Metastatic niche formation

A guide towards improved stem cell homing

Master Thesis

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Biology of disease

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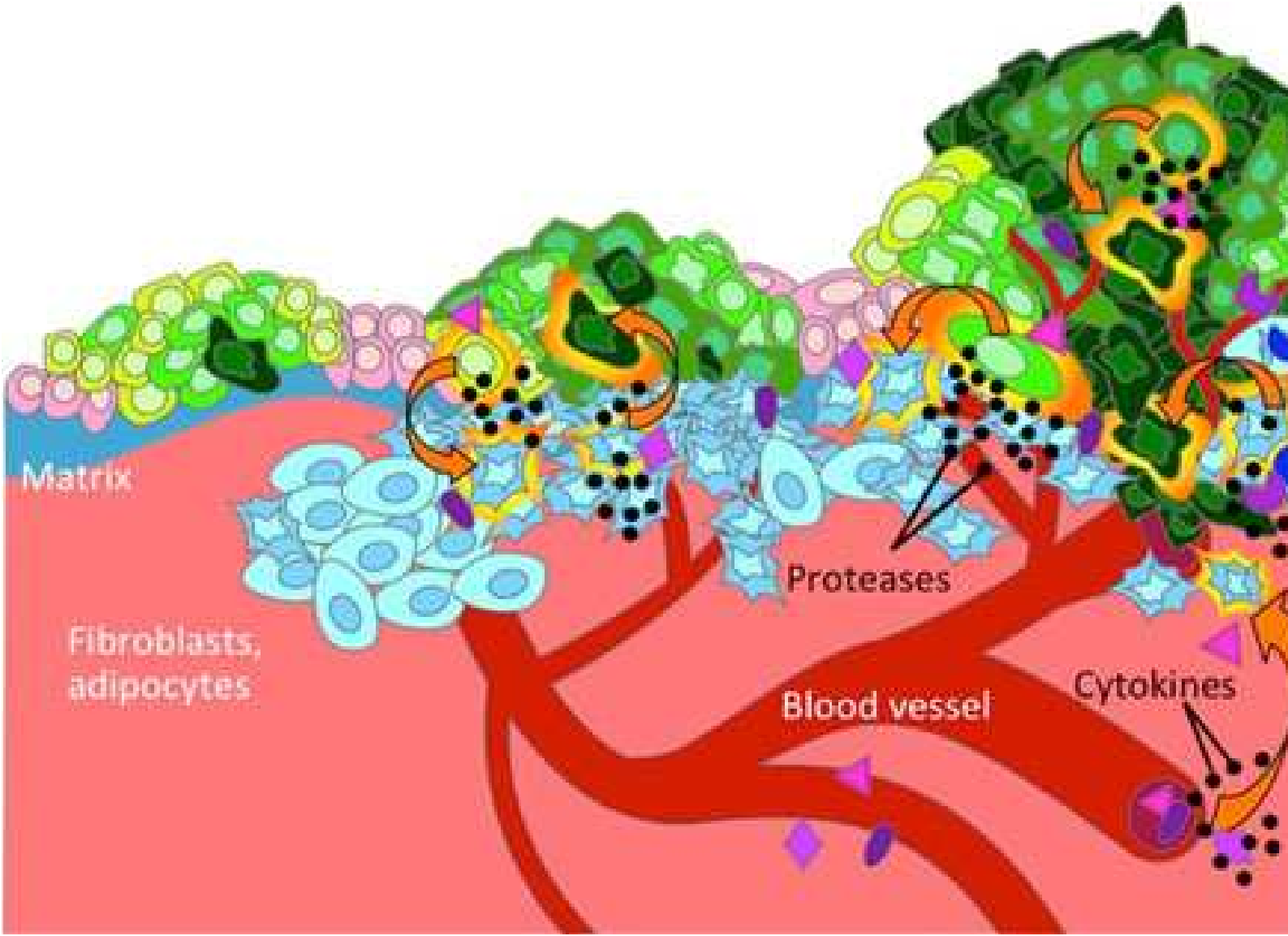

Outline

Abstract

Upon injury, stem cells can either repair or regenerate the damaged tissue. Repair results in non-functional fibrous tissue whereas with regeneration all former cells and functions revert. The major ingredients for tissue regenerations are an intact extracellular matrix (ECM) providing structure to the regenerate, proliferating stem cells to repopulate the damaged area, and surrounding cells that support the stem cells by providing growth factors. Not all tissues are able to regenerate themselves. Regenerative medicine attempts to stimulate regeneration by providing stem cells and ECM. However, homing of stem cells towards the injured tissue is not always an efficient process. By stimulating the implanted stem cells we might be able to improve tissue regeneration.

Which proteins or factors should be used, remains largely unknown. However, a little more is known about the migration of cancer stem cells towards pre-metastatic niches. Because cancer stem cells have great similarity with normal stem cells (e.g. CXCR4 expression), cancer stem cell attractive proteins (e.g. CXCRL21) might be useful in attracting normal stem cells towards injured tissue.

List of abbreviations

Ang1	Angioprotein 1
BMDC	Bone marrow derived cell
BMP	Bone morphogenic pritein
CSC	Cancer stem cell
CCL21	(C-C-motif) ligand 21
CCR7	C-C chemokines receptor 7
CXCL2	(C-X-C-motif) ligand 2
CXCL12	(C-X-C-motif) ligand 12
CXCR4	C-X-C chemokine receptor 4 (or: Stromal cell-derived factor 1)
ECM	Extracellular matrix
G-CSF	Granulocyt colony stimulating factor
GFs	Growth gaftors
HIF1	Hypoxia induced factor 1
HSC	Hematopoietic stem cell
JAG1	Jagged 1
LLC	Lewis lung carcinoma cell
LOX	Lysys oxidase
MI	Myocardial infarction
MSC	Mesenchymal stem cell
PlGF	Placental growth factor
SAA3	Serum amyloid A
STAT3	Signal transducer and activator of transcription 3
TCM	Tumor conditioned medium
TGFβ	Transforming growth factor-β
TKR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α
VCAM1	Vascular cell adhesion protein 1
VEGFA	Vascular endothelial growth factor A
VLA4	Very late antigen 4 (Integrin $\alpha_4\beta_1$)

Introduction

Regenerative medicine: the generation of new body parts and complete organs. Half a century ago, when the first organs transplantations were conducted, the idea of growing patient specific organs was just science fiction. However, from the moment that stem cells were discovered, studies on regeneration and regenerative medicine emerged quickly. In no time clinical trials were set up and the first patients were treated with stem cells. Some researchers say that it is just a matter of time until we can buy new body parts or organs like we can buy new parts for our cars. Others predict the future less optimistic, due to the many hurdles and unforeseen difficulties that arise and have to be overcome.

In this thesis we will review the current progress and hurdles of regenerative medicine. One of these hurdles, the one on which we will focus, is efficient homing of transplanted stem cells towards the injured tissue. Since cancer cells seem to home to distinct organs, we hypothesize that there is some mechanism of efficient homing in there. We will discuss whether we can use current research results on malign cancer cell homing to approach the difficulties in stem cell homing in regenerative medicine.

1 Healing damaged tissue

1.1 Repair versus regeneration

There are two intrinsic ways to heal a damaged tissue termed repair and regeneration. These terms should not be confused with each other, since they describe two distinct mechanisms of healing. Repair is the main mechanism of healing in mammalian adult tissues, in which the wound is first closed by constriction and the damaged tissue is replaced by fibrous scar tissue (Yannas, 2005). After a myocardial infarct, for instance, damaged tissue is replaced by fibrous tissue. This method of healing is efficient –it prevents rupture in the first place- but the repaired tissue is not able to function like healthy myocardium, resulting in eventual heart failure (Kumar *et al*., 1999; Gurtner *et al.,* 2008).

The second mechanism of healing, called regeneration, is the mechanism by which damaged tissue is replaced by new tissue. The new, regenerated tissue, regains its old morphology and functional capacities. Regeneration was already observed in many species in the 18th century. Prime examples of regeneration are off course the regeneration of amphibian limbs and sea star arms. But regeneration is also found in mammals/humans, although to a lesser extent. In the early 20th century the regeneration of a human fingertip was first described (Wicker *et al.,* 2009). Human organs with remarkable regeneration capacities are the liver and kidneys. Also most surface skin wounds can regenerate without the formation of fibrous scar tissue (Kumar *et al*., 1999). Unfortunately, not all mammalian organs or tissues have regeneration capacities and the ability to regenerate seems to deteriorate during life in mammals (Kumar *at al.*, 1999).

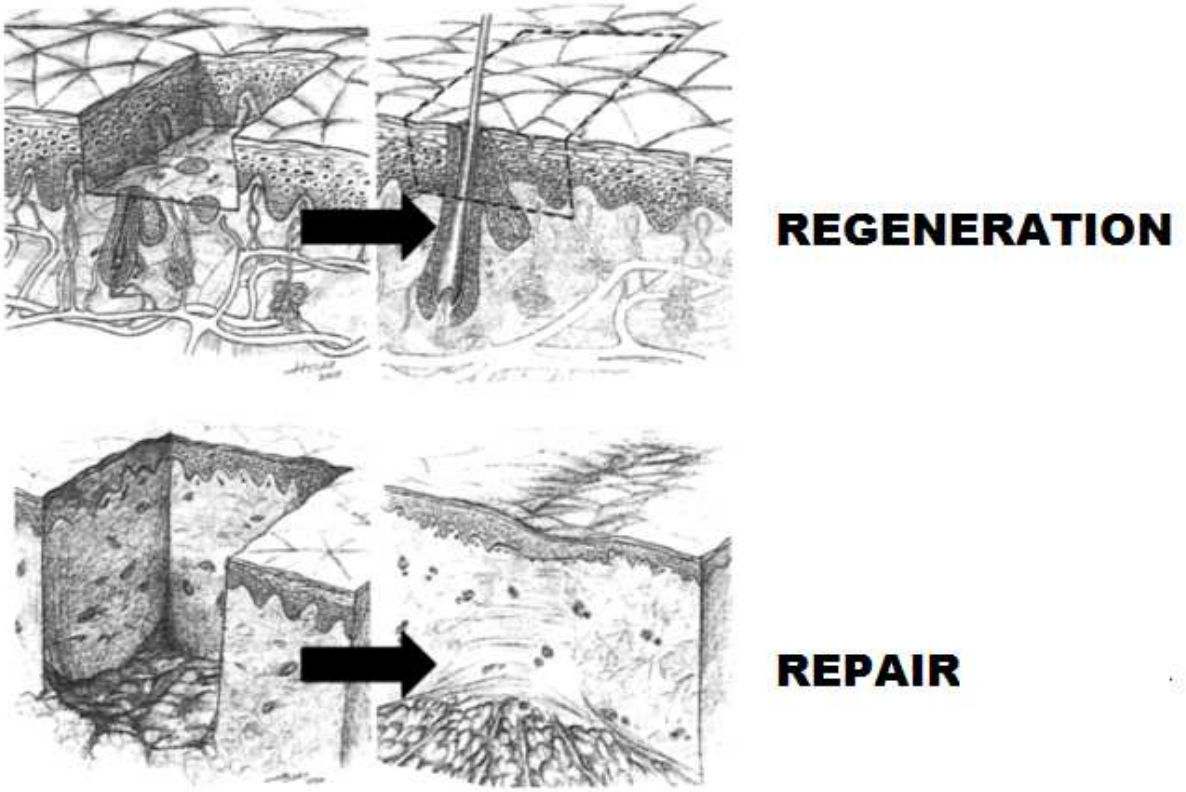

Figure 1 - Regeneration versus repair. The skin is one of the organs that often regenerates. When a skin wound is superficial and both stem ells and ECM are intact, regeneration can take place. However, when a wound cuts through the stem cell layer and the ECM is ruptured, the skin will heal following the repair mechanism, leaving a scar of fibrous tissue (figure adapted from Yannis, 2005).

1.2 Extracellular matrix and Stem cells: ingredients for regeneration

A rule of thumb for regeneration is that the extracellular matrix must be intact (Figure 1). Moreover, undamaged, tissue specific stem cells must be present in order to restore the tissue (Yannis, 2005; Kumar *et al.,* 1999). The extracellular matrix (ECM) consists of fibrous components, such as collagen, fibronectin and elastin to provide structural support to the cells within a tissue and enables them to migrate throughout damaged tissue (Zheng *et al.,* 2012; Singh *et al.,* 2010). Polysaccharides such as heparin and hyaluronan, play a role in hydratation and trapping of growth factors (GFs) (Unterman *et al.,* 2012).

1.2.1 Stem cells

Stem cells are the major players in regeneration. While supported by the ECM, stem cells are the ones who give rise to new cells that will repopulate the damaged tissue. Stem cells are defined by the ability of self-renewal and to give rise to differentiated daughter cells. The potency of a stem cell indicates the divergence of daughter cells that arise from this stem cell. Adult stem cells, which can be isolated from all kinds of tissues, are multipotent: they are only able to differentiate into the cells of their own specific organ (Lander *et al.,* 2012). These cells are the ones who maintain the tissue in homeostasis and (potentially) regenerate damaged tissue. Also distant stem cells, like bone marrow - derived mesenchymal stem cells (MSCs) have been shown to migrate towards the injured tissue to help regeneration. MSCs are the only adult stem cell that can differentiate into cells of all three germ layers and therefore can be classified as pluripotent (Jiang *et al*., 2002). They can be harvested from several tissues, including bone marrow, adipose tissue, muscle, and periosteum (Kisiel *et al.,* 2012). In the Bone marrow MSCs are localized perivascular and therefore have the possibility to migrate towards injured tissue to support regeneration (Kolf et al., 2007; Patel *et al.,* 2011).

After a myocardial infarction SDF-1 levels increase in the borderzone of the infarction, but decease in serum and the amount of MSCs inside the heart increased with 15%. Gene expression analysis of mice hearts after MI reveals that besides SDF-1, also MMP-9, VCAM-1 and ICAM-1 are upregulated upon myocardial damage, indicating that these molecules also play a role in MSC recruitment (Abbott *et al.,* 2004). Figure 2 shows the VLA4/VCAM1-mediated entrance of MSCs into damaged tissue.

Endothelial progenitor cells (EPCs) are also triggered to home towards injured tissue. EPCs that migrated to the damaged tissue in a pig model of atherosclerotic renal artery stenosis showed increased expression of CXCL12, Ang1, Tie2, and c-Kit. In the damaged kidney tissue c-kit ligand and integrin β_2 were upregulated. Ang1 and CXCR4, however, were not upregulated in the damaged tissue (Chade *et al.,* 2010).

1.2.2 Stem cell niches

Stem cells are surrounded by supporting cells. The role of this 'niche' of supporting cells, however, remains only partly understood. Stromal cells that reside in the ECM play a very important role in tissue regeneration by supporting stem cells with growth factors such as EGF, VEGF, FGF, and TGFβ (Kumar *et al.,* 1999). Apart from the general supportive features, the role of the stem cell niche seems to differ among stem cell types (Lander *et al.,* 2012).

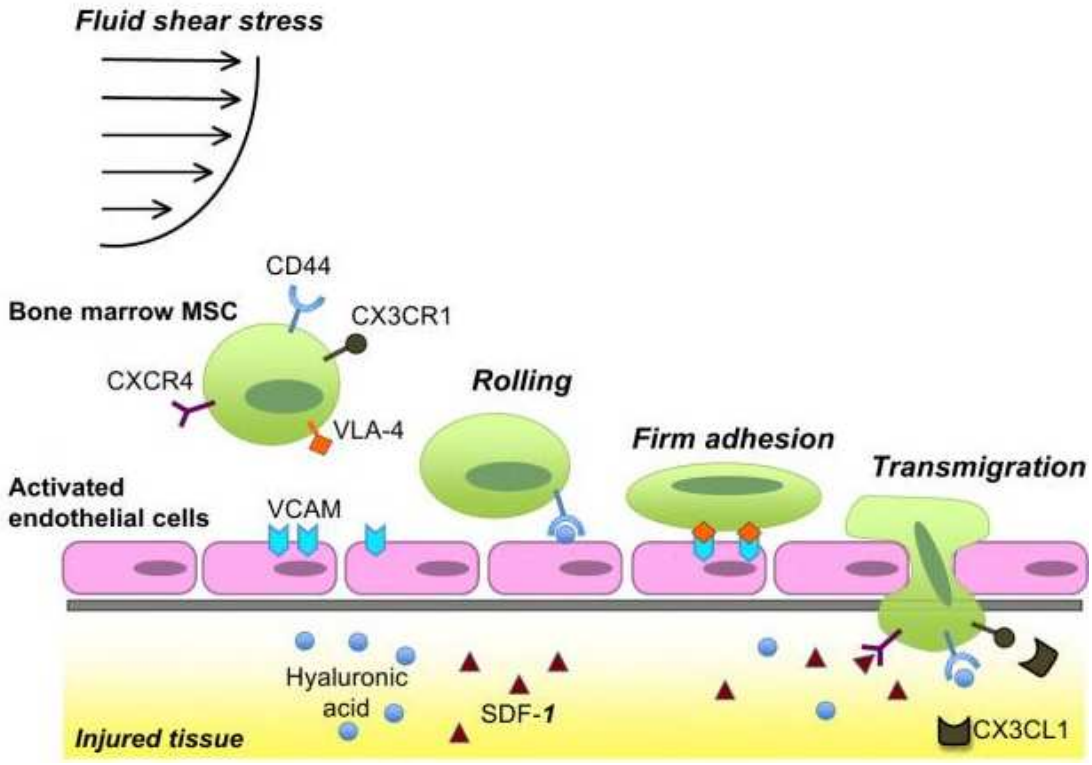

Figure 2 - MSC entrance to injured tissue. MSCs are recruited to injured tissue by released chemokines. Once at the injured area MSCs bindt to VCAM1 and transmigrate into the tissue. Figure from Goligorsky *et al.,* 2010.

In the gut, where the tissue is almost continuously replaced, the main role of the niche is to control the amount of non-differentiated stem cells and differentiated daughter cells, while in less active tissues, like muscles, the niche mainly regulates dormancy and activation of stem cells upon injury. Growth factors and cytokines involved in stem cell control are summarized in table 1.

The best characterized niches are those in bone marrow, which resides hematopoietic stem cells (HSCs) and MSCs. Two main microenvironments are described in literature: the endosteal niche and the vascular niche. In the endosteal niche HPCs are preserved so that long-term repopulation ability is maintained (Arai *et al.,* 2004). The endosteal niche consists of osteoblasts, witch stimulate HSC proliferation and inhibit differentiation by JAG1/notch signaling (Calvi *et al.,* 2003) and enhance quiescence via Ang1/Tie2 interaction (Arai *et al.,* 2004). Osteoblasts also secrete CXCL12 (also known as SDF-1), a chemoattractant for $CXCR^{+}$ HSCs (Petit *et al.,* 2002). TGF-β, BMP2 and BMP7, secreted by osteoclasts, also play a role in quiescence of HSCs (Lilly *et al.,* 2011).

In the vascular niche, endothelial cells play a major role in HSC differentiation (Yao *et al.,* 2005). MSCs in the vascular niche are also important residents of the HSC niche. Depletion of MSCs lead to a decrease of HSCs, in part explained by increased migration toward other tissues (Mendez-ferrer *et al.,* 2010). Also in the vascular niche CXCL12/CXCR4 signaling is important for homing of HSCs and MSCs. Treatment with G-CSF decreases CXCL12 and thereby stimulates MSCs and HSCs to leave their niches and enter the circulation, to home towards damaged tissues (Petit *et al.,* 2002; Hannoush *et al.,* 2011).

Tabel 1 - Growth factors and cytokines involved in stem cell regulation

Molecule	Function	Niche	Reference
EGF	Proliferation	Gut	Lander *et al.,* 2012**,** Bjerknes*et al.*, 1981; Barker *et al.,* 2007
TGFα	Proliferation	Gut	
Wnt3	Proliferation	Gut	
Dll4	Proliferation	Gut	
G-CSF	Quiescence	HSC/MSC	Mendez-ferrer *et al.,* 2010; Liu *et al.,* 1009
Wnt	Differentiation	MSC	Kolf *et al.,* 2007
parathormone	Proliferation	HSC	Mendez-ferrer *et al.,* 2010
CXCL12	Activation & homing	HSC/MSC	Mendez-ferrer *et al.,* 2010; Liu *et al.,* 1009
Osteoponin	Quiescence	HSC	Stier *et al.,* 2005
VLA4	Homing & adhesion	HSC/MSC	Williams *et al.,* 1991; Liu *et al.,* 1009
Ang1/Tie2	Quiescence	HSC	Arai *et al.,* 2004
Cdc42	Quiescence	HSC	Yang *et al.,* 1007

2 Induced regeneration: regenerative medicine

While some tissues are capable of repairing damage and regeneration, other tissues cannot intrinsically be regenerated. Regenerative medicine aims to induce or enhance regeneration or, according to Chris Mason and Peter Dunnill, “regenerative medicine replaces or regenerates human cells, tissue or organs, to restore or establish normal function” (Mason *et al.,* 2008). This ranges from injection of functional cells to stimulate stem cells, to *in vitro* engineering of complete new organs for transplantation. Basically, regenerative medicine could be considered as: inducing regeneration by providing all necessary components: ECM, stem cells, and growth factors.

2.1 Scaffolds: an artificial ECM

Already in 1989 an artificial ECM, consisting of collagen and glycosaminoglycan was used to improve regeneration of deep skin wounds (Yannas *et al.,* 1989). Later, scaffolds were made of decellularized material, or synthetic polymers. Whereas decellulized scaffolds are less immunogenic, scaffolds made of synthetic polymers can be produced in large quantities and might provide more strength or a better structure (Olson *et al.,* 2011; Zheng *et al.,* 2012; Unterman *et al.,* 2012; Atala, 2012). The ideal scaffold should, besides providing strength and structure, be biodegradable and not immunogenic.

To prevent antigenicity, the first heart valve scaffolds were decellularized and directly transplanted into patients. In the first clinical experiments using decellularized, re-cellularization did not, or hardly, occur (Simon *et al.,* 2003; Elkins *et al.,* 2001). Transplantation of scaffolds seeded with autologous cells, prior to implantation, led to better results (Shinoka, 2002). However, isolation and culturing of cells and seeding them onto a scaffold is a time-consuming process. Being able to enhance *in vivo* seeding of scaffolds, for instance by adding compounds of proteins that attract stem cells, would greatly enhance the application of heart valve scaffolds.

2.2 Stem cell Therapy

Stem cell therapy is based on induction of regeneration by application of stem cells, to replace the damaged tissue. Several types of stem cells have been tested and can be used for this purpose. This can be autologous cells, obtained from a biopsy and expanded *in vitro* prior to transplantation, adult stem cells, isolated from various tissues, embryonic stem cells, of induced pluripotent stem cells.

One area in which stem cell therapy is applied is the myocardium after a myocardial infarction (MI). About a decade ago the first studies in mice were performed, using stem cells to regenerate the myocardium after a MI. Min *et al.* showed that MSCs, implanted into the damaged myocardium of MI-induced pigs, can differentiate into cardiomyocytes, stimulate angiogenesis and improve myocardial perfusion (Min *et al.,* 2002)**.** Soon thereafter the first clinical trials were set up. Bone marrow derived stem cells were implanted intracoronary during PTCA procedure and in a 6 months follow up, patients had a better cardiac performance (ejection fraction and end systolic volume) (Wollert *et al.,* 2004; Chen *et al.,* 2004). Administration of MSC must take place soon after the myocardial event. A large clinical trial in which patients were treated 2-3 weeks post-MI showed no significant increase in cardiac performance of MSC-treated patients compared to controls (Traverse *et al*., 2011). Since the injected stem cells probably do not stay in the heart for a long time, clinical improvement might decline in time. Therefore also long-term follow up studies were done, of which the first are evaluated nowadays.

One pitfall of circulatory administration of stem cells is that a lot of stem cells have to be applied in order to be successful. This is caused by unsuccessful homing of the cells towards the infarcted area (Kraitchman *et al.,* 2005). To generate enough cells for successful therapy takes a long time and, moreover, tumorgenicity might increase. Therefore it is of great importance that stem cell homing to damaged tissue is improved. In order to improve this homing, the factors that might attract the correct cells to the correct place must be found.

3 Lessons from cancer

Circulating tumor cells seem to home to specific tissues, as predicted by the primary tumor. Because tumour cells and stem cells have a lot properties in common, they might also share specific homing mechanisms. Adding attractive molecules to scaffolds and/or injured tissue will in turn result in better regenerative medicine strategies. Therefore, we will evaluate the migration and homing factors that play a role in cancer metastasis.

3.1 Cancer stem cells and their niche

The cancer stem cell theory states that cancer derives from a tumor-initiating cell, and only a small population of tumor cells can initiate tumor formation (Bonnet & Dick, 1997; Al Hajj *et al.,* 2003). Cancer stem cells (CSCs) have been isolated by numerous tumor types (Tysnes, 2010). These cells behave like stem cells: they can self-renew and give rise to differentiated daughter cells. CSCs do not devide not that often, protecting their repopulation ability (Figure 3). When injected into an animal

model, CSCs were far more efficient in forming metastases than non-CSCs. Therefore these cells are often called tumor-initiating cells (Al Hajj *et al.,* 2003; Pang *et al.,* 2010). The differentiating daughter cells are termed transient amplifying cells, because these are the cells that rapidly replicate and undergo (epi-)genetic changes. Therefore the transient amplifying cells are more tissue-specific but have less longevity (Liu *et al.,* 2011; Sneddon & Werb, 2007).

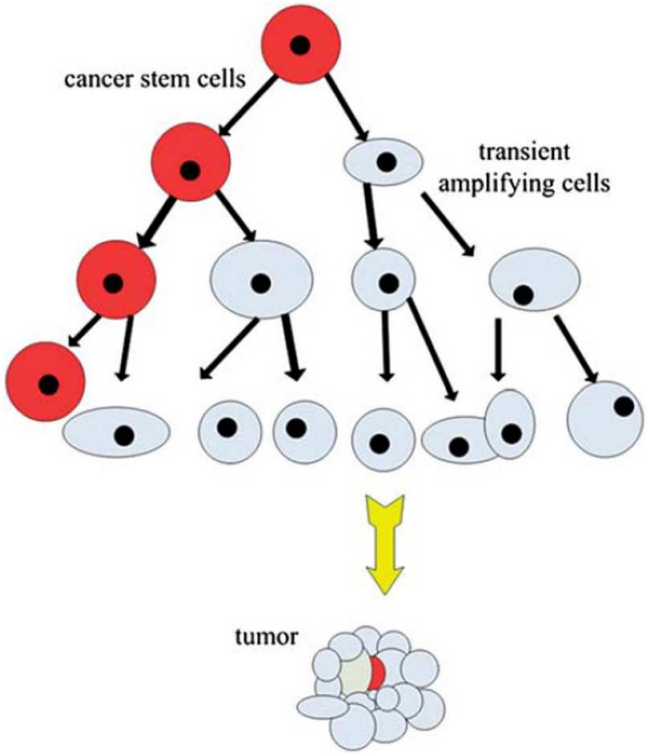

Figure 3 - Cancer stem cell therapy. The cancer stem cell therapy states that a select population of cells within a tumor has stem cell properties (red cells). These are the cells that are dormant and have capacity to induce a new tumor. The transient amplifying cells (grey) are the cells that continuously replicate, undergoing rapid mutagenesis and are better adapted to a specific tissue. Figure from Liu *et al*., 2011.

3.2 Metastatic niches

Like stem cells need a niche to remain 'stemness', CSCs also need a niche to be able to survive and proliferate. This niche includes non-CSCs, a specialized ECM, (myo-) fibroblasts, endothelial cells, and bone marrow-derived cells (Liu *et al.,* 2011; Sneddon & Werb, 2007).

3.2.1 directed metastasis

Over a century ago it was already observed that metastases arise mainly in distinct organs: lungs, liver, brains, and bone, while other organs seemed to remain unaffected. The general believe that the site of metastasis was solely determined by vessel width was replaced by two new theories that described this phenomenon. First, there was the 'seed and soil' theory, proposed by Steven Paget. He stated that the microenvironment of specific organs in some way are more conductive (are a better 'soil') for circulating cancer cells to 'seed' (Paget, 1889). James Ewing, on the other hand stated that the location of metastatic outgrowths was determined by the anatomy of draining blood and lymph vessels. The organ next to the primary tumor would have the highest chance of getting metastasized. Ewings' theory was generally accepted and cancer research mainly focussed on primary tumors and their surroundings (Khamis *et al.,* 2012; Psaila & Lyden, 2009).

When it became clear that the metastases, instead of the primary tumor itself, were the cause of death in cancer patients, investigation on metastatic disease was resumed and the existence of 'soil' or a '(pre)metastatic niche' became part of the debate again. More convincing research to support

the seed and soil theory came in 1980, when Fidler and Hart reported that circulating cancer cells do reach all organs, but not give rise to metastases in every organ and thus indeed search for the best 'soil' to grow. It became clear that circulating CSCs, like primary tumor cells, need a niche in order to form a metastatic outgrowth. Without such a niche the CSC will become dormant, doesn't have an angiogenic switch or is not even able to exit the bloodstream (Langley & Fidler., 2011).

Two types of pre-metastatic niches are described; abused or adapted pre-existing stem cell niches and primary tumor-induced pre-metastatic niches.

3.2.2 Pre-existing metastatic niches

A prime example of the abuse of pre-existing stem cell niches is found in breast cancer- and prostate cancer-derived bone marrow metastases. Bone marrow is one of the most common organs for metastasis, probably due to the many stem cell binding sites that are already existing (Langley & Fidler, 2011). $CXCR4^+$ and $CCR7^+$ breast cancer cells are known to use haematopoietic stem cell niches as their (pre-) metastatic niches. Due to the expression of CXCL12 and CCL21 at the HPC niche, circulating $CXCR4^+$ and $CCR7^+$ CSCs can easily bind to that niche. CXCL12 is also up-regulated in lung and liver; the two organs in which breast cancer is also often reported to metastasize (Müller *et al.,* 2001). In a similar way prostate cancer stem cells (expressing sLe^x) home to stem cell niches in the bone marrow that express E-selectin, the binding partner of sLe^x (Barthel *et al.,* 2009).

3.2.3 Tumor-induced metastatic niches

The primary tumor-induced formation of pre-metastatic niches is best studied in breast cancer-derived lung metastases. Lungs are the second most common organ for metastasis. Due to their architecture of very small capillaries, the lungs are very likely to give rise to metastases, since circulating tumor cells easily get stuck in the lung (Langley & Fidler, 2011). The formation of a pre-metastatic niche is a complex process, involving distance signalling, cell recruitment, activation. A schematic overview of important metastasis-associated proteins is shown in figure 4. Besides the role of secreted proteins, exosomes and micro RNAs are heavily investigated for their roles in metastasis and metastatic niche formation (Feng *et al.,* 2012; Ngora *et al.,* 2012; Peinado *et al.,* 2012).

First, the future site of metastasis is determined by factors secreted by the primary tumor. Evidence for this hypothesis was provided by Kaplan *et al*. in 2005, when they were able to invert metastatic patterns in mice by pre treatment with tumor conditioned medium (TCM). They pre-treated mice with TCM of Lewis lung Carcinoma cells (LLCs), which only metastasize to the lungs, or TCM from B16 melanoma cells, which are more aggressive and metastasize to Kidneys, intestine, spleen and oviduct. After injection, the LLCs redirected metastasis to the kidneys, spleen, intestine and oviduct. Comparison between the two conditioned media revealed differences in VEGFA and PlGF, which were more abundantly present in B16 melanoma conditioned medium (Kaplan *et al.,* 2005).

Besides VEGFA and PlGF, analysis of LLC TCM revealed the secretion of TGF-β, TNF-α, and STAT3 (Kaplan *et al.,* 2005; Hiratsuka *et al.,* 2006; Deng *et al.,* 2012**;** Tu *et al.,* 2012). Under hypoxic conditions LOX and C4.4A secretion was observed, of which the latter was associated to exosomes (Erler *et al.,* 2009; Ngora *et al.,* 2012). Also, MET-containing exosomes were isolated from metastatic B16 TCM (Peinado *et al.,* 2012). All factors play a role in inducing pre-metastatic niche formation in the lung.

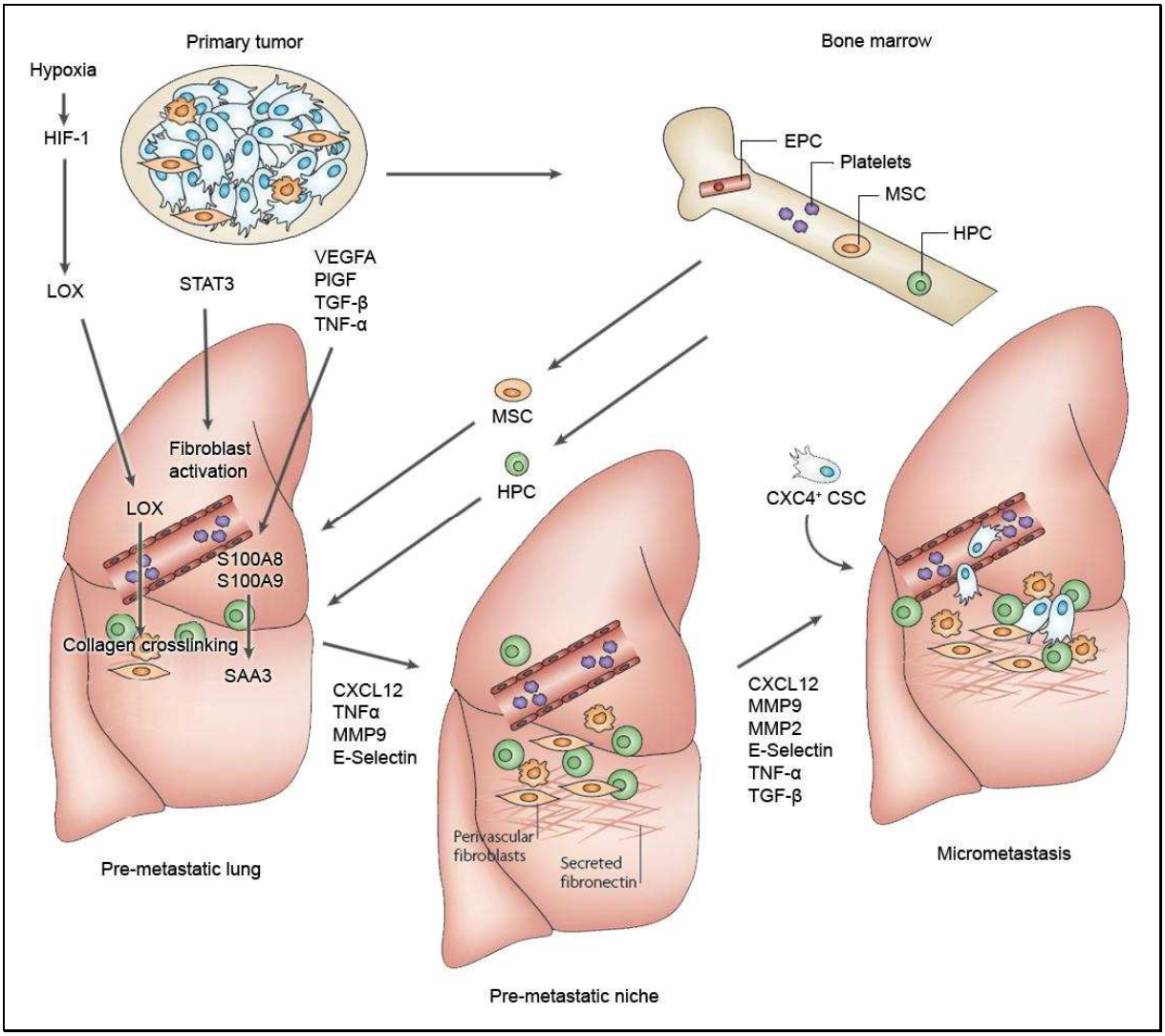

Figure 4 - Pre-metastatic niche formation. Primary tumors secrete proteins LOX, STAT3, VEGFA, PlGF, TGF-β, and TNF-α, that initiate pre-metastatic niche formation in the lungs. Within the lung, these proteins activate lung cells, which secrete and upregulate various factors that attract BMDCs. The BMDCs upregulate CXCL12, TNF- α, and MMPs that in term attract tumour cells. Figure adapted from Psaila and Lyden, 2009

More specifically, VEGFA, TGF-β, and TNF-α, have been shown to induce local expression of the inflammatory chemoattractants S100A8 and S100A9 (Hiratsuka *et al.,* 2006) and STAT3 activates fibroblasts at the pre-metastatic site, leading to increase fibronectin expression and the formation of fibronectin patches (Deng *et al.,* 2012). LOX secretion is hypoxia (HIF-1) dependent and migrates to the lungs, where it cross-links collagen IV (Erler *et al.,* 2009; Wong *et al.,* 2012).

These initial changes in the lungs result in the attraction of several cells. First, $CD11b^+$ BMDCs are attracted by the cross-linked collagen. In reaction they start to produce MMP2, which facilitates the (enhanced) recruitment of $CD11b^+$ BMDCs and $c\text{-}Kit^+$ cells **(Erler *et al.,* 2009**). The fibronectin patches attract VLA^+-$VEGFR^+$ BMDCs. VLA^+-$VEGFR^+$ BMDCs in turn increase MMP9 secretion and CXCL12 expression, attracting VCAM1 expressing circulating CSCs and facilitating their infiltration and survival (Deng *et al.,* 2012; Chen *et al.,* 2012). Upregulation of S100A8 and S100A9 lead to attraction of $mac1^+$ BMDCs following an SAA3-TLR4 dependent interaction. $Mac1^+$ BMDCs inside the niche start secreting TNFα, TGFβ andCXCL2, which attract circulating CSCs (Hiratsuka *et al.,* 2008).

4. Enhanced stem cell therapy

MSCs are promising tools for regenerative therapy. However, this success is only obtained when huge amounts of MSCs are applied, due to inefficient homing and binding of the MSCs. Malignant, primary tumor cells can efficiently create a metastatic niche, which attracts both CSCs and stromal cells. There indeed are similarities between normal stem cells and cancer stem cells. Similar strategies to regulate and enhance homing might thus be applied and useful to improve stem cell homing in stem cell therapy.

One of the similarities is the expression of CXCR4 on both CSCs and MSCs and the upregulation of CXCL12 in the pre-metastatic niche and injured tissues, leading to homing to CSCs towards the niche. Inducing an increase of CXCL12 at the site of injury thus might enhance the attraction of MSCs towards damaged tissues. Hannoush *et al.* applied this strategy and injected CXCL12 in the lungs of rats after unilateral lung contusion. Within 5 days, increased MSC homing and improved wound healing were observed (Hannoush *et al.* 2011). Another, less invasive approach is to increase CXCR4 expression in MSCs. *In vitro* essays of CXCR4 overexpressing HSCs revealed a 10-fold increase of migration towards CXCL12 (Brenner *et al.,* 2004). Moreover, mice treated with CXCR4 overexpressing MSCs after MI showed less collagen and better preservation of the ventricles, compared to unmodified MSC treated animals. Also, more modified MSCs homed to the myocardium (Cheng *et al.,* 2008).

Upon hypoxia, primary tumor cells increase HIF1 expression, which lead to transcription and secretion of factors that facilitate in metastatic niche formation and BMDC attraction. Similarly, in injured tissue hypoxia plays a role in stem cell recruitment. However, while regenerating, stem cells are in need of oxygen and might die due to the hypoxia. Cerrada *et al* demonstrated that 'pre-treating' MSCs to hypoxia by overexpressing HIF1 in MSCs, prior to injection into the damaged tissue of infracted rat hearts, resulted into better cardiac regeneration, without hypertrophy and fibrotic tissue (Cerrada *et al.,* 2012).

Pretreatment of MSCs with the signals that attract them towards the metastatic niches might also mobilize MSCs towards injured tissues. These signals include fibronectin, VCAM1, S100A8 &9, SAA3, VEGFA, MMPs, TNFα, and TGFβ. Treatment of MSCs *in* vitro with TNFα leads to enhanced migration capacity of MSCs. Even more, *in* vitro treatment with TNFα makes MSCs more sensitive to other cytokines (Ponte *et al.,* 2007). This suggest that treatment of MSCs with TNFα prior to transplantation increases homing towards the injured tissue.

In the case of the pre-metastatic niche, BMDCs homed to collagen and fibronectin patches. Recently, Weeks *et al.,* simulated these patches by coating a scaffold with fibronectin or collagen. Moreover, they also used VCAM-1 and CXCL12 to coat scaffolds. All coated scaffolds had increased MSC adhesion and the combination of VCAM1 and CXCL12 enhanced the MSC adhesion even more, indicating that coating of scaffolds with metastatic niche components indeed enhances stem cell homing (weeks *et al.,* 2012).

Conclusion

Because CSCs, MSCs, metastatic niches and damaged tissue share many properties, research on these topics can build on each other. Studying metastatic niche formation provides insights into the mechanism of stem cell recruitment, which can enhance regenerative medicine strategies. However, mimicking metastasis remains like playing with fire. Care must be taken when these strategies are applied, since one does not want circulating CSCs to colonize damaged tissues or scaffolds. Such safety standards might be the biggest hurdles in de coming years of improved regenerative medicine.

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