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PTTG-1 knockout gene leads to structural and functional changes in peripheral blood cells in mice

Usunięcie genu PTTG-1 prowadzi do zmian strukturalnych i funkcjonalnych w komórkach krwi obwodowej myszy

INTRODUCTION

Pituitary Tumor Transforming Gene (PTTG1) was first discovered in 1997. Human Pituitary Tumor Transforming gene 1 (PTTG1) encodes a securin protein that is critically important inregulating chromosome separation. Securin proteins together with separin and cohesin regulate sister chromatid separation during M phase of the cell cycle [4]. Securin accumulation and binding to separin during interphase and early mitosis prevents premature separin activation. During the normal cell cycle, the anaphase promoting complex eventually degrades securin, thus activating separin to facilitate equal chromosome segregation. In this sense, securin functions as an inhibitor of chromatid separation during anaphase [4].

Pttg-KO mice are viable but exhibit a variety of abnormalities, including splenic hypoplasia, thymic hyperplasia, small testes, thrombocytopenia, and sexually dimorphic diabetes mellitus [3]. These mice also develop organ-specific abnormal cell nuclear morphology, such as macronuclei in the pancreatic b-cells and the hepatocytes [3]. Thus, PTTG seems to be associated with tumorigenesis, angiogenesis and cancer progression. Recently, it has been shown that the level of PTTG1 mRNA, normally undetectable in T-lymphocytes, is markedly increased in the activated T-lymphocytes [2]. In this report, we show that securin is indispensable for haemopoesis in these animals.

MATERIAL AND METHODS

M o d e l f o r r e s e a r c h . Blood samples were collected from the wild-type and knockout mice (both of them – BL6/C57 line of mice). The homozygotes of *pttg*-WT and *pttg*-KO mice was

obtained by breading of the *pttg* -geterozygotes (*pttg* +/–) or gomozygotes *pttg*-KO. Genotyping of the mice were performed by PCR, using specific for the *pttg* gene (WT) and for the insert (*pttg*-KO) primers [4].

Cell counting. Murine blood samples from the wild-type and knockout mice were collected from the tail vein in heparinized tubes. 2 μl of blood was added to 2 ml and cells were counted in Goryayev's chamber.

Fractionation of erythrocytes on a discontinuous sucrose gradient. All steps were performed on ice and all reagents precooled to 4 °C. Sucrose was dissolved in water to obtain a 30% sucrose solution. The following concentration solutions from stock solution were prepared: 26%, 22%, 18%, 14%, 10%, 6% of sucrose. 2 ml of 30% sucrose solution was carefully layered in tube with erythrocytes suspensions. Afterwards, 2ml of each solutions was carefully put on the top (starting with 30% concentrations at the bottom and ending with 6% at the top). Erythrocytes were separated according to the density. Each fraction of erythrocytes was collected and the number of cells in each of them was counted.

Erythrocyte aggregation. RBCs were washed three times with 0.9% NaCl. RBC suspensions (10^6cells / ml) in polycarbonate test tubes (1.5 ml/tube) were kept at 37 ^{0}C for 1 min before being introduced into the flow channel of aggregation analyzer. After having been reacted with Alcian Blue and lectin of *Maakia amurensis*, the registration of optical density and aggregate mean radius began [1].

RESULTS

We analysed leukocytes, red blood cells, haematocrit, mean cellular haemoglobin concentration, platelets, neutrophiles, lymphocytes, monocytes, eosinophiles and basophiles. In these tests of the whole blood we found only a change in the platelet, erythrocyte and neutrophile numbers whereas all other haematological parameters were not affected.

Therefore, we tested the erythrocyte function and performed aggregometry measurements with erythrocyte rich plasma. The analysis of the transmission values of aggregation curves after erythrocyte activation by Alcian Blue showed that both curves differed significantly after erythrocyte initiation (Fig. 1). *Pttg*-KO erythrocytes showed a slightly lowered aggregation velocity. When we compared the aggregation curves after erythrocyte activation by Alcian Blue with the ones after lectin of *Maakia amurensis*, we found that the difference in the curves was much more pronounced (Fig. 2). Additionally, *pttg*-KO erythrocytes demonstrated decreased osmotic fragility (Fig. 3). Similar properties can be observed in red blood cells with lowered membrane permeability. The acid resistance of *pttg*-WT red blood cells is significantly increased compared to *pttg*-KO cells. 50% haemolysis is achieved at 3.5 min for wild type cells versus 5.5 min for knock out cells.

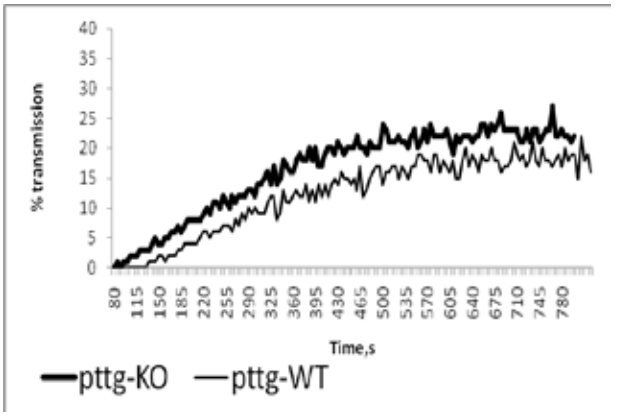

Fig. 1. Typical Alcian Blue – induced aggregation curve

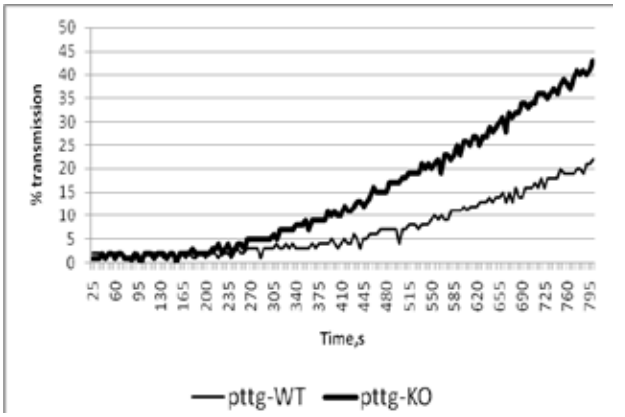

Fig. 2. Typical lectin – induced aggregation curve

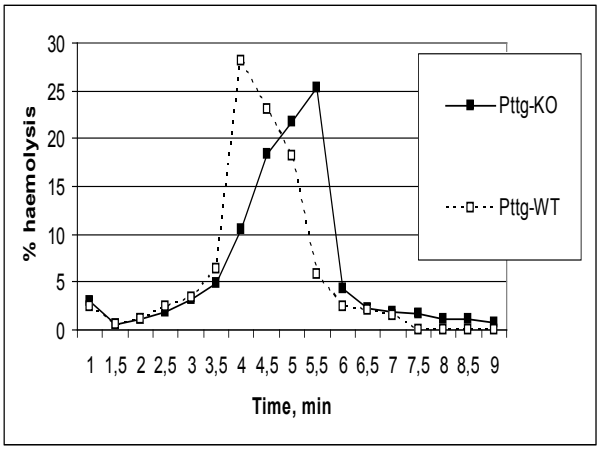

Fig. 3.Typical acid erythrohram

It suggests *pttg*-KO-induced increase in the number of young erythrocytes in peripheral blood. These data were confirmed by means of erythrocyte density fractionation. However, an additional fraction of old erythrocytes in peripheral blood of *pttg*-KO mice was detected. Such erythrocytes were not identified in the peripheral blood of WT-mice.

Serum alanine amino-transferase (ALT), aspartate amino-transaminase (AST) and total bilirubin (TBil) were tested. The concentration of serum bilirubin in plasma was elevated without defection of liver function. It testifies to the violation of old erythrocyte elimination.

Histological and electron-microscopy studies were used to establish the effect of *pttg*-KO upon spleen development. We revealed a significant increase in the number of apoptotic lymphocytes and a decrease in the number of active macrophages in both *pttg*-KO spleen (Fig 4).

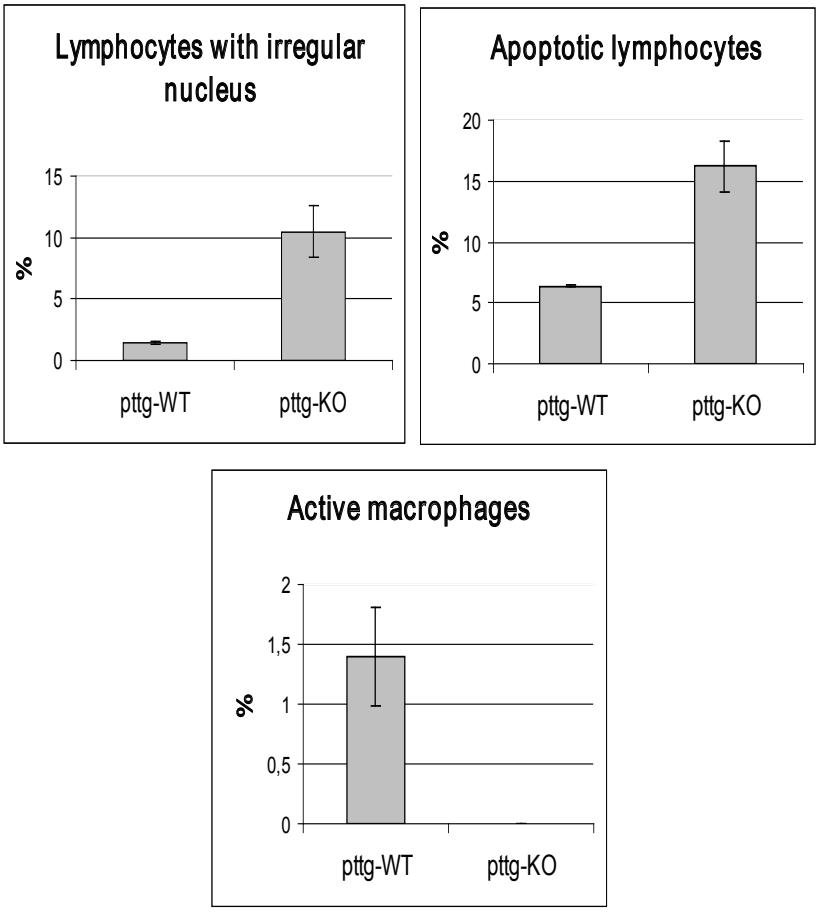

Fig. 4. Spleen: pttg-WT versus *pttg*-KO (electron microscopy study)

DISCUSSION AND CONCLUSIONS

Securin is an integral component of the mitotic mechanism, and conceptually mitotic chaos should ensue when securin function is absent [4]. Because cells and mice devoid of securin are indeed viable, a second mechanism for sister chromatid cohesion has been suggested. In this report, we demonstrate that securin-knockout mice have profound defects on spleen and thymus development and abnormal haemopoesis thus illustrating that any compensatory mechanisms for sister chromatid separation are not sufficient for maintaining beta cell proliferation and subsequent intact islet cell function.

It was found that *pttg*-KO leads to changing physical, biochemical, morphological properties of erythrocytes, which fall into the bloodstream in reticulocyte stage. Changes related to the features of erythrocytes erythropoiesis. It was found that *pttg*-KO has a stimulative effect on proliferation of erythroid bone marrow cells. During the study acid erythrohram conditions found displacement of the curve to the right of hemolysis, indicating the increasing stability of erythrocytes, due to the growing number of young forms of erythrocytes. This can be explained by slow maturation of erythrocariocytes and rapid elimination of reticulocytes in the peripheral blood. It can be concluded that *pttg*-1 might play an important role in regulation of haemopoesis.

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SUMMARY

Pituitary tumor transforming gene-1 is involved in the regulation of mitotic sister chromatid separation. We studied the effect of *pttg*-KO upon the production of new blood cells and elimination of erythrocytes. It can be concluded that *pttg*-1 might play an important role in the regulation of haemopoesis.

Keywords: pituitary tumor transforming gene-1, Pttg-1, gene knockout, mouse, blood cells

STRESZCZENIE

Gen transformujący guzów przysadki-1 (*Pttg*-1) bierze udział w regulacji podziałów mitotycznych chromatyd siostrzanych. Zbadano wpływ usunięcia genu *pttg*-1 na tworzenie nowych komórek krwi i eliminację erytrocytów. Stwierdzono, że *pttg*-1 może odgrywać ważną rolę w regulacji hemopoezy.

Słowa kluczowe: gen transformujący guzów przysadki-1, pttg-1, usunięcie genu, mysz, komórki krwi