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Erythrocyte adenosine deaminase levels are elevated in Diamond Blackfan anemia but not in the 5q-syndrome

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Keywords

Diamond Blackfan anemia; GATA-1; eADA; 5q-syndrome

Introduction

Diamond Blackfan anemia (DBA) is a congenital bone marrow failure syndrome characterized by red cell aplasia and a predisposition to cancer. It was the first disease linked to ribosomal dysfunction with 70% of patients having haploinsufficiency of a ribosomal protein (RP) gene with *RPS19* being the most frequently mutated[1]. Recently, several DBA patients without RP mutations have been shown to have mutations in *GATA-1*[2]. The 5q-syndrome, a subtype of myelodysplastic syndrome (MDS), is also characterized by a severe anemia that is caused by heterozygous loss of *RPS14* on chromosome 5q[3]. MDS patients also have a predisposition to cancer. The reciprocal relationship between these two diseases, which are now collectively known as the ribosomopathies[1], has spurred interest in how we may be able to better understand the pathophysiology of these disorders to broaden therapeutic options, and to improve diagnosis.

Erythrocyte adenosine deaminase (eADA) activity has been useful for the last three decades in the diagnosis of DBA based on the finding in 1983 that eADA enzyme levels are significantly elevated in 75% of DBA patients[4]. Furthermore, elevated eADA activity has a sensitivity of 84%, specificity of 95% and positive and negative predictive values of 91% for the diagnosis of DBA compared with other inherited bone marrow failure syndromes[5]. The

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Contributions: A.N. and B.G. developed the research idea and wrote the paper. N.L.D. analyzed the data. C.L. assisted with sample preparation/procurement. C.W. performed eADA assays. All authors reviewed and approved the final version of the paper.

goal of the present study is to measure the eADA activity in patients with the 5q-syndrome and DBA patients with GATA-1 mutation in order to determine whether the eADA levels are elevated thereby indicating eADA may be specific to ribosomal haploinsufficiency.

Methods

Under a Stanford University approved IRB protocol, an additional peripheral blood sample was obtained from patients with the 5q-syndrome who were undergoing routine blood draws for clinical purposes. The diagnosis of myelodysplastic syndrome (MDS) was determined by the local physician and the presence of del(5q) was confirmed by cytogenetics. Patients were also confirmed to not have received a blood transfusion within the previous 3 months, which could mask the true eADA value. The patients with Diamond Blackfan anemia are either followed at our center at Stanford University or were previously referred to us for eADA testing with accompanying clinical information. This includes DBA patients with known RP mutations, GATA-1 mutations and some with no identified mutations.

Erythrocyte ADA levels were measured according to previously described standard methods[5].

Analysis was performed using SAS 9.3 (SAS Institute, Carey, NC). Continuous variables were presented as means and standard deviations (SD) and analyzed using 2-tailed T-Test.

Results

A total of 8 patients with the diagnosis of del(5q) MDS confirmed by cytogenetics were analyzed and in this group, the mean (+/− SD) eADA was 0.6188 +/− 0.2378 (normal 0.33–0.96).

Among the 52 DBA patients, 35 of whom had a documented mutation in one of 7 ribosomal protein (RP) genes, the mean eADA level was elevated at 1.7527 +/− 0.8158 (normal 0.33–0.96).

In the three DBA patients with GATA-1 mutations but no RP gene mutations[2], the mean eADA was normal at 0.58 +/− 0.12288 (normal 0.33–0.96).

Complete results by mutation type are summarized in Table 1.

Discussion

In a disorder with the marked genotypic and phenotypic variability observed in DBA, it is critical to have sensitive tests to aid with the diagnosis. Erythrocyte adenosine deaminase (eADA) levels have long been used as a supporting diagnostic criteria for the disease. The test has been especially useful in distinguishing DBA from other causes of anemia and other inherited bone marrow failure syndromes[5]. However, the reason for the elevation of eADA in DBA remains elusive.

Our goal in this study was to assess eADA activity in the 5q-syndrome, characterized by an acquired ribosomal haploinsufficiency (loss of RPS14 on chromosome 5q), which contrasts

to the congenital haploinsufficiency seen in DBA. Of interest, we observed that the eADA activity was normal in 8 patients with the 5q-syndrome. This observation suggests that eADA may be specific for DBA. We also studied eADA activity in DBA patients due to mutations in GATA-1 and without RP mutations. In this subset, eADA activity also was normal suggesting that elevated eADA is a specific feature of DBA associated with RP mutations.

It is noteworthy that patients with *RPS19* mutations, who tend to have a milder clinical phenotype with fewer congenital anomalies, had lower (but still elevated) levels of eADA when compared to other RP mutations, in particular *RPL11* and *RPL5*. In our cohort, the difference in mean eADA between *RPS19* and *RPL11* was statistically significant (p=0.0150) and a trend towards *RPL5* (p=0.1264). DBA patients with mutations in *RPL11* and *RPL5* have been shown to have a higher association with physical malformations[6]. A larger number of patient samples in future studies should improve the statistical power to compare eADA levels between individual RP mutations.

This is the first study to assess the value of eADA in another disorder of ribosomal haploinsufficiency [del(5q) MDS] and in DBA associated with GATA-1 mutations . In summary, we found that an elevated eADA strongly suggests the diagnosis of DBA although a normal eADA does not exclude the diagnosis, particularly in the setting of GATA-1 mutations. There does not appear to be a utility for using eADA in the diagnosis of the 5q-syndrome despite the connection of the disease with impaired ribosome function. The reason for elevated eADA activity in DBA with RP mutations remains to be determined.

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Table 1

eADA values from 5q-patients and from patients with GATA-1 mutations as compared to each specific DBA mutation including unknown.

Patient Info	eADA Mean	eADA Standard Deviation	p-value (vs. 5q)	p-value (vs. GATA-1)
5q- (n=8)	0.62	0.24	n/a	n/a
RPL5 (n=7)	1.84	0.71	0.0032	0.0030
RPL11 (n=8)	2.67	1.08	0.0009	0.0008
RPS7 (n=3)	1.87	0.71	0.0873	0.0364
RPS10 (n=1)	2.9	-	-	-
RPS19 (n=12)	1.46	0.35	<.0001	0.0010
RPS24 (n=3)	2.2	1.25	0.1579	0.1525
RPS26 (n=1)	1.5	-	-	-
Known mutation (allabove, n=35)	1.95	0.86	<.0001	<.0001
Unknown (n=17)	1.34	0.53	0.0001	0.0267
GATA-1 (n=3)	0.58	0.12	n/a	n/a