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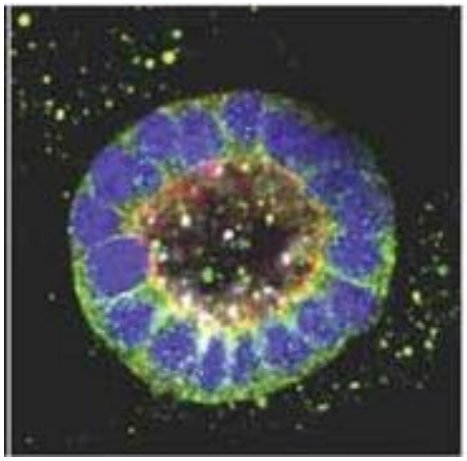
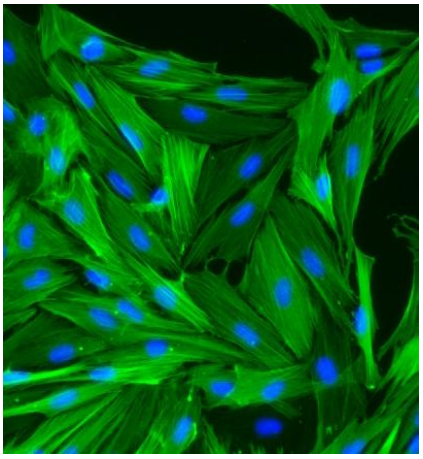
The Differentiation Choice of Bone Marrow-derived Cells





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Source stem cell: http://www.immortalhumans.com/inspecting-stem-cell-therapies/
Source renal tubule: http://www.nature.com/ng/journal/v42/n10/fig_tab/ng.662_F6.html
Source myofibroblasts: https://shop.lonza.com/shop/prd/intestinal-myofibroblasts/lonza_b2b/7.0-7_2_86_69_76_10_13/2/E01EA9CA2952BFF1A4D278E7D1E88A34/#

Index

Abstract

Bone marrow derived cells (BMDC) have shown to engraft renal tubuli and constitute part of the interstitial myofibroblast population after kidney damage. It is unknown which BMDC are responsible for the differentiation towards tubular epithelial cells (TEC) or myofibroblasts. Mesenchymal stem cells, haematopoietic stem cells, fibrocytes, peripheral blood mononuclear cells, fibroblasts and pericytes are all resident or peripheral BMDC. They each show in vitro or in vivo differentiation capability towards either TEC or myofibroblasts, creating a complicated web of cells that can contribute to renal recovery. With the help of literature research the signalling molecules that affect this differentiation are identified. In the damaged kidney, inflammation and wound healing create a specific microenvironment. The composition of this microenvironment will determine the differentiation choice of arriving BMDC towards TEC or myofibroblast. By studying the damaged kidney's gene transcription and protein levels it is possible to predict the microenvironment and hypothesize which differentiation choice the BMDC will make. At the moment of BMDC arrival the microenvironment is predominantly pro-fibrotic, therefore BMDC will mainly choose to differentiate towards myofibroblasts.

Introduction

Bone marrow-derived cells (BMDC) have been shown to engraft renal tubule after kidney damage and obtain an epithelial phenotype (1-4). BMDC are not only involved in repair, but also play a role in normal renal epithelial turnover (5). The same involvement of BMDC in epithelial engraftment is found in other organs such as the lung, liver, skin and gastrointestinal tract in both cellular turnover (6)(7) and after damage (8-11). Renal damage is sometimes reversible (12), but severe or chronic renal damage can lead to the accumulation of myofibroblasts. Myofibroblasts foremost produce extracellular matrix (ECM) proteins. An excessive and imbalanced deposition of these ECM proteins can lead to fibrosis (13). Studies have shown that BMDC can differentiate into myofibroblasts and hereby contribute to the development of fibrosis in the kidney (12), in the liver (14) and in other organs (15). Thus cells derived from the bone marrow can migrate to the kidney and other organs and differentiate in both tubular epithelial cells (TEC) and myofibroblasts, potentially contributing to recovery or fibrosis.

The cell mainly produced during recovery is the TEC. TEC compose the lining of the renal tubule. They are responsible for the reabsorption of water and solutes. The TEC are attached to a basal membrane and border the kidney's lumen filled with pre-urine. There are small differences between the epithelial cells found in the different parts of the tubule. For example proximal TEC have a brush border to increase the area for reabsorption and are more 'leaky' than distal TEC (16). Cells contributing to fibrosis are the myofibroblasts. They are large cells with long processes and are identified by their expression of α-smooth muscle actin (αSMA) (17), although this marker is expressed by some other cell types as well like pericytes and vascular smooth muscle cells (18). αSMA is a cytoskeletal protein and is used by the myofibroblasts to exert contractile forces on the surrounding tissue. Myofibroblasts in the kidney are traditionally thought of as an activated population of resident fibroblasts (19). During fibrosis they are the main producers of the excessive ECM deposition that characterizes this condition (13,20).

Both myofibroblasts and TEC can be produced by cells originating from the bone marrow. The bone marrow consists of several different cell populations that give rise to a high variety of cell types. An example is the haematopoietic stem cell (HSC) population that produces all cells within the haematopoietic system, such as lymphocytes, myelocytes and erythrocytes (16). Upon damage cytokines and chemokines, like for instance interleukin-8 (IL-8) and stromal derived factor-1 (SDF-1), regulate the homing of BMDC to damaged tissues (21). I suggest that the signalling molecules present in the damaged kidney's microenvironment will determine the differentiation direction of these migrating BMDC.

Several researchers have stated that BMDC can form TEC and myofibroblasts in vivo (1-4,12). At the moment it is not precisely clear which BMDC populations contribute to their formation in the kidney. It is conceivable that a single specific BMDC population serves as the progenitor for both TEC and myofibroblasts and that the microenvironment in the kidney determines the differentiation direction (see figure 1A). Another possibility is that one BMDC population gives rise to TEC and another gives rise to myofibroblasts. It now becomes important which of the BMDC populations is recruited to the kidney, because within this scenario the recruitment will determine if TEC or myofibroblasts will be formed (see figure 1B). The last explanation is that multiple BMDC populations can differentiate into both tubular epithelial cells and myofibroblasts. This would mean that the composition of the microenvironment will direct the multiple BMDC populations to differentiate to either TEC or myofibroblast (see figure 1C).

Most studies on BMDC differentiation in the kidney are observational and study just the appearance or engraftment of BMDC derivatives. After identification with surface markers, such as E-cadherin (1) or aquaporin 2 (2) for epithelial cells and αSMA (12) for myofibroblasts, the studies end. The issue that these studies do not address is what signals trigger the differentiation to either TEC or myofibroblast and if these signals are different between the BMDC populations. By obtaining this knowledge it is possible to speculate on how the microenvironment in the damaged kidney will affect the BMDC differentiation choice.

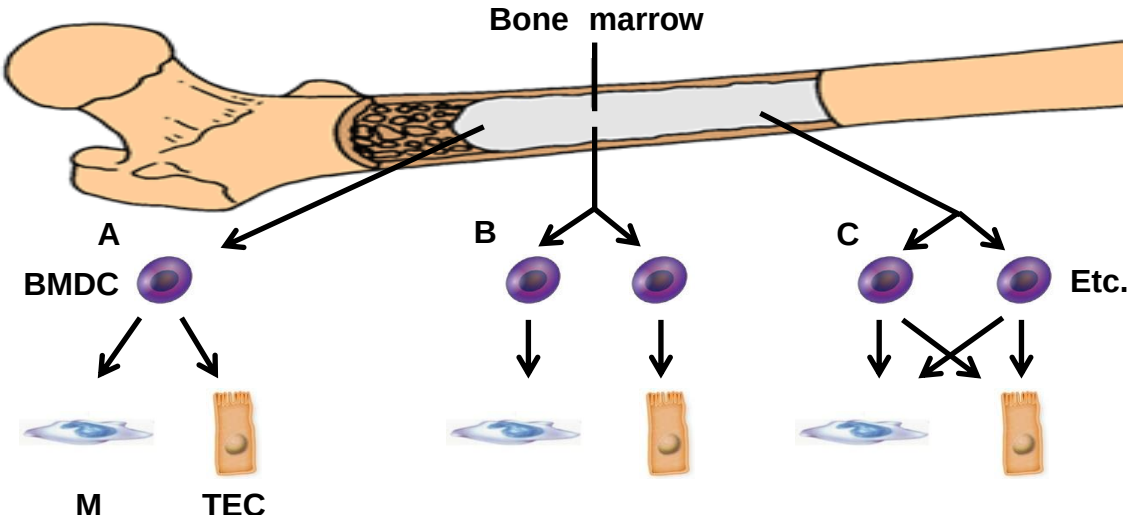

Figure 1. Multiple options for BMDC contribution to tubular epithelial cells and myofibroblasts. One BMDC population can produce both cell types (A). Two BMDC populations serve as progenitor for a single cell type each (B). Both myofibroblasts and TEC can be produced by two or more BMDC populations (C). Bone marrow-derived cell (BMDC), Myofibroblasts (M), tubular epithelial cell (TEC).

In this thesis I will first give an overview on the different bone-marrow derived cell populations that have the right differentiation potential. I will not only discuss resident BMDC populations but also briefly point out peripheral cell types with a bone marrow background. This will provide more insight on which BMDC populations might be able to produce TEC or myofibroblasts in the damaged kidney. Secondly I will clarify which signals trigger the differentiation of migrating BMDC. Finally by looking at the damaged kidney's microenvironment I will hopefully be able to determine which differentiation choice the BMDC will make when they arrive at the kidney.

BMDC with differentiation potential towards TEC or myofibroblast

Resident bone marrow-derived cells

Cells from the bone marrow can go to the damaged kidney and directly differentiate into TEC (22) or myofibroblasts (12). There are multiple BMDC populations that can execute this process. I divide them into two groups: 1) Cells resident to and originating directly from the bone marrow, termed “resident BMDC”. 2) Cells that are a descendant of a bone marrow progenitor but do not reside in the bone marrow any longer. In this thesis these cells are called “peripheral BMDC”. There are two types of resident BMDC that have shown differentiation to either TEC or myofibroblasts which will be discussed below.

Mesenchymal stem cell (MSC)

The MSC was first thought of as only a supportive stromal cell that creates the stem cell niche required by the HSC to retain its “stemness” or balance between self-renewal and reproduction (23-25). The first hint to their multipotent character was discovered by Alexander Friedenstein (26). He seeded bone marrow cells in liquid cultures and after two weeks colonies of plastic-adherent, non-phagocytic, elongated cells of fibroblastic morphology started to form. He then implanted these colonies under the renal capsule of semi syngeneic animals and after a few weeks the colonies gave rise to fibrous tissue, bone and bone containing recipient bone marrow. This last result, although obtained in a different study of Friedenstein, provided supporting evidence for the hypothesis of a HSC niche created by stromal cells or MSC (27). Later studies found that MSC could differentiate to osteoblasts, chondrocytes and adipocytes, which further shows the ability of the MSC to differentiate to multiple mesenchymal cell types (28).

MSC, TEC and myofibroblasts do not seem to differ much in origin. MSC and myofibroblasts are both part of the mesenchyme, which originates -for the greater part- from the mesoderm. During kidney development mesenchymal cells differentiate into the epithelial cells that constitute the renal tubule (29). The ability of epithelial cells to attain a mesenchymal phenotype when confronted with fibrosis stimulating factors further shows the kinship between these three cell types (30). In vivo studies with mice demonstrate the capability of MSC to differentiate to TEC after the induction of renal damage (31,32). Morigi et al injected MSC from male bone marrow origin into cisplatin-treated syngeneic female mice. Y chromosome-containing cells were located at the tubular epithelial lining and displayed binding sites for the lectin *Lens culinaris*, which is in indication that MSC differentiate into TEC (31). These results were supported by in vitro research. MSC were co-cultured with injured murine TEC, but separated by a physical barrier to prevent contact between the two cell types. After 7 days the MSC differentiated into a TEC-like phenotype, expressing both kidney-specific cadherin and aquaporin-1 (33). Both the in vitro and in vivo studies were also performed in a rat model and similar results were obtained (22).

The capability of MSC to differentiate directly into myofibroblasts has not been defined for the kidney in vivo. Research suggests different origins for myofibroblasts found in damaged tissues, such as fibrocytes, pericytes and fibroblasts (34). These cell types will be discussed later on. It must be noted though that some of these other myofibroblast sources have MSC as a progenitor (35,36). There are however in vitro studies that show MSC differentiation to a myofibroblast under the right culture conditions. Nedeau et al used bone marrow derived MSC co-cultured with platelet-derived growth factor-B-activated fibroblasts (PDGF-B-aFBs) in a 3D culture model and showed that PDGF-B-aFBs induced differentiation of MSC to myofibroblasts. This effect was most likely mediated by basic fibroblast growth factor (bFGF) and epithelial neutrophil activating peptide-78 (CXCL5) (37). If the differentiation was direct or through a fibroblast intermediate was not established.

Based on these findings I can conclude that MSC are one of the BMDC populations with the potential to differentiate into TEC in vitro and in vivo. The differentiation potential to myofibroblast is less clear. It seems possible under the right culture conditions, but this might not be the correct reflection of

the in vivo situation. In damaged tissue MSC are most likely not the main producer of myofibroblasts, but they can give rise to other progenitor populations that can differentiate to myofibroblasts in vivo.

Haematopoietic stem cell (HSC)

As stated previously the HSC is the progenitor of the haematopoietic system and thus produces all the different blood and immune cells in the body. Studies have confirmed the capability of HSC to differentiate into non-haematopoietic cells, one of which is the epithelium of the gastrointestinal tract, hereby confirming their pluripotency (11). An in vivo mouse study performed by Lin et al in 2003 showed that HSC might be able to form TEC as well. They used a highly purified fraction of HSC from β-galactosidase expressing male Rosa26 mice to make sure no MSC could serve as progenitor for TEC. The HSC were injected into female non-transgenic mice after induction of ischemic kidney damage. The results showed β-galactosidase and Y-chromosome positive cells in the renal tubule of female mice following kidney damage. After staining for Fx1A (brush border antigen on TEC) and sodium/phosphate co-transporter the researchers concluded that HSC can contribute to tubular epithelial regeneration after ischemic damage (38). This study provides a first clue on the ability of HSC to differentiate into TEC in vivo. These results were later supported by another, similar in vivo study (39). In vitro evidence is still lacking thought. Further research on HSC differentiation towards TEC is thus needed.

The group of Ogawa et al studied the possibility that myofibroblasts are derived from HSC. They transplanted a HSC culture from a single male cell which expressed enhanced green fluorescent protein (eGFP) into a female non-transgenic recipient (40). HSC-derived myofibroblasts were detected in the form of glomerular mesangial cells in the kidney (41), microglia cells and perivascular cells (pericytes) in the brain (42), hepatic stellate cells in the liver (43), fibroblasts/myofibroblasts in the adult heart valve (44) and as myofibroblast in the heart following myocardial infarction (45). The same group performed in vitro studies on HSC from both mice and humans and found that they were a possible precursor for fibroblasts, but direct differentiation to a myofibroblast was not shown (46,47). Myofibroblasts are thought of as an activated form of fibroblasts, so the fibroblasts derived from the HSC can be seen as an intermediate step to a myofibroblast. These in vitro and in vivo results on HSC differentiation to myofibroblasts are however obtained by one laboratory and or not yet reproduced by other research groups.

When taking all these results together it seems that the HSC can contribute to the myofibroblast population during normal turnover and after damage, more so then the MSC. It is unclear if this is due to direct differentiation or that there is another progenitor in between the HSC and the myofibroblast. Contrary to these findings the HSC is less likely to produce TEC then the MSC. Although some clues supporting this differentiation pathway have been found during in vivo research, further proof is needed to fully acknowledge the HSC as one of the BMDC populations capable of producing both TEC and myofibroblasts. It is now safe to assume that the theory of one BMDC populations being responsible for the production of both TEC and myofibroblasts is false and can be discarded (see figure 1A). It is at this point unknown which of the other two theories (see figure 1B and 1C) will prove correct. To solve this dispute I will look at the peripheral bone marrow-derived cell populations and their differentiation.

Peripheral bone marrow-derived cells

Peripheral bone marrow-derived cells are cell populations that have a progenitor from the bone marrow, but do not reside there themselves any longer. However, they might have retained the ability to differentiate to either TEC or myofibroblasts. There are four types of peripheral BMDC which will be

discussed below. They consist of circulating cells and cells local to the kidney, but with a potential bone marrow background.

Fibrocytes

Fibrocytes are circulatory cells derived from the bone marrow (48) and comprise around 0,1-1% of the nucleated cells in the peripheral blood (49-51). Fibrocytes perform important functions in wound repair and inflammation. They secrete pro-inflammatory cytokines (52), pro-angiogenic factors (53) and chemokines for the regulation of inflammatory cells and have antigen presenting capabilities (54). Ebihara et al performed single HSC tracing studies and found an indication that fibrocytes are derived from HSC (46). Other researchers have expanded this observation, stating that a CD14+ monocyte progenitor serves as a precursor for fibrocytes (55-58). More conclusive lineage tracing studies are still needed though.

Research on differentiation to an epithelial cell has not been performed on fibrocytes. However, in 2003 Schmidt et al used an in vivo mouse asthma model to provide the first evidence that fibrocytes can differentiate into myofibroblasts (59). Later studies showed the same differentiation in a wound healing model (60). Also in the damaged kidney fibrocytes contribute to the myofibroblast population and fibrosis (61). In vitro research on purified fibrocytes confirmed these in vivo findings (62). It is now safe to conclude that fibrocytes are one of the BMDC that can form myofibroblasts, both in vitro and in vivo.

Peripheral blood mononuclear cells (PBMC)

Within the category of PBMC fall the peripheral blood monocyte, lymphocytes and the macrophage, among other inflammatory cells. They are produced during haematopoiesis and are descendants of the myeloid and lymphoid progenitors. Studies have only been performed on in vitro differentiation towards normal epithelial cells after stimulation with the right factors or culture medium (63,64). PBMC are a very heterogeneous population and need to be purified very well to determine which cells differentiate to epithelial cells. In a study performed by Zhao et al a subset of monocytes appeared to be responsible for the differentiation to epithelial cells (63). Another study only excluded the circulating precursor cell population and used the remaining heterogeneous PBMC population. This meant that it was not possible to determine the precise origin of the epithelial cells they found (64). Further research on this differentiation pathway is called for.

Differentiation of PBMC to myofibroblasts is backed by some in vivo studies involving the foreign body response (FBR) and the formation of a fibrous capsule. After implantation of a sterile foreign object the body reacts with an inflammation response resulting in an influx of immune cells. When the foreign object cannot be removed by immune cells a fibrous capsule is formed around it (65). The researchers used labelled mononuclear cells to investigate the FBR to peritoneal implanted tubes. They observed the appearance of labelled mononuclear cells at the tubes in the earlier stages of the FBR, corresponding with the acute inflammatory response described in literature (65). As the fibrous capsule developed the labelled cells obtained a spindle-shaped morphology and began to express αSMA, pointing to the formation of a myofibroblast-like cell (66,67).

The in vitro differentiation of PBMC to epithelial cell shows potential. Maybe under the right culture conditions a further differentiation to TEC is achievable, but until now no proof of this differentiation pathway has been provided. The in vivo differentiation of PBMC to myofibroblasts during the FBR does not mean that the same can happen in a damaged kidney. It does however hint at the existence of a differentiation pathway straight from PBMC to myofibroblast. Further research on which cell types from the PBMC population can accomplish the differentiation to epithelial cell or myofibroblast is needed, but according to these results it is unlikely that this BMDC population contributes to recovery or fibrosis in the damaged kidney by means of differentiation to TEC or myofibroblast.

Pericytes

Pericytes consist of a heterogeneous population of cells residing in the kidney, among other organs. They are located in the perivascular niche and are in close contact with the vascular endothelial cells. Two of their many functions are maintaining vessel stabilisation and vessel permeability. During embryogenesis pericytes develop mostly from mesodermal mesenchymal stem cells, although those found in the central nervous system or cardiac tract can arise from the neurocrest (ectoderm). Pericytes can be seen as progenitors and as descendant cells already resident in the tissue. They can develop from other pericytes, from fibroblasts/myofibroblasts, from MSC (35) and from HSC (42).

The ability of pericytes to form TEC or other epithelial cells has not been established yet. Myofibroblast formation is on the other hand a much seen differentiation pathway. Humphreys et al performed lineage analysis studies in Cre/Lox knock-in mice and found that during nephrogenesis FoxD1+ (renal stromal cell marker) mesenchymal cells gave rise to adult CD73+ (MSC marker), platelet derived growth factor receptor β+, αSMA- interstitial pericytes. In the event of kidney fibrosis these pericytes expand, differentiate and constitute for the majority of αSMA+ myofibroblasts (68). These and other (69) results support the fact that pericytes can differentiate into myofibroblasts.

Fibroblasts

Fibroblasts are a heterogeneous population of mesenchymal cells found in stromal tissues throughout the body (70) and perform a function in wound healing and inflammation (71). They synthesize many components of the extracellular matrix, such as fibronectin and collagen I, III and V (17,72), and produce matrix metalloproteinases (MMP) which break down the ECM, underlining their role in ECM turnover and homeostasis (73,74). There seem to be various sources of fibroblasts. Studies on the developing kidney suggest the mesenchyme as a source for interstitial kidney fibroblasts (75). As discussed earlier in this thesis Alexander Friedenstein found fibroblastic cells after seeding bone marrow cells, giving a first clue to their origin (26). An in vitro study performed by Lee et al in 2010 showed the capability of MSC to form fibroblasts when stimulated with connective tissue growth factor (36). A HSC origin was also shown by single HSC lineage studies performed by Ogawa et al (76) and by in vitro differentiation studies (46). However due to the heterogeneous population of fibroblasts and their wide distribution within the body it remains difficult to elucidate all their possible origins.

The fibroblast is recognized as the classical progenitor cell for myofibroblast formation within tissues. The myofibroblast is viewed as an “activated” fibroblast. During tissue injury the resident interstitial fibroblasts are mechanically challenged by the destruction of their microenvironment. As a response they acquire contractile stress fibers and attain a transitional phenotype called a protomyofibroblast (17). Further stimulation with transforming growth factor- β (TGF-β) and the presence of specialized ECM products is needed to start the production of αSMA and to become a differentiated myofibroblast (34).

All this information together points to theory C as being the most likely theory (see figure 1C). There seem to be multiple BMDC, both resident and peripheral, with the potential to differentiate to TEC, myofibroblast or both. The MSC and HSC populations can directly give rise to both TEC and myofibroblasts but also produce progenitor cells that reside outside the bone marrow, creating a complicated image (see figure 2). These progenitor cells especially possess the ability to form myofibroblasts. A few remarks are in order however. Differentiation of MSC to myofibroblast, PBMC to epithelial cell and HSC to fibroblast have only been established in vitro and are no guarantee that the same can happen in vivo. Also it is not clear if the HSC population can directly produce myofibroblasts and TEC or if this process involves an intermediate cell type. More lineage tracing and cell culture studies are needed to asses with certainty that HSC themselves can directly differentiate into TEC or myofibroblasts.

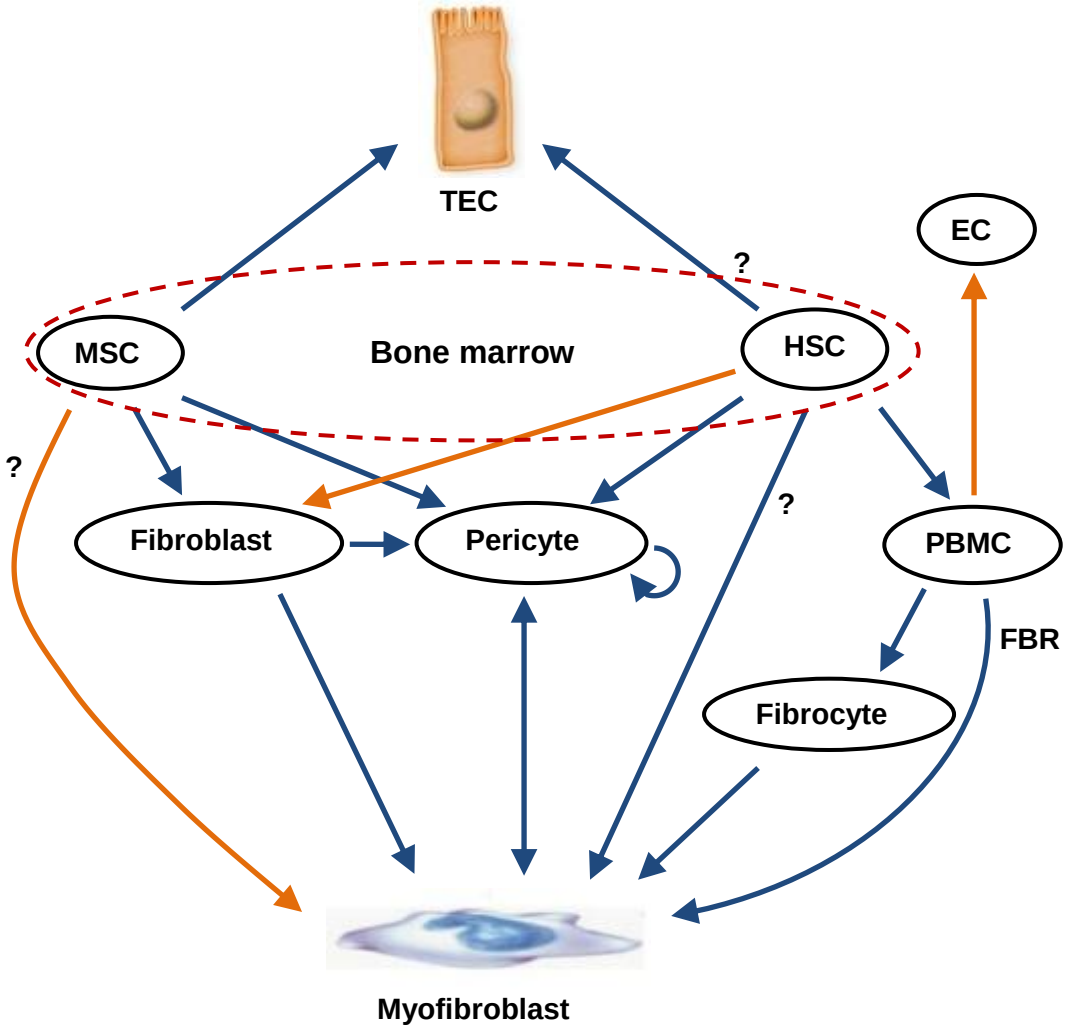

Figure 2. The differentiation potential of migrating and resident BMDC populations. HSC and MSC reside within the bone marrow and produce TEC, myofibroblasts, fibroblasts and pericytes. HSC also produce the heterogeneous population of PBMC which can create fibrocytes and EC. Fibroblasts, pericytes, fibrocytes and PBMC can in vivo differentiate into myofibroblasts under the right conditions. Blue arrow = in vivo differentiation, orange arrow = in vitro differentiation, ? = not certain. Mesenchymal stem cell (MSC), Haematopoietic stem cell (HSC), Peripheral blood mononuclear cells (PBMC), Tubular epithelial cell (TEC), Epithelial cell (EC), Foreign body response (FBR).

Which signals trigger BMDC differentiation?

In this chapter I will focus on the MSC, HSC and fibrocytes. These three cell populations are completely derived from the bone marrow and migrate to the kidney in response to damage (21,55,61,77). The kidney resident pericytes and fibroblasts are only partly produced by the bone marrow, but are primarily derived from other sources or created during embryogenesis. The very heterogeneous PBMC population showed no differentiation potential to myofibroblasts or TEC in the damaged kidney. Therefore these last three BMDC populations are not further discussed.

After establishing which BMDC can differentiate, the next step is to determine what signals trigger the differentiation. Before the BMDC can differentiate they first have to migrate towards the kidney. Chemotactic molecules, like for example SDF-1, are produced in the damaged kidney and create a chemotactic gradient to which HSC and MSC respond by entering the blood circulation and migrating to the kidney (21,77). Migrating BMDC arrive at the kidney vessels and encounter activated vascular endothelial cells which express adhesion molecules. The BMDC recognise these adhesion molecules and adhere to the endothelial monolayer. After a series of molecular events the BMDC transmigrate the endothelial and enter the interstitium of the damaged kidney (21,78). Here they encounter the signalling molecules that can determine their differentiation fate. Which molecules trigger the differentiation of migrating BMDC will be discussed below.

Differentiation to TEC

Haematopoietic stem cell (HSC)

Only the HSC and MSC populations can differentiate to TEC (see figure 2). There has not been a lot of research on which signals trigger this differentiation. For the HSC only in vivo results are available as a proof of principle, but in vitro evidence and knowledge about the mechanisms is lacking. Chemokines, cytokines, growth factors, adhesion molecules and ECM products can all play a role in directing differentiation. During liver damage or fibrosis HSC repair the hepatocyte epithelium (79,80). In a recent in vitro study the differentiation from human umbilical cord-derived HSC to hepatocyte was found to be driven by hepatocyte growth factor (HGF) and fibroblast growth factor 4 (FGF 4) (81). A similar mechanism could drive HSC differentiation in the kidney.

Mesenchymal stem cell (MSC)

More is known on MSC differentiation to TEC, thanks to several in vitro studies. Păunescu et al cultured human MSC with epidermal growth factor (EGF), keratinocyte growth factor (KGF), HGF and insulin-like growth factor-2 (IGF-2) simultaneously. The MSC started to express epithelial markers and attained an epithelial morphology (82). A TEC phenotype was not reached though, but maybe by stimulating with additional signalling molecules this differentiation could be accomplished. When co-culturing MSC with injured murine TEC, separated by a physical barrier, the MSC differentiated into a tubular epithelial-like phenotype. Apparently the injured TEC express renotypic factors that direct MSC to differentiate into TEC (33). Another study supported this hypothesis by showing that adipose tissue-derived MSC, which have very similar characteristics to bone marrow-derived MSC (83-86), differentiated to an epithelial cell type when treated with conditioned medium from TEC (87). The TEC seem to express soluble molecules that influence the differentiation of MSC. A possible mode of action of these molecules is down-regulation of the Wnt/β-catenin pathway. Wang et al showed that during co-culture of airway epithelial cells and MSC, blocking of Wnt/β-catenin signalling promoted MSC to differentiate towards lung epithelial cells (88).

Intercellular crosstalk between cells of mesenchymal and epithelial origin via nephrogenic factors is described as a fundamental process in nephrogenesis and maintenance of organ integrity in the adult

(89-91), therefore investigating the effect of these nephrogenic factors on MSC differentiation is a logical step. Nephrogenic factors such as basic fibroblast growth factor (bFGF), retinoic acid (RA), leukaemia inhibitory factor (LIF) and bone morphogenic protein-7 (BMP-7), which are involved in nephrogenesis and the embryonic development of TEC (92-95), however did not elicit TEC differentiation in MSC in vitro (33).

The right combination between growth factors and the renotypic factors expressed by surviving TEC might direct arriving MSC and HSC to engraft as new TEC in the damaged kidney. Most of the studies mentioned above are however not very specific, but only conclude that molecules expressed by TEC direct the differentiation. Only the study performed by Păunescu examines which growth factors have a specific effect on MSC differentiation. The growth factor HGF might have an effect on both MSC and HSC. Later we will discuss which growth factors and other molecules are up-regulated in the damaged kidney. More studies on which signalling molecules direct the differentiation of BMDC towards TEC are needed, because with the current understanding there is not enough information to draw a definitive conclusion.

Differentiation to myofibroblast

Haematopoietic stem cell (HSC)
HSC, MSC and fibrocytes can form myofibroblasts after migrating to the damaged kidney. The mechanisms and signalling molecules involved in myofibroblast differentiation have been studied more extensively than for TEC differentiation. The exception is the HSC population. Just like described earlier for the TEC, there have been no studies on which factors direct HSC differentiation towards myofibroblasts.

Mesenchymal stem cell (MSC)
On the other hand MSC differentiation has been studied more thoroughly. Both differentiation through an intermediate cell type and direct differentiation have been examined in vitro. Connective tissue growth factor (CTGF) induces MSC to differentiate to fibroblasts. After stimulation with TGF-β these fibroblasts differentiate towards myofibroblasts (36). TGF-β is one of the molecules that activate resident fibroblasts to become myofibroblasts as well, by stimulating the expression of αSMA (96). Likewise, TGF-β is capable of inducing αSMA expression in MSC, hinting at a more direct differentiation mechanism of MSC towards a myofibroblast-like cell (97,98).

Nedeau et al studied direct MSC differentiation in a wound model. They found that PDGF-B-activated fibroblasts induced myofibroblast differentiation in MSC and that this effect was most likely mediated by the secretion of bFGF and CXCL5 (37). Direct stimulation of MSC with PDGF-B decreased the expression of αSMA, hereby inhibiting differentiation (97). PDGF-B can stimulate both PDGF-receptor-α (PDGFR-α) and PDGFR-β (99). PDGFR-α stimulation promotes the expression and polymerization of αSMA. In contrast, PDGFR-β stimulation promotes the depolymerisation of αSMA filaments (100). PDGF-B seems more prone to stimulate to PDGFR-β on MSC, even when the PDGFR-α is more expressed on the surface of MSC (100). The reason for this is unknown, but it might be that different culture conditions influence the expression of receptors on the MSC surface.

Another molecule capable of influencing the MSC is bradykinin (BK). BK is a vasoactive peptide produced during tissue injury. It induces αSMA expression in adipose tissue-derived MSC by an extracellular-signal-regulated kinase (ERK)-dependent activation of the autocrine TGF-β1-Smad2 pathway (101). The same results were found in a comparative study on the effect of lysophosphatidic acid (LPA) on adipose tissue-derived MSC (102).

Fibrocyte

Fibrocytes are derived from a CD14+ subset of monocytes and migrate to the injured kidney in response to damage (55,61). When they arrive they can differentiate into fibroblasts and myofibroblasts. In an in vitro study performed by Schmidt et al it was found that TGF-β1 or endothelin-1 (ET-1) could both promote fibrocyte differentiation towards a myofibroblast (59). TGF-β1 stimulates αSMA expression in fibrocytes through the TGF-β1-Smad2/3 pathway (62), which is similar to the pathway described above in adipose tissue-derived MSC.

Pro-fibrotic cytokines also play a role in fibrocyte differentiation. The T helper (Th) type 2 cells secrete the cytokines IL-4 and IL-13. These cytokines promote fibrocyte differentiation to myofibroblast. The anti-fibrotic cytokines interferon-γ (IFN-γ) and IL-12 produced by Th-1 cells inhibit this differentiation (103). This demonstrates how the composition of the local microenvironment decides if fibrocytes differentiate to myofibroblasts. It also shows the important role of the inflammatory response, because the reaction if the immune system to kidney damage determines the microenvironment.

HSC differentiation factors are not found yet, but this cell population might respond to the same molecules that trigger myofibroblast differentiation in MSC and fibrocytes. More knowledge exists on which signalling molecules direct the differentiation choice of MSC and fibrocytes towards myofibroblast. The growth factor TGF-β and its intracellular Smad2 pathway seem to be the major determinants of myofibroblast formation due to their effect on αSMA expression. Which pathways the other signalling molecules activate is not known. It might be possible that they stimulate non-canonical TGF-β pathways or activate downstream second messenger proteins through another receptor, which lead to αSMA expression. The presence αSMA in a cell points to a myofibroblast, but other factors are needed for a positive identification such as ECM production and morphology, because αSMA alone is not discriminatory enough. However, it is known that TGF-β also stimulates the expression of collagen type 1 (104,105). High levels of TGF-β inhibit this production, suggesting the existence of a negative feedback loop (106).

Multiple cells from the bone marrow arrive at the damaged kidney and the local microenvironment determines the differentiation choice of these BMDC. These results show that theory C, proposed in the beginning of this thesis, seems even more likely (see figure 1C). In figure 3 the molecules that guide the choice of arriving BMDC have been summarized. In the next chapter I will discuss which signalling molecules are up-regulated in the damaged kidney. With this information I can propose a composition for the local microenvironment and give a prediction which differentiation choice the arriving BMDC will make and why they make it.

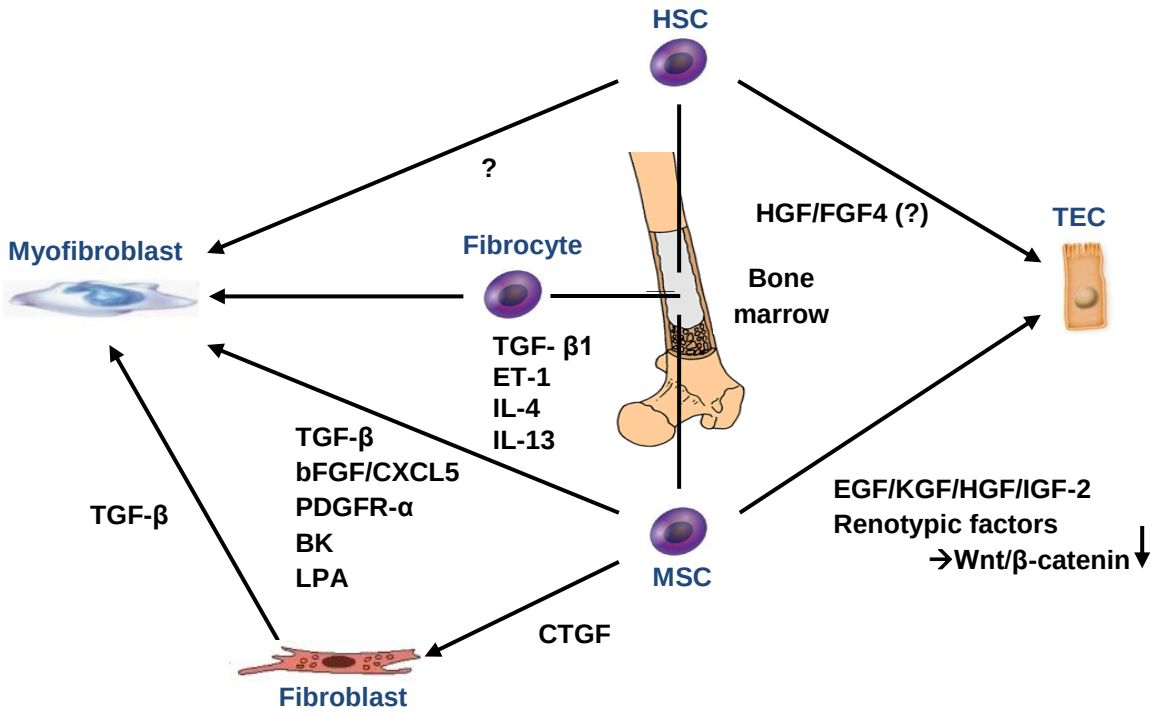

Figure 3. Signalling molecules that direct the differentiation of migrating BMDC. MSC, HSC and fibrocytes migrate to the kidney in response to damage. Multiple growth factors and other signalling molecules can influence the differentiation of these three BMDC populations to TEC or myofibroblast. Hepatocyte growth factor (HGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), insulin-like growth factor (IGF), connective tissue growth factor (CTGF), transforming growth factor β (TGF-β), Endothelin (ET), interleukin (IL), basic (b), epithelial neutrophil activating peptide-78 (CXCL5), platelet derived growth factor receptor-α (PDGFR-α), bradykinin (BK), lysophosphatidic acid (LPA), ? = uncertain.

Available signalling molecules in the damaged kidney

In this chapter I will look at the microenvironment of acute kidney injury (AKI), because at the acute phase of damage arriving BMDC will make their differentiation choice. When the disease shifts to chronic kidney injury the choice has been long since made for myofibroblast differentiation and if the kidney recovers the epithelial direction has been chosen. There are several models to investigate AKI and the ischemia/reperfusion (I/R) model is one of the most extensive studied. I will first discuss the time course of the inflammation response and the time point of BMDC arrival. After this I will give an overview on which signalling molecules are being transcribed or excreted in the ischemic kidney at the time the BMDC arrive and how these molecules can affect the BMDC differentiation choice.

Time course of inflammation and BMDC arrival

I/R causes ATP depletion and the production of reactive oxygen species (ROS) by cells within the kidney. These processes will lead to apoptosis, necrosis and cell injury through several pathways (107). As a response, vascular endothelial cells up-regulate adhesion molecules (108,109) and surviving ischemic TEC start to produce pro-inflammatory cytokines (TNF-α, IL-6, IL-1, TGF-β) and chemokines (monocyte chemoattractant protein-1 (MCP-1), IL-8). This results in the accumulation of leukocytes in the kidney at the early phase of I/R (107,110). Neutrophils are the first leukocytes to arrive at the damaged kidney. They start to arrive 3 hours after I/R injury, are most abundant on day 1 and decrease again after 7 days. Macrophages and T cells arrive after 7 days, when TEC apoptosis is at its highest (111). These arriving leukocytes further contribute to the inflammatory response by expressing cytokines. It has become clear that over time the post-ischemic kidney does not fully restore from damage and animal models display a reduction in renal microvasculature, interstitial fibrosis, tubular hypercellularity and atrophy and persistent inflammation (107,112-117). This makes the time point of BMDC arrival very important, because the microenvironment differs between the early inflammatory phase and the late persistent inflammation, wound healing reaction and sub-optimal recovery of damage.

The migratory BMDC populations (HSC, MSC, fibrocyte) respond to some of the secreted chemokines and journey to the damaged kidney. BMDC enter the circulation after 3 days and the peak of arriving BMDC in the kidney interstitium is found on day 7 and decreases after day 28 (12). Broekema et al found that BMDC engraftment in the renal tubule started at day 7 and peaked on day 14 (1). These results seem to be supported by Ohnishi et al, which showed an increase in GFP+ BMDC tubular engraftment at day 8, although no significant comparison was made with a control group (118). On the other hand interstitial myofibroblast populations peaked on day 7 after I/R injury (12).

These studies show that myofibroblasts and TEC are both produced on day 7, but BMDC are most probably not solely responsible for their formation (12,119,120). BMDC arrive around the same day and will differentiate according to the local microenvironment. This microenvironment is produced by infiltrating macrophages and T cells among others. TEC apoptosis also peaks on day 7, simultaneously with TEC proliferation, both adding their molecules to the microenvironment (111). The differentiation choice of BMDC will eventually be determined by the signalling molecules present in the local microenvironment on day 7.

Available factors for BMDC differentiation

It is very hard to determine which proteins are secreted at the time BMDC arrive at the damaged kidney. There are multiple studies on gene expression in mice, but they do not target the genes for all the

proteins known to be involved in BMDC differentiation (see figure 3) or do not state the time point of gene expression. Next to that they determine the relative transcription levels in the whole kidney, which cannot always be extrapolated to a microenvironment. Nevertheless I will discuss some of the results obtained from these microarray studies and combine them with protein information from multiple articles to see if it is possible to determine which differentiation choice the BMDC will make.

Early after I/R the genes of the growth factors HGF and TGF-β1 are up-regulated (121). The gene Zf9 is up-regulated 3 hours after I/R and is thought to be responsible for the early higher activity of the TGF-β1 gene. Both Zf9 and TGF-β protein levels are higher and seem to follow a parallel pattern (122). How the Zf9 gene is activated is unknown though. Another inducer of TGF-β activity is thrombospondin-1 (TSP-1). TSP-1 is expressed 3 hours after I/R and returns to baseline after 48 hours (123). TSP-1 activates latent TGF-β by inducing conformational changes in the latent TGF-β complex (124,125). I can conclude from this information that TGF- β is excreted and activated almost directly after I/R. Information on the transcription of other BMDC differentiation molecules is not available and declaring the early microenvironment as pro-fibrotic is a bit premature. It is therefore farfetched to state that resident fibroblast will be activated to become myofibroblasts without extensive knowledge on other signalling molecules present in the early ischemic kidney.

After 10 days the initial ischemic damage and inflammatory reaction are starting to diminish and the wound healing reaction has started. Ko et al (126) did a transcriptional analysis during the repair phase of AKI. They found a higher transcription of MMP-14, which points to the degradation of ECM. Also they found a higher transcription of ET-1, the gene of the protein needed for fibrocyte differentiation to a myofibroblast (see figure 3). The increased transcription does not necessarily mean a higher protein excretion. An increase in pro-collagen type 1α1 transcription however seems to point to a more active ECM production, which could mean the presence of myofibroblasts differentiated from fibrocytes.

There is literature on which proteins are expressed after ischemic kidney injury, but there is a lack of information about their time course. HGF secretion is higher after damage (107). HGF stimulates the breakdown of ECM by stimulating MMP's and reducing MMP-inhibitor proteins (127). It also blocks the TGF-β/SMAD pathway, hereby possibly reducing the pro-fibrotic effects of TGF-β (128). EGF is produced by TEC in response to ischemia (91,127). Both HGF and EGF have positive effects on epithelial cell survival and proliferation, but if they can direct the differentiation of BMDC to TEC is uncertain.

There is also an increased expression of pro-fibrotic proteins. BK is produced in reaction to injury and stimulates the expression of TGF-β (101). The presence of TGF-β and ET-1 together with a hypoxic state stimulates TEC to produce CTGF (129). CTGF can induce fibroblast differentiation in MSC whereupon TGF-β stimulation produces a myofibroblast (see figure 3). CTGF also stimulates TEC in vitro to produce TGF-β, pointing to a possible positive feedback loop in vivo (130).

7 days after I/R the BMDC arrive at the damaged kidney, myofibroblast formation reaches its peak and TEC engraftment is starting to rise. Macrophages and T cells infiltrate the kidney and the neutrophil population starts to diminish. All of these processes create a complicated and variable microenvironment for BMDC differentiation in which the pro-fibrotic factors seem to predominate (see figure 4 for a hypothetical pro-fibrotic microenvironment). The production of TGF-β, ET-1 and CTGF seems to outweigh the contribution of HGF and EGF. If the arriving BMDC differentiate it will presumably be towards a myofibroblast.

There are however a lot of unknown factors which do not make the above a strong conclusion. One of the unknown factors is the composition of the cellular inflammatory response. Macrophages exist out of several subpopulations all with their own expression pattern. M1 macrophages are pro-inflammatory and their accumulation leads to fibrosis. On the other hand M2 macrophages secrete trophic factors and promote ECM remodelling and cellular regeneration (131). This does not mean that they will not promote myofibroblast formation, because myofibroblasts are also needed for ECM remodelling. Both M1 and M2 macrophages can be present simultaneously and their balance determines the outcome of

the ischemic kidney. The same applies for the T-cell population. They can be divided into Th1 and Th2 cells, again both with their own excretion patterns. Th2 cells produce IL-4 and IL-13, causing fibrocytes to differentiate to myofibroblasts (see figure 3). Th1 and Th2 each produce cytokines that suppress the other, so the balance will in all probability shift to one subset (132). Literature on which Th-cell predominates in the ischemic kidney injury is not available. This means that it is not known if the inflammatory cells in the interstitium are pro-fibrotic (M1 and Th2) or anti-fibrotic (M2 and Th1) at the time the BMDC arrive.

The route of BMDC migration also plays a role in differentiation. Migratory BMDC leave the blood vessels and enter the interstitium. Here they encounter activated fibroblasts, macrophages and T cells which mostly produce factors that direct BMDC differentiation towards the myofibroblasts. To encounter TEC directing molecules the BMDC have to migrate through the ECM towards the damaged tubuli were they come across EGF produced by the surviving TEC. Another option is that HGF produced by MSC themselves (133) directs the differentiation, although HGF alone is probably not enough to cause BMDC to differentiate towards TEC (see figure 3). How it is possible that BMDC are able to differentiate towards TEC when they have to cross a pro-fibrotic interstitium is unknown. The distance from a vessel towards a tubuli is short, which could explain the appearance of BMDC derived TEC. Also when M2 and Th1 cells predominate the inflammatory reaction the microenvironment found in the interstitium might not be so pro-fibrotic. Next to that there might be molecules in the interstitium that inhibit the BMDC to differentiate towards myofibroblast. As stated before, there are a lot of unknown factors. However in vivo studies suggest that the majority of BMDC differentiate towards myofibroblasts (2,5,12,38), which is most likely because of the pro-fibrotic microenvironment found in the damaged kidney.

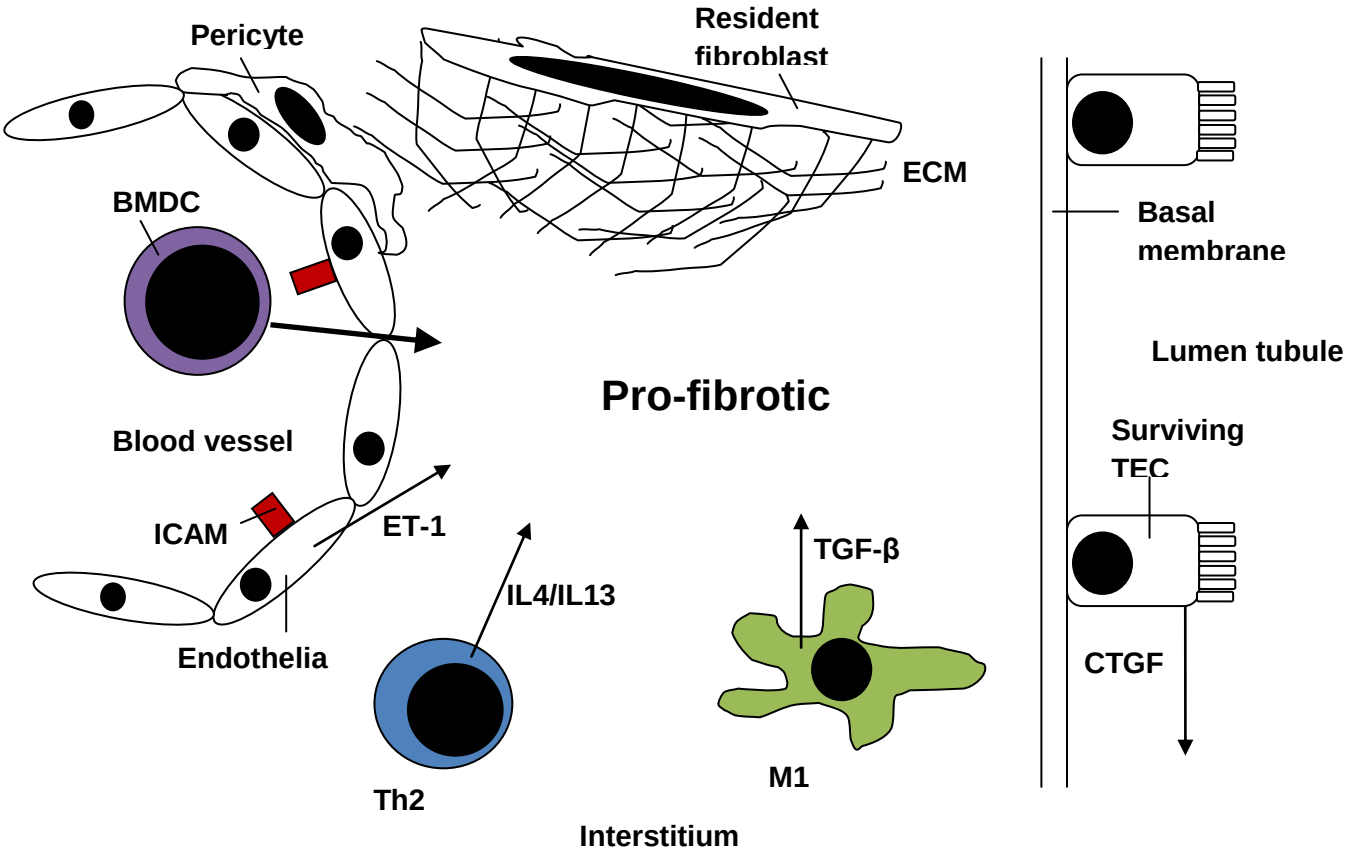

Figure 4. Hypothetical pro-fibrotic microenvironment for BMDC differentiation in the ischemic kidney. 7 days after I/R the BMDC arrive at the damaged kidney. It is conceivable that a pro-fibrotic microenvironment awaits them. Activated epithelial cells produce ET-1 and ICAM. BMDC transmigrate the endothelial monolayer to enter the interstitium were they encounter TGF-β produced by M1 cells and IL4/IL13 produced by Th2 cells. Also the surviving TEC cells produce pro-fibrotic factors like CTGF and TGF-β. All these molecules direct BMDC to differentiate towards myofibroblasts. Information from microarray and protein studies point to this microenvironment, although they are missing exact time indication. Also the presence of Th2 and M1 cells is uncertain, because the balance could shift to Th1 and M2 cells that produce anti-fibrotic molecules. Bone marrow-derived cells (BMDC), intracellular adhesion molecule (ICAM), endothelin-1 (ET-1), interleukin (IL), transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), tubular epithelial cells (TEC), extracellular matrix (ECM), macrophage subset (M), T-helper cell subset (Th).

Conclusion and perspectives

Different cell populations with a bone marrow origin can differentiate towards TEC or myofibroblast. The HSC and MSC are populations resident in the bone marrow and migrated towards the periphery in reaction to injury. Both cell types have the ability to form TEC and myofibroblasts when stimulated with the right molecules. Fibrocytes are derived from the bone marrow through a CD14+ monocyte intermediate and constitute about 0,1-1% of the nucleated cell population in the peripheral blood. PBMC are the lymphoid and myeloid cells created during haematopoiesis and are also present in the circulation. They respond to inflammatory queues and can form fibrocytes, epithelial cells and during a FBR they can differentiate towards myofibroblasts. Two kidney-resident cell populations are partly derived from the bone marrow. Both fibroblasts and pericytes form during embryogenesis, but during normal tissue turnover they can be derived from bone marrow precursor cells, giving them an indirect bone marrow origin. During tissue damage they are very capable to differentiate towards myofibroblasts after the right stimulation. All these different cell populations create a complex image. In the introduction I stated three theories on BMDC differentiation towards TEC and myofibroblasts. It seems that theory C proves to be most fitting, for multiple BMDC populations can form tubular epithelial cells and myofibroblasts. A combination of the right recruitment and microenvironment will determine the differentiation choice towards either TEC or myofibroblast.

The focus narrows to which cells can migrate and differentiate in the kidney as a response to ischemic injury. HSC, MSC and fibrocytes can do both. They respond to certain chemokines by entering the circulation and transmigrate the vascular endothelial at the site of kidney damage. Here they differentiate towards TEC or myofibroblasts. Information on which signalling molecules trigger the differentiation of these migrating cells is mainly provided by in vitro research, but if these results can be extrapolated towards an in vivo microenvironment remains to be seen. The next step is to see which of these signalling molecules are present in the ischemic kidney at the time of BMDC arrival. 7 days after ischemic kidney injury the BMDC arrive at the damaged kidney. This is simultaneously with macrophages and T cells and with the peak of myofibroblast formation. Results from microarray and protein studies point to a pro-fibrotic microenvironment at the time of BMDC arrival. TGF-β, CTGF and ET-1 production seem to overrule HGF and EGF production. If these are the only factors present in the ischemic kidney the BMDC differentiation choice will predominantly go towards the myofibroblast.

However, the amount of unknown contributors that can influence the differentiation choice is huge. The arriving macrophages and T cells consist of several subpopulations that can direct BMDC to either TEC or myofibroblast differentiation. It is unknown what subsets are more prominent in the ischemic kidney. Both genetic and epigenetic make-up might be able to influence this, together with the microenvironment in which the macrophages and T cells arrive. Also there might be other signalling molecules influencing BMDC differentiation then the ones found with in vitro research. The microarray studies performed do now study all the genes and the amount of protein at the moment of BMDC arrival in the ischemic kidney. This shows how much information is missing, so drawing a solid conclusion on BMDC differentiation choice is not possible. Research has stated however that BMDC more often differentiate towards myofibroblasts then they do towards TEC.

The clinical relevance of BMDC differentiation to TEC or myofibroblasts appears small. Studies found that between 1-10% of the restored TEC are from bone marrow origin (2,5,38). From the information gathered in this thesis I can conclude that MSC and HSC are the most likely source. However, the main part of renal tubule regeneration is performed by surviving TEC. After damage they dedifferentiate to a more mesenchymal phenotype, proliferate and differentiate back to TEC, hereby re-colonizing the damaged tubule (134). The formation of myofibroblasts from BMDC is more prominent. Around 32% of the interstitial myofibroblast population in the rat ischemic kidney is of bone marrow origin (12). Which BMDC contributes primarily to the myofibroblast formation is not known, but fibrocytes seem a likely candidate. In a wound healing study performed by Mori et al it was found that 40% of the myofibroblast population was derived from fibrocytes (60). Recent lineage tracing studies have revealed that resident pericytes contribute to most, if not all, myofibroblasts formed after kidney damage (119,120). The resident pericytes are however not all of bone marrow origin. They are predominantly formed during embryogenesis, but can also be derived by self-renewal and from MSC, HSC and fibroblasts. An explanation for the differences between these studies is not provided.

There is even a controversy about the renal tubular engraftment of BMDC. Some researchers state that this does not happen at all and that BMDC only perform a paracriene protective function in the damaged kidney by excreting anti-inflammatory and anti-fibrotic proteins (135-137). The techniques used by the studies that found BMDC engraftment are said to produce false positive results, although I believe this is not always the case. The variety in results might be explained by varying methods of inducing kidney damage and different animal models.

In this thesis I described that HSC, MSC, fibrocytes, PBMC, fibroblasts and pericytes are completely or partly derived from the bone marrow and that they are capable of differentiating towards a TEC or myofibroblast. In vivo, migrating BMDC seem to contribute to the regeneration or fibrosis of the ischemic kidney, but the local microenvironment determines the differentiation choice. With the current knowledge it appears that the microenvironment is mostly pro-fibrotic when BMDC arrive. Further studies on protein presence and interactions are needed to better predict the differentiation choice of arriving BMDC.

References

(1) Broekema M, Harmsen MC, Koerts JA, Petersen AH, van Luyn MJ, Navis G, et al. Determinants of tubular bone marrow-derived cell engraftment after renal ischemia/reperfusion in rats. Kidney Int 2005 Dec;68(6):2572-2581.

(2) Fang TC, Alison MR, Cook HT, Jeffery R, Wright NA, Poulsom R. Proliferation of bone marrow-derived cells contributes to regeneration after folic acid-induced acute tubular injury. J Am Soc Nephrol 2005 Jun;16(6):1723-1732.

(3) Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. J Clin Invest 2003 Jul;112(1):42-49.

(4) Nishida M, Kawakatsu H, Shiraishi I, Fujimoto S, Gotoh T, Urata Y, et al. Renal tubular regeneration by bone marrow-derived cells in a girl after bone marrow transplantation. Am J Kidney Dis 2003 Nov;42(5):E10-2.

(5) Poulsom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnarasah S, et al. Bone marrow contributes to renal parenchymal turnover and regeneration. J Pathol 2001 Sep;195(2):229-235.

(6) Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001 May 4;105(3):369-377.

(7) Krause DS. Engraftment of bone marrow-derived epithelial cells. Ann N Y Acad Sci 2005 Jun;1044:117-124.

(8) Suratt BT, Cool CD, Serls AE, Chen L, Varella-Garcia M, Shpall EJ, et al. Human pulmonary chimerism after hematopoietic stem cell transplantation. Am J Respir Crit Care Med 2003 Aug 1;168(3):318-322.

(9) Wang X, Ge S, McNamara G, Hao QL, Crooks GM, Nolta JA. Albumin-expressing hepatocyte-like cells develop in the livers of immune-deficient mice that received transplants of highly purified human hematopoietic stem cells. Blood 2003 May 15;101(10):4201-4208.

(10) Verstappen J, Katsaros C, Torensma R, Von den Hoff JW. Bone marrow-derived cells in palatal wound healing. Oral Dis 2010 Nov;16(8):788-794.

(11) Okamoto R, Yajima T, Yamazaki M, Kanai T, Mukai M, Okamoto S, et al. Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract. Nat Med 2002 Sep;8(9):1011-1017.

(12) Broekema M, Harmsen MC, van Luyn MJ, Koerts JA, Petersen AH, van Kooten TG, et al. Bone marrow-derived myofibroblasts contribute to the renal interstitial myofibroblast population and produce procollagen I after ischemia/reperfusion in rats. J Am Soc Nephrol 2007 Jan;18(1):165-175.

(13) Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008 Jan;214(2):199-210.

(14) Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright NA, et al. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. Gastroenterology 2004 Apr;126(4):955-963.

(15) Direkze NC, Forbes SJ, Brittan M, Hunt T, Jeffery R, Preston SL, et al. Multiple organ engraftment by bone-marrow-derived myofibroblasts and fibroblasts in bone-marrow-transplanted mice. Stem Cells 2003;21(5):514-520.

(16) Petersen OH. Lecture notes. Human physiology. 5th ed. Malden, Mass.: Blackwell Pub.; 2007.

(17) Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002 May;3(5):349-363.

(18) Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006 May;6(5):392-401.

(19) Zeisberg M, Strutz F, Muller GA. Role of fibroblast activation in inducing interstitial fibrosis. J Nephrol 2000 Nov-Dec;13 Suppl 3:S111-20.

(20) Qi W, Chen X, Poronnik P, Pollock CA. The renal cortical fibroblast in renal tubulointerstitial fibrosis. Int J Biochem Cell Biol 2006 Jan;38(1):1-5.

(21) Chavakis E, Dimmeler S. Homing of Progenitor Cells to Ischemic Tissues. Antioxid Redox Signal 2011 Feb 3.

(22) Qian H, Yang H, Xu W, Yan Y, Chen Q, Zhu W, et al. Bone marrow mesenchymal stem cells ameliorate rat acute renal failure by differentiation into renal tubular epithelial-like cells. Int J Mol Med 2008 Sep;22(3):325-332.

(23) Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 1977 Jun;91(3):335-344.

(24) Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 1978;4(1-2):7-25.

(25) Moore KA, Lemischka IR. Stem cells and their niches. Science 2006 Mar 31;311(5769):1880-1885.

(26) Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 1970 Oct;3(4):393-403.

(27) Charbord P. Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther 2010 Sep;21(9):1045-1056.

(28) Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997 Apr 4;276(5309):71-74.

(29) Dressler GR. The cellular basis of kidney development. Annu Rev Cell Dev Biol 2006;22:509-529.

(30) Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009 Nov 25;139(5):871-890.

(31) Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. J Am Soc Nephrol 2004 Jul;15(7):1794-1804.

(32) Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med 2004 Dec;14(6):1035-1041.

(33) Singaravelu K, Padanilam BJ. In vitro differentiation of MSC into cells with a renal tubular epithelial-like phenotype. Ren Fail 2009;31(6):492-502.

(34) Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol 2007 Jun;170(6):1807-1816.

(35) Diaz-Flores L, Gutierrez R, Madrid JF, Varela H, Valladares F, Acosta E, et al. Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol 2009 Jul;24(7):909-969.

(36) Lee CH, Shah B, Moioli EK, Mao JJ. CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. J Clin Invest 2010 Sep 1;120(9):3340-3349.

(37) Nedeau AE, Bauer RJ, Gallagher K, Chen H, Liu ZJ, Velazquez OC. A CXCL5- and bFGF-dependent effect of PDGF-B-activated fibroblasts in promoting trafficking and differentiation of bone marrow-derived mesenchymal stem cells. Exp Cell Res 2008 Jul 1;314(11-12):2176-2186.

(38) Lin F, Cordes K, Li L, Hood L, Couser WG, Shankland SJ, et al. Hematopoietic stem cells contribute to the regeneration of renal tubules after renal ischemia-reperfusion injury in mice. J Am Soc Nephrol 2003 May;14(5):1188-1199.

(39) Fang TC, Otto WR, Rao J, Jeffery R, Hunt T, Alison MR, et al. Haematopoietic lineage-committed bone marrow cells, but not cloned cultured mesenchymal stem cells, contribute to regeneration of renal tubular epithelium after HgCl 2 -induced acute tubular injury. Cell Prolif 2008 Aug;41(4):575-591.

(40) Ogawa M, Larue AC, Watson PM, Watson DK. Hematopoietic stem cell origin of connective tissues. Exp Hematol 2010 Jul;38(7):540-547.

(41) Masuya M, Drake CJ, Fleming PA, Reilly CM, Zeng H, Hill WD, et al. Hematopoietic origin of glomerular mesangial cells. Blood 2003 Mar 15;101(6):2215-2218.

(42) Hess DC, Abe T, Hill WD, Studdard AM, Carothers J, Masuya M, et al. Hematopoietic origin of microglial and perivascular cells in brain. Exp Neurol 2004 Apr;186(2):134-144.

(43) Miyata E, Masuya M, Yoshida S, Nakamura S, Kato K, Sugimoto Y, et al. Hematopoietic origin of hepatic stellate cells in the adult liver. Blood 2008 Feb 15;111(4):2427-2435.

(44) Visconti RP, Ebihara Y, LaRue AC, Fleming PA, McQuinn TC, Masuya M, et al. An in vivo analysis of hematopoietic stem cell potential: hematopoietic origin of cardiac valve interstitial cells. Circ Res 2006 Mar 17;98(5):690-696.

(45) Fujita J, Mori M, Kawada H, Ieda Y, Tsuma M, Matsuzaki Y, et al. Administration of granulocyte colony-stimulating factor after myocardial infarction enhances the recruitment of hematopoietic stem cell-derived myofibroblasts and contributes to cardiac repair. Stem Cells 2007 Nov;25(11):2750-2759.

(46) Ebihara Y, Masuya M, Larue AC, Fleming PA, Visconti RP, Minamiguchi H, et al. Hematopoietic origins of fibroblasts: II. In vitro studies of fibroblasts, CFU-F, and fibrocytes. Exp Hematol 2006 Feb;34(2):219-229.

(47) Shirai K, Sera Y, Bulkeley W, Mehrotra M, Moussa O, LaRue AC, et al. Hematopoietic stem cell origin of human fibroblasts: cell culture studies of female recipients of gender-mismatched stem cell transplantation and patients with chronic myelogenous leukemia. Exp Hematol 2009 Dec;37(12):1464-1471.

(48) Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1994 Nov;1(1):71-81.

(49) Metz CN. Fibrocytes: a unique cell population implicated in wound healing. Cell Mol Life Sci 2003 Jul;60(7):1342-1350.

(50) Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest 2004 Aug;114(3):438-446.

(51) Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol 2004 Apr;36(4):598-606.

(52) Chesney J, Metz C, Stavitsky AB, Bacher M, Bucala R. Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. J Immunol 1998 Jan 1;160(1):419-425.

(53) Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, et al. Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. FASEB J 2001 Oct;15(12):2215-2224.

(54) Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. Proc Natl Acad Sci U S A 1997 Jun 10;94(12):6307-6312.

(55) Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J Immunol 2001 Jun 15;166(12):7556-7562.

(56) Yang L, Scott PG, Giuffre J, Shankowsky HA, Ghahary A, Tredget EE. Peripheral blood fibrocytes from burn patients: identification and quantification of fibrocytes in adherent cells cultured from peripheral blood mononuclear cells. Lab Invest 2002 Sep;82(9):1183-1192.

(57) Pilling D, Buckley CD, Salmon M, Gomer RH. Inhibition of fibrocyte differentiation by serum amyloid P. J Immunol 2003 Nov 15;171(10):5537-5546.

(58) Varcoe RL, Mikhail M, Guiffre AK, Pennings G, Vicaretti M, Hawthorne WJ, et al. The role of the fibrocyte in intimal hyperplasia. J Thromb Haemost 2006 May;4(5):1125-1133.

(59) Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J Immunol 2003 Jul 1;171(1):380-389.

(60) Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S. Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. Exp Cell Res 2005 Mar 10;304(1):81-90.

(61) Sakai N, Wada T, Yokoyama H, Lipp M, Ueha S, Matsushima K, et al. Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. Proc Natl Acad Sci U S A 2006 Sep 19;103(38):14098-14103.

(62) Hong KM, Belperio JA, Keane MP, Burdick MD, Strieter RM. Differentiation of human circulating fibrocytes as mediated by transforming growth factor-beta and peroxisome proliferator-activated receptor gamma. J Biol Chem 2007 Aug 3;282(31):22910-22920.

(63) Zhao Y, Glesne D, Huberman E. A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. Proc Natl Acad Sci U S A 2003 Mar 4;100(5):2426-2431.

(64) Medina A, Kilani RT, Carr N, Brown E, Ghahary A. Transdifferentiation of peripheral blood mononuclear cells into epithelial-like cells. Am J Pathol 2007 Oct;171(4):1140-1152.

(65) Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. Semin Immunol 2008 Apr;20(2):86-100.

(66) Jabs A, Moncada GA, Nichols CE, Waller EK, Wilcox JN. Peripheral blood mononuclear cells acquire myofibroblast characteristics in granulation tissue. J Vasc Res 2005 Mar-Apr;42(2):174-180.

(67) Mooney JE, Rolfe BE, Osborne GW, Sester DP, van Rooijen N, Campbell GR, et al. Cellular plasticity of inflammatory myeloid cells in the peritoneal foreign body response. Am J Pathol 2010 Jan;176(1):369-380.

(68) Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol 2010 Jan;176(1):85-97.

(69) Rajkumar VS, Howell K, Csiszar K, Denton CP, Black CM, Abraham DJ. Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. Arthritis Res Ther 2005;7(5):R1113-23.

(70) Phan SH. Biology of fibroblasts and myofibroblasts. Proc Am Thorac Soc 2008 Apr 15;5(3):334-337.

(71) Eckes B, Zigrino P, Kessler D, Holtkotter O, Shephard P, Mauch C, et al. Fibroblast-matrix interactions in wound healing and fibrosis. Matrix Biol 2000 Aug;19(4):325-332.

(72) Rodemann HP, Muller GA. Characterization of human renal fibroblasts in health and disease: II. In vitro growth, differentiation, and collagen synthesis of fibroblasts from kidneys with interstitial fibrosis. Am J Kidney Dis 1991 Jun;17(6):684-686.

(73) Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, et al. Diversity, topographic differentiation, and positional memory in human fibroblasts. Proc Natl Acad Sci U S A 2002 Oct 1;99(20):12877-12882.

(74) Simian M, Hirai Y, Navre M, Werb Z, Lochter A, Bissell MJ. The interplay of matrix metalloproteinases, morphogens and growth factors is necessary for branching of mammary epithelial cells. Development 2001 Aug;128(16):3117-3131.

(75) Ekblom P, Weller A. Ontogeny of tubulointerstitial cells. Kidney Int 1991 Mar;39(3):394-400.

(76) Ogawa M, LaRue AC, Drake CJ. Hematopoietic origin of fibroblasts/myofibroblasts: Its pathophysiologic implications. Blood 2006 Nov 1;108(9):2893-2896.

(77) Togel F, Isaac J, Hu Z, Weiss K, Westenfelder C. Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. Kidney Int 2005 May;67(5):1772-1784.

(78) Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 2007 Sep;7(9):678-689.

(79) Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000 Nov;6(11):1229-1234.

(80) Zhan Y, Wang Y, Wei L, Chen H, Cong X, Fei R, et al. Differentiation of hematopoietic stem cells into hepatocytes in liver fibrosis in rats. Transplant Proc 2006 Nov;38(9):3082-3085.

(81) Sellamuthu S, Manikandan R, Thiagarajan R, Babu G, Dinesh D, Prabhu D, et al. In vitro trans-differentiation of human umbilical cord derived hematopoietic stem cells into hepatocyte like cells using combination of growth factors for cell based therapy. Cytotechnology 2011 May;63(3):259-268.

(82) Paunescu V, Deak E, Herman D, Siska IR, Tanasie G, Bunu C, et al. In vitro differentiation of human mesenchymal stem cells to epithelial lineage. J Cell Mol Med 2007 May-Jun;11(3):502-508.

(83) Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. J Cell Physiol 2001 Oct;189(1):54-63.

(84) De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003;174(3):101-109.

(85) Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005 Nov;33(11):1402-1416.

(86) Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006 May;24(5):1294-1301.

(87) Baer PC, Bereiter-Hahn J, Missler C, Brzoska M, Schubert R, Gauer S, et al. Conditioned medium from renal tubular epithelial cells initiates differentiation of human mesenchymal stem cells. Cell Prolif 2009 Feb;42(1):29-37.

(88) Wang Y, Sun Z, Qiu X, Li Y, Qin J, Han X. Roles of Wnt/beta-catenin signaling in epithelial differentiation of mesenchymal stem cells. Biochem Biophys Res Commun 2009 Dec 25;390(4):1309-1314.

(89) Stuart RO, Nigam SK. Development of the tubular nephron. Semin Nephrol 1995 Jul;15(4):315-326.

(90) Karihaloo A, Nickel C, Cantley LG. Signals which build a tubule. Nephron Exp Nephrol 2005;100(1):e40-5.

(91) Baer PC, Geiger H. Mesenchymal stem cell interactions with growth factors on kidney repair. Curr Opin Nephrol Hypertens 2010 Jan;19(1):1-6.

(92) Karavanova ID, Dove LF, Resau JH, Perantoni AO. Conditioned medium from a rat ureteric bud cell line in combination with bFGF induces complete differentiation of isolated metanephric mesenchyme. Development 1996 Dec;122(12):4159-4167.

(93) Suzuki A, Iwatani H, Ito T, Imai E, Okabe M, Nakamura H, et al. Platelet-derived growth factor plays a critical role to convert bone marrow cells into glomerular mesangial-like cells. Kidney Int 2004 Jan;65(1):15-24.

(94) Barasch J, Yang J, Ware CB, Taga T, Yoshida K, Erdjument-Bromage H, et al. Mesenchymal to epithelial conversion in rat metanephros is induced by LIF. Cell 1999 Nov 12;99(4):377-386.

(95) Zeisberg M, Shah AA, Kalluri R. Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney. J Biol Chem 2005 Mar 4;280(9):8094-8100.

(96) Wang W, Koka V, Lan HY. Transforming growth factor-beta and Smad signalling in kidney diseases. Nephrology (Carlton) 2005 Feb;10(1):48-56.

(97) Kinner B, Zaleskas JM, Spector M. Regulation of smooth muscle actin expression and contraction in adult human mesenchymal stem cells. Exp Cell Res 2002 Aug 1;278(1):72-83.

(98) Wang D, Park JS, Chu JS, Krakowski A, Luo K, Chen DJ, et al. Proteomic profiling of bone marrow mesenchymal stem cells upon transforming growth factor beta1 stimulation. J Biol Chem 2004 Oct 15;279(42):43725-43734.

(99) Fredriksson L, Li H, Eriksson U. The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev 2004 Aug;15(4):197-204.

(100) Ball SG, Shuttleworth CA, Kielty CM. Platelet-derived growth factor receptor-alpha is a key determinant of smooth muscle alpha-actin filaments in bone marrow-derived mesenchymal stem cells. Int J Biochem Cell Biol 2007;39(2):379-391.

(101) Kim YM, Jeon ES, Kim MR, Lee JS, Kim JH. Bradykinin-induced expression of alpha-smooth muscle actin in human mesenchymal stem cells. Cell Signal 2008 Oct;20(10):1882-1889.

(102) Jeon ES, Moon HJ, Lee MJ, Song HY, Kim YM, Cho M, et al. Cancer-derived lysophosphatidic acid stimulates differentiation of human mesenchymal stem cells to myofibroblast-like cells. Stem Cells 2008 Mar;26(3):789-797.

(103) Shao DD, Suresh R, Vakil V, Gomer RH, Pilling D. Pivotal Advance: Th-1 cytokines inhibit, and Th-2 cytokines promote fibrocyte differentiation. J Leukoc Biol 2008 Jun;83(6):1323-1333.

(104) Inagaki Y, Truter S, Ramirez F. Transforming growth factor-beta stimulates alpha 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. J Biol Chem 1994 May 20;269(20):14828-14834.

(105) Ponticos M, Harvey C, Ikeda T, Abraham D, Bou-Gharios G. JunB mediates enhancer/promoter activity of COL1A2 following TGF-beta induction. Nucleic Acids Res 2009 Sep;37(16):5378-5389.

(106) Fragiadaki M, Ikeda T, Witherden A, Mason RM, Abraham D, Bou-Gharios G. High doses of TGF-{beta} potently suppress type I collagen via the transcription factor CUX1. Mol Biol Cell 2011 Jun;22(11):1836-1844.

(107) Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol 2006 Jun;17(6):1503-1520.

(108) Kelly KJ, Williams WW,Jr, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, et al. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. J Clin Invest 1996 Feb 15;97(4):1056-1063.

(109) Singbartl K, Green SA, Ley K. Blocking P-selectin protects from ischemia/reperfusion-induced acute renal failure. FASEB J 2000 Jan;14(1):48-54.

(110) Bajwa A, Kinsey GR, Okusa MD. Immune mechanisms and novel pharmacological therapies of acute kidney injury. Curr Drug Targets 2009 Dec;10(12):1196-1204.

(111) Stroo I, Stokman G, Teske GJ, Raven A, Butter LM, Florquin S, et al. Chemokine expression in renal ischemia/reperfusion injury is most profound during the reparative phase. Int Immunol 2010 Jun;22(6):433-442.

(112) Sikorski EM, Hock T, Hill-Kapturczak N, Agarwal A. The story so far: Molecular regulation of the heme oxygenase-1 gene in renal injury. Am J Physiol Renal Physiol 2004 Mar;286(3):F425-41.

(113) Pagtalunan ME, Olson JL, Tilney NL, Meyer TW. Late consequences of acute ischemic injury to a solitary kidney. J Am Soc Nephrol 1999 Feb;10(2):366-373.

(114) Pagtalunan ME, Olson JL, Meyer TW. Contribution of angiotensin II to late renal injury after acute ischemia. J Am Soc Nephrol 2000 Jul;11(7):1278-1286.

(115) Forbes JM, Hewitson TD, Becker GJ, Jones CL. Ischemic acute renal failure: long-term histology of cell and matrix changes in the rat. Kidney Int 2000 Jun;57(6):2375-2385.

(116) Basile DP, Donohoe D, Roethe K, Osborn JL. Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. Am J Physiol Renal Physiol 2001 Nov;281(5):F887-99.

(117) Basile DP, Donohoe DL, Roethe K, Mattson DL. Chronic renal hypoxia after acute ischemic injury: effects of L-arginine on hypoxia and secondary damage. Am J Physiol Renal Physiol 2003 Feb;284(2):F338-48.

(118) Ohnishi H, Mizuno S, Nakamura T. Inhibition of tubular cell proliferation by neutralizing endogenous HGF leads to renal hypoxia and bone marrow-derived cell engraftment in acute renal failure. Am J Physiol Renal Physiol 2008 Feb;294(2):F326-35.

(119) Lin SL, Kisseleva T, Brenner DA, Duffield JS. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 2008 Dec;173(6):1617-1627.

(120) Duffield JS, Humphreys BD. Origin of new cells in the adult kidney: results from genetic labeling techniques. Kidney Int 2011 Mar;79(5):494-501.

(121) Supavekin S, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia/reperfusion. Kidney Int 2003 May;63(5):1714-1724.

(122) Tarabishi R, Zahedi K, Mishra J, Ma Q, Kelly C, Tehrani K, et al. Induction of Zf9 in the kidney following early ischemia/reperfusion. Kidney Int 2005 Oct;68(4):1511-1519.

(123) Thakar CV, Zahedi K, Revelo MP, Wang Z, Burnham CE, Barone S, et al. Identification of thrombospondin 1 (TSP-1) as a novel mediator of cell injury in kidney ischemia. J Clin Invest 2005 Dec;115(12):3451-3459.

(124) Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. J Cell Biol 1993 Aug;122(4):923-932.

(125) Murphy-Ullrich JE, Poczatek M. Activation of latent TGF-beta by thrombospondin-1: mechanisms and physiology. Cytokine Growth Factor Rev 2000 Mar-Jun;11(1-2):59-69.

(126) Ko GJ, Grigoryev DN, Linfert D, Jang HR, Watkins T, Cheadle C, et al. Transcriptional analysis of kidneys during repair from AKI reveals possible roles for NGAL and KIM-1 as biomarkers of AKI-to-CKD transition. Am J Physiol Renal Physiol 2010 Jun;298(6):F1472-83.

(127) Flaquer M, Romagnani P, Cruzado JM. Growth factors and renal regeneration. Nefrologia 2010;30(4):385-393.

(128) Dai C, Liu Y. Hepatocyte growth factor antagonizes the profibrotic action of TGF-beta1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF. J Am Soc Nephrol 2004 Jun;15(6):1402-1412.

(129) Phanish MK, Winn SK, Dockrell ME. Connective tissue growth factor-(CTGF, CCN2)--a marker, mediator and therapeutic target for renal fibrosis. Nephron Exp Nephrol 2010;114(3):e83-92.

(130) Phanish MK, Wahab NA, Hendry BM, Dockrell ME. TGF-beta1-induced connective tissue growth factor (CCN2) expression in human renal proximal tubule epithelial cells requires Ras/MEK/ERK and Smad signalling. Nephron Exp Nephrol 2005;100(4):e156-65.

(131) Ricardo SD, van Goor H, Eddy AA. Macrophage diversity in renal injury and repair. J Clin Invest 2008 Nov;118(11):3522-3530.

(132) Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology. 6th ed. Philadelphia: Saunders Elsevier; 2007.

(133) Meirelles Lda S, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009 Oct-Dec;20(5-6):419-427.

(134) Humphreys BD, Bonventre JV. The contribution of adult stem cells to renal repair. Nephrol Ther 2007 Mar;3(1):3-10.

(135) Herzog EL, Bucala R. Fibrocytes in health and disease. Exp Hematol 2010 Jul;38(7):548-556.

(136) Duffield JS, Park KM, Hsiao LL, Kelley VR, Scadden DT, Ichimura T, et al. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. J Clin Invest 2005 Jul;115(7):1743-1755.

(137) Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, et al. Intrinsic epithelial cells repair the kidney after injury. Cell Stem Cell 2008 Mar 6;2(3):284-291.