Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Turkey

Molecular pathology of glucose-6-phosphate dehydrogenase (G6PD) was studied in 50 unrelated red cell G6PD deficient Turkish males. DNA analysis of G6PD genes revealed that 40 of the 50 subjects (80%) had G6PD B⁻ 563T, 2 subjects (4%) A⁻ (376G/202A) and one subject (2%) G6PD Chatam (1003A). The remaining 7 subjects (14%) did not have any of these three mutations.

Sir,

Red cell glucose-6-phosphate dehydrogenase (G6PD) deficiency is not rare in Turkey. The frequency of this enzyme deficiency in Turkish males was reported to vary between 0.5-11.4% depending upon geographical areas and/or ethnic groups.[1,2] Molecular studies of red cell G6PD enzyme revealed the presence of about 122 mutations which were recently reviewed by Vulliamy *et al*.[3] Enzyme deficiency in Turkey is rarely associated with chronic hemolytic anemia with only one such subject having been reported. In this patient, enzyme deficiency resulted from the 823G mutation.[4] No detailed molecular study has been conducted in Turkey to search for the frequency of polymorphic deficient mutations. The study presented below was designed in order to fill this gap. A total of 50 unrelated male subjects with red cell G6PD deficiency were the subjects of this study. Subjects were referred to our center from various parts of Turkey. The diagnosis of enzyme deficiency was made in the neonatal period in 40 of 50 subjects during screening studies for hyperbilirubinemia. In the remaining 10 subjects, enzyme deficiency was diagnosed at the time of a hemolytic crisis. Genomic DNA was obtained from peripheral blood using standard methods. PCR was used to amplify the portions of coding region of the G6PD gene as described previously.[5] All DNA samples were screened for common known mutations by using appropriate restriction endonuclease enzymes.[6] For mutations which could not be determined by restriction endonuclease analyses such as G-A at nt 202 and G-A at nt 1003 (Chatam), direct sequencing of PCR products was performed. The study revealed that the enzyme deficiency was associated with Mediterranean type of G6PD deficiency B⁻ (563T) in 40 of 50 subjects (80%), two patients had A-(376G/202A) type of G6PD deficiency (4%) and one patient had 1003 A mutation (G6PD Chatam)(2%). In the remaining 7 subjects (14%) none of the above mentioned mutations was found to be responsible for the deficiency (Table 1). Two patients with A⁻ were living in different villages in the region of Ankara. Our study indicated that although the major molecular pathology in G6PD deficiency is G6PD B⁻ (563T), G6PD A⁻ (376G/202A) and G6PD Chatam (1003A) mutations are also present in the Turkish population. Population screening studies showed that the frequency of G6PD enzyme deficiency was 6.5-11.4% in Çukurova region where HbS of Benin type is also prevalent.[1,2,7] Therefore, one may expect G6PD deficiency of A⁻ to be present in this population. Contrary to this expectation, none of the A⁻ patients originated from this region suggesting that G6PD mutation had occurred sometime after the introduction of the HbS gene into this population. The presence of isolated G6PD A⁻ in central Anatolia indicates that in Turkey, like other Mediterranean countries, the African G6PD genes were introduced by slavery and not by large population movements. G6PD Chatam was diagnosed in a patient from central Anatolia. This pathology was first diagnosed in Asian Indians followed by a Syrian and later in several Mediterranean countries.[8] It seems that this mutation is the third most common polymorphic deficiency mutation after B⁻ and A⁻ in Mediterranean countries.

Table 1. Frequency of glucose-6-phosphate dehydrogenase mutations in the enzyme deficient males studied.

	No. of affected chromosomes	*Mutations*	*%*
	40	G6PD B⁻ (563T)	80
	2	G6PD A⁻ (376G/202A)	4
	1	G6PD Chatam (1003A)	2
	7	Unknown	14
Total	50		100

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Expression of transmembrane and soluble forms of CD44H in human myeloid cell lines and its regulation by hyaluronic acid

Using myeloid cell lines, it has been demonstrated that hyaluronan modulates transmembrane and soluble CD44H expression in immature (KG1a) but not mature (KG1) progenitors. Concomitantly, cell proliferation is decreased. This effect suggests that the specific interaction of CD44H with its ligand not only delineates a specific cell-matrix adhesive system, but also a proliferation signal.

Sir,

CD44, a protein involved in many cell to matrix adhesive events, exists as transmembrane (tm) and soluble (s) isoforms. In myeloid cells, CD44H (the common isoform) is the receptor for several ligands, including hyaluronate (HA). HA in addition, is a differentiation and activation signal in a variety of cells.[1-4] Since, marrow stromal cells produce and organize HA for deposit and matrix assembly,[5] myeloid progenitors while in the marrow stroma should be continuously exposed to an HA-enriched environment. By using established myeloid cells lines, expressing defined differentiation patterns (KG1a, KG1 and HL-60), we tested whether cell exposure to HA may affect CD44H expression and release and cause a concomitant effect on cell proliferation and differentiation.

As judged by flow cytometric analysis, the three cell lines uniformly express tmCD44H (Table 1,A); however, variants CD44v4/5 are not expressed and CD44v6 is expressed with only low intensity (MFI=5). In turn, production of sCD44H was lower in KG1a, as compared to KG1 and HL-60 (Table 1, A). Thus, these results confirm that tmCD44H expression is

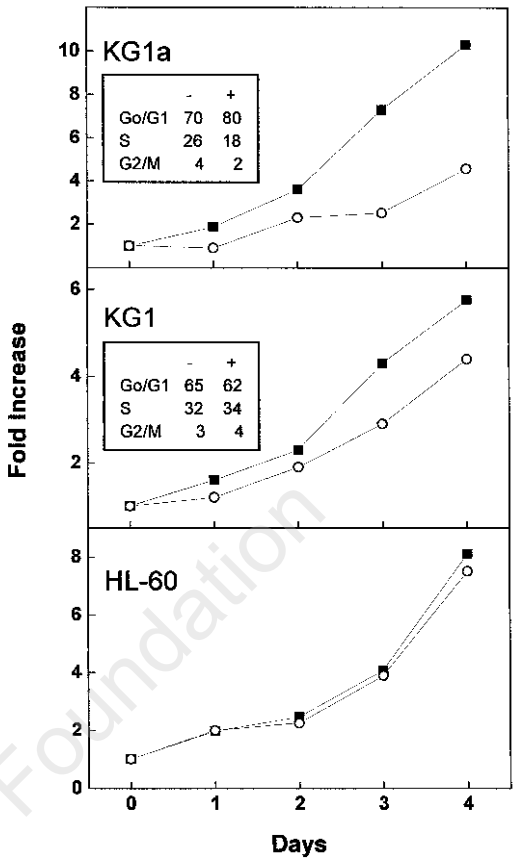

Figure 1. Effect of hyaluronan on proliferation and cell cycle status of myeloid cells. Cells (20×10³ cells/mL) were seeded in culture medium alone (■) or containing 10 µg/mL HA (○). At the indicated culture times, the total number of viable cells was counted and expressed as fold increase over the respective starting cell number, which was set to 1. Data shown are representative of 4 experiments. SEM (not shown), was in all cases less than 5 %. Insets show percentages of control (–) or HA-exposed (+) cells at the indicated cell cycle phase, as determined (day 4) by flow cytometric analysis of DNA content, after propidium iodine and RNAase treatment.

Table 1. Expression of transmembrane and soluble CD44H in myeloid cells and the effect of hyaluronan.

	A: control cells		*B: HA-treated cells*	
	tmCD44 (MFI)	*sCD44 (ng/10⁶ cells)*	*tmCD44* (ratio)*	*sCD44° (ratio)*
KG1a	80	1.3±0.1	1.6±0.5	13.4±1.7
KG1	65	3.7±1.6	1.0±0.4	2.2±0.2
Hl-60	8	5.5±1.4	1.0±0.1	2.0±0.5

*Cells were cultured (3 days) in medium (RPMI 1640 containing 10 % fetal calf serum) (control) or containing 10 µg/mL hyaluronic acid (HA-treated). Transmembrane CD44H (tmCD44) was estimated by flow cytometry after labeling cells with anti-human CD44H or FITC-conjugated mouse IgG_{wm}. Data shown is the mean fluorescence intensity (MFI), which was calculated as the ratio of MFIs for first antibody/control antibody. Soluble CD44H (sCD44) in the culture medium, was measured by an immunoenzymometric assay (Parameter, R&D Systems). Values are mean±SEM for 3 separate experiments performed in duplicate. *Ratio of MFI for HA-treated to control cells. °Ratio of sCD44 produced by HA-treated to control cells. Control cells were also assessed (3) for their capacity to bind to immobilized or soluble hyaluronic acid. For KG1a and KG1 respectively, the values were: to immobilized HA: 39 and 37% (± 10 %); to soluble HA: 3.4 and 4 (relative shift in MFI)*