Univerzita Karlova v Praze

1. lékařská fakulta

Autoreferát disertační práce

Exprese a funkce buněčného prionového proteinu na krevních buňkách

Expression and function of cellular prion protein in blood cells

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2012

Doktorské studijní programy v biomedicíně

Univerzita Karlova v Praze a Akademie věd České republiky

Obor: Molekulární a buněčná biologie, genetika a virologie

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Školicí pracoviště: Ústav imunologie a mikrobiologie

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

Obsah

Abstrakt

Buněčný prionový protein (PrPc) je nezbytný pro patogenezi neurodegenerativních prionových onemocnění. V současné době jsou známy čtyři případy přenosu variantní Creutzfeldt-Jakobovy choroby krevní transfuzí. Objasnění vlastností a množství PrPc na krevních buňkách je předpokladem pro vývoj testu pro detekci prionových chorob v krvi. Průtoková cytometrie představuje potenciální detekční metodu, nicméně výsledky studií zabývajících se množstvím PrPc na lidských krevních buňkách jsou rozporuplné.

V této práci jsme ukázali, že většina PrPc v klidových destičkách se nachází intracelulárně a je přítomna v α-granulích. Lidské destičky a červené krvinky nesou významné množství PrPc a mohou tak hrát roli při přenosu prionů krevní transfuzí. Naše výsledky naznačují, že PrPc na červených krvinkách je kovalentně modifikován. Podobná modifikace patologického prionového proteinu by mohla ovlivnit detekci prionových chorob v krvi. Dále jsme ukázali, že skladování krve před vlastní analýzou a výběr prionových protilátek výrazně ovlivňuje detekci PrPc metodou průtokové cytometrie a že destičkový satelitismus ovlivňuje detekci PrPc na krevních buňkách. Prokázali jsme, že část PrPc přítomná na leukocytech primátů *cynomolgus monkey (Macaca fascicularis)* je s buňkami volně asociovaná a uvolní se během izolace buněk. Dále jsme studovali možnou funkci PrPc v erytropoéze. Dokázali jsme, že povrchová exprese PrPc na myších erytroidních prekurzorech v kostní dřeni a slezině je regulována v průběhu zrání buněk. Podobně je regulován PrPc během *in vitro* diferenciace MEL buněk. To naznačuje úlohu PrPc v časných stádiích erytroidní diferenciace. Exprese PrPc navíc vedla k efektivnější erytropoéze po experimentálně vyvolané anémii u inbredních myší.

Výsledky této studie přispívají k objasnění vlastností PrPc a jeho distribuce v krvi a jsou důležité pro vývoj testu pro detekci prionových chorob v krvi.

Klíčová slova:

buněčný prionový protein, průtoková cytometrie, erytroidní diferenciace, fyziologická role

Abstract

The cellular prion protein (PrPc) is essential for pathogenesis of fatal neurodegenerative prion diseases. Recently reported four cases of vCJD transmission by blood transfusion raise concerns about the safety of blood products. Proper understanding of PrPc in blood is necessary for development of currently unavailable blood screening tests for prion diseases. Flow cytometry is an attractive method for prion detection, however, the reports on the quantity of PrPc on human blood cells are contradictory.

We showed that the majority of PrPc in resting platelets is present in the intracellular pool and is localized in α-granules. We demostrated that both, human platelets and red blood cells (RBC) express significant amount of PrPc and thus may play an important role in the transmission of prions by blood transfusion. Our results suggest a unique modification of PrPc on human RBC. Such modification of pathological prion protein could distort the results of blood screening tests for prions. Further we showed that the storage of blood prior to analysis and the choice of the anti-prion antibody greatly affect the detection of PrPc by flow cytometry and we identified platelet satellitism as a factor contributing to the heterogeneity of PrPc detection in blood cells. Moreover, we demonstrated existence of washable pool of PrPc on the leukocytes of cynomolgus monkeys. Next we studied the role of PrPc in erythropoiesis. We demonstrated that the surface expression of PrPc on mouse erythroid precursors in bone marrow and spleen follows similar pattern during the cells' maturation. PrPc expression in differentiating MEL cells mimics the pattern seen *in vivo* suggesting PrPc importance in early stages of erythroid differentiation. Moreover, PrPc expression accelerated recovery from stress erythropoiesis in inbred mice.

In conclusion, this study contributes to the knowledge about the PrPc distribution and function in blood cells which is important for development of blood screening test for prion disease.

Key words:
cellular prion protein, flow cytometry, erythroid differentiation, physiological role

1. Introduction

Human prion diseases (Transmissible spongiform encephalopathies, TSE) cause severe neuronal degeneration and are invariably fatal. No routine diagnostic test or treatment is available so far. The most common human prion disorder is sporadic form of Creutzfeldt-Jakob disease (CJD). Recently, a variant form of CJD (vCJD) which was transmitted to humans by consumption of food contaminated with bovine spongiform encephalopathy (BSE) prions, has attracted much attention.

The main, if not only, constituent of the infectious prion particle is a pathological form of prion protein (PrPtse), which can propagate upon contact with normal cellular prion protein (PrPc) by changing its conformation [1]. Importantly, PrPtse is the only known marker of prion diseases.

Recently described transmissions of vCJD by blood transfusion [2,3] highlight the need for development of non-invasive blood screening tests. However, the PrPtse detection is complicated by limited knowledge about the nature of PrPtse in the blood and the unavailability of reliable PrPtse -specific antibodies. Moreover, the large amount of poorly characterized PrPc present in blood may interfere with the test. Interestingly, several flow cytometry studies utilizing antibodies recognizing PrPc reported differences in their binding to blood cells of prion-infected individuals [4-6]. However, flow cytometry data usually demonstrate relatively large variations in PrPc levels within a particular cell type even in healthy donors.

In human blood, PrPc has been found in plasma as well as on blood cells, including T and B lymphocytes, NK cells, monocytes, dendritic cells, follicular dendritic cells [7-10] and platelets [7,11]; lower levels of PrPc have been detected in red blood cells (RBC) [12,13]. In contrary, granulocytes express little to no PrPc [7,14]. Although PrPc expression on human blood cells has been studied by several groups, considerably different levels of PrPc have been reported. These discrepancies may be caused by the choice of antibodies for PrPc detection or by a diverse processing of the blood before analysis.

Although PrPc is ubiquitously expressed and conserved throughout the species its physiologic function is unclear. It was suggested that PrPc can act as

copper binding protein, antioxidant, receptor for various molecules, signal transductor, and may protect against apoptosis [15]. PrPc seems to be important for normal CNS function as Prnp-/- mice show altered circadian rhythm, increased susceptibility to neuronal damage by oxidative stress and motor and cognitive abnormalities [16,17]. PrPc role in hematopoiesis has recently been suggested. PrPc is expressed by hematopoietic stem cells (HSC) [12] and is necessary for their self-renewal [18]. Prnp-/- mice have impaired erythropoiesis after induction of experimental anemia [19]. Moreover, PrPc mediates iron uptake and transport in vitro and in vivo [20,21] and is necessary for iron homeostasis [22]. However the involvement of PrPc in erythropoiesis is still not elucidated.

Understanding the PrPc role and determination of its amount on blood cells together with its distribution in blood are important for development of blood screening tests for prion disease and prevention of prions transmission by blood transfusion.

2. Aims and hypothesis

The overall objective of this work was to enhance the understanding of the cellular prion protein (PrPc) in blood cells and its possible involvement in TSE pathogenesis. The knowledge of the PrPc levels expresed by blood cells and its distribution in blood is essential for development of currently unavailable non-invasive detection methods for prion disease.
The aims of the study are as follows:

1. Analysis of PrPc levels on blood cells and its localization
    - Assessment of the intracellular pool of PrPc in human platelets and analysis of its localization.
    - Characterization of PrPc on human red blood cells and revelation of the reason for disproportions in published data concerning quantity of PrPc associated with human red blood cells.
    - Clarification of the origin of the discrepancies in PrPc levels on human peripheral blood leukocytes reported in the literature.
    - Verification of the PrPc levels expressed on leukocytes of cynomolgus monkeys used as a model of human prion disease.

2. Study of the importance of PrPc expression for erythroid differentiation utilizing murine models.
    - Analysis of PrPc regulation on erythroid precursors *in vivo* in murine bone marrow and spleen and *in vitro* in MEL cells.
    - Description of the effect of PrPc silencing on erythroid differentiation *in vitro* in model system of MEL cells.
    - Analysis of PrPc relevance for normal and stress-induced erythropoiesis in mice

3. Material and Methods

Isolation of platelets and red blood cells ghosts was done from whole blood as described in [13]. Platelets from plasma apheresis were isolated by gel filtration
Isolation of mouse bone marrow and spleen erythroid precursors was performed as described previously [23].
Isolation of lymphocytes from peripheral blood using Ficoll was done according to manufacturer's instructions.
Platelet activation (TRAP) and Proteinase K protection assay were performed as described in [24].
Biotin-labeling of mouse RBC *in vivo* with Sulfo-NHS-biotin, i.v. application.
SDS-PAGE and western blot of platelet and red blood cell (RBC) PrPc
Densitometry was done using GS-800 Calibrated Densitometer with integrated Quantity One software (Bio-Rad).
ELISA of RBC PrPc and erythropoietin in plasma.
Flow cytometry was done using either BD FACS Canto II flow cytometer equipped with BD Diva v6 software or BD FACScan equipped with CELLQuest^{TM} software (Becton Dickinson).
Quantitative multicolor flow cytometry was performed using Quantum RP-MESF beads (BangsLabs) and the numbers of PrPc molecules expressed per cell were calculated as described previously [12].
qRT-PCR (M. Panigaj) - relative fold expression levels were calculated using the 2-ΔΔCt method [25] normalized to the reference *Gapdh* gene.
RNAi (M. Panigaj) - retrovirus produced by HEK293 GP2 packaging cells was delivered to target cells by spinfection or co-cultivation. Upon puromycin selection cells expressing LP1 or LP2 shRNA targeting PrP mRNA and control nonsilencing LN shRNA were obtained.
Hemoglobin content was measured by TMB assay as described previously [26]
Laboratory mouse techniques – *i.p.* application of phenylhydrazine, *i.v.* application of biotin, cardial puncture, venipuncture, organ isolation
Statistical analyses - performed in SigmaStat v3.5 (Systat Software Inc.) and GraphPad v5.03 (GraphPad Software Inc.) using either paired or unpaired student's t-test, two-way anova, significance $P < 0.05$

4. Results

4.1. The size and localization of intracellular pool of PrPc in platelets

We analyzed the amount of PrPc accessible for Proteinase K (PK) digestion on the surface membrane of resting and activated platelets. Most (69%) of the PrPc in resting platelets was localized intracellulary and translocated on the cell membrane upon platelet activation (Fig. 1). The correlation of agonist dose-dependent membrane upregulation of PrPc and P-selectin (α-granular protein) on platelets suggested that PrPc is up-regulated from the same compartment as P-selectin.

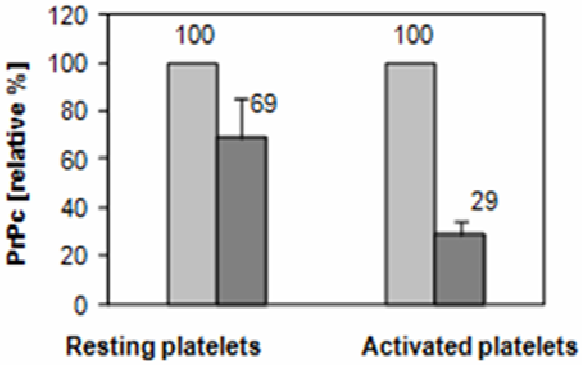

Figure 1. Most platelet PrPc resides in the intracellular pool.

Intact resting and TRAP-activated platelets or platelets solubilized by Triton X-100 were treated with proteinase K (PK) to cleave accessible PrPc. PrPc was detected on western blots with a mixture of mAb 6H4 and AG4. Densitometric quantification of PrPc is presented. Light gray bars: non-treated platelets, dark gray bars: PK-treated platelets. Approximately 70% and 30% of platelet PrPc reside in the intracellular pool of resting and activated platelets, respectively.

4.2. Analysis of PrPc levels on RBC

In our previous studies we detected low levels of PrPc on RBC, however, no PrPc on RBC was generally reported in literature. To clarify the discrepancies we performed the quantitative flow cytometry which demonstrated that RBC express around 300 molecules of PrPc per cell, assuming equimolar binding of

mAb 6H4 to PrPc. Antibody 3F4 and FH11 bound to RBC significantly less than 6H4 (Fig. 2A). In contrast, the binding of 3F4 and 6H4 to PLT in identical samples was roughly equivalent, around 600 molecules of PrPc per cell. Next we compared the PrPc levels on the membranes of PLT and RBC using western blot and one RBC expressed four times less PrPc than one PLT (Fig. 2B-C). Given the number of RBC, the total amount of PrPc associated with RBC is approximately five times higher than the level associated with PLT.

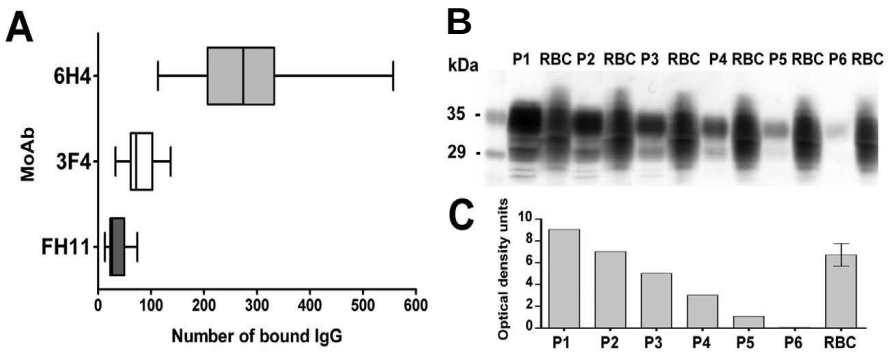

Figure 2. Quantification of PrPc expression on human RBC.

(**A**) Binding of mAbs to RBC of healthy donors measured by quantitative flow cytometry (n = 8). The range of the data, 50% percentile (box), and median are shown. (**B**) WB comparison of PrPc levels in representative standardized samples of RBC ghosts ($1x10^{10}$ RBC/ mL) and PLTs (P1-P6 = $5x10^{9}$, $2.5x10^{9}$, $1.25x10^{9}$, $6.25x10^{8}$, $3.13x10^{8}$, and $1.56\ x10^{8}$ PLT/mL) developed with mAb 6H4. (**C**) Densitometry of bands on blot (B) demonstrates that RBC contain four times less PrPc levels than an equal number of PLT.

4.3. PrPc modification in RBC

Flow cytometry demonstrated that mAbs 3F4 and 6H4 bind equally well to transferrin receptor-positive (CD71+) erythroid precursors in human cord blood. This suggests that the modification of the 3F4 epitope (KTNMKHM) occurs after the release of RBC into circulation (Fig. 3).

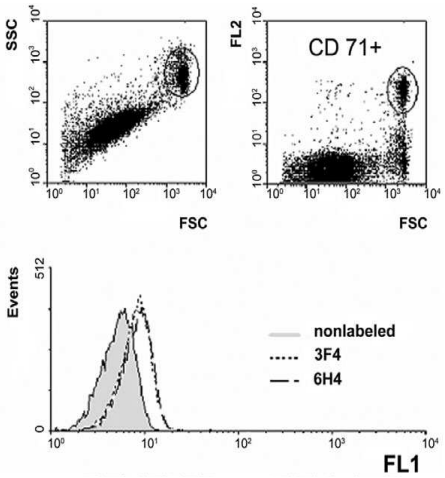

Figure 3. PrPc epitope for 3F4 is modified on mature human RBC.

MAbs 3F4 and 6H4 bind equally well to transferrin receptor–positive (CD71+) erythroid precursors in human cord blood (n = 3). The SSC/FSC plot shows the RBC gate (top left). CD71+ cells were gated on a FL2/FSC plot (top right) and their 3F4 and 6H4 fluorescence were compared (histogram overlay).

4.4. PrPc expression on human WBC

Since discrepancies in PrPc levels detected on WBC in human peripheral blood are reported in literature we performed a flow cytometry study to evaluate the impact of the preanalytical variables such as anticoagulant type and storage temperature on the detection of PrPc on those cells. Our results show that the choice of antibody greatly affects the detected levels of PrPc; the mAb AG4 (PrP 31-51) recognizing the N-terminal part of PrPc generally detected lower levels than the C-terminal-recognizing mAb AH6 (PrP 159-175) and the differences were cell population-specific. The highest numbers of PrPc molecules were detected on NK cells followed by T cells, monocytes, and dendritic cells (DC), whereas B cells expressed low levels of PrPc. This pattern was not affected by the choice of anticoagulant with exception of monocytes on which we detected significantly decreased levels of PrPc in fresh blood anticoagulated with citrate. Blood storage preceding analysis led to a cell type-specific decrease in PrPc detected on most leukocytes (Fig. 4).

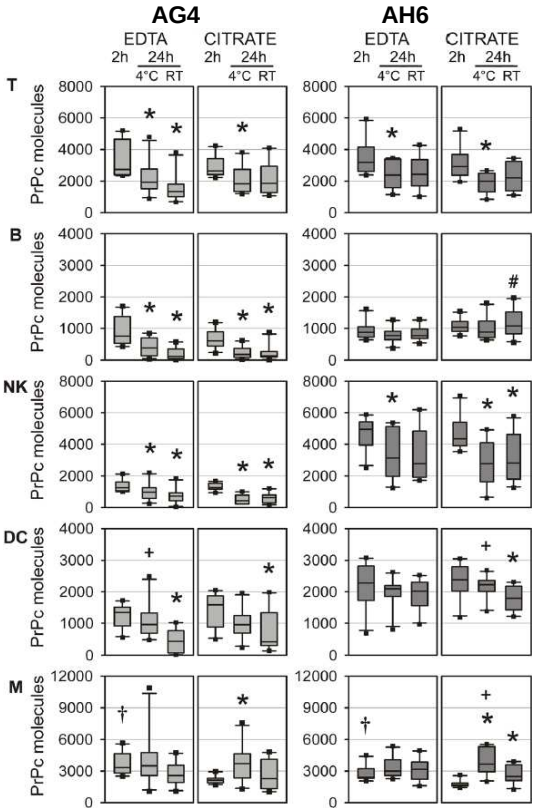

Figure 4. PrPc levels on T cells, B cells, NK cells, DC and monocytes in fresh blood and following overnight storage under different conditions.

The medians of the number of IgG molecules of anti-prion mAb bound per cell are presented. Assuming an equimolar binding of mAb to PrPc molecules, these data represent the number of PrPc molecules expressed by a single cell. *P < 0.05 vs. samples processed within 2 h after blood collection (2 h). +P < 0.05 vs. samples stored 24 h at RT. #P < 0.05 vs. EDTA samples stored 24 h. †P < 0.05 vs. EDTA samples processed within 2 h after blood collection (2 h), (n = 10).

4.5. Platelet satellitism in human leukocytes

An important finding of our study was the identification of platelet satellitism (Fig. 5). We report that $CD41^{pos}$ (PLT-associated) monocytes and granulocytes in fresh blood exhibited significantly increased levels of PrPc compared with their $CD41^{neg}$ counterparts. The storage of blood usually led to a further

increase of PrPc levels on CD41^{pos} cells but with large individual differences among donors. Interestingly, we also detected significantly increased levels of PrPc on CD41^{pos} lymphocytes in citrate. In these cells the existence of platelet satellitism is currently disregarded.

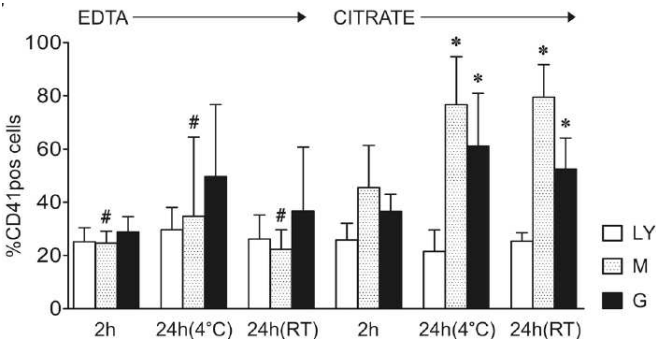

Figure 5. Evaluation of platelet satellitism by leukocytes populations.

The percentages of CD41pos (platelet-associated) lymphocytes (LY), monocytes (M), and granulocytes (G) were assessed in samples stored under different conditions. Data are the mean ± SD (n = 6).*P < 0.05 vs. samples processed within 2 h after blood collection (2 h). #P < 0.05 vs. citrate anticoagulated samples.

4.6. PrPc expression on WBC of cynomolgus monkeys

Although granulocytes of cynomolgus monkeys were shown to express very low to no PrPc similar to humans, high PrPc levels were reported recently. We determined that granulocytes of cynomolgus monkeys express negligible levels of PrPc (Fig. 6).

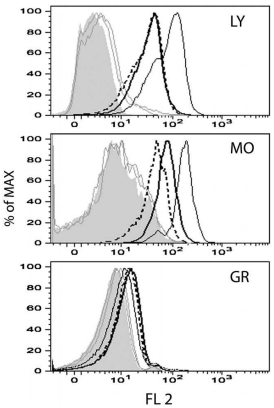

Figure 6. The binding of prion antibodies to granulocytes of healthy cynomolgus monkeys is negligible.

Representative fluorescence histograms of lymphocytes (LY), monocytes (MO) and granulocytes (GR) of a single monkey are shown. AG4 = thick dashed black line; 3F4 = thin black line; AH6 = thick black line; IgG2a and IgG2b isotypic control = thin gray lines; fluorescence of unlabeled cells = gray filled peak.

Ficoll separation of peripheral blood mononuclear cells led to decreased binding of mAbs 3F4 and AG4 to lymphocytes. This may be explained by washing out loosely associated PrPc from the cell surface (Fig. 7).

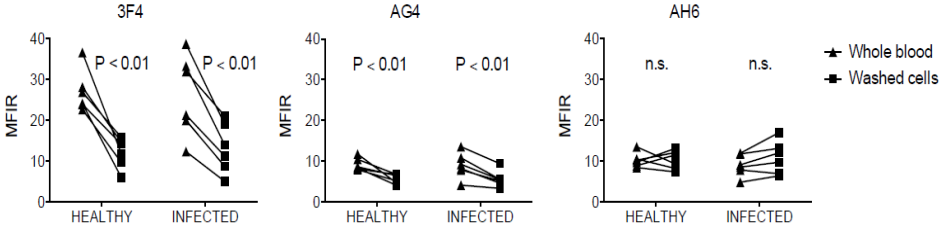

Fig. 7. Isolation of lymphocytes using Ficoll leads to decreased binding of prion antibodies.

Healthy (n = 6) and BSE infected (n = 6) cynomolgus macaques were analyzed. MFIR was calculated as median fluorescence of prion antibody divided by median fluorescence of appropriate isotypic control. Both AG4 and 3F4 detected significantly less PrPc on washed lymphocytes ($p < 0.01$). No significant difference was detected with AH6 (n.s.).

4.7. Regulation of PrPc expression in murine erythroblasts

The expression of PrPc on both mouse bone marrow (BM) and spleen erythroid precursors is upregulated on early basophilic erythroblasts (EryA) and decreases with the cells' maturation to late basophilic and polychromatic erythroblasts (EryB) and most mature small precursors (EryC) (Fig. 8).

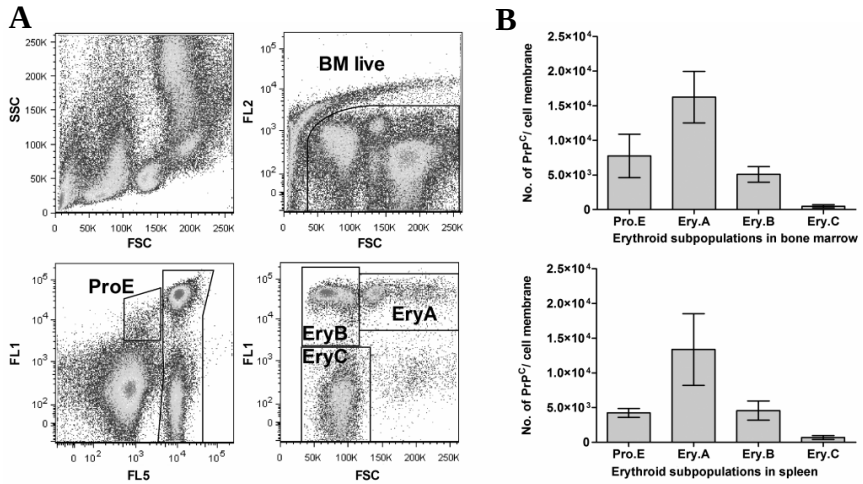

Figure 8. PrPc expression on mouse bone marrow (BM) and spleen erythroid precursors.

(**A**) Gating strategy for erythroid precursors: upper left – scattergram of BM cells; upper right – gating of viable 7-AAD negative cells (BM live); lower left – live BM cells labeled with CD71-FITC (FL1) and Ter119-eFluor450 (FL5): ProE = CD71+Ter119+; Ter119+ cells were gated on CD71-FITC (FL1) and FSC plot (lower right): EryA = large CD71+, EryB = small CD71+, EryC = small CD71- cells. (**B**) PrPc expression (number of mAb AH6 IgG molecules /cell) measured by quantitative flow cytometry on erythroid precursors in mouse BM (upper panel) and spleen (lower panel). PrPc expression on EryA is significantly higher ($p < 0.005$, $n = 5$) than on proerythroblasts (ProE) in BM and in the spleen.

4.8. Regulation of PrPc expression during MEL cells' differentiation

The amount of PrPc protein in differentiating MEL cells is highest 24–48 h after HMBA induction and then is downregulated (Fig. 9).

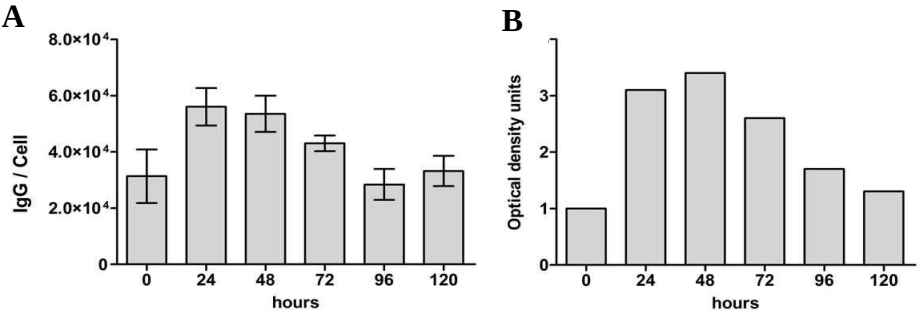

Figure 9. Surface expression of PrPc on differentiating MEL cells

PrPc expression (number of mAb AH6 IgG molecules /cell) measured by quantitative flow cytometry (**A**) correlates with the levels detected by WB (performed M. Panigaj) (**B**). Blots were developed with a mix of PrPc mAbs (AH6, AG4, 6H4) and an actin antipody was used as a loading control.

4.9. RNAi effectively silenced PrPc expression

Silencing of PrPc (performed M. Panigaj) was stable during the entire differentiation. PrPc expression in LP1 and LP2 cells was inhibited on average down to ~ 20% and 5% at time 0 and 120 h, respectivelly in comparison to MEL LN line (Fig. 10).

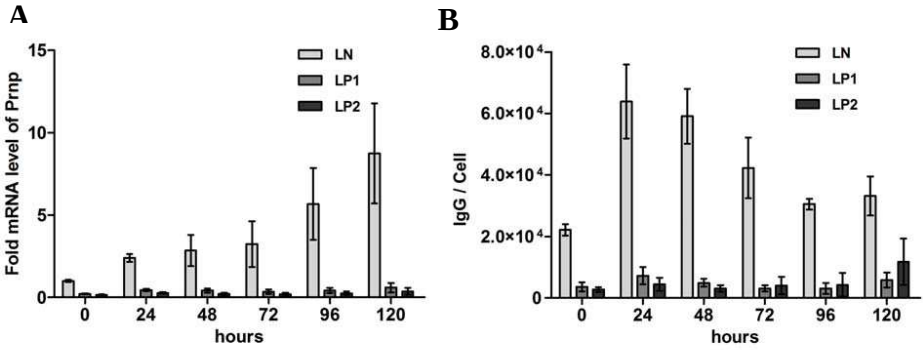

Figure 10. PrPc expression is stably supressed in differentiating MEL cells.

(**A**) PrP mRNA level measured with qRT-PCR in cell lines stably expressing anti-Prnp shRNAmir (LP1 and LP2) is downregulated in comparison with control (LN) during the cell differentiation. (**B**) The effect of silencing on the level of PrPc on the cell membrane estimated by quantitative flow cytometry.

4.10. PrPc silencing does not affect MEL cell differentiation

Erythroid differentiation of MEL cells with silenced PrPc expression (LP1, LP2) (performed M. Panigaj) was not altered compared to non-silenced control (LN) as demonstrated by similar level of both hemoglobin (performed M. Panigaj) and transferrin receptor (Fig. 11).

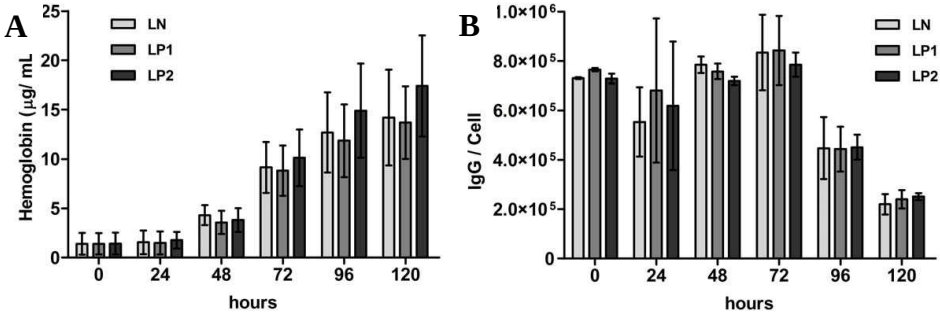

Figure 11. Differentiating MEL cells display similar characteristics irrespective to the level of PrPc expression.

(**A**) Concentration of total hemoglobin in the cells per 100 mg/mL of total cell proteins. (**B**) Number of CD71+ (transferrin receptor) molecules per cell, analyzed by quantitative flow cytometry, based on assumption that one anti-CD71 mAb IgG binds one molecule of transferrin receptor.

4.11. PrPc relevance for normal and stress erythropoiesis in mice

We studied challenged and unchallenged erythropoiesis in mice with manipulated levels of PrPc. Induction of anemia in inbred mice resulted in lower hematocrit and enhanced erythropoietin levels in Prnp-/- (KO) mice. Reintroduction of Prnp gene in Tga20 mice rescued the animals from severe anemia (Fig. 12). WT mice exhibited enhanced turnover of red blood cells in circulation under physiologic conditions compared to Prnp-/- mice. In more stringent models of outbred mice, the effect of PrPc on recovery from acute anemia was less pronounced or even it appeared to be compensated. The physiologic erythropoiesis in outbred mice was not significantly affected by PrPc expression. Nevertheless, we observed slightly lower relative numbers of erythroid precursors in bone marrow of outbred Prnp-/- mice while in spleen they were slightly elevated in comparison to Prnp+/+ mice.

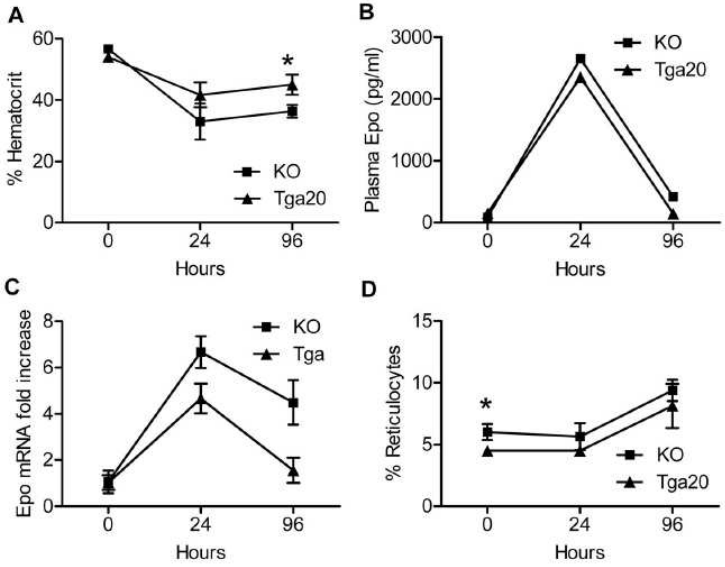

Figure 12. Reintroduction of Prnp gene in Tga20 mice rescued the animals from severe anemia.

After induction of anemia Prnp-/- (KO) mice had significantly lower HCT (**A**) elevated plasma erythropoietin (**B**) and increased levels of Epo mRNA in kidneys (**C**) compared to Tga20 mice. (**D**) Reticulocyte counts were already higher in KO at 0 h (*$p < 0.05$). Values are mean ± SD (n=3).

5. Discussion

Since PrPc serves as a template for PrPtse propagation during pathogenesis of prion disease, the knowledge of PrPc distribution in blood cells and its physiological function is necessary for the development of both diagnostic test and prevention of the disease transmission by blood transfusion. This work contributes to the current knowledge about PrPc and its possible physiologic role.

We demonstrate that the majority of PrPc in resting platelet is present in the intracellular pool and localized in α-granules, as was suggested using electron microscopy [27,28]. Platelets from deer suffering from chronic wasting disease (CWD), which similarly to human platelets express PrPc, are able to transmit the disease [29]. Hypothetically, PrPtse could be uptaken by platelets and delivered to α-granules where it could propagate without being detected in blood.

The estimated overall quantity of PrPc associated with RBC is at least two times higher than in platelets. We imply that the PrPc levels on RBC were deeply underestimated in previous studies [7,9,30-32] due to use of mAb 3F4 which failed to detect RBC PrPc due to modification of its epitope as we demonstrate here. Modification of PrPc on RBC may result in unique properties of the molecule affecting its interaction with PrPtse. Although RBC harbour the majority of cell associated PrPc in human blood, they are unlikely to propagate prions as they are not able to synthesize new molecules of PrPc.

The choice of the antibody significantly affects detection of PrPc on human blood leukocytes, as the N-terminus-recognizing mAb generally detected lower levels than the C-terminus-recognizing mAb, consistent with the previous study in non-human primates [33]. The pre-analytical variables such as blood storage, anticoagulant and storage temperature may also significantly affect the detected levels of PrPc and may contribute to the heterogeneity of results obtained by flow cytometry. Importantly, we identified platelet satellitism [34,35] as a currently unappreciated factor contributing to the heterogeneity of PrPc detection in blood cells. Since platelets were shown to

harbor infectivity, platelet satellitism may affect the distribution of prion infectivity in blood.

The granulocytes of healthy cynomolgus monkeys express negligible levels of PrPc in accordance with a previous study [33]. We propose that recently reported high PrPc levels [5] are introduced artificially. Importantly, Ficoll isolation of the cells decreased PrPc/PrPtse on lymphocytes of both healthy and BSE-infected animals suggesting existence of washable pool of PrPc on the cells. Similar behavior of PrPtse would significantly complicate its detection.

The regulation of surface PrPc expression on murine erythroid precursors *in vivo* and *in vitro* follows a similar pattern, although the PrPc levels on MEL cells are higher. PrPc was significantly up-regulated in basophilic erythroblasts and decreased with the cells' maturation suggesting involvement of PrPc in the early stages of erythroid differentiation. Inhibition (~80%) of the PrPc expression by RNAi did not affect the differentiation of MEL cells nor the viability, although the anti-apoptotic effect of PrPc was reported in several studies [36]. These results suggest that PrPc is dispensable for unchallenged erythropoiesis *in vitro.*

After induction of anemia inbred Prnp-/- mice show deeper anemia with lower hematocrit than Prnp+/+ mice, in agreemenet with a previous report [19]. Higher levels of erythropoietin in plasma and retarded recovery to normal hematocrit suggest that the absence of PrPc negatively affects stress erythropoiesis. Physiological erythropoiesis seemed to be more efficient in inbred WT mice with higher turnover of RBC in circulation comparing to Prnp-/- mice. The relative numbers of erythroid precursors were slightly increased in the spleen compared to bone marrow (BM) of outbred Prnp-/- mice. As steady state erythropoiesis in mice occures in BM whereas stress erythropoiesis in the spleen [37], our results suggest a compensation mechanism for impaired basal erythropoiesis. PrPc might be important in stress erythropoiesis in inbred mice whereas its role seems to be compensated during unchallenged erythropoiesis and the effect diminished in outbred mice. Hovewer, elucidation of the exact role of PrPc in erythropoiesis requires further experimentation.

6. Conclusions

The results of the work contribute to our knowledge about the nature and function of PrPc on blood cells and are important for the development of blood screening test for prions. The results are summarized as follows:

1. Two thirds of platelet PrPc are present in the intracellular pool.
2. PrPc within platelets is located in α-granules.
3. Platelet activation leads to translocation of intracellular PrPc onto the cell membrane, together with an α-granular marker P-selectin.
4. One platelet express twice the amount of PrPc found on red blood cells, however when total cell number is taken into account red blood cells possess majority of the cell-associated PrPc in human blood.
5. PrPc on human red blood cells undergoes a posttranslational modification in circulation which results in the loss of epitope for widely used prion mAb 3F4.
6. The choice of anti-prion antibody greatly affects the PrPc detection on leukocytes in peripheral blood.
7. Blood storage preceding analysis leads to a decrease in PrPc detection on most leukocytes.
8. Platelet satellitism contributes to the heterogeneity of PrPc detection in blood cells as the platelet association with leukocytes may significantly increase the levels of PrPc detected on leukocyte subsets.
9. Granulocytes of cynomolgus monkeys express negligible levels of PrPc and cell isolation using Ficoll leads to decreased binding of prion antibodies to lymphocytes.
10. PrPc expression is regulated during erythroid differentiation of murine erythroid precursors *in vivo* and *in vitro*.
11. PrPc is dispensable for erythroid differentiation under unchallenged conditions *in vitro*.
12. PrPc might be important during stress erythropoiesis *in vivo* whereas its absence seems to be compensated during unchallenged erythropoiesis.

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Glierová H, Holada K. Úloha imunitního systému v prionových chorobách. Alergie 2/2006, 143-148

Manuscript in preparation for publication

Glier H, Panigaj M, Janouskova O, Semberova J, Hudakova A, Holada K. Impairment of erythropoiesis in inbred cellular prion protein deficient mice.