SOHC
SWISS ONCOLOGY & HEMATOLOGY CONGRESS

Molecular mechanisms of regulation of apoptosis and autophagy in pediatric immune thrombocytopenia

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Experimental Hematology / Oncology

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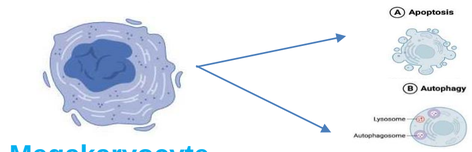
Introduction

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low platelets counts. Most treatments are effective in a limited number of patients only. Previous studies, also from our group, have demonstrated a role of platelet apoptosis in the pathogenesis of pediatric ITP, while the role of the autophagic machinery is far less characterized.

Aim of the study

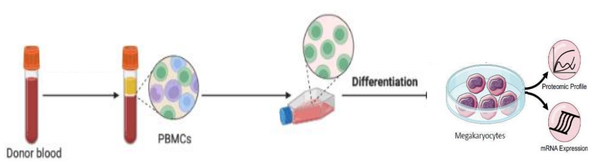
We aimed to investigate the gene expression and activation mechanisms of key proteins of the apoptosis and autophagy/ER-phagy signaling pathways in ITP disorder at megakaryocytes (MKs) level, as they are the precursors of platelets.



Megakaryocyte

Methods

We used the MEG-01 cell line and MKs differentiated from PMBCs derived from healthy controls (HC), which were treated with plasma isolated either from HC or ITP. Plasma was heat inactivated (HI) at 53°C for 30 min. We investigated the expression of specific regulatory genes both at mRNA level by qRT-PCR and at protein level by Western blotting. We further performed transmission electron microscopy (TEM) on primary MKs derived from PBMCs either from ITP patients or age matched healthy individuals.



Results

1. Effects of heat inactivation of control or ITP plasma on apoptosis and autophagy in MEG-01 cell line

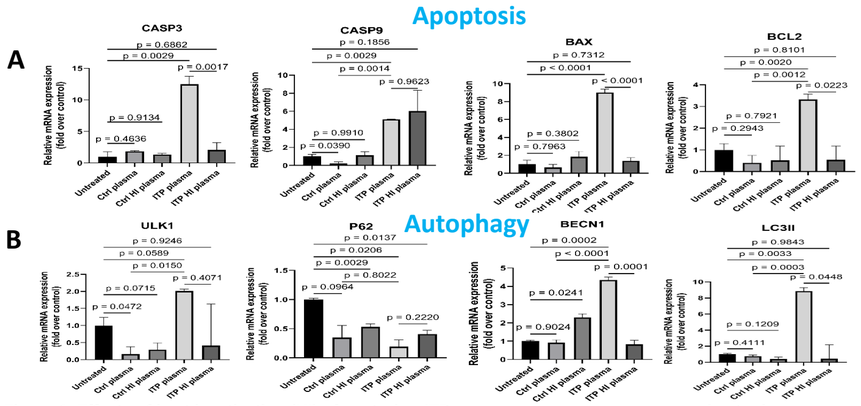


Figure 1. Effects of heat inactivation (HI) of control or ITP plasma plasma on apoptosis and autophagy genes in MEG-01 cell line. Relative mRNA levels of apoptosis and autophagy ER-phagy markers were determined by qRT-PCR. Statistical analysis was performed using one-way ANOVA followed by multiple comparisons tests to compare the mean ranks between the groups. Error bars show SD. Significance is shown as $p < 0.05$.

2. Effects of heat inactivation of control or ITP plasma on ER-phagy in MEG-01 cell line

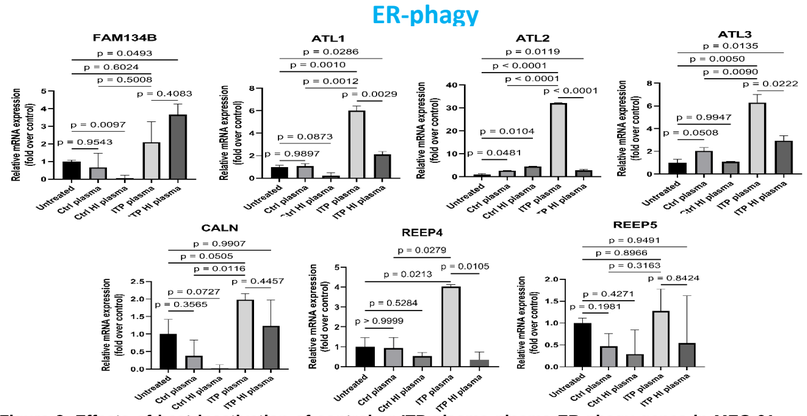


Figure 2. Effects of heat inactivation of control or ITP plasma plasma ER-phagy genes in MEG-01 cell line Relative mRNA levels of apoptosis and autophagy ER-phagy markers were determined by qRT-PCR. Statistical analysis was performed using one-way ANOVA followed by multiple comparisons tests to compare the mean ranks between the groups. Error bars show SD. Significance is shown as $p < 0.05$.

3. Effects of ITP plasma on autophagy/ER-phagy in healthy MKs at distinct time points

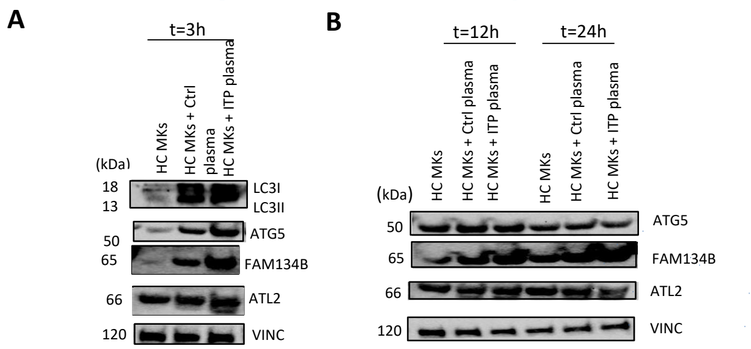


Figure 3. Effects of ITP plasma on autophagy and ER-phagy genes in MKs differentiated from PBMCs derived from healthy individuals. Western blot for healthy control MKs treated either with control or ITP plasma at A) 3h and B) 12h and 24h before cell harvesting. Vinculin served as an internal loading control.

4. Ultrastructural analysis of ITP derived MKs compared to MKs derived from healthy individuals

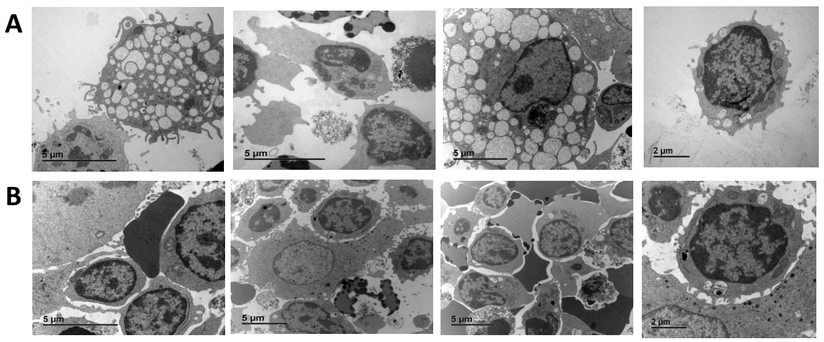


Figure 4. Ultrastructural analysis of ITP-derived MKs generated in a 3-phase model in *vitro*. By TEM, atypical morphological changes were identified in MKs differentiated from PBMCs isolated from **(A)** ITP patients (vacuolization of the cytoplasm, nuclear condensation and fragmentation) compared to **(B)** MKs differentiated from PBMCs derived from healthy individuals.

Conclusions

1) ITP plasma can induce **caspase-dependent apoptosis** in MKs

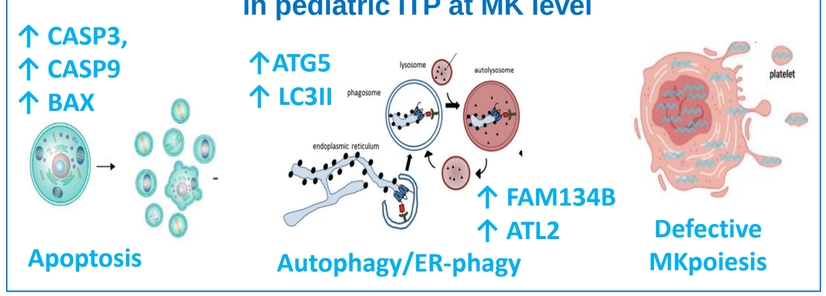
2) **Plasma heat inactivation** could reverse the upregulatory effect of almost all the investigated markers except for FAM134B and CASP9.

3) ITP plasma has modulatory effects on **autophagy/ER-phagy signaling** at MK level confirmed both at mRNA and protein level

4) **Abnormal morphology** was observed in MKs differentiated from PBMCs of ITP patients compared to HCs.

Summary

Apoptosis and autophagy/ER-phagy involved mechanisms in pediatric ITP at MK level



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