panied by a reduction in circulating ET-1 and, as a conse-
quency, by the blunting of a vasoconstrictive stimulus
which could partly account for the beneficial effects
of HU. The decrease of circulating ET-1 is consistent
with the HU-induced decreased expression of the ET-1
gene in endothelial cells in culture. However, it has also
been shown that the number of pro-adhesive RBCs and
the expression of adhesion molecules on lymphocytes,
monocytes and neutrophils are decreased in patients
with HU. Thus, it is probable that all these pleiotropic
effects of HU concur to reduce the aggressiveness of cir-
culating cells towards the endothelium, and thereby ET-1
production, thus conferring the clinical benefits.

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Key words: sickle cell disease, hydroxyurea, endothelin-1.

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References
1. Hebbel R. Adhesive interactions of sickle erythrocytes
2. Graido-Gonzalez E, Doherty JC, Bergreen EW, Organ G,
Teller M, et al. Plasma endothelin-1, cytokine, and prostag-
landin E2 levels in sickle cell disease and acute vaso-occlu-
3. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB,
Eckert SW, et al. Effect of hydroxyurea on the frequency of pain-
f ul crises in sickle cell anemia. Investigators of the Multi-
4. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK,
Kutlar A, et al. Effect of hydroxyurea on mortality and mor-
bidity in adult sickle cell anemia: risks and benefits up to 9
5. Maier-Redelsperger M, Labie D, Elion J. Long-term hydroxy-
urea treatment in young sickle cell patients. Curr Opin He-
R, Lapouméroulie C. Hydroxyurea down regulates endothe-
lin-1 gene expression and upregulates ICAM-1 gene expres-
sion in cultured human endothelial cells. Pharmacogenomics
7. Styles L, Lubin B, Vichinsky E, Lawrence S, Hua M, Test S, et
al. Decrease of very late activation antigen-4 and CD56 on
reticulocytes in sickle cell patients treated with hydroxyurea.
decreases the in vitro adhesion of sickle erythrocytes to
Relationship between the clinical manifestations of sickle
cell disease and the expression of adhesion molecules on
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clear neutrophils from patients with sickle cell anemia. Blood

Molecular Hematopoiesis

Changes in expression of WT1 isoforms during
induced differentiation of the NB4 cell line

The levels of expression of WT1 gene and
WT1+17AA isoforms rapidly decreased during the
differentiation of NB4 cells induced by all-trans
retinoic acid; this decrease was conversely related
to the dynamic changes of CD11b positive cells,
indicating that the abnormally high expression of
WT1 gene and WT1+17AA isoforms was associat-
ed with a block of NB4 cell differentiation.

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The Wilms’ tumor gene (WT1) produces four major
distinct isoforms due to two alternative splicing events
within this gene. The first alternative splice corresponding
to exon 5 causes the presence or absence of a 17-
amino acid insertion between the trans-regulatory and
ZF domain while a second splice results in gain or loss of
a 9 bp insertion encoding 3 amino acids (KTS) between
the third and fourth ZF DNA-binding domain; this produc-
tes four distinct isoforms designated as -17AA/-KTS,
+17AA-KTS, -17AA+KTS and +17AA+KTS. WT1 iso-
forms are proposed to have distinct functions. The
changes in the ratio of these four isoforms in cells is thus
thought to render different phenotypes.

In this study, a real-time quantitative reverse tran-
scription polymerase chain reaction (RQ-RT-PCR) method
was established for detecting the expression levels of
WT1 gene, WT1+17AA isoforms and GAPDH in NB4 cells
induced by all-trans retinoic acid (ATRA 0.5 uM) using
LightCycler. RNA extraction, cDNA conversion, standard
preparation for RQ-RT-PCR, and the composition and
condition of the PCR reaction mixture were as described
previously. All primers and the TaqMan probe were
designed by Primer Priemier software (version 5.0) and
their positions referred to the WT1 sequence are shown in
Figure 1. Detailed sequences of the sense (SP1), antisense
primers (AP1) and fluorescent probe of total WT1 were

References
1. Hebbel R. Adhesive interactions of sickle erythrocytes
2. Graido-Gonzalez E, Doherty JC, Bergreen EW, Organ G,
Teller M, et al. Plasma endothelin-1, cytokine, and prostag-
landin E2 levels in sickle cell disease and acute vaso-occlu-
3. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB,
Eckert SW, et al. Effect of hydroxyurea on the frequency of pain-
f ul crises in sickle cell anemia. Investigators of the Multi-
4. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK,
Kutlar A, et al. Effect of hydroxyurea on mortality and mor-
bidity in adult sickle cell anemia: risks and benefits up to 9
5. Maier-Redelsperger M, Labie D, Elion J. Long-term hydroxy-
urea treatment in young sickle cell patients. Curr Opin He-
R, Lapouméroulie C. Hydroxyurea down regulates endothe-
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Figure 1. Position of the primers and probe of total WT1 gene
and WT1+17AA isoforms. SP1: sense primer of total WT1 gene, locat-
ed on exon 6; AP1: antisense primer of total WT1 gene, located
on exon 7; SP2: sense primer of WT1+17AA isoforms located on
exon 5; the probe was designed to hybridize at the sense strand
of exon 6/7.
The down-regulation may be a relatively early event which blocks NB4 cell differentiation, indicating that WT1 expression may functionally block blood cell differentiation. Here our results showed that the levels of expression of WT1 gene and WT1+17AA isoforms rapidly decrease during the differentiation of NB4 cells induced by ATRA, which is in accordance with our previous competitive RT-PCR results. During the induced differentiation of NB4 cells, the events of down-regulated WT1 gene expression and up-regulated CD11b antigen expression occurred much earlier than the reduction of NBT rate, suggesting that WT1 down-regulation may be a relatively early event which blocks NB4 cell differentiation. Which of the four isoforms contributes predominantly to blockade of NB4 leukemic cell differentiation needs to be further investigated.

Figure 2. A comparison of the time courses of WT1 and WT1+17AA isoforms expression and CD11b positive rates during the ATRA-induced differentiation of NB4 cells. The red square, blue prism and yellow triangle represent WT1+17AA and CD11b expression level respectively. The expression levels of WT1 gene and WT1+17AA isoforms rapidly decrease during the differentiation of NB4 cells induced by ATRA; in contrast, the rate of CD11b expression increases gradually.

References

7. Sekiya M, Adachi M, Hinoda Y, Imai K, Yachi A. Down-
regulation of Wilms’ tumor gene (WT1) during myelomonocytic differentiation in HL60 cells. Blood 1994;83:1876-82.


**Expression of polycythemia rubra vera-1 decreases the dependency of cells on growth factors for proliferation**

An increase in the level of polycythemia rubra vera-1 (PRV-1) mRNA has been reported in some myeloproliferative disorders. We have studied the effects of PRV-1 on cell proliferation and cell survival. In cell growth assays, the number of heterologous cells expressing PRV-1 increased faster than sham-transfected cells, a difference that was more pronounced in serum-free media. Even after 5 days of exposure to serum-free media, cells expressing PRV-1 continued to proliferate, whereas the control cells ceased to proliferate. We conclude that PRV-1 is a pro-proliferation molecule, and hypothesize that its overexpression may have a role in the pathogenesis of myeloproliferative disorders.

Polycythemia rubra vera-1 (PRV-1, also known as CD177 or NB1) is a member of Ly-6/uPAR protein family, whose members have been shown to play a role in cell proliferation. In normal subjects, PRV-1 mRNA is expressed on a subpopulation of neutrophils, on myelocytes and on metamyelocytes. An increase in the level of mRNA encoding PRV-1 has been reported in neutrophils from patients with polycythemia vera (PV) and essential thrombocythemia (ET). However, the functional role of PRV-1 is unknown. We have conducted in vitro studies investigating the function of PRV-1 in a heterologous cell line stably expressing this molecule. After transfecting either PRV-1 cDNA or empty plasmid to Chinese hamster ovary (CHO) cells, we established a stable CHO cell line expressing PRV-1 (CHO-PRV-1) and a control cell line (CHO-Sham). Flow cytometry studies with MEM-166, a monoclonal antibody to CD177 (BD Pharmingen, San Diego, CA, USA), showed that CHO-PRV-1 cells, but not CHO-Sham cells, expressed PRV-1 (data not shown). In the cell growth assay, the number of CHO-PRV-1 cells increased faster than CHO-Sham cells in the presence of 10% fetal bovine serum (FBS, Sigma-Aldrich) in the growth media (Figure 1A).

The difference in the number of cells was statistically significant at each time-point, except at 0 and 24 hr, and persisted throughout the 5-day follow-up. This difference in growth rates between CHO-PRV-1 and CHO-Sham cells was further increased when the cells were grown in the absence of FBS. The growth curves diverged further with time, and the difference in the cell number was most prominent on the fifth day (Figure 1B).

We studied cell proliferation by measuring the percentage of bromodeoxyuridine (BrdU)-incorporating cells. On the first day after exposure to serum-free media, the percentage of BrdU (+) cells was similar between CHO-PRV-1 and CHO-Sham cells; however, starting on the 4th day, there was a significantly higher percentage of proliferating cells among CHO-PRV-1 cells than among control cells (8.8±0.8% vs 0.8±0.1%, respectively; p=0.02) (Figure 2A). Additionally, we compared the rate of apoptosis and necrosis between the two cell lines by measuring binding of annexin V and incorporation of SYTOX green stain, using the Vybrant Apoptosis Assay Kit (Molecular Probes, Eugene, OR, USA). We found that although apoptosis was not different between CHO-PRV-1 and CHO-sham cells (Figure 2B), the percentage of necrotic cells was higher in sham-transfected cells than in PRV-1-expressing cells (Figure 2C).

There are clinical observations that support a pro-proliferative role for PRV-1. Clinical settings that are associated with elevated PRV-1 expression, such as administration of granulocyte colony-stimulating factor, sepsis, pregnancy, and polycythemia vera are also known to be associated with higher neutrophil counts. Several important questions on the expression of PRV-1 are unanswered. Do early hematopoietic progenitor cells