Figure B). However, in HL-60/ADR resistant cells, DNR fluorescence was mainly distributed in periphery region of cytoplasm and membrane, the granule was not homogeneous, and fluorescence signal was hardly seen in the nucleus (Figure C). Nevertheless, as concentration of quercertin increased, fluorescence signal was gradually increased in the nucleus and cytoplasm. When the concentration of quercertin increased up to 40 μmol/L, the fluorescence intensity almost reached level of that in sensitive cells with diffuse granule distribution(Fig. D). Altered subcellular distribution of DNR in resistant cell line was related to MDR gene formation in tumor cells. Quercetin could inhibit MRP1 function and restore the sbucellular distribution of DNR *in vitro*.



1311

RETINOIC ACID AFFECTS THE RESPONSE OF V-MYB-TRANSFORMED MONOBLASTS TO OKADAIC ACID

P. Benes,[1] V. Maceckova,[1] Z. Andrysik,[2] J. Zatloukalova,[2] J. Smarda[1]

[1]*Masaryk University, BRNO, Czech Republic;* [2]*Institute of Biophysics ASCR, BRNO, Czech Republic*

Background. Okadaic acid (OA) inhibits serine/threonine protein phosphatases 1 (PP1) and 2A (PP2A), thus inducing differentiation and/or apoptosis of various leukemic cell lines in dose-dependent manner. This suggests that PP1 and PP2A phosphatases actively participate in regulation of these processes. Moreover, retinoic acid (RA) affects expression and activity of the PP2A. *Aims*. The aim of this study was to explore the functional interactions of RA- and OA-driven pathways in v-myb-transformed monoblasts BM2. We have previously described that BM2 monoblasts ectopically expressing Jun, RA-receptor (RAR) or retinoid X receptor (RXR) proteins differentiate to macrophage-like cells upon treatment with RA while wild-type BM2 cells do not respond to RA. *Results*. In this study we found that 10 nM OA induces adherency, cell cycle arrest, phagocytic activity, production of reactive oxygen species and expression of vimentin in BM2 cells. These features that mark differentiation along monocyte/macrophage pathway are enhanced in BM2 cells upon simultaneous treatment with OA and RA. Interestingly, the 20nM OA induces rather apoptosis than differentiation of BM2 cells as documented by analysis of cell morphology, chromatin condensation, internucleosomal DNA fragmentation and fosfatidylserine translocation. This proapoptotic effect of OA in BM2 cells was inhibited by RA. *Conclusions*. These results indicate that pro-differentiation and pro-apoptotic effects of OA on BM2 monoblasts are differently regulated by RA.

Funding: This work was supported by the grant 301/03/D022 of the Grant Agency of the Czech Republic and by the grant MSM0021622415 of the Ministry of Education, Youth and Sports of the Czech Republic.

1312

STUDIES OF PNAS-2, AN ANTI-APOPTOSIS GENE

H.R. Wang, C.H. Gu, J.Y Zhu, J.Y. Han, F.Y. Chen, R.R. Ou Yan

RenJi Hospital, SHANGHAI, China

We use gene chip and find PNAS-2 (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As4S4 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4, but PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. Moreover, the results of microarray are validated by RT-PCR in a time course, the expression of PNAS-2 decreased after treated with As4S4 in NB4 cells, and this effect is time dependent. We even performed similar experiment on primary leukemia cells from leukemia patient. Again PNAS-2 expression decreased significantly in APL samples after the treatment of As4S4, while no change in M4 primary cells upon the same treatment. The hypothetic protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, no studies has characterized this gene. To learn whether *PNAS-2* has a function associate with apoptosis. pRNAT U6.1/NEO and pTracer-CMV/Bsd were used to construct both shRNA express plasmids and over-express plasmids, stable tansfected recombinant plasmids to U937 cell lines respectively. Used both antibiotics and GFP^+ subpopulations cell sorting through FCM to purify $GFP(^+)$ tansfected cells. Annexin V-APC and 7-AAD were used to stain cells, applied FCM and confocal microscopy to detect apoptosis. After antibiotic selected and GFP^+ subpopulations cell sorting through FCM, confocal microscopy confirmed we had got almost pure transfected U937 cells (more than 90%). The expressions of PNAS-2 in shRNA groups decreased after RNA interference had occurred, the PNAS-2 inhibition rates were 78.1% in shRNA group, 75.4% in shRNAII group and 51.4% in shRNA group. The results of apoptosis ratio from confocal microscope were: in shRNA control group was 7.3%, in shRNA I group was 14.7%, 13.8% in shRNAII group and 10.3% in shRNA III group. Mean apoptosis ratios by FCM were 3.52% in shRNA control group, 9.23% in siRNA I group, 8.85% in siRNAII group and 7.19% in shRNA III group. Paired t test showed p values were 0.0088, 0.014 and 0.1788 respectively in shRNAI, II and III group paired compared with shRNA control group. In our PNAS-2 over-express experiment, the expression of PNAS-2 in PNAS-2-pTracer recombinant plasmid transfected group increased 1.73 times than control groups. The results of apoptosis ratio by confocal microscope were: 6.90% in control-pTracer group, 3.76% in PNAS-2-pTracer group. Apoptosis ratio detected by FCM was 4.07_±0.30% in PNAS-2-pTracer group, while in control-pTracer group was 5.51%±0.12% (p=0.0096). We find inhibition of PNAS-2 by RNA interference will increase cell apoptosis both detected by confocal microscopy and FCM, and show statistical significance in shRNA I group and in shRNA II group compared with control group. While no statistical significance in shRNA III group (p=0.1788), we think it may attribut to partail inhibition of PNAS-2, and it also shows a tendence of increased apoptosis ratio (7.19% while in shRNA control group is 3.52%). Over-expression study has showed cell apoptosis ratio statistical significantly decrease when PNAS-2 gene is over-expressed. We reveal the biologic effects of the expression level of the PNAS-2 transcript are associated with cell apoptosis, PNAS-2 is an anti-apoptosis gene.

1313

PNAS-2, A NOVEL GENE PROBABLY PARTICIPATE IN LEUKEMOGENESIS

H.R. Wang

RenJi Hospital, SHANGHAI, China

We use gene chip and find *PNAS-2* (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As4S4 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4. Moreover, PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. The hypothetic protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, there are no studies characterizing this gene. To obtain 5' unknown sequence of *PNAS-2* in NB4 cell line; To know whether *PNAS-2* is a pseudogene and its expression-spectrum both in multi-tissue and patients. 5'RACE was used to obtain 5' unknown sequence of *PNAS-2*; PNAS-2-GFP-fusion proteins express plasmid was constructed, after transfected to U937 cell line, Western blot analysis was applied to detect GFP fusion proteins; Northern Blot was used to detected the expression of *PNAS-2* gene in the multi-tissue; Real-Time PCR was applied to detected *PNAS-2* expression in patients.

After 5'RACE, we found two splice patterns of *PNAS-2* in NB4 cell lines, as F1 *PNAS-2* and F2 *PNAS-2*; both were more than 98% homology to *CHMP5*, *CGI-34* and *HSPC177*, these genes had a same open reading frame (Figure 1). After tranfected GFP fusion protein expression plasmid to U937 cells, we applied Western blot analysis. The results confirmed *PNAS-2* could be translated into protein and it was not a pseudogene (Figure 2). Northern Blot was applied in the multi-tissue including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver, we found no expression of PNAS-2 gene in majority tissues except in placenta (Figure 3, 4).After Real-Time PCR, we found PNAS-2 expression statistically higher in 77 cases of acute leukemia (AL) include 71 *de novo* and 6 relapse when compare with 8 complete remission (CR) patients (p=0.0001) or 37 non-tumorous disease patients (p=0.0003). (Figure 5). There was no statistic difference between each subtype of AL.