Cellular Transitions and Tissue Engineering

Aaron Schindeler^{1,2} Mille Kolind¹ and David G. Little^{1,2}

Abstract

Epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndMT) describe complex changes in progenitor lineage, cell morphology, and gene expression. Stimulated by environmental cues, these cellular transitions are essential for elements of embryonic development and can be pathologically dysregulated in disease states. EMT occurs in biological processes such as gastrulation, cardiogenesis, and fibrosis. EndMT is involved in development and tissue fibrosis, but recent studies have implicated this process in musculoskeletal biology and pathology. Tissue engineering and regenerative medicine typically rely on endogenous progenitors or progenitors expanded *ex vivo* to repair damaged or impaired tissues or organs. The processes of EMT and EndMT may aid in elucidating new methods for reducing fibrosis and identifying novel plastic progenitor populations for tissue repair. This review will discuss the potential for EMT and EndMT to impact on tissue engineering and regenerative medicine.

Introduction

THE END GOAL IN TISSUE engineering and regenerative medicine is to restore tissue function or heal tissue defects in situations where the tissue is diseased or damaged. This can be achieved by: (1) Supplying exogenous progenitor cells, *e.g.*, via cell seeded scaffolds or; (2) activating resident stem cells; or (3) inducing endogenous cells to dedifferentiate and thereby regain the properties of stem cells or progenitor cells.

The classical mechanism for cell differentiation is for a committed progenitor to proceed down a well-ordered pathway of an immature cell type to a more mature cell type. The terminally differentiated cell will carry out its specialized function, and at a fixed time the cell undergoes apoptosis. However, to maintain tissue mass and function, differentiated mature cells will constantly be supplied from the committed progenitor cell pool. Although this is a common mechanism for natural tissue regeneration or rejuvenation, there are instances where biological systems rely on alternative cell lineages for repair.

An extreme example of this is seen in the newt, where after loss of a limb the cells adjacent to the wound dedifferentiate (Jopling et al., 2011) and form a blastema. These cells then proliferate and are able to repattern and differentiate to reform the lost limb (Brookes, 1997). Mammals cannot regenerate in such a way, but dedifferentiation can still occur, for example, in Schwann cells in neural repair (Mirsky

et al., 2008). After neural injury, the mature Schwann cells lose contact with the axon that they are myelinating. This triggers dedifferentiation, re-expression of genes associated with immature Schwann cells, followed by proliferation, and finally regeneration of the neuron (Chen et al., 2007).

In the context of tissue engineering, intervention can allow committed progenitors to participate in processes to which they would not normally contribute. One approach is cellular reprogramming, a more absolute form of dedifferentiation, where an adult cell is induced to take on a pluripotent state. Reprogramming involves resetting many epigenetic markers, including microRNAs (miRNAs) and histone modifications. This occurs in induced pluripotent stem cell (iPSC) generation with the transduction or transfection of Yamanaka transcription factors, such as OCT3/4, SOX2, KLF4, and MYC (Takahashi and Yamanaka, 2006). Following reprogramming, the cells can be stimulated to undergo differentiation into various types of somatic cells. Ectopic expression of the transcription factor Pax7 stimulates iPSCs to differentiate down the myogenic lineage, where they can contribute to muscle fibers and populate the satellite cell niche (Darabi et al., 2011a; Darabi et al., 2011b). The capacity to generate iPSCs is emerging as a promising method for creating progenitor cells capable of making specific tissues in the field of tissue engineering. However, a caveat for the therapeutic use of these cells is their potential to form tumors (Knoepfler, 2009).

¹Department of Orthopaedic Research & Biotechnology, the Children's Hospital at Westmead, Sydney, Australia.

²Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Sydney, Australia.

An alternative tissue engineering approach is transdifferentiation, where one mature differentiated cell type can be encouraged to take on features of another mature cell type. Natural transdifferentiation of mature mammalian cells remains a controversial concept, although it can be induced via growth factor treatment or by forced expression of specific genes (Jopling et al., 2011). Several studies have reported on successful transdifferentiation of one adult somatic cell type directly into another (Ieda et al., 2010; Szabo et al., 2010; Vierbuchen et al., 2010; Zhou et al., 2008). When this concept is applied to committed progenitors rather than mature cells, it is referred to as a cellular transition and can occur without exogenous stimulation (Kalluri and Neilson, 2003). This is an attractive approach to change the cellular program in an efficient and possibly less tumorigenic way as compared to the iPSCs (Asuelime and Shi, 2012). The two most common examples of this are epithelial-to-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndMT). This review will focus on these cellular transitions and their potential implications for normal and aided regeneration.

Epithelial-to-Mesenchymal Transitions

EMTs occur in three very different biological situations, and accordingly EMTs are split up in categories reflecting their very different outcomes (Kalluri and Weinberg, 2009). The common denominator is generation of motile cells with a mesenchymal phenotype. Type 1 EMTs occur during implantation, embryogenesis, and organ development. Prior to gastrulation, the epiblast displays a columnar epithelial morphology that is tightly connected by tight/adherens/gap junctions and sitting upon a basal lamina. Transition coincides with the breakdown of the basement membrane by matrix metalloproteinases (MMPs), which in turn releases cytokines such as epidermal growth factor (EGF), insulin-like growth factor-II (IGF-II), fibroblast growth factor-2 (FGF-2), and transforming growth factor- β (TGF- β). This leads to changes in SNAIL1, SNAIL2, and TWIST1 that can lead to suppression of E-cadherin and mesenchymal transition (Kalluri and Neilson, 2003).

Expression of the *Snail* genes has also been linked to increased MMP expression, suggesting a potential positive feedback loop for degradation of the basal lamina once EMT has been initiated (Miyoshi et al., 2004). In addition, SNAIL overexpression has been shown to be sufficient for induction of EMT (Cano et al., 2000). The mechanism and involvement of MMPs, breakdown of the basement membrane, and SNAIL factors appear to be common mechanisms for the various types of EMT (Nakamura and Tokura, 2011). Type 2 EMT takes place during tissue regeneration and fibrosis. It is induced by tissue injury and/or inflammation and will cease to continue once the infection has been removed or the tissue has been repaired (Kalluri and Neilson, 2003). In regard to fibrosis, type 2 EMT has received lots of attention, and studies have reported on EMT giving rise to fibrosis of the lung, kidney, and liver (Choi and Diehl, 2009; Willis and Borok, 2007). Other studies finding no evidence of EMT in kidney (Humphreys and Bonventre, 2007) and liver (Taura et al., 2010; Chu et al., 2011) suggest contributions from other cell types such as pericytes and endothelial cells. To some extent, the different outcomes of the studies can be explained

by the use of different and sometimes unspecific markers. The expression of fibroblast-specific protein 1 (FSP1) has, for instance, been used to define EMT *in vivo*, whereas other studies are questioning its specificity and suggest that FSP1 is a marker of leukocytes and other nonfibroblastic cell types (Lin et al., 2008; Le et al., 2005). Identification of more specific biomarkers of EMT will overcome these problems in the future. Type 3 EMTs are involved in tumor growth and cancer progression. The cancer cells induce epithelial cells at the invasive front to become invasive and spread to other organs via an EMT transition (Thiery, 2002; Yang and Weinberg, 2008).

In tissue engineering, an understanding of EMT may impact on therapies where EMT may be a desired or an undesirable outcome. For epithelial cell transplantation where the end stage tissue is epithelial, such as corneal cell grafting and skin grafting, EMT is associated with fibrosis and a suboptimal response (Nakamura and Tokura, 2011). EMT is also reported as a major hurdle for the *ex vivo* expansion of human pancreatic β -cells for the treatment of type 1 diabetes (Montgomery and Yebra, 2011). However, the potential for harnessing EMT to recapitulate development events and regenerate mesenchymal tissues makes it an appealing area for study. In particular, this is an area of study for cardiac valve replacement (Sewell-Loftin et al., 2011), but the reprogramming of cells from the skin (skin precursors, or SKPs) may be beneficial for repairing a range of mesenchymal tissues (Zipori, 2004). An intriguing progenitor that has been described is the mesothelial cell, which shares qualities of epithelial and mesenchymal cells (Herrick and Mutsaers, 2004). Although this cell type is mesodermal in origin, it has been reported to undergo an EMT-like transition where it loses its epithelial character in response to cytokines, including TGF- β 1 and interleukin-1 β (IL-1 β), and induction of *Snail* expression (Yanez-Mo et al., 2003). The tissue engineering potential of mesothelial cells has been broadly explored with vascular grafting as well as omental grafts and nerve grafts (Herrick and Mutsaers, 2004).

Endothelial-to-Mesenchymal Transitions

There are many parallels between EMT and EndMT, including the fundamental involvement of TGF- β and SNAIL factors (van Meeteren and ten, 2012). TGF- β factors signal through canonical type II/type I receptor complexes and are transduced via the SMAD transcription factors. TGF- β -induced upregulation of SNAIL is required for EndMT. Cell culture studies have shown that forced expression of SNAIL can induce EndMT independently of TGF- β signaling and that small interfering RNA (siRNA) suppression of SNAIL can repress EndMT (Kokudo et al., 2008). While TGF- β is implicated as the primary regulator of EndMT, other pathways such as the Wnt signaling pathway may be important. In the TOPGAL mouse line, EndMT was observed in response to myocardial infarction and this was associated with increased canonical Wnt signaling (Aisagbonhi et al., 2011). Like EMT, EndMT has been shown to be important for development, particularly in the heart (Kovacic et al., 2012). In the heart, Notch has been implicated in the EndMT signaling process (Chang et al., 2011), although Notch may primarily act via the protein SLUG rather than SNAIL (Niessen et al., 2008).

In addition to mechanistic similarities with EMT, EndMT also has a critical role in tissue fibrosis and disease (Kalluri and Neilson, 2003). Targeted induction of EndMT may allow for endothelial progenitors to be broadly used in regenerative medicine. The suppression of fibrosis and the promotion of EndMT for tissue engineering will be focused in the remaining discussion.

EndMT in tissue fibrosis

In most tissues, regeneration is associated with some degree of scarring/fibrosis, and in tissue engineering fibrosis is a negative but often unavoidable consequence. EndMT is emerging as a key mechanism for scar formation, and understanding this process may allow fibrosis to be minimized and maximal function to be maintained.

In the kidney, EndMT has emerged as an important mechanism in the development of pathological fibrosis. Examination of fibroblasts and myofibroblasts (FSP1⁺ or α -SMA⁺ cells) in mouse models of nephropathy show 30–50% of cells to co-express the endothelial cell marker CD31, implying an endothelial origin. This was further confirmed by lineage-tracking experiments using conditional reporter mice (Humphreys and Bonventre, 2007; Zeisberg et al., 2008). The involvement of EndMT in a range of animal models of kidney fibrosis suggests this to be a common mechanism for all forms of kidney disease.

Cardiac fibrosis can lead to compromised heart function, hypertrophy, and eventually congestive heart failure. In an elegant study using Tie1-Cre and FSP1 reporter mice, it was demonstrated that Tie1-lineage cells could be induced to express the mesenchymal/fibroblastic marker FSP-1 in models of cardiac fibrosis (Zeisberg et al., 2007). Furthermore, it was shown that this used a TGF- β 1-dependent pathway, and that recombinant bone morphogenetic protein-7 (BMP-7) could antagonize TGF- β 1 signaling and impair EndMT. Emerging data from cardiac models also suggests that aging and cell division may impact on the propensity of cells to undergo EndMT (Fleenor et al., 2012).

While the majority of the literature concentrates on these two organs, EndMT has been suggested to contribute to idiopathic pulmonary fibrosis (Nataraj et al., 2010) and intestinal fibrosis (Rieder et al., 2011). In addition, there is emerging evidence that circulating cells may also contribute to dermal fibrosis, and it was observed that subdermal application of the vasoconstrictive peptide angiotensin II could induce inflammation and EndMT (Stawski et al., 2012).

To facilitate repair while limiting fibrosis, future tissue engineering strategies targeting these organs will need to consider the impact of EndMT. For example, a recent study looking at tubulointerstitial fibrosis used vascular endothelial growth factor (VEGF) treatment to downregulate TGF- β 1 and SMA in the early stages of fibrosis in an attempt to reduce EndMT and fibrosis (Lian et al., 2011). The development of new tissue engineering strategies that consider EndMT and TGF- β may enable improved tissue and organ regeneration that ameliorate the fibrotic response.

EndMT in musculoskeletal tissues and tissue engineering

In addition to its involvement with fibrotic pathologies, there are several emerging bodies of research that have

explored the capacity of endothelial cells to contribute to muscle regeneration and heterotopic bone formation. Normal muscle regeneration is attributed to a population of quiescent mesenchymal mononuclear progenitors known as satellite cells (Chen and Goldhamer, 2003). Following injury, these cells are activated, differentiate, and fuse together and with existing myofibers to repair the damage. Although endothelial cells are generally not regarded to play a role in the repair of muscle tissue, recent work from David Goldhamer has shown that the intermuscular fat likely arises via the process of EndMT (Starkey et al., 2011).

Satellite cells can be challenging to culture and become depleted with age. For tissue engineering, a cell type with endothelial qualities—the mesoangioblast—has emerged as a potent vehicle for cell-based therapy for muscle disease. Mesoangioblasts are associated with the walls of large vessels and they express a combination of mesenchymal and endothelial markers (Cossu and Bianco, 2003). These cells have been shown to be able to rescue muscular dystrophy in α -sarcoglycan null mice (Sampaolesi et al., 2003; Guttinger et al., 2006) and in dystrophic dogs (Sampaolesi et al., 2006). Mesoangioblasts can be considered more mesenchymal or endothelial, and whether they undergo a process of EndMT remains to be explored; but as a cell type, they show unique potential for the treatment of congenital muscle disease.

The role of endogenous endothelial cells in bone formation is a topic of evolving interest. In the context of genetic disease, EndMT has an important role in the genetic disease fibrodysplasia ossificans progressiva (FOP) (Shore and Kaplan 2008). This disorder, caused by mutations in *ACVR1*, features progressive ossification of muscle and connective tissues. Mechanistically, this causes constitutive activation of ALK2, a member of the TGF- β superfamily (Fukuda et al., 2009). In a series of published studies, Shore and Kaplan's group showed that endothelial cells of the Tie2 lineage and not myogenic or mesenchymal cells of the *MyoD* or *Smmhc* (smooth muscle myosin heavy chain) lineages contribute to heterotopic bone formation via EndMT (Lounev et al., 2009). TIE-2 is the receptor tyrosine kinase for the angiopoietins, is important for angiogenesis and vasculogenesis, and is a marker of all early endothelial precursors. Later work by the same group showed that endothelial cell lines could be induced to take on mesenchymal stem cell markers and become multipotent upon activation by recombinant TGF- β 2 or BMP-4 but not BMP-7 (Medici et al., 2010). This also occurred when they overexpressed mutant R206H ALK2, possessing an activating mutation associated with FOP. This work demonstrates a key role for EndMT in the FOP disease process, but also suggests a potential capacity to use EndMT as a mechanism to exploit vascular endothelial cells in bone tissue regeneration.

However, a recent study from the Goldhamer group questions the veracity of using Tie2 as an endothelial cell marker (Wosczyzna et al., 2012). It is already well recognized that Tie2 is expressed in hematopoietic cell lineages (Tang et al., 2010), but a comparison of Tie2-Cre R26^{NG/+} conditional reporter mice with VE-Cadherin-Cre R26^{NG/+} mice indicated that there may be a subpopulation of Tie2-lineage cells that lack other endothelial cell markers that behave with mesenchymal cell properties. When transplanted with rhBMP-2 and Matrigel, the Tie2⁺ CD31⁺ cells formed vessels only, while Tie2⁺ CD31[−] cells formed cartilage and bone

(Wosczyzna et al., 2012). These data argue against EndMT being a key mechanism in heterotopic bone formation outside of the FOP genetic disease.

The capacity of non-FOP cells to undergo EndMT to participate in normal repair, as well as be induced to aid in tissue regeneration, reflects issues that have yet to be addressed. In a rat bone defect model, a tissue engineering solution where endothelial progenitors were introduced led to improved outcomes, although EndMT was not specifically demonstrated (Li et al., 2011). More considered studies will be required to examine the ability of endogenous and transplanted endothelial cells to contribute to bone repair models, such as fracture healing and treatment of critical defects (Matsumoto et al., 2008).

Conclusions

Tissue engineering approaches often involve isolation and transplantation of progenitor cells to repair or regenerate the target tissue. The underlying principle is that committed progenitors will unilaterally form the desired tissue either due to local environmental factors or exogenous stimuli. However, dedifferentiation, transdifferentiation, and, as discussed, cellular fate transitions (EMT, EndMT) can lead to alternative fate decisions. Depending on the local cytokine milieu, particularly TGF- β and other inflammatory signals, cells can take on fibrogenic or other undesirable features.

Conversely, there are opportunities for using vessel-associated cells, including mesoangioblasts, mesothelial cells, and vascular endothelial cells as progenitors for mesenchymal tissues. Heterotopic bone formation is an example of how endothelial cells can be coerced to express chondrogenic or osteogenic expression profiles and produce cartilage and bone. It could be imagined that for bone tissue engineering and potentially for other tissue types, these progenitors could be an alternative cell source for regeneration and repair.

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Address correspondence to:

Aaron Schindeler

Orthopaedic Research & Biotechnology

Research Building

The Children's Hospital at Westmead

Locked Bag 4001

Westmead, NSW 2145, Australia

E-mail: Aaron.Schindeler@sydney.edu.au