DIFFERENTIAL REGULATION OF CERULOPLASMIN ISOFORMS EXPRESSION IN MACROPHAGES AND HEPATOCYTES

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INTRODUCTION

Ceruloplasmin (Cp) is a multicopper oxidase implicated in iron (Fe) metabolism and protection against free radical–driven cell injury [1]. Through oxidation of Fe (II) to Fe (III), Cp assists the sole identified mammalian iron exporter ferroportin (Fpn) for transporting iron out from the cells 2-3]. Cp can be expressed as a soluble secreted protein (sCp) or as a membrane GPI–anchored protein (GPI–Cp) as a result of alternative splicing [4-5]. sCp is abundant in serum and is known to be mostly expressed by hepatocytes while GPI–Cp has been shown to be mostly expressed in astrocytes, leptomeningeal cells, and sertoli cells [1]. Previously, we reported the mRNA expression of both sCp and GPI–Cp in human lymphocytes and in the hepatocarcinoma cell line HepG2 [6]. Herein, we clarified the protein expression of both Cp isoforms in different immune cells as well as in HepG2 cells.

MATERIAL & METHODS

The subcellular localization of Cp isoforms was analyzed by immunofluorescence and immunoblotting of resting human peripheral blood lymphocytes (PBL) and monocytes (PBMN), mouse bone marrow derived macrophages (BMDM) and HepG2. Cells were treated with PI–PLC, an enzyme that specifically cuts GPI–proteins, followed by analysis of Cp expression at cell surface by immunofluorescence. BMDM and HepG2 were treated with iron (Fe-NTA) and expression of Cp and Fpn was studied by immunoblotting of subcellular fractions (cytosol, membrane and lipid rafts). Colocalization of Cp and Fpn was investigated by immunofluorescence in iron-treated BMDM. Cp antibodies: anti-Cp FITC (BIOTREND), anti-human Cp (Koma Biotech) and anti-mouse Cp (BD Bioscience). Fpn antibody: anti-mouse Fpn (Alpha Diagnostic). For identification of lipid rafts/DRM (detergent resistant membrane) fractions, Flotilin-1 (Flot) and caveolin-1 (Cav) were used as lipid rafts markers.

ANALYSIS OF RESULTS

Both Cp isoforms are expressed in human PBL and PBMN

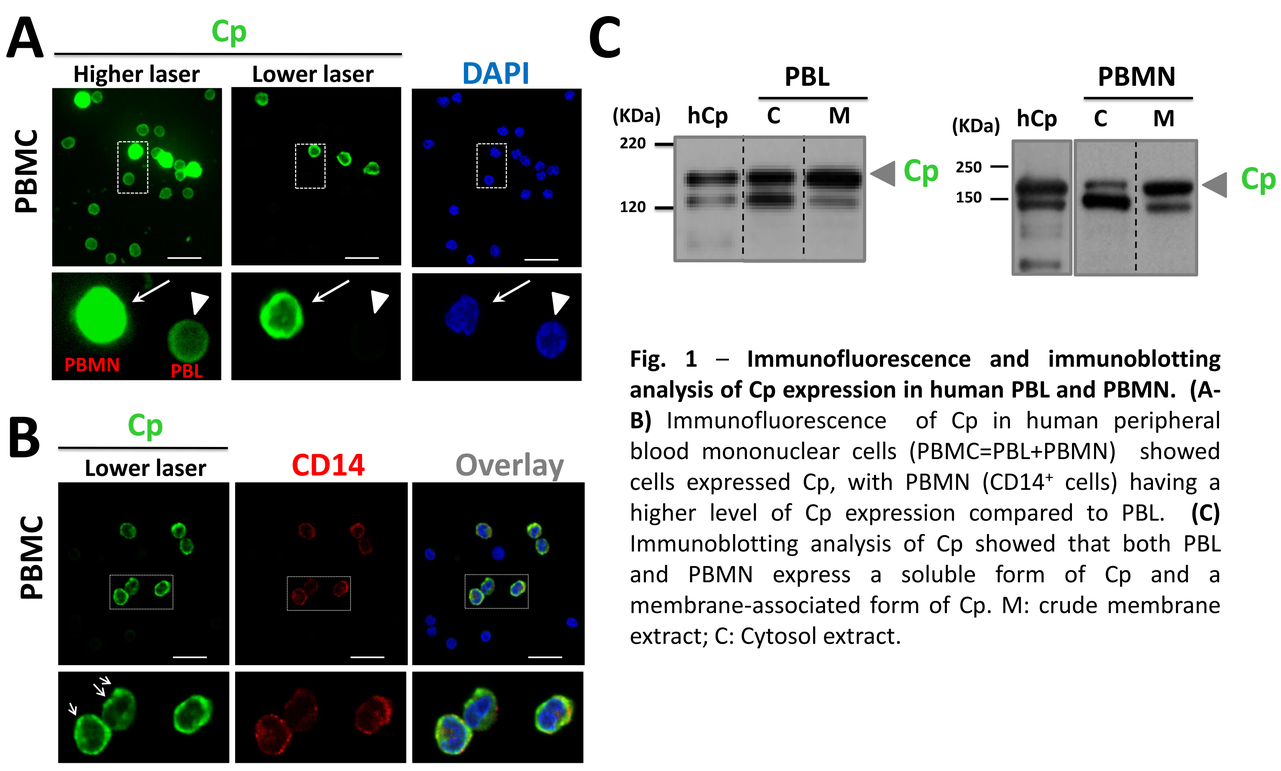

Fig. 1 – Immunofluorescence and immunoblotting analysis of Cp expression in human PBL and PBMN. (A-B) Immunofluorescence of Cp in human peripheral blood mononuclear cells (PBMC=PBL+PBMN) showed cells expressed Cp, with PBMN ($CD14^+$ cells) having a higher level of Cp expression compared to PBL. **(C)** Immunoblotting analysis of Cp showed that both PBL and PBMN express a soluble form of Cp and a membrane-associated form of Cp. M: crude membrane extract; C: Cytosol extract.

Mouse BMDM and human HepG2 express sCp and GPI-Cp

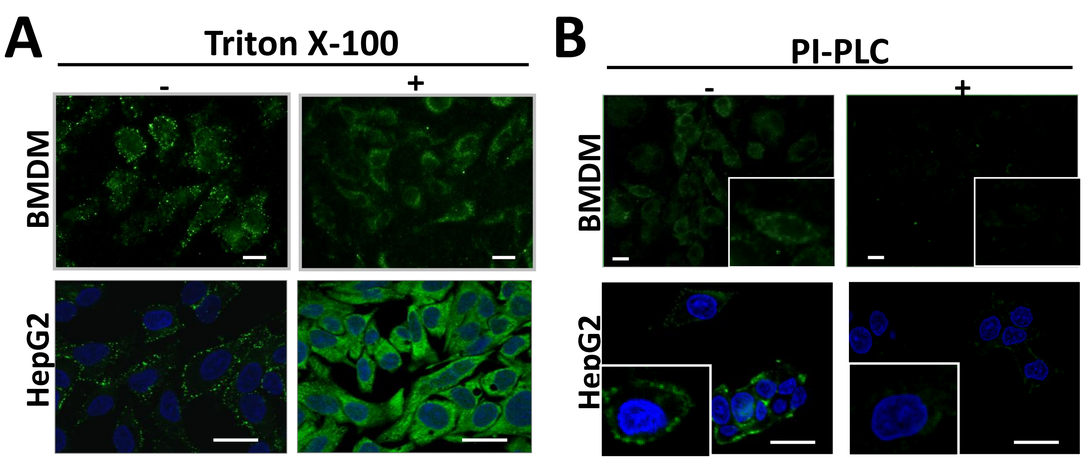

Fig.2 – Immunofluorescence analysis of Cp expression in human HepG2 and mouse BMDM. (A) BMDM and HepG2 show a ponctuated pattern of Cp staining in non-permeabilized cells (-) suggestive of lipid rafts localization, contrasting to the cytoplasmic distribution of Cp in permeabilized cells (+). **(B)** Decrease of Cp staining after PI-PLC treatment in non-permeabilized BMDM and HepG2 show that these cells express the GPI-Cp isoform at cell surface.

Cp is upregulated by iron in BMDM but not in HepG2 and is localized in lipid rafts at cell surface

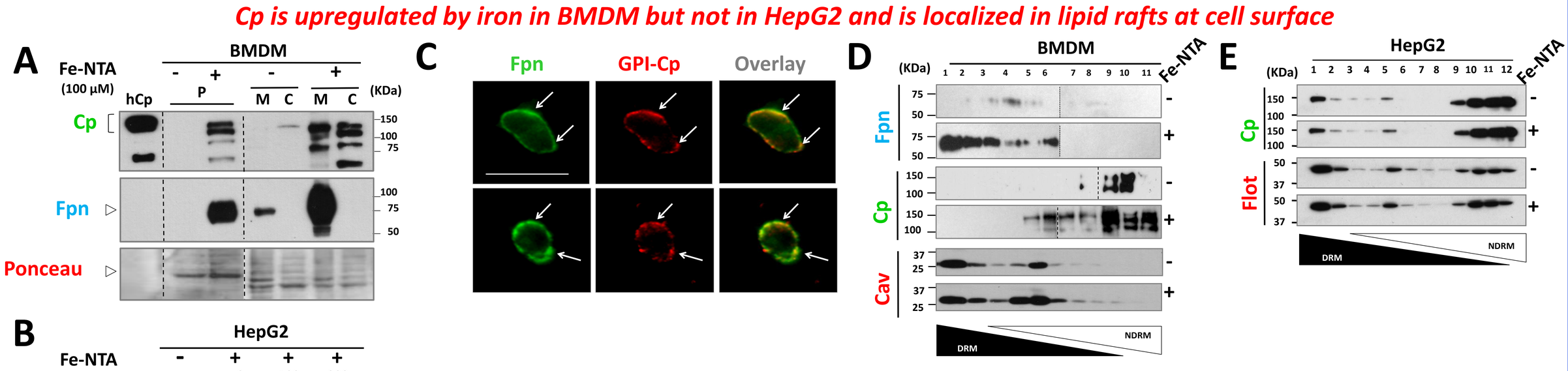

Fig.3 – Immunoblotting and immunofluorescence analysis of Cp and Fpn expression and localization in human HepG2 and mouse BMDM. (A-B) Western blot analysis of cytosolic (C) and crude membrane (M) fractions show that both Cp isoforms expression is upregulated by iron (Fe-NTA) in BMDM but not in HepG2. **(C)** Partial colocalization of GPI-Cp and Fpn lipid rafts (fractions 5-6) is also confirmed by immunofluorescence. **(D)** Immunoblotting analysis of BMDM iodixanol fractions show that Cp and Fpn distribution in lipid rafts/DRM fractions in increased by Fe-NTA. **(E)** GPI-Cp distribution in HepG2 iodixanol fractions overlaps with flotilin in DRM, confirming its localization in lipid rafts. Also, no effect of Fe-NTA in Cp expression or distribution is observed in HepG2. P: Post-nuclear supernatant; DRM: Detergent Resistant Membrane; NDRM: non-DRM.

CONCLUSIONS

In this study, we showed that PBL, PBMN, BMDM and HepG2 express both a soluble form of Cp and a membrane-associated form that was shown to be correspond to GPI-Cp. Analysis of immunofluorescence data for Cp in non-permeabilized PBMN, BMDM and HepG2 was suggestive of GPI-Cp localization in lipid rafts microdomains, which was confirmed by immunoblotting analysis of iodixanol-gradient fractions of BMDM and HepG2. Also, our results revealed that iron overload conditions upregulate the expression of both Cp isoforms in BMDM but not in HepG2 cells, suggesting that Cp expression is under distinct regulatory mechanisms in these cells. Such observation that likely reflects a cell-type specific function of Cp. Interestingly, partial colocalization of GPI-Cp and Fpn was observed in lipid rafts microdomains in iron-treated BMDM, indicating a possible role for GPI-Cp/Fpn interaction in iron metabolism in mouse macrophages.

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