



4 25 kD

Alex Osnis, PhD<sup>1</sup>, Donna Ron, MD<sup>1</sup>, Martin Ellis, MD<sup>1,2</sup> and Osnat Ashur-Fabian, PhD<sup>1,3</sup>

<sup>1</sup> Translational Hemato-Oncology Laboratory, Hematology Institute and Blood Bank<sup>2</sup> Sackler Faculty of Medicine, Tel Aviv University<sup>3</sup> Dept. of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University

## INTRODUCTION

Blood-donor derived platelets are critical for a variety of clinical implementations. Room temperature storage limits the shelf life and availability of platelet concentrates. Redox control of cellular homeostasis is a key determinant of cell destiny. This is particularly true for platelets. Selenoproteins, an essential family of enzymes containing selenium in their catalytic domain, are involved in maintaining the redox state in a large variety of cells. Selenoproteins considered new and promising players in a wide range of processes, yet very little is known regarding their expression in platelets.

MEIR MEDICAL CENTER

#### **OBJECTIVES**

This research examines, for the first time in platelets, the expression pattern of a series of selenoproteins throughout the shelf life of donor platelets and after they are considered expired. Insights from this work may generate fundamental novel biological information with implications regarding mechanisms involved in platelet function and survival.

## METHODS

Donor platelets with various expiration dates were collected, extracted after different time periods and stored (-80°C) until use. Total proteins were extracted and quantified. Expression of selenoproteins and other thyroid hormone related proteins was analyzed by Western Blot. The optical density of the bands obtained was measured and normalized to protein loading amounts .Statistical analysis and interpretation of the results was performed using Unpaired Students T test.

## RESULTS

Expression levels of **Glutathione Peroxidase** (**GPRx**), a well known antioxidant, decreased over time (**Fig. 1**). Expression levels of **Selenoprotein B** (**SelB**), a translation elongation factor that participates in selenoprotein production, also decreased over time (**Fig. 2**). Expression levels of **Iodothyronine Deiodinase 1** (**DIO1**), converter of T4 to the bioactive form T3 and participant in a wide range of cellular processes, increased during storage (**Fig. 3**). A significant decrease in **DIO3** expression levels, a thyroid hormone inactivator (**Fig. 4**).

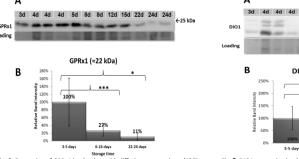


Fig. 1: Expression of GPRx1 in platelets with differing storage times (A) The number of days of storage is shown above the Western Blot image. Ponceau S is shown as loading control. (B) Bar graph showing statistically significant (p<0.0005, p<0.05) decrease in expression of GPRx1.

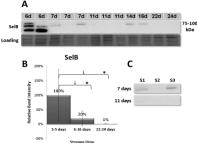


Fig. 2: Expression of SelB in platelets with differing storage times (A) Storage time (in days) is shown above the Western Blot image. Ponceau S is shown as loading control. (B) Bar graph showing statistically significant (pc0.05) decrease in SelB expression. (C) Paired samples showing SelB expression at 7 days of storage compared with expression in the same platelet sample after 11 days of storage at RT.

Fig. 3: DIO1 expression in platelets with differing storage times (A) The number of days each sample was stored is shown above the Western Blot image. Ponceau S is shown as loading control. (B) Bar graph showing statistically significant (p<0.005) increase in DIO1 expression.

6-16 days

Storage time

22-24 days

DIO1 (28-34 kDa)

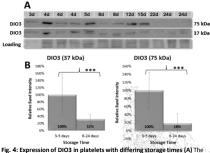


Fig. 4: Expression of DIO3 in platelets with differing storage times (A) The number of days each sample was stored is shown above the Western Biot image. Ponceau S is shown as loading control. (B) Bar graph showing statistically significant (p<0.00005) decrease in expression of both DIO3 forms.

# **CONCLUSIONS**

This study sheds light on the expression levels of a series of selenoproteins throughout platelet shelf life and thereafter. This information may have important implications regarding donor platelet quality, function and shelf life and may prevent the wasteful discarding of platelets that are currently considered expired.