Tier 2 Research Proposal

The metabolic control of tissue integrity and function in health and disease

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Background and Significance

Western society enjoys almost unrestricted access to food, but the combination with low levels of physical exercise results in a condition termed the metabolic syndrome (Grundy et al., 2006). Typical features associated with this syndrome are an increase in diabetic patients as well as a high risk for cardiovascular complications and enhanced potential for developing cancers. Although many research proposals are currently addressing the impact of metabolic changes caused by an excess availability of sugar and fat in our diet on the survival and proliferation of cells in our tissues, almost none of these studies is asking the question how tissues and their functional properties are changing under such conditions.

A functional tissue is composed of cells held in place by an extracellular scaffold, which is called an extracellular matrix. This matrix determines the shape and mechano/physical properties of the tissue. These mechano/physical properties are exemplified by the rigidity of bones, tensional strength of tendons and ligaments, the elasticity of arterial walls and muscle, as well as the flexibility of sheet-like epithelia lining our body surface, the intestinal tract, or kidney tubules. In addition to a mechanical support to maintain the shape of an organ or tissue, the scaffold formed by the extracellular matrix provides an extracellular reservoir for growth factors and stimulates the survival of cells embedded within and interacting with this dynamic scaffold (Engler et al., 2009). However, we have only limited information about the biological mechanisms that control cell growth and survival in response to extracellular matrix. Furthermore, even though the matrix is produced and assembled by the cells that interact with it, very little is known about the processes that control this assembly or about how cells rearrange and remodel this extracellular scaffold. Importantly for this proposal, the effects of the metabolic syndrome on cell-matrix interactions during assembly have yet to be investigated. It is critical to understand matrix remodeling by cells, if we want to curb pathological processes that destroy tissue function due to excessive deposition of extracellular matrix, a process termed fibrosis.

In order to meet the increasing demands in the clinics to understand and treat metabolic diseases, molecular information is needed about the effects of metabolites such as glucose on the amount and organization of the extracellular matrix, and on the activity of integrin cell surface receptors for extracellular matrix. Importantly, recent bio-medical research conducted by groups in Geneva (Pinon et al., 2014) and Princeton (Miller et al., 2014) has revealed key mechanistic interactions between an increased uptake of metabolites and the deterioration of tissue function through fibrosis. In addition, research conducted at the level of the integrin receptors (in the Wehrle-Haller lab) proposes that the metabolic state of a cell, exemplified by either aerobic or anaerobic decomposition of glucose, strongly influences the function of these integrin receptors. The consequences are either strongly-adherent and highly-signaling cells under low glucose conditions, or the enhanced deposition and remodeling of extracellular matrix under high glucose conditions. Independently, research in the Schwarzbauer lab showed that high glucose conditions stimulate assembly of the extracellular matrix protein fibronectin and that this fibronectin matrix serves as a template for accumulation of type IV collagen, which is a hallmark of fibrosis in vivo.

The goal of this application is for our research teams to join forces in order to explore the mechanistic details of integrin receptor function in response to the metabolic state of cells, and to link glucose-induced changes in integrin function to the pathological deposition of extracellular matrix. This research will identify new regulatory mechanisms acting at the cell (cytoplasmic adapter for integrins) and tissue levels (functional state of the matrix) and suggest new therapeutic targets, diagnostic markers and potentially new drugs designed to revert or identify pathological

changes in the tissue. Metabolic effects on cell-matrix interactions are directly related to diseases such as diabetes, chronic inflammation, and nerve and muscle degeneration. Thus, our joint project will have a great socio-economic impact for our society and will hopefully serve as a seed to attract other research groups from our universities to investigate the importance of this mechanism in cancer and cardiovascular disease.

Hypothesis and Specific Aims

Based on our preliminary results described above, we propose that high glucose conditions, as occur in diabetes and other metabolic diseases, cause abnormal accumulation and organization of extracellular matrix fibrils through increases in fibronectin expression, modification of integrin receptors, and stimulation of integrin receptor activity. We propose to apply the complementary expertise of the Wehrle-Haller lab in integrin biology with that of the Schwarzbauer lab in the extracellular matrix to address two specific aims.

1. Determine the mechanism by which metabolic modifications of integrin cytoplasmic domains lead to an increase in extracellular matrix assembly.

Mutation or acetylation of a specific site in the integrin cytoplasmic domain changes integrin activity and ability to bind to the extracellular matrix protein fibronectin. We will screen for and identify drugs and enzymes that affect this acetylation state. Rates of fibronectin assembly will be measured and compared between mutants, acetylated, and wild type integrins. TGFβ activation, which affects integrin and fibronectin levels, will also be measured. A screen for differentially recruited intracellular integrin adapter proteins will be performed to understand how cytoplasmic domain acetylation affects intracellular interactions.

2. Use fluorescently tagged integrin receptors, intracellular adapters and extracellular fibronectin for live cell imaging.

Receptor-fibronectin interactions and glucose-induced integrin modifications will be correlated with cytoplasmic adapter protein recruitment and its impact on formation of fibronectin fibrils. We will use fluorescence recovery after photobleaching (FRAP) or photo-conversion imaging techniques to analyze the dynamic assembly of fibronectin fibrils and the recruitment of intracellular adapter proteins to differentially acetylated integrins. Drugs that affect acetylation will be tested for their impact on adapter recruitment and fibronectin fibril formation in culture.

Rationale for Princeton-Geneva collaboration

Wehrle-Haller and Schwarzbauer have many complementary skills, methods, reagents, and expertise that support the development and success of this collaboration. The major strength is that they are among the international elite of research in the mechanisms that control the pathological deposition of extracellular matrix (JS) and the regulation and control of the interaction of a cell with the extracellular matrix (BWH). As such, each group has unique and state-of-the-art techniques and reagents including fluorescently-tagged proteins, mutant cells, and well-established protocols for studying integrins and extracellular matrix. Furthermore, colleagues on the campus in Geneva provide strength in diabetes while Princeton Molecular Biology has a Nikon Center of Excellence imaging facility with the latest equipment for cell and matrix imaging studies. In order to support active exchange of knowledge, we have included a travel budget for students and/or postdocs to go on short-term scientific missions between Princeton and Geneva. In addition, the PI's will travel to ensure on-site teaching and technology exchange with the local faculty to foster new interactions in related clinical domains. The Consumables budget covers supplies for cell

culture, biochemical analyses of extracellular matrix assembly and integrin acetylation, TGFβ studies, and reagents used in cell experiments. Use of facilities for proteomics and for confocal microscopy at both Princeton and Geneva will be supported by the Facilities budget.

Specific Aim 1

Fluorescent proteins are used routinely by the Wehrle-Haller lab to analyze the dynamics of cell adhesion and migration. Among others, they have pioneered the analysis of fluorescently-tagged integrin receptors (Wehrle-Haller and Imhof, 2002). Relevant to this proposal, tools to study β1-integrin dependent functions have been developed and these will be crucial for the proposed experiments since α5β1-integrin has the unique capacity to polymerize fibronectin fibrils at the cell surface. In addition, Wehrle-Haller has shown that in high glucose conditions, integrin cytoplasmic domains are modified by acetylation and that this modification modulates their fibronectin-binding affinity. They have developed culture conditions that induce acetylation of integrins and site-specific mutations that target sites of acetylation on integrins. The Schwarzbauer lab has shown that high glucose conditions stimulate fibronectin matrix assembly and lead to an accumulation of excess fibronectin and type IV collagen, a hallmark of fibrosis (Miller et al., 2014). They have well-established methods and reagents for analyzing and quantifying matrix assembly rates and amounts of matrix. This aim will combine the integrin reagents from Geneva with the matrix assembly methods of Princeton to determine how changes in the metabolic state affect cell-matrix activities.

In Princeton: Cells will be cultured in conditions that lead to integrin acetylation. In parallel, cells expressing integrins that have mutations at acetylation sites so that they mimic an acetylated state or are unable to be acetylated will be cultured. Fibronectin and collagen IV will be added to the cultures and their assembly will be followed microscopically and biochemically as described (Miller et al., 2014). Imaging will show the organization and number of fibrils that are assembled under various conditions. Biochemical analyses will allow quantification so that the effects of different conditions can be compared directly. In parallel experiments, the activation of TGFβ will be measured. One mechanism of TGFβ activation depends on integrin activity and thus levels of this growth factor could be modulated by integrin acetylation. Active TGFβ increases integrin and fibronectin levels and thus could affect fibronectin matrix formation in response to acetylation. We will use established procedures to monitor changes in TGFβ (Park and Schwarzbauer, 2014). Drugs that affect integrin activity (as identified in Geneva, see below) will be incorporated in the fibronectin matrix experiments as they become available. Together these experiments will determine whether acetylation or mutation affect the rate or amount of fibronectin matrix and will provide information about integrin and TGFβ activities that will inform the studies in Geneva.

In Geneva: We will use a cell-based fibronectin binding assay by incubating fluorescently labeled soluble fibronectin or the integrin-binding fragment thereof, with cells. Preliminary data have demonstrated strong binding to de-acetylation mimetic integrins and weaker interaction with acetylated integrins. We will use this assay to screen a library of acetylases and de-acetylases (enzymes that modify the acetylated states of proteins) to identify the intracellular proteins responsible for integrin modification in response to altered glucose levels. We will also use a panel of drugs directed against de/acetylases in order to modulate acetylation so we can measure its effects on integrin-mediated fibronectin fibril formation. Quantitative imaging of cell migration as well as fibronectin remodeling will be used to analyze the impacts of acetylation-modulating enzymes in living cells.

The identification of the intracellular adapter proteins that recognize the de/acetylated integrin peptide is a key step in the development of new therapeutic targets. We will use biochemical assays to measure the binding of cytoplasmic proteins to differentially acetylated integrin peptides. In addition to screening of candidate adapters that are already known to bind to the integrin cytoplasmic

tail, we will also use proteomics to identify proteins differentially recruited to this regulatory region. The University of Geneva possesses a core facility in which such analysis can be performed.

Specific Aim 2

Live cell imaging provides unique information about cell and molecular interactions and organization over time. However, this approach has not been used extensively in studies of cell-fibronectin interactions, primarily because of the limited availability of fluorescent integrins and the difficulty in generating fluorescent fibronectin. The PI's have cells expressing fluorescent integrins (Wehrle-Haller) and purified fluorescent fibronectin (Schwarzbauer) and therefore are uniquely qualified to apply this approach to determine how cell receptors and matrix rearrange in response to metabolic challenges.

We will exchange cells that are stably expressing fluorescent integrin variants (generated in Geneva), in order to be analyzed for fibronectin matrix formation. Wehrle-Haller has introduced fluorescent β1-integrin into cells that lack endogenous integrin. For expression of fluorescent β1-integrin in other cell types, we will use shRNA strategies to suppress endogenous wildtype receptors. A major advantage of using fluorescently-tagged protein is that it allows us to get dynamic information about fibronectin binding to receptors and about the initial steps in fibronectin fibril formation, as well as about the acetylation state-dependent recruitment of intracellular adapter proteins. To quantify these dynamics, we will use FRAP (Cluzel et al., 2005) for the spatio-temporal analysis of fluorescence converted receptors, fibronectin and adapter proteins. Drugs that modulate integrin acetylation will be incorporated into these experiments and, in combination with live cell imaging, will allow us to define spatio-temporal roles for integrin modifications in fibronectin matrix assembly.

Both Universities have well equipped facilities, which enhance our ability to perform these experiments. Initially the emphasis will be on studying the extracellular part in Princeton and intracellular aspect in UNIGE. However, this initiative will also allow us to create a route for technology and knowledge transfer by sharing protocols and data and exchanging students so that studies of integrin adapter proteins can be carried out in Princeton and matrix assembly in UNIGE.

Project Summary: Points 7 – 11.

7. Accumulating evidence indicates that the metabolic syndrome and the effects of metabolites on cell and tissue structure/function have major roles in the progression of a number of prevalent diseases including diabetes, chronic inflammation, and cancer, among others. Studying highly complicated, multilayered metabolic processes requires combined efforts from different disciplines and universities. This initiative will unite two research groups with different specialities to create synergism enabling cooperative, in-depth analysis in a newly emerging field of clinical biology.

8. This initiative will be directed by two PI's whose international visibility is already significant in their respective fields. The proposed collaboration will form an initial scaffold of scientific interactions, which should lead to recruitment of students at both universities to work on the project. The budget includes funds for travel of students to visit the collaborating labs so they can participate in experiments and learn new techniques. We also hope to incorporate scientists from other departments on campus into a highly multidisciplinary effort.

9. Metabolic effects on cells and tissues are very complex and the proposed experiments will only test a small aspect of the problem. However, results of these experiments will support additional studies and establish a working relationship between the PI's that can easily be developed into a more extensive project with both basic and clinical applications. For example, if results suggest that receptors or extracellular matrix present potential therapeutic targets, then a clinical

collaborator could be recruited to help take the work in that direction. It is also possible that results from the joint experiments will attract new investors who are interested in developing diagnostics for use in this newly emerging socio-economic field. These and other potential outcomes would continue and expand the proposed collaboration.

10. We (Professor Schwarzbauer and PD Dr. Wehrle-Haller) have known each other for about ten years through interactions at conferences focused on integrins and extracellular matrix. However, we have not worked together previously so this is a completely new collaboration. In addition, the effects of metabolites such as glucose on integrin functions and extracellular matrix assembly are brand new directions in our labs with one paper published (Schwarzbauer) and one paper submitted (Wehrle-Haller) from our labs. Thus, the theme of the project is new and we will be addressing new questions in the field. Further, we anticipate developing methodological and biological tools that can be used in disease diagnostics, which we hope will lead to additional collaborations with experts in tool development.

11. The "Department of Cell Physiology and Metabolism" in Geneva has already a strong commitment to metabolic modifications leading to diabetes or cancer and "Molecular Biology" in Princeton has strength in basic cell and cancer biology and biochemistry. Both departments will profit from this new research axis dealing with cell-extracellular matrix interactions and pathological modifications of the tissue scaffold. We plan to use the data obtained with this funding to develop more substantial research grant proposals for submission to agencies like the NIH, the American Diabetes Association, and the Swiss National Science Foundation as collaborative initiatives. Publication of our initial results will help us to get further funding to firmly establish this new research axis. In addition, we will look into possible mechanisms to raise new financial support from intellectual property through licensing novel reagents or approaches or through development of our own tools that can be marketed to the scientific community.

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