Elucidating Ferritin Localization in Murine Macrophages

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Abstract

This research aims to reveal the location of one of the most well-known iron proteins, ferritin, in macrophage cells. According to our results, ferritin is partially located in the lysosome and late endosome organelles. However, additional locations of ferritin are still unknown and require further investigation.

Introduction

Ferritin, a protein found in most known cells, is essential for iron storage and detoxification and has the ability to store and release iron when there is cellular need (Meyron-Holtz 2011). Shaped as a hollow sphere, ferritin's shell is composed of 24 subunits. Most of the utilized body iron is recycled by white blood cells called macrophages. Macrophages contain ferritin and iron (Koorts 2007, Meyron-Holtz 2011). However, the exact location of ferritin within macrophages is still unknown, thus leading to the objective of our project. The goal of our research is to locate ferritin in murine macrophages using immunofluorescent techniques to stain these cells with antibodies against specific cellular markers (Fig.1) and tissue embedding.

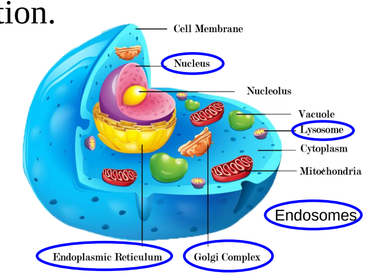


Figure 1: Cellular organelles

Methods and Materials

A. Isolation of bone marrow derived macrophages:
14 week-old C57Bl/6J male mice were sacrificed in CO_2. The skin was removed from the lower part of the body to expose the legs. The tibia and femur were broken and put into DMEM medium. Using a needle and syringe filled with medium, bone marrow was expelled from the bone. After centrifugation at 200g for 5 minutes, supernatant was removed and cells were re-suspended in media with MCS-F growth factor. Cultures were maintained for 6 days at 37°C under 5% CO_2 and 95% humidity. After 6 days, cells were plated on culture plates with complete DMEM medium for the growth of macrophages.

B. Tissue embedding & staining:
Bone marrow extracted from the tibia and femur was fixed in formaldehyde and embedded in paraffin. 5-µm tissue sections were manually sliced with a microtome and mounted on a slide. Hematoxylin & Eosin (H&E) staining was performed to visualize the nuclei and cytoplasm.

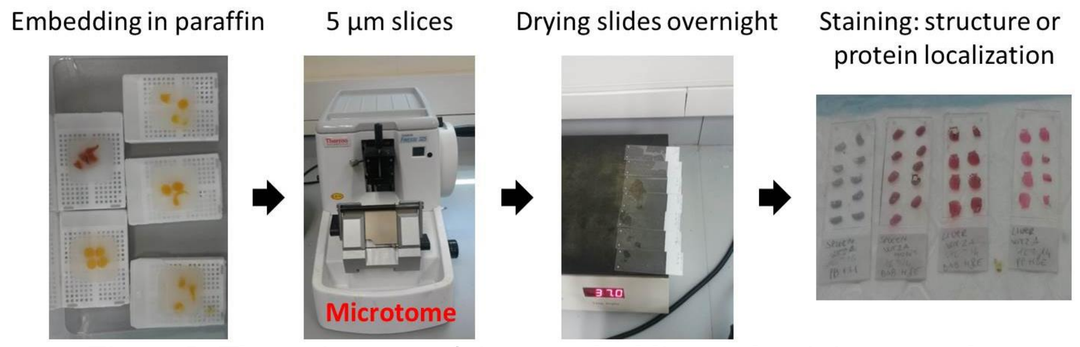
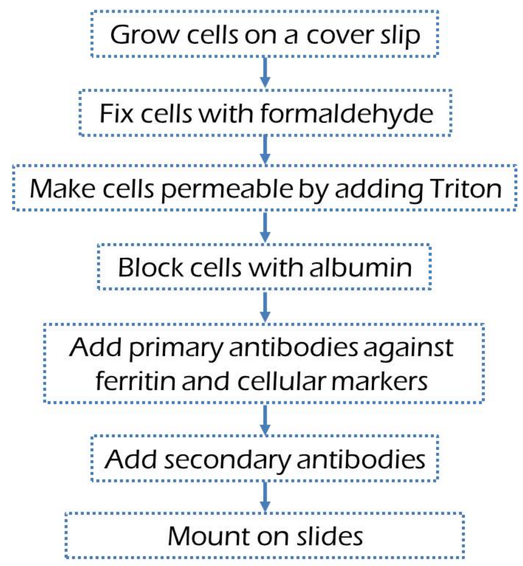


Figure 2: The main steps of tissue embedding and staining procedure

C. ImmunoFluorescent (IF) Staining:



Results

A. H&E staining of murine bone marrow

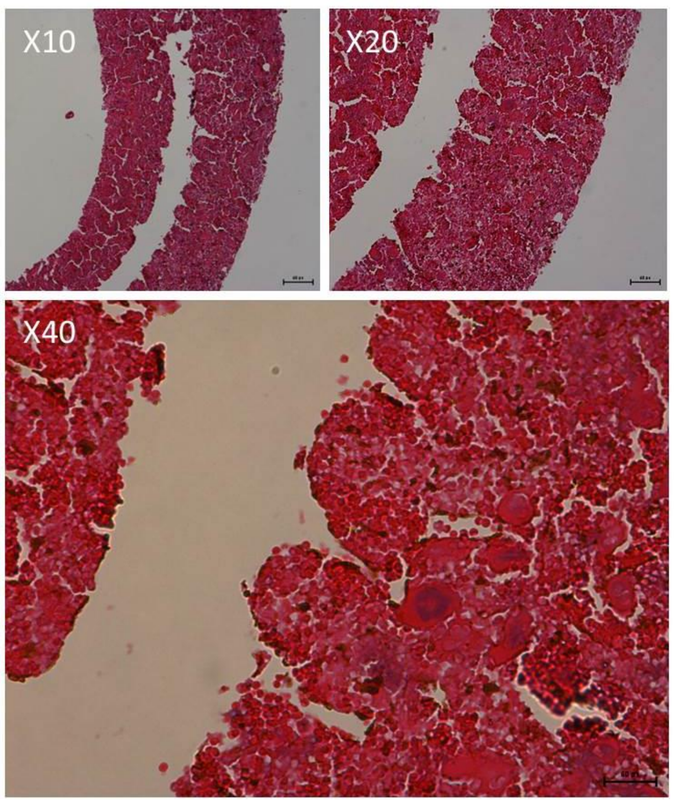


Figure 3: Murine bone marrows were stained with hematoxylin (stains nuclei in blue) and eosin (stains cytoplasm in pink). Scale bar: 60 px.

B. Immunofluorescent staining of murine macrophage cells

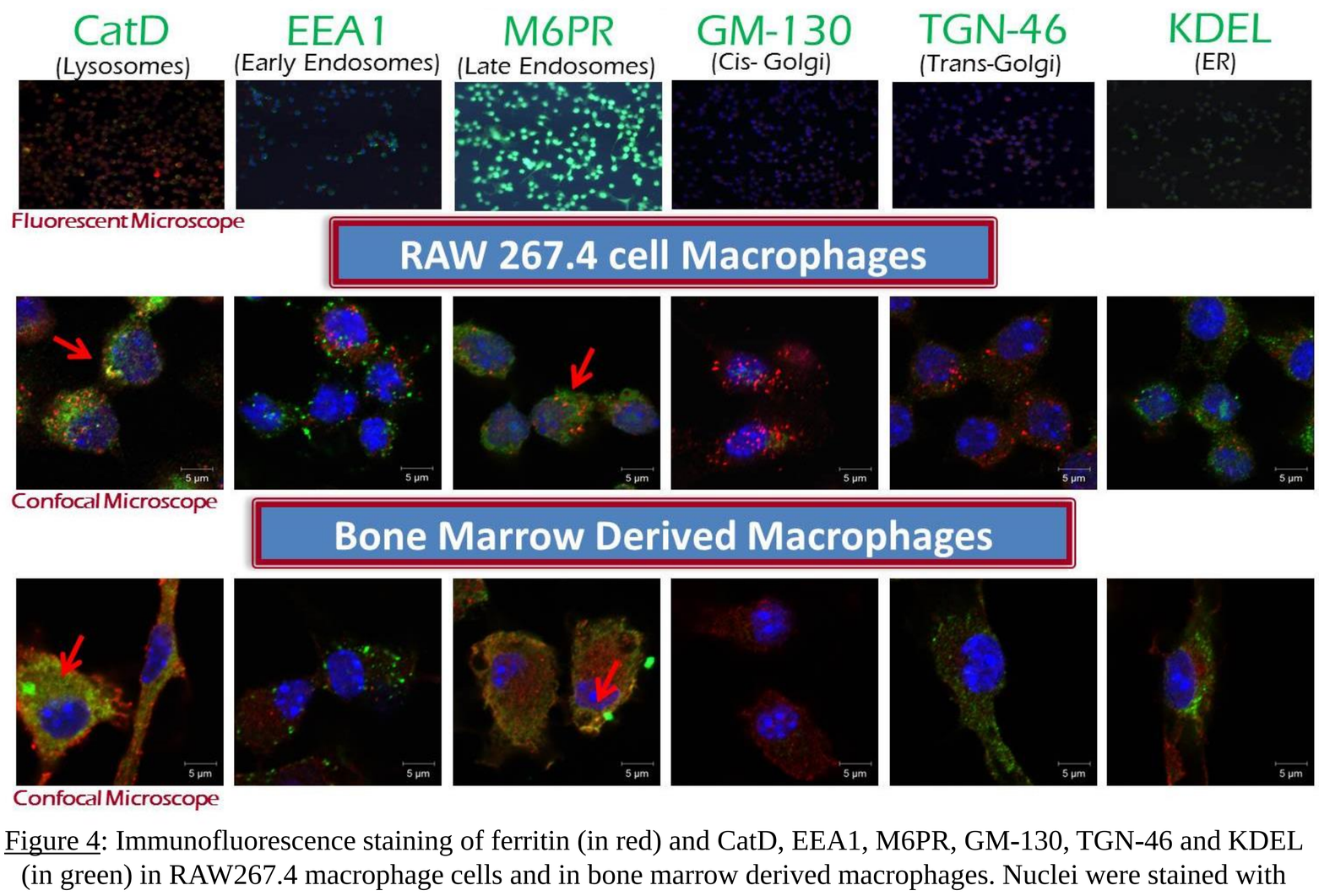


Figure 4: Immunofluorescence staining of ferritin (in red) and CatD, EEA1, M6PR, GM-130, TGN-46 and KDEL (in green) in RAW267.4 macrophage cells and in bone marrow derived macrophages. Nuclei were stained with DAPI (in blue). Upper Line: Fluorescent microscope. Two lower lines: Confocal Microscope. Negative controls were done with secondary antibodies only and with one primary anitbody followed by both secondary antibodies, to exclude channel leakage (data is not shown). Scale bar: 5 µm.

C. 3D view of murine macrophages stained with ferritin and the lysosomal marker CatD

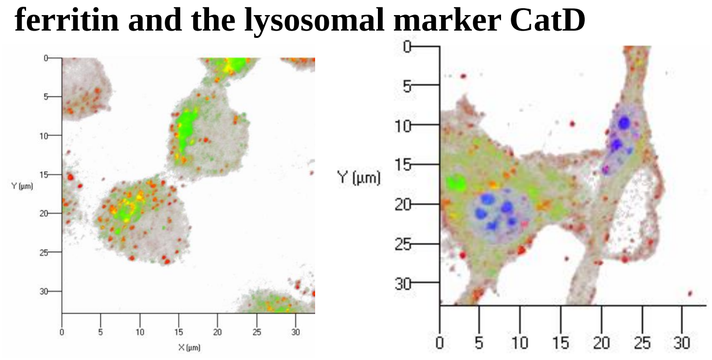


Figure 5: 3D view of murine macrophages stained with ferritin (in red) and with the lysosomal marker CatD (in green). Co-localization is represented in yellow. Nuclei were stained with DAPI (in blue)

Discussion and Conclusions

The goal of this research was to reveal the location of one of the most well-known iron proteins, ferritin, in macrophage cells. With the help of immunofluorescent staining, an immunofluorescence microscope, and confocal microscopy we were able to determine where ferritin is located within macrophages (Figure 4). The results from this project show that the immunofluorescence microscope may not be sufficient for this project and in the future we must use the confocal microscopy to obtain the most accurate results. According to our results, ferritin is partially located in the lysosome (Figures 4-5) and late endosome organelles (Figure 4). However, additional locations of ferritin are still unknown and require further investigation.

References

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