

How does the regulator Heparin affect iron trafficking in a model cell system representing the kidney?

Idan Lavian, Lin Zhao, Dr. Michael Garrick

Department of Biochemistry, State University of New York at Buffalo, 140 Farber Hall, 3435 Main Street, Buffalo, NY 14214



Abstract

This project's goal is to discover how both the metal importer Divalent Metal Transporter 1 (DMT1) and the opposite iron exporter, Ferroportin (FPN), respond to a regulator, Heparin (Hepc), in the renal tubules of the kidneys.

Hepc gets filtered out of the blood in the kidney along with other small molecules. It is either taken up and degraded in the proximal tubule or exits the body intact in the urine. If Hepc is absent or barely expressed, the kidney will experience an iron overload. The expected result is that both transporters are present in kidney cells and help recover filtered iron from the renal filtrate as they are responsive to Hepc. What I intend to try to gain from my experiments is understand iron regulation in more detail inside kidney cells. Hepc binds to FPN not DMT1 but breaks induces FPN and DMT1 turnover within kidney cells.

Introduction

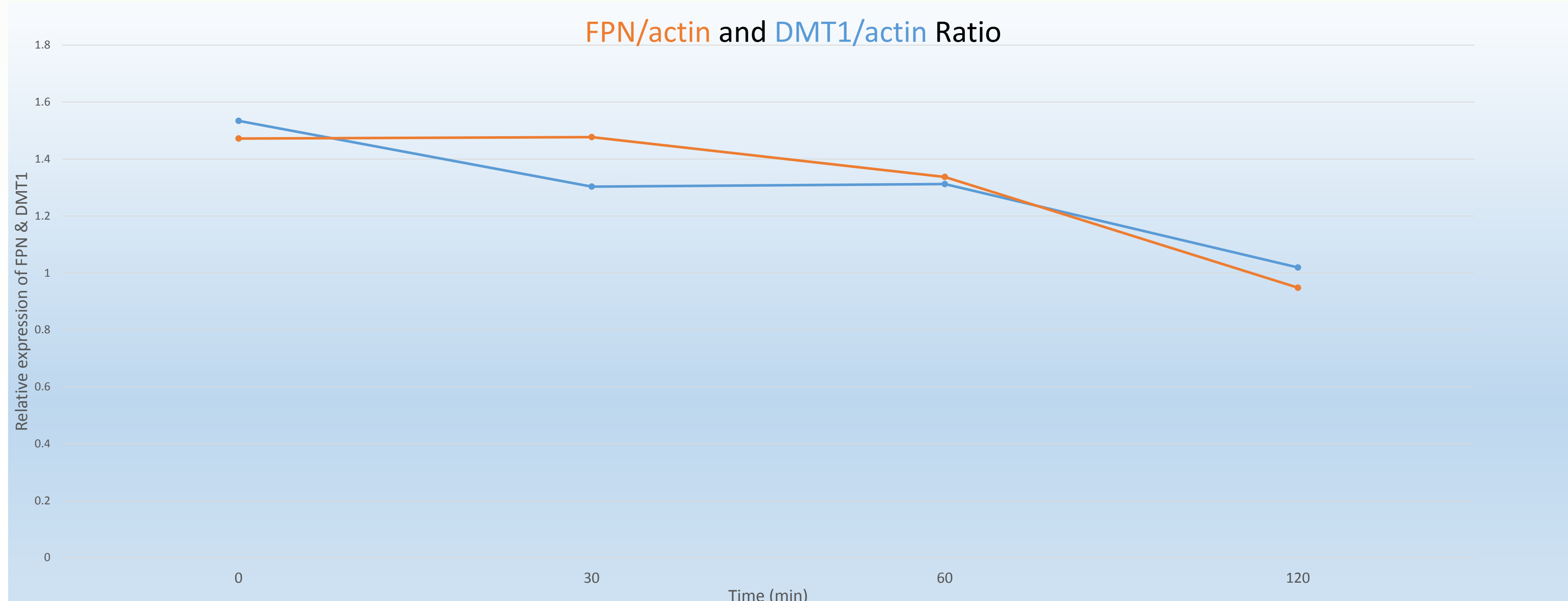
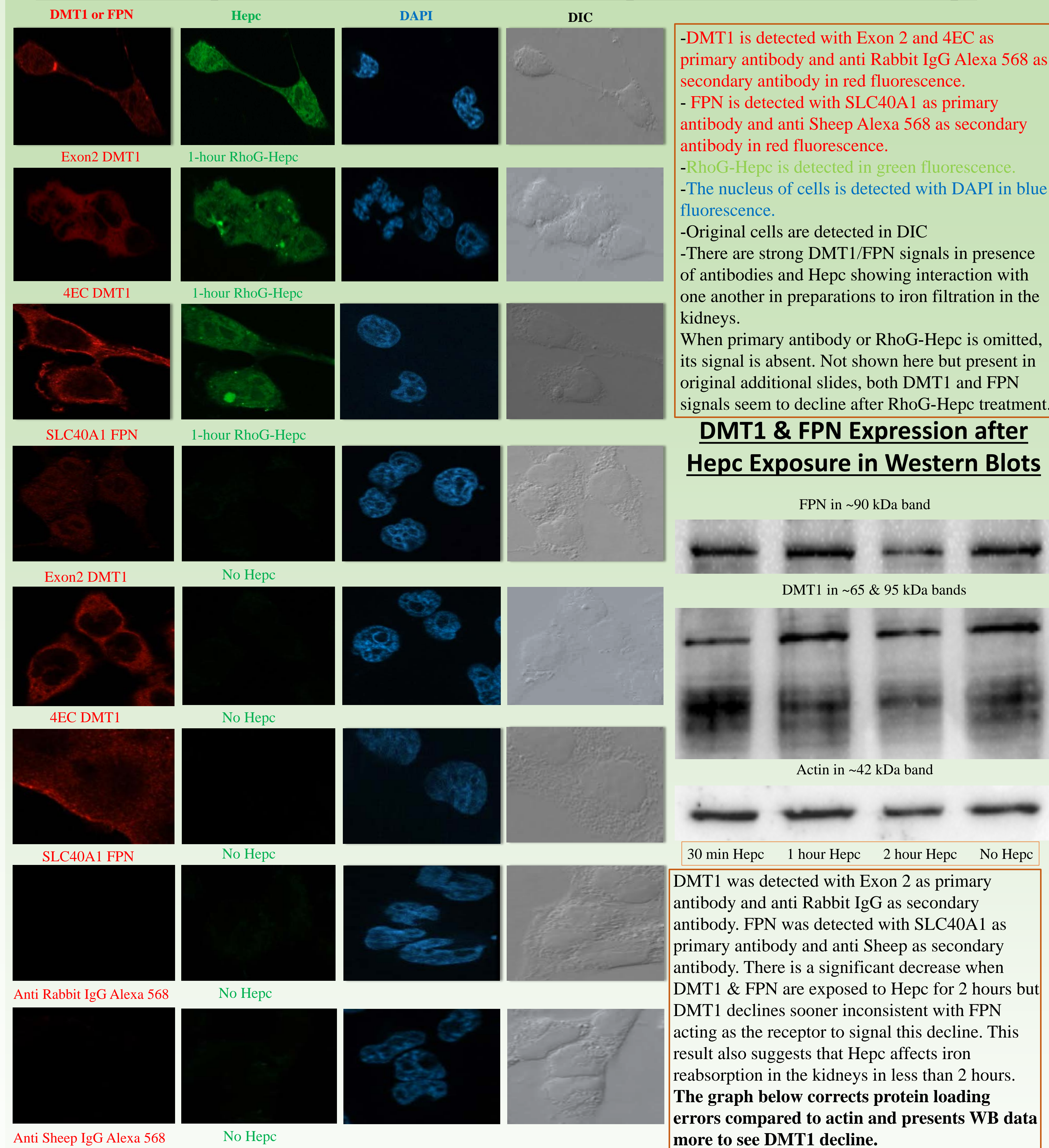
Iron is transported by specific transport proteins. The common importer for iron into the cells is divalent iron transporter (DMT1) while a protein called ferroportin (FPN) exports iron out of them. Their expression in the body varies with the dietary iron content consumed. DMT1 has an inverse correlation to large iron intake where FPN has a direct correlation instead [8]. These transport proteins are wide spread among the cells in the body. DMT1 is a major apical iron transporter protein that controls the balance of iron in the body [2,8]. However, it could easily contribute to bringing toxicity into the body by the uptake of Cd or other toxic DMT1 ligands [2]. FPN is also common in a spectrum of tissues and important since many of its mutations cause hemochromatosis, an iron overload disease [1]. There is high expression of those proteins and their mRNA in the kidneys, especially in the inner medulla [4,5].

However, their function in iron handling is still speculative in that organ and needs to be clarified [1,2,5,8]. It has been recently discovered that Heparin (Hepc) levels in the urine correlate to the non-heme iron concentration in the liver[3] and Hepc ordinarily binds to FPN leading to FPN turnover. In the GI tract, DMT1 turnover also develops after Hepc levels rise. What I would like to do in my experiment is detect the response of DMT1 and FPN in a kidney cell model to Hepc to gain insight into the response there. I will be using a kidney cell line called WKPT-0293-Cl.2 → that represents proximal tubule kidney cells from an adult rat. The cells it represents are those that are responsible for the majority of solute reabsorption from the filtrate excreted in the distal tubules [1] that would otherwise be lost in the urine.

Purpose of Experiments

The purpose of this project is to discover how both the metal importer (DMT1) and the opposite iron transporter, FPN, respond to a regulator Hepc, in the renal tubules of the kidneys. The expected result is that both transporters are present in kidney cells and are probably used in recovering filtered iron from the renal filtrate as they are responsive to Hepc. I also want to learn how they respond to Hepc.

DMT1/FPN Expression with and without RhoG-Hepc under Confocal Microscope



Methods

Confocal Experiment

- ❖ The cell line WKPT-0293-Cl.2 that has been passaged 10-30 times will be used and grown 80% to 100% confluence on a 12 well plate over the course of 4-6 days.
- ❖ The medium of each well then will be replaced with their specific experimental groups. (Green Fluorescent Hepc, primary and secondary antibodies).
- ❖ The cells will then be fixed, permeabilized to add antibodies for specific proteins, and prepared to be viewed for the next day under confocal microscope.

WB Experiment

- ❖ The cell line WKPT-0293-Cl.2 that have been passaged 10-30 times will be used and grown to 100% confluence on a 6-well plate over the course of one week.
- ❖ The medium of each well then will be replaced with non-fluorescent Hepc with their individual time of exposure for cells to be collected, lysed and centrifuged under 10,000g to extract their proteins.
- ❖ Denatured protein samples were run through electrophoresis, transferred onto a membrane, and exposed to their specific antibodies to observe antibody attachment to membrane bands.

Conclusion and Future Experiments

Results show that both transporters are present in kidney cells and could help recover filtered iron from the renal filtrate as they are responsive to Hepc. As Sam Poon in the lab showed Hepc binds to FPN not DMT1 but induces FPN and DMT1 turnover within another cell line, the quicker response of DMT1 implies another Hepc receptor. What I intend to try in future experiments is to understand iron regulation in kidney cells more detail inside via other methods of protein detection and protein concentration.

References

- Natascha A. Wolff, Wei Liu, Robert A. Fenton, Wing-Kee Lee, Frank Thevenod, Craig P. Smith (2011) "Ferroportin 1 is expressed basolaterally in rat kidney proximal tubule cells and iron excess increases its membrane trafficking" J Cell Mol Med. 2011 Feb; 15(2): 209-219
- Abouhamed M, Gburek J, Liu W, Torchalski B, Wilhelm A, Wolff NA, Christensen EL, Thevenod F, Smith CP (2006) "Divalent metal transporter 1 in the kidney proximal tubule is expressed in late endosomes/lysosomal membranes: implications for renal handling of protein-metal complexes" Am J Physiol Renal Physiol. 2006 Jun; 290(6): F1525-33
- Robert staron, Rachel P. L. Van Swelm, Pawel Lipinski, Anna Gajowiak, Malgorzata Lenartowicz, Aleksandra Bednarz, Malgorzata Gajewska, Marek Piezka, Coby M. M. Laarakkers, Dorine W. Swinkels, Rafal R. Starzynski (2015) "Urinary Heparin Levels in Iron-Deficient and Iron-Supplemented Piglets Correlate with Heparin Hepatic mRNA and Serum Levels and with Body Iron Status" PLoS One. Aug 2015; 10(8): e0136695
- Veuthey T, D'Anna MC, Roque ME (2008) "Role of the kidney in iron homeostasis: renal expression of Prohepcidin, Ferroportin, and DMT1 in anemic mice" Am J Physiol Renal Physiol, Oct 2008; 295(4): F1213-21
- Cannone Hergaux F, Gros P (2002) Expression of the iron transporter DMT1 in Kidney from normal and anemic mice" Kidney Int, July 2002; 62(1): 147-56
- Martines AM, Masereeuw R, Tjalsma H, Hoenderop JG, Wetzels JF, Swinkels DW (2013) "Iron metabolism in the pathogenesis of iron induced Kidney injury" Nat Rev Nephrol Jul 2013; 9(7): 385-98
- Moulouel B, Houamel D, Delaby D, Tchernitchko D, Vaulont S, Letteron P, Thibaudeau O, Puy H, Gouya L, Beaumont C, Karim Z (2013) "Heparin regulates intrarenal iron handling at the distal nephron" Kidney Int, Oct 2013; 84(4): 756-66
- Wareing M, Ferguson CJ, Delannoy M, Cox AG, McMahon RF, Green R, Riccardi D, Smith CP (2003) "Altered dietary iron intake is a strong modulator of renal DMT1 expression" Am J Physiol Renal Physiol, Dec 2003; 285(6): F1050-9