Advancing regenerative medicine by generating knowledge about the nature of cadherins in human mesenchymal stem cells

8

Impact

INTRODUCTION

The concept of tissue regeneration is not new; one could even say it is ancient. Skin grafts were first employed in the Indian subcontinent as early as 2500 BC to treat mutilations of the ear.[1] Since then, our fascination with the ability to regenerate tissues and organs has been unstoppable. It has led to the development of *ex vivo* products in the mid-1990s and, more recently, the *in vivo* constructs that are ushering in the new era of regenerative medicine. The field of regenerative medicine promises to improve health and quality of life by repairing or regenerating cells, tissues, and organs as a way to meet the demand for worn-out body parts as the world's population lives longer. One method of progressing in this area is to effectuate collaboration between developmental biologists and tissue engineers. In doing so, ideas regarding the specification and correct positioning of the cells of our tissues can be shared, and consequently used to repair damage caused by injury or disease. Over time, we have come to realize that the interactions that cells have with one another and with their environments are very complex, and their behavior is difficult to control.

The work in this thesis is conducted primarily on mesenchymal stem cells (MSCs), a type of adult stem cell. This thesis also focuses on cadherins, which are an adhesion receptor that can influence MSC behavior. The most commonly understood impact of cadherins lies in their contribution to the preservation of cell-to-cell cohesion in tissues. Understanding cadherins in the context of regeneration of tissue can give us the advantage we need to manipulate cell behavior. In the following sections, I explore how the knowledge generated by this thesis can be used to create an impact.

A QUESTION OF ETHICS

MSCs are adult stem cells that can be isolated from numerous sources including bone marrow, fat, and placental tissue. Their relative ease to culture *in vitro*, their ability to differentiate into several different cell types that are in short supply, and their immunomodulatory properties make them a powerful cell source for regenerative medicine.[2] According to the website *www.clinicaltrials.gov*, as of March 2021, more than 1300 trials using MSCs are underway worldwide, 390 of which are completed trials, and 20 of those completed trials were phase 3 trials. Due to the increase of degenerative diseases in the globally aging population, there is both a huge scientific and public interest to see regenerative medicine succeed. As a result, this has created a surge in the interest for MSC-based therapies.

When looking into Google Trends data of the past decade, the search count for MSC is more than double that of the other stem cells. This is in line with the increase in the number of private clinics that advertised and sold autologous-based MSC therapies to patients.[3–5] These therapies have not been published in scientific literature and hence are untested and unproven. Furthermore, there is a significant gulf between the public expectation of MSC therapies fueled by media coverage and the reality of progress that is made by early phase clinical trials.[6] Vulnerable patients and their families have bought into these therapies and have incurred the exorbitant costs as well as the uncertain risk associated with it. Although MSCs have remarkable potential, our understanding of their behavior is not necessarily ready for medical application and widespread use. Education and information generated by scientists should be targeted at the public to empower people to take responsibility for their healthcare choices.

To a large extent, education in regenerative medicine is also lagging behind the scientific advances.[7] This leaves the physicians ill-equipped to address the changing needs of patient care. Early incorporation of next-generation healthcare tools into mainstream medical education is essential in order to deliver validated and regulated regenerative medicine solutions. This could potentially prevent patients from seeking stem cell treatments outside of regulated clinics.

NEED FOR FUNDAMENTAL RESEARCH

MSC-based approaches:

Early-stage MSC trials have demonstrated safety and efficacy, but only a small number of MSC products have be commercialized, indicating that the therapeutic market for MSCs remains at an early stage. Several meta-analysis studies of these trials have revealed that MSCs therapies were effective in certain patients but not all, and the reason for this is unclear. Like with most cell-based therapies in regenerative medicine, the physical, phenotypic, and functional properties of MSCs remain ill-defined. Acknowledging the complexity of MSCs behavior and therefore a need for a better understanding of MSC biology is essential to temper the expectations placed on MSC therapies. The work discussed in Chapters 3, 4, and 5 deepens our knowledge of the inner workings of MSCs, as we have explored the effect of cadherin-11 on their differentiation and proliferation.

Differentiation and proliferation are the properties of stem cells that are of considerable interest to the field of regenerative medicine. Importantly, proliferation and differentiation, if unchecked, can be dangerous to patients, therefore the quality of their regulation is crucial. In Chapter 4, we tried to understand the mechanism that helps MSCs differentiate and commit to becoming a fat cell over a bone cell,

which could help to fine-tune MSC fate commitment for regenerative medicine applications. In Chapter 5, we showed that cadherin-11 is essential for MSC proliferation, which could help further our understanding of the mechanisms that preserve the undifferentiated stem state of the MSCs. However, additional research is required to understand if the mechanisms described in Chapters 3, 4, and 5 are robust in MSCs from various donors and if they are reproducible across different laboratories. Ultimately, the knowledge generated by these chapters indicates that cadherin-11 can be used as a tool to control MSC behavior.

Material-based approaches:

Tissue engineering literature describes a diverse selection of scaffold materials that aim to promote specific cell behavior and advance regenerative medicine. Yet, the field is puzzled over the question of why some materials succeed in directing cell behavior while others fail. For example, in the case of bone regeneration, many bone graft materials fail to replicate the fracture healing exhibited by autografts. The current toolbox used by tissue engineers has not been enough, and a better understanding of how cells respond to cues is needed for improved material design. Thanks to the research conducted in this thesis and by others, it is slowly becoming a reasonable reality that new knowledge can be used to advance the field.

We have seen in Chapters 3, 4, and 5 that MSCs lacking cadherin-11 do not proliferate and differentiate like normal MSCs, establishing the importance of cadherin-11 in MSC behavior. Proteins such as cadherin-11 can then be harnessed by tissue engineers to improve material-based therapies by incorporating this information into the design of engineered constructs to control MSC cell fate. In Chapter 2, we summed up various material-based approaches that have used cadherins to improve material design. The results in Chapter 4 suggest that the

timing of certain signaling molecules is essential for extracellular matrix deposition and the eventual MSC fate commitment. This therefore points to the importance of nuanced material design to guide cell behavior.

3D models

Many results obtained *in vitro* do not correlate to results obtained in an *in vivo* setting. We have shown in Chapter 3 that MSC behavior differs based on the cell culture dimensionality. Moreover, in Chapter 6, we explored different 3D culture methods which better mimic the *in vivo* setting and observed a dramatic decrease in cell number over long-term culture. This shows that great effort is necessary to prepare MSCs for the *in vivo* environment, which may eventually pay big dividends, as it will enhance their clinical efficacy. The findings of Chapters 4 and 5 need to be validated in a 3D model, as 3D culture has profound effects in MCSs and the results are needed for *in vivo* applications.

OUTLOOK FOR REGENERATIVE MEDICINE

Regenerative medicine solutions are meant to address the need for the replacement of damaged tissues and organs. However, while much of the current research in the field of regenerative medicine is confined to the bench rather than the bedside, clinical translation is becoming increasingly apparent. When it comes to clinical translation of fundamental research, tissue engineering should be to developmental biology what drug development is to molecular biology. Tissue engineers cannot do what they do without the knowledge of specific molecular regulators, and similarly, biologists are looking for newer tools to answer questions that cannot be done using the existing toolbox. The main focus of this thesis was to try and improve tissue engineering by taking lessons from cadherin biology. We have the technology in our arsenal, but basic biology still needs to be deciphered. Stem cell–based approaches to repair and regenerate tissue are far from being successful in the clinic because our understanding of the basic biology underlying tissue repair is still far from exhaustive. This is despite all the advancements that have been made by fundamental biologists in our understanding of biology. A strong alliance between tissue engineers and developmental biologists can catapult the field towards new discoveries. This is possible if fundamental biology questions are framed within the context of tissue engineering.

The knowledge generated by this thesis is a start to give scientists a better understanding of the microenvironment they wish to control. Furthermore, fields such as cancer biology could also use the knowledge for their questions, as there are many similarities between tissue stem cells and cancer stem cells.

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