SYNERGISTIC INTERACTIONS BETWEEN UTEROFERRIN AND SECRETED PHOSPHOPROTEIN 1 IN HEMATOPOIETIC NICHES

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Synergistic Interactions between Uteroferrin and Secreted Phosphoprotein 1 in Hematopoietic Niches. (May 2014)

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Synthesis of red blood cells is vital to maintaining health in organisms; however, current therapeutic procedures to correct inadequate levels of erythropoiesis for production of red blood cells are not sufficient to improve the situation effectively. For this reason, further research is needed in order to implement new therapeutic methods to ameliorate conditions such as anemia. Uteroferrin [aka UF, phosphatase, acid, type 5, tartrate-resistant (ACP5) and tartrate-resistant acid phosphatase (TRAP)] and secreted phosphoprotein 1 (SPP1) are proteins that are proposed to co-localize in sites of erythropoiesis which are referred to as hematopoietic niches. The placental yolk sack, as well as liver, spleen, and bone of fetal mice are the major hematopoietic centers during fetal development. Therefore, this research was to obtain those fetal tissues at different stages of gestation and perform immunohistochemistry, in situ hybridization analysis, western blotting, and biological assays to determine co-localization of UF and SPP1. Subsequent research will be to gain further insight into the roles of UF and SPP1 and their possible synergistic interactions with respect to stimulation of erythropoiesis.

DEDICATION

I dedicate this research to my Mom and Dad. I am so incredibly blessed to receive their continued support and encouragement. Without them, this opportunity would not have been possible.

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I would like to thank Dr. Fuller W. Bazer for his gracious support. His guidance and teaching has been foundational to the production of this thesis and my advancement as a student. I would also like to thank the graduate students in the laboratory, James “Will” Frank and Chelsie Burroughs for providing their time and advice in the laboratory setting, without their assistance, progress would have been much more arduous.

NOMENCLATURE

UF	Uteroferrin
ACP5	Phosphatase, Acid Type 5, Tartrate-Resistant
TRAP	Tartrate-Resistant Acid Phosphatase
SPP1	Secreted Phosphoprotein 1
LAP	Lysomal Acid Phosphatase
HSPC	Hematopoietic Stem and Progenitor Cells
GE	Glandular Epithelium

CHAPTER I

INTRODUCTION

Problems in current treatments for anemia

Erythropoiesis is the process by which hematopoietic stem and progenitor cells (HSPC) differentiate into erythrocytes or red blood cells. A decreased concentration of O_2 in blood is detected by the kidneys, which in turn secrete erythropoietin to stimulate erythropoiesis and production of red blood cells. Erythrocytes are crucial to sustaining life in all vertebrate organisms, and are essential in all stages of life: embryonic, fetal, neonatal, adolescent, and adult [1]. When the organism is not experiencing proper red blood cell formation, severe health consequences are likely to arise. One of the possible consequences is anemia. Anemia is a disorder in which the level of healthy, thriving red blood cells in the body is lower than required to carry the appropriate amount of oxygen to all cells in the body. One of the most prevalent forms of anemia involves iron deficiency. A crucial protein inside a red blood cell is known as hemoglobin. Hemoglobin is rich in iron and is the part of the RBC that binds oxygen and enables them to carry oxygen to the rest of the body. Multi-organ, such as brain, heart, and kidney, injuries present increased risk factors for patients suffering from acute and chronic anemia. To make matters worse, current treatments for anemia such as transfusions, erythropoiesis stimulating agents, and blood substitutes have not been proven to improve health of the patient sufficiently. This highlights the demand to develop new therapeutics to stimulate erythropoiesis [2]. The findings obtained from the proposed research will lay a foundation for formulating new therapeutic approaches for treating anemia that will compliment or replace transfusions and use of blood substitutes.

UF is a progesterone-induced protein secreted by uterine glandular epithelium (GE) of pigs and other ungulates, as well as human placenta, spleen and osteoclasts [3]. In previous investigations, UF-lysomal acid phosphatase (LAP) double knockout mice were produced and found to have severe skeletal defects [4]. So it is clear that this protein is crucial to bone development. However, these researchers did not investigate its effects on erythropoiesis, which occurs in flexible tissue known as bone marrow in the interior of certain bones. More recent results indicate that mutations in UF reduce or eliminate acid phosphatase activity which increases the presence of hyperphosphorylated SPP1 associated with skeletal defects, neurological problems, developmental delays, increased production of interferon alpha that leads to systemic autoimmunity and impaired erythropoiesis [5]. Based on preliminary results from Dr. Bazer's laboratory, it is proposed that UF, due to its acid phosphatase activity, interacts with SPP1 to reduce its state of phosphorylation and thereby increase the bioactivity of SPP1 within the hematopoietic niche to stimulate hematopoiesis/erythropoiesis [5]. Specifically, this research tested the hypothesis that UF co-localizes with SPP1 in both fetal and adult "erythropoietic niches" to stimulate erythropoiesis.

CHAPTER II

METHODS

Research to test the hypothesis was conducted in a laboratory setting in which required techniques are established and published [7]. Utilizing the advanced equipment and methods provided in the laboratory of the faculty advisor, Dr. Fuller Bazer, I determined temporal and spatial (cell-specific) changes in co-localization of UF and SPP1 in yolk sac, liver, spleen and bone of fetuses from mice in which the major hematopoietic niches are located. The primary technique performed on the mouse tissue is known as immunohistochemistry. Immunohistochemistry is a technique that identifies specific targeted proteins in tissues. The tissue is treated with specific antibodies that bind to the protein of interest. The antibody binds to the molecule under study and subsequently, when treated with a color reaction molecule, forms a visual precipitate that can be viewed under a microscope. Immunohistochemistry can be used to aid in disease diagnosis, detect the presence of microorganisms, and, in basic research, to understand how cells grow and become specialized [8]. In this study, immunohistochemistry was used to determine the co-localization of UF and SPP1 proteins in liver, bone, placenta, and yolk sac of fetal tissues from mice. Further experiments will shine more light on the results and confirm the synergistic effects in greater detail. In situ hybridization will determine temporal and cell-specific changes in expression of UF and SPP1 mRNAs in hematopoietic centers of the mouse conceptus during gestation. In addition, western blotting will confirm the presence of UF and SPP1 in tissues of interest.

CHAPTER III

RESULTS

After successful immunohistochemical reactions were conducted on liver, bone, placenta, and yolk sac of fetal tissues from mice, results were analyzed to determine if our hypothesis was indeed valid. The specific tissue staining detected both UF and SPP1 proteins in hematopoietic niches including allantois, bone marrow, and yolk sac (Figure 1). This indicates that UF and SPP1 are in fact co-localized with the potential to interact and play a dual role in mediating molecular and biochemical reactions in these tissues that likely affect hematopoiesis and, in particular, erythropoiesis. Confirmed co-localization of SPP1 and UF was established for yolk sac, bone marrow, and allantois of the placenta of fetal mice. As shown in Figure 1, the

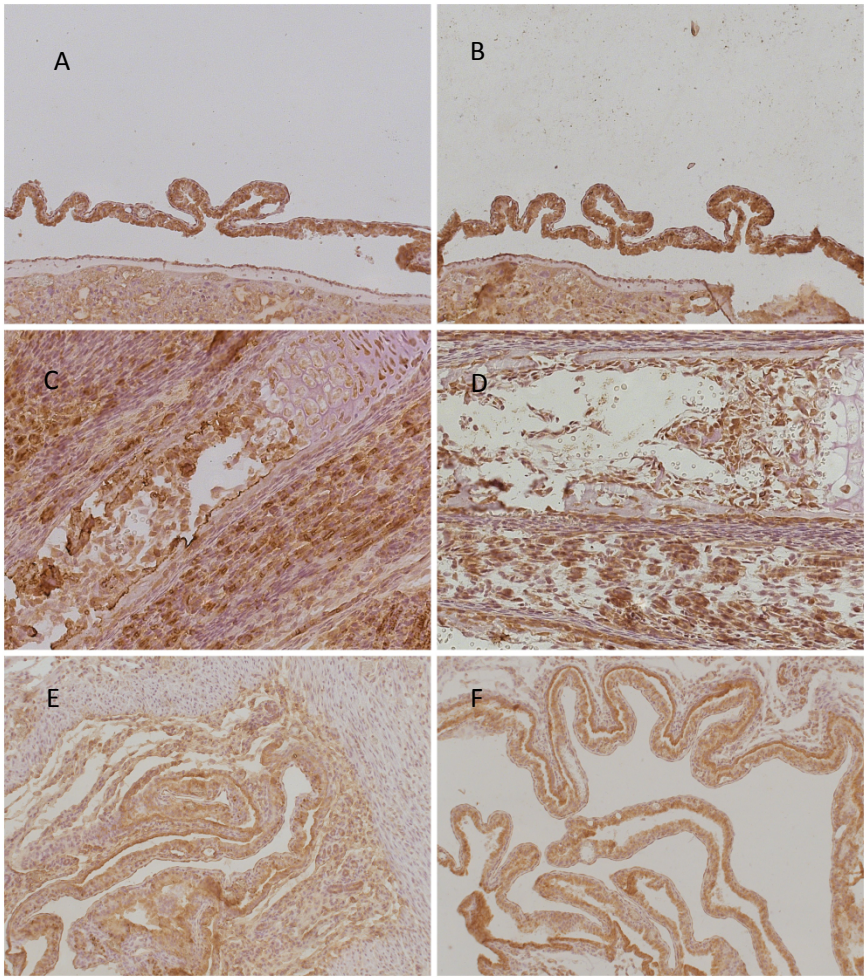

Figure 1. Immunohisotchemistry demonstrating co-localization of UF and SPP1 proteins in fetal-placental tissues from mice: A. allantois SPP1; B allantois UF; C bone marrow SPP1; D bone marrow UF; E volk sac SPP1 and F volk sac UF

specific proteins were successfully detected (brown precipitate) and determined to co-localize in common niches. In addition, it was previously determined that there is co-localization of UF and SPP1 in erythroid islands of pig liver. The mechanism that we are proposing leads to synergistic effects on erythropoiesis based on co-localization of the two proteins and involves actions of UF to decrease the amount of phosphorylation of SPP1 which increase its biological activity. As mentioned previously, UF decreases phosphorylation of SPP1 to allow the bioactivity of SPP1 to have a more stimulatory effect. The action of SPP1 is to control the number of hematopoietic progenitor cells that move into the various differentiated states, such as erythrocytes. After erythropoiesis starts, UF stimulates hemoglobin synthesis and differentiation of erthyroid cells to mature red blood cells, rather than differentiating into megakaryocytes that would form platelets. UF up-regulates genes for the transcription factors that direct the pathway of differentiation of the erythroid progenitor cells toward formation of mature red blood cells which represents part of the synergy between UF and SPP1 to enhance erythropoiesis.

CHAPTER IV

CONCLUSION

The culmination of this research ended in the successful determination that UF and SPP1 are in fact co-localized in fetal-placental tissues of mice such as the allantoic membrane and yolk sac of the placenta, as well as bone marrow. The presence of UF and SPP1 is common to these niches which gives rise to their potential to interact and play a dual role in mediating molecular and biochemical reactions in the organism which is likely stimulatory to erythropoiesis. The mechanism that we are proposing to result in increased erythropoiesis is based on the acid phosphatase activity of UF which cleaves phosphate groups from SPP1 and subsequently increases its bioactivity and diminishes the presence of hyperphosphorylated SPP1 which is associated with severe skeletal defects and impaired erythropoiesis in an organism. Further experiments are required to solidify the results and allow advancement in potential strategies for treatments to improve erythropoiesis. In situ hybridization will determine temporal and cell-specific changes in expression of *UF* and *SPP1* mRNAs in hematopoietic centers of the mouse conceptus (embryo/fetus and placenta) during gestation. In addition, western blotting will confirm the presence of UF and SPP1 in tissues of interest.

Impact on Treatments

The goal of this research was to provide new knowledge to inform translational research for development of therapeutic approaches to increase erythropoiesis and reduce morbidity and mortality associated with anemia. These two proteins, UF and SPP1, exist in all species; therefore, the association between SPP1 and UF is not novel or unique to the mouse, and results

can be transferred in development of new strategies to enhance erythropoiesis in humans and animals. As a result, our findings are highly relevant to further narrow the research to be done toward developing new therapeutic approaches to mitigate human anemia through complimenting or replacing transfusions and use of blood substitutes.

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