



# STUDIENBRIEF

## STAMMZELLEN UND REGENERATIVE MEDIZIN

Modul im geplanten weiterbildenden Masterstudiengang  
„Biopharmazeutisch-Medizintechnische Wissenschaften (BM-Wiss)“  
der Fakultät für Naturwissenschaften der Universität Ulm,  
der Medizinischen Fakultät der Universität Ulm  
und der Fakultät für Biotechnologie  
der Hochschule für Angewandte Wissenschaften Biberach  
mit dem Abschluss  
„Master of Science (M. Sc.)“  
an der Universität Ulm und der Hochschule Biberach

## MODULBESCHREIBUNG Stammzellen und Regenerative Medizin

MODULNUMMER	3.5.
MODULTITEL	Stammzellen und Regenerative Medizin
STUDIENGANG	Biopharmazeutisch-Medizintechnische Wissenschaften (M.Sc.)
MODULVERANTWORTLICHKEIT	<b>Prof. Dr. Uwe Knippschild</b>
LEHRENDE	Prof. Dr. Uwe Knippschild, Dr. Joachim Bischof, Dr. Pengfei Xu, PD Dr. Markus Hönicka, PD Dr. Timo Burster, PD Dr. Cagatay Gunes
SEMESTER (EMPFOHLEN)	Wintersemester
ART DER VERANSTALTUNG	Präsenzveranstaltung im Labor, Wochenendseminar und E-Learning-Elemente
VEANSTALTUNGSSPRACHE	Deutsch und Englisch
ECTS-CREDITS	6 Credits  <b>Arbeitsaufwand:</b> Präsenzzeit: Seminarwochenende und Praktikum (60 Stunden) Selbststudium: Nachbereitung der Vorlesungen (überwiegend E-Learning basiert), schriftliche Ausarbeitung der Hausarbeit, Vortrags- und Praktikumsvorbereitung (120 Stunden)
PRÜFUNGSFORM UND –UMFANG	Seminararbeit (mit Präsentation) Praktikumsprotokoll  <b>Notenbildung:</b> Die Abschlussnote ergibt sich aus der Bewertung der schriftlichen Seminararbeit und dem zugehörigen Vortrag (50%) sowie dem Laborprotokoll (50%). <u>Seminar:</u> Die Notenvergabe erfolgt aufgrund der Bewertung der schriftlichen Seminararbeit (66,6%) und des Vortrags (33,3%) <u>Praktikum:</u> Die Notenvergabe erfolgt aufgrund der Bewertung des Praktikumsprotokolls.
LERNZIELE	<u>Fachkompetenz:</u>

## MODULBESCHREIBUNG

	<ul style="list-style-type: none"> <li>- Studierende kennen und verstehen die gesetzlichen Regelungen zur Stammzellforschung.</li> <li>- Studierende verstehen die Physiologie von Stammzellen.</li> <li>- Studierende verstehen Signalnetzwerke und können Zellzyklusregulation in Stammzellen analysieren.</li> <li>- Studierende verstehen Stammzelltherapiekonzepte und können diese anwenden.</li> </ul> <p><u>Methodenkompetenz:</u></p> <p>Studierende können folgende Techniken anwenden und beurteilen:</p> <ul style="list-style-type: none"> <li>- <i>Tierexperimentelle Arbeiten</i> Isolierung und Charakterisierung hämatopoetischer Stammzellen aus der Maus mit Hilfe chromatographischer Techniken</li> <li>- <i>Proteinchemische Techniken</i> (SDS-PAGE, Western Blot, Kinaseassays, Enzymkinetik, IC50 Bestimmungen von Kinaseinhibitoren, Aufreinigung GST-Fusionsproteinen)</li> <li>- <i>Zellbiologische Techniken</i> Zellviabilitätsbestimmungen (MTT Assays), Differenzierung von Stammzellen, Immunfluoreszenzanalysen</li> <li>- <i>Molekularbiologische Techniken</i> RNA Isolierung, cDNA Synthese, PCR, qRT-PCR</li> <li>- <i>Immunhistologische Techniken</i> HE-Färbungen IHC-Färbungen</li> </ul> <p><u>Selbst- und Sozialkompetenz:</u></p> <ul style="list-style-type: none"> <li>- Studierende kennen die üblichen Verfahren und Grundsätze wissenschaftlichen Arbeitens in der Stammzellforschung und können diese anwenden.</li> <li>- Studierende können selbstständig wissenschaftliche Arbeiten auf dem Gebiet der Stammzellforschung verfassen.</li> <li>- Studierende können komplexe Aufgaben in Teams gemeinsam lösen und strukturiert bearbeiten.</li> </ul>
LEHRINHALTE	In diesem Modul werden folgende fachliche Inhalte vermittelt (6 Leistungspunkte):

## MODULBESCHREIBUNG

	<ul style="list-style-type: none"> <li>• <b><u>Vorlesung (1SWS):</u></b> <ol style="list-style-type: none"> <li>1. Eigenschaften von Stammzellen</li> <li>2. Stammzellnischen und Stammzellkultur</li> <li>3. Leberstammzellen und Tumorstammzellen</li> <li>4. Molekulare Mechanismen der Stammzellalterung</li> <li>5. Signaltransduktion und Stammzellen</li> <li>6. „Small molecule“ Inhibitoren (HDAC- und Kinaseinhibitoren)</li> <li>7. Stammzelltherapie</li> </ol> </li>   <li>• <b><u>Seminar (1 SWS): Seminarwochenende in Rettenberg</u></b> <ol style="list-style-type: none"> <li>1. Erstellen einer schriftlichen Seminararbeit über ein Stammzell-relevantes Thema</li> <li>2. Powerpointpräsentation der Seminararbeit (während der Wochenendexkursion)</li> </ol> </li>   <li>• <b><u>Praktikum (ganztägig 1 Woche, 2 SWS)</u></b> <ol style="list-style-type: none"> <li>1. Isolierung und Charakterisierung von hämatopoetischen Stammzellen</li> <li>2. Analyse von Signaltransduktionswegen</li> <li>3. Bestimmung der Expression und Aktivität verschiedener Kinasen</li> <li>4. Charakterisierung von potentiellen Kinaseinhibitoren (IC<sub>50</sub> Bestimmungen)</li> <li>5. Nachweis der Expression von Tumorsuppressoren und Stammzellmarkern (Western-Blot-Analysen, Immunfluoreszenzanalysen, Immunhistochemie, quantitative Realtime PCR)</li> <li>6. Aufreinigung von rekombinanten Proteinen</li> </ol> </li> </ul>
LITERATUR	<ol style="list-style-type: none"> <li>[1] Bischof J, Muller A, Fander M, Knippschild U, Fischer D. Neurite outgrowth of mature retinal ganglion cells and PC12 cells requires activity of CK1delta and CK1epsilon. PLoS One 2011;6(6):e20857.</li> <li>[2] Brack AS, Munoz-Canoves P. The ins and outs of muscle stem cell aging. Skelet Muscle 2015;6:1.</li> <li>[3] Brouwer M, Zhou H, Nadif Kasri N. Choices for Induction of Pluripotency: Recent Developments in Human Induced Pluripotent Stem Cell Reprogramming Strategies. Stem Cell Rev 2016;12(1):54-72.</li> <li>[4] Buske P, Przybilla J, Loeffler M, Galle J. The intestinal stem cell niche: a computational tissue approach. Biochem Soc Trans 2014;42(3):671-7.</li> <li>[5] Chen KG, Mallon BS, McKay RD, Robey PG. Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics. Cell Stem Cell 2014;14(1):13-26.</li> <li>[6] Desai N, Rambhia P, Gishto A. Human embryonic stem cell cultivation: historical perspective and evolution of xeno-free culture systems. Reprod Biol Endocrinol 2015;13:9.</li> </ol>

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## ▲ Vorlesungsplan

Kapitel 1: Gesetzeslage der Stammzellforschung

Kapitel 2: Allgemeine Grundlagen, Embryonale-, Adulte- und Tumor-Stammzellen

Kapitel 3: Stammzellnischen

Kapitel 4: Signaltransduktion

Kapitel 5: Stammzellkulturen und Methoden in der Stammzellbiologie

Kapitel 6: Stammzellalterung

Kapitel 7: Stammzelltherapie

## ▲ Kapitel 1 - Gesetzeslage der Stammzellforschung



In diesem Kapitel können Sie sich mit der nationalen und internationalen Gesetzeslage zur Stammzellforschung vertraut machen.



Skript: Gesetzliche Richtlinien

## ▲ Kapitel 2 - Allgemeine Grundlagen, Embryonale-, Adulte- und Tumor-Stammzellen



In diesem Kapitel werden allgemeine Grundlagen rund um das Thema Stammzellen sowie embryonale und adulte Stammzellkulturen vermittelt. Des Weiteren wird die Rolle der Tumorstammzellen bei der Entstehung von Tumorerkrankungen dargestellt. Nach Abschluss dieses Kapitels sollen die Studierenden in der Lage sein die Charakteristika der verschiedenen Stammzelltypen benennen zu können sowie die Begriffe der Selbsterneuerung und Potenz beschreiben können. Den Studierenden wird ein Einblick in die Stammzelltypen und die zugehörigen Modelle für verschiedene Gewebe gegeben. Ferner wird auch das Konzept der Tumorstammzellen eingeführt.

# The Cancer Stem Cell Niche

*How Essential Is the Niche  
in Regulating Stemness of Tumor Cells?*

by Natalie Nilson

edited by Uwe Knippschild

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## 1. Introduction

In the recent years, cancer therapy has been improved. Nevertheless, many cancer patients still fail therapy resulting in disease progression, recurrence and decreased overall survival. These factors are suggested to be influenced by intratumoral heterogeneity (Hanahan, Weinberg 2011). The cancer stem cell (CSC) and the clonal evolution models have been proposed to explain the development mechanisms of this heterogeneity. However, the most recent theory, the phenotypic plasticity model, has added additional complexity to the existing models as it considers the observed bidirectional conversion of non-CSCs to CSCs (Hanahan, Weinberg 2011; Cabrera et al. 2015).

CSCs exhibit stemness that is defined as the self-renewal and differentiation ability of stem cells (Gupta et al. 2009). The self-renewal capacity of CSCs has been demonstrated by serial transplantations of selected tumor cell populations in immune-deficient mice or by serial passages of colonies *in vitro* (Beck, Blanpain 2013; Al-Hajj et al. 2003; Bonnet, Dick 1997). According their name, CSCs share many features with somatic stem cell like the expression of tissue-specific “stem cell markers”, activation or deregulation of signaling pathways associated with stem cell function like Wnt, Hedgehog and Notch and the phenotypic plasticity (Pardal et al. 2003; Reya et al. 2001).

Another parallel of CSCs to normal stem cells is their residence in niches. For example, recent publications showed that glioblastoma multiform, an aggressive type of primary brain tumor in humans, depends on a similar niche as neural stem cells (Calabrese et al. 2007; Charles et al. 2010). The validity of the niche concept has been also demonstrated in other cancers including leukemia, skin, colon and breast cancers (Kise et al. 2016). The cellular heterogeneity is also influenced by the niche as multiple niches can coexist in a tumor giving rise to various different CSCs.

The CSC niche maintains the CSC properties to self-renew and to generate progeny while staying in an undifferentiated state themselves (Calabrese et al. 2007). Thus, the tumor microenvironment (TME) comprising the CSC niche contributes to tumor initiation and progression. Depending on the specific tumor the TME, also known as tumor stroma, can include cancer cells, CSCs, mesenchymal stem cells (MSCs), adipocytes, activated fibroblasts, endothelial cells, pericytes and immune cells which interact through networks of cytokines and growth factors and are surrounded by an extracellular matrix (ECM) (Korkaya et al. 2011; Hanahan, Coussens 2012).

## Introduction

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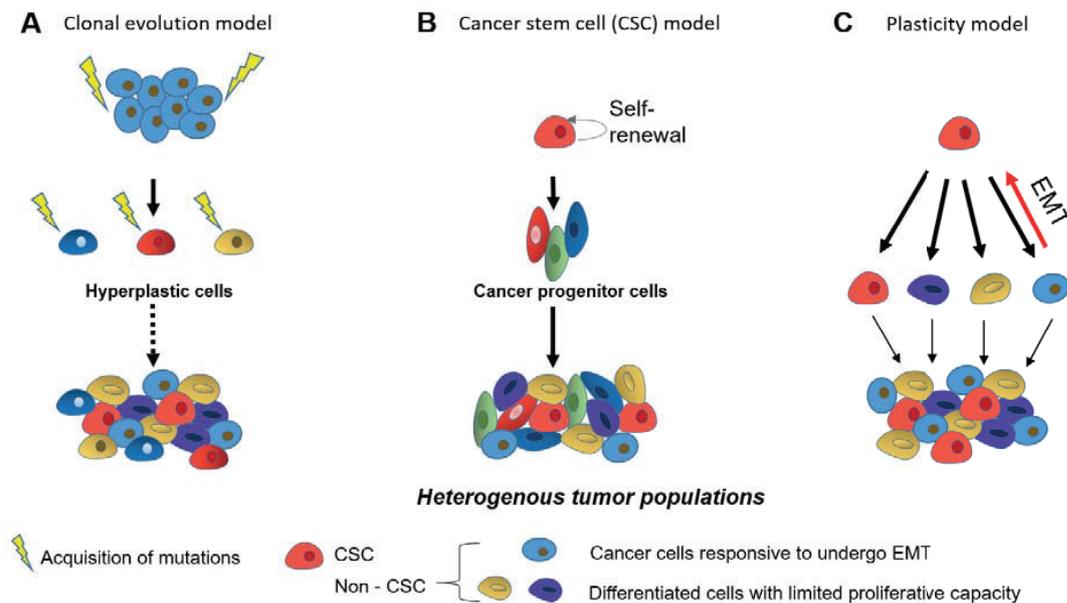
It is widely accepted that the TME and CSCs are a dynamic network (Hanahan, Weinberg 2011) as for instance CSCs can transform neighbor fibroblasts into cancer-associated fibroblasts (CAFs) that have increased proliferation rates (Kalluri, Zeisberg 2006). CSCs can also serve as a vascular progenitor for angiogenesis (Alvero et al. 2009). On the other hand, for example, endothelial cells can support the stemness of CSCs (Calabrese et al. 2007).

Furthermore, the TME is assumed to preserve the phenotypic plasticity of CSC, protect them from the immune system and facilitate their metastatic potential (Plaks et al. 2015). Consequently, the TME and the CSCs are important therapeutic targets as it is assumed that the eradication of CSCs most likely could prevent cancer relapse (O'Brien et al. 2007; Borovski et al. 2011; Ma et al. 2008).

The focus of this seminar paper is on the CSC and the impact of the TME on their stemness potential. First, the theories of tumor heterogeneity and the CSC plasticity are described followed by the role of the TME in tumor initiation, tumor progression and formation of metastasis. Eventually, examples of TME targets for cancer therapy are given to highlight its clinical potential.

## 2. Three Models for Cancer Stem Cell Heterogeneity

Cancer stem cell heterogeneity has been hypothesized to contribute to disease progression and therapy efficacy (Hanahan, Weinberg 2011). Two models, the clonal evolution model and the CSC model, have been postulated describing the mechanisms of cancer development (Figure 1). However, these models are based on different considerations regarding CSCs they are not mutually exclusive. In the recent years, a third theory, the cancer stem cell plasticity, has been described which combines the other two theories into one model (Plaks et al. 2015).



**Figure 1: Different models of carcinogenesis and tumor heterogeneity**

(A) Clonal evolution model or stochastic model suggests that serial acquisition of mutations generates tumor cell heterogeneity and that the cells are capable of renewal and tumorigenesis. (B) Cancer stem cell (CSC) or hierarchical model assumes that heterogenous tumor populations are organized into a hierarchy; CSC with self-renewal and tumor growth initiating capacity as the apex. (C) The plasticity model states that differentiated tumor cells can dedifferentiate via the epithelial-to-mesenchymal transition (EMT) to CSC and therefore can regain the stem-cell like properties.

### a. Clonal Evolution Model

The stochastic model or the clonal evolution model states that tumor cells are equipotent and the accumulation of somatic mutations lead to cancer development (Vogelstein, Kinzler 1993). This sequential acquisition of oncogenic mutations which can be caused by genetic instability can lead to the formation of hyperproliferating cells which can further develop to tumor cells by acquiring additional mutations (Nowell 1976).

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### Three Models for Cancer Stem Cell Heterogeneity

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Most of these mutant cells are eliminated because of metabolic disadvantage or immunologic destruction. But one mutant which gained a selective advantage becomes a precursor of a predominant tumor subpopulation (Nowell 1976).

According to this model, tumor heterogeneity is caused by the stochastic proliferation and self-renewal capacity of some tumor cells while other tumor cells differentiate (Beck, Blanpain 2013). As the intrinsic variation in cancer genomes have been suggested to be the “driving” force of tumor cell activities tumor genome sequencing revealed two to eight “driver” mutations regulating cell fate, cell survival, and genome maintenance in a typical tumor (Vogelstein et al. 2013). For example, some colorectal cancers most likely adhere to the stochastic model as molecular alterations in the tumor accumulated corresponding to the tumor progression (Vogelstein et al. 1988).

In general, the clonal evolution model postulates that any tumor cell can be involved in tumor progression and that tumor heterogeneity and therapy resistance is achieved due to the evolution of tumor cells along the Darwinian selection line (Campbell, Polyak 2007).

#### **b. Cancer Stem Cell Model**

Based on the hierarchical model, also called CSC model, CSC are the cells which generate and propagate malignant tumor cells. This model closely resembles the hematopoietic paradigm of stem cell biology as multi-potent CSCs with self-renewal capacity build the apex of unidirectional differentiation hierarchy of carcinogenesis. Consequently, tumor heterogeneity is achieved due to a small CSC subpopulation with unlimited growth and self-renewal capacity and a big population of tumor cells which are descendants of CSC.

CSCs are widely assumed to develop from normal stem or progenitor cells (Plaks et al. 2015). Propagation of CSCs lead to formation of cancer progenitor cells. These cells are more committed tumor cells that are part of the tumor system and are able to further differentiate to tumor cells with low proliferative capacity (Bonnet, Dick 1997; Kreso, Dick 2014).

The CSCs were first observed in acute myeloid leukemia. Bonnet and Dick found that leukemic cells expressing stem cell markers (CD34<sup>+</sup>, CD38<sup>-</sup>) showed self-renewal, propagation and differentiation potential in immune-deficient mice models defining those cells as leukemic stem cells (Bonnet, Dick 1997). The first solid tumor in which CSCs were identified was breast cancer (Al-Hajj et al. 2003). Many studies have reported that tumor initiating cells (TICs) or CSCs were isolated from tumors based on stem cell surface markers which were able to initiate cancer on transplantation into immunocompromised mice (Eramo et al. 2008; O'Brien et al. 2007; Vermeulen et al. 2008; Ricci-Vitiani et al. 2007). Generally, CSCs are assumed to

### Three Models for Cancer Stem Cell Heterogeneity

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generate a tumor, lead to tumor reestablishment and metastasis due to its self-renewal capacity (Bonnet, Dick 1997; Al-Hajj et al. 2003). Therefore, the eradication of CSCs during cancer treatment is of great importance (O'Brien et al. 2007).

However, studies showed that neither the clonal evolution model nor the CSC model are exclusive and its combination might be “the theory” of carcinogenesis und tumor heterogeneity (Campbell, Polyak 2007; Tang 2012). Especially, the “extension of the CSC model” to a “bidirectional model” is widely accepted as studies has revealed that differentiated cells can de-differentiate to generate CSCs (Chaffer et al. 2011). This additional complexity of the CSC model is considered in the third model of tumor heterogeneity, the cellular plasticity.

#### **c. Cellular Plasticity**

The phenotypic plasticity may be the model explaining the observed heterogeneity in solid tumors. This theory posits that CSCs are a dynamic population as non-CSC like differentiated cells can be converted back to CSC (Marjanovic et al. 2013). Due to this conversion, the generated undifferentiated cancer cells gain stemness traits (Mani et al. 2008).

Cellular plasticity is given by the activation of latent embryonic program, the epithelial to mesenchymal transition (EMT) (Singh et al. 2004). The EMT involves phenotypic changes including the loss of cell adhesion, cell polarity and the acquisition of migratory and invasive properties (Thiery et al. 2009). This physiological process occurs during embryonic development enabling the migration of mesodermal cells during gastrulation or the delamination of the neural crest cells from the dorsal neural tube. Several rounds of the conversion of mesenchymal to epithelial cells, the mesenchymal to epithelial transition (MET), and EMT are needed for the final differentiation of specialized cell types and the development of the complex three-dimensional structure of internal organs; thus, these conversions are reversible processes (Thiery et al. 2009). The generation of CSC-like cells by EMT is demonstrated by studies in different tumor types including breast (Mani et al. 2008), pancreatic (Wang et al. 2009) and colorectal tumors (Fan et al. 2012). These evidences reveal that not all cancer cells adhere to the unidirectional hierarchical model but rather have a dynamic ability to shift from a non-CSC state to a CSC state and *vice versa* building a plastic and dynamic CSC population. Further, tumor relapse after initial successful T-cell therapy is suggested to be caused by phenotypic plasticity of melanoma cells in an inflammatory environment (Landsberg et al. 2012). Several external signals are able to trigger EMT in cancer pointing out the importance of the TME and the cross talk between its heterogenous cell populations (Thiery et al. 2009).



**a. Cancer-associated fibroblasts**

Many studies have postulated that fibroblasts in the TME showing a modified phenotype similar to the activated fibroblasts during wound healing have an important role in cancer progression (Kalluri, Zeisberg 2006; Kalluri 2016). These cancer-associated fibroblasts (CAFs) support tumorigenesis as they trigger angiogenesis, cancer cell proliferation, invasion and mediate tumor-enhancing inflammation and resistance to antiangiogenic therapy (Erez et al. 2010; Crawford et al. 2009).

Within tumor tissue the persistent accumulation of cancer cells causes continuous tissue injury. This stimulates a chronic host repair response involving activated fibroblasts forming cancer fibrosis, tumor stroma or TME (Kalluri 2016). In contrast to the fibroblast activation in wound healing, the activation of CAFs in cancer is irreversible. This difference might be caused by epigenetic alterations (Mrazek et al. 2014; Hu et al. 2005). The observed epigenetic changes in CAFs compared to normal activated fibroblasts (NAFs) may also explain the increased tumorigenesis being associated with presence of CAFs in a tumor. For example, CAFs show in contrast to NAFs an enhanced migratory potential and an increased secretome. The TME milieu most likely also contributes to the tumorigenesis enhancement by CAFs (Kalluri 2016).

Kalluri proposed a classification of subtypes of CAFs based on their function because tumor-restraining (F1 subtype) and tumor-promoting (F2 subtypes) CAFs are observed in tumors. Further, they speculated that specialized CAFs with enhanced growth factor secretome activity affecting tumor progression may represent the F3 subtype and that the F4 subtype may constitute ECM-producing CAFs remodeling the ECM content of the TME (Kalluri 2016).

In general, the fibroblasts recruitment to tumor tissue are assumed to be stimulated by growth factors secreted by cancer cells and the infiltrating immune cells (Kalluri 2016) like the transforming growth factor (TGF- $\beta$ ), platelet-derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2) known as fibroblasts activators in chronic fibrosis (Elenbaas, Weinberg 2001). For example, TGF- $\beta$  stimulates the proliferation of CAFs and correlates with the increased deposition of collagen (Aoyagi et al. 2004; Lohr et al. 2001). In lung cancer, the expression of Thrombospondin 2 (THBS2) by metastatic cancer cells have been correlated to the activation of lung fibroblasts (Kalluri 2016).

CAFs secrete several molecules that have a pro-tumorigenic role (Kalluri 2016). It has been shown that the paracrine signaling of CAFs and CSCs maintains the stemness of lung cancer and supports tumor growth (Chen et al. 2014). More precisely, the insulin-like growth factor-II/IGF1 receptor/Nanog pathway of CAFs and CSCs was revealed to regulate the plasticity of lung cancer stemness (Chen et al. 2014). Furthermore, CAFs trigger stemness of CSCs via the

activation of Wnt and Notch pathways. For example, the secretion of hepatocyte growth factors (HGFs) of myofibroblasts has been postulated to stimulate de-differentiation of differentiated cancer cells to CSC-like cells by reactivating the Wnt pathway (Vermeulen et al. 2010). Canonical Wnt is a major pathway regulating CSCs and stemness in many cancers. Interestingly, it was stated that in some cancers the elevated Wnt signaling was mainly caused by increased expression of secreted Wnt ligands (autocrine signaling) indicating the importance of external factor (Saito-Diaz et al. 2013).

CAFs have been also observed to induce EMT. CAFs-derived galectin-1 was found that it binds to  $\beta 1$  integrin in the ECM. This leads to the induction of EMT in gastric cancer through  $\beta 1$  integrin-mediated upregulation of the glioma-associated oncogene 1 in gastric cancer (Chong et al. 2016).

Other features of CAFs contribute to the CSC plasticity and tumor progression as well; for example their capacity to remodel the ECM. Therefore, CAFs are considered as the primary source of type I collagen (Kalluri 2016) which inhibited differentiation of CSCs and promoted a stem-cell like phenotype in human colorectal carcinoma cells (Kirkland 2009). In general, CAFs remodel the ECM as they produce different matrix metalloproteinase and therefore contribute to the generation and maintenance of the CSC niche (Kalluri 2016).

Further, CAFs are also correlated to the drug resistance of CSCs. A study suggests that drug resistance of CSCs is not only caused by their inherent resistance capacity but can be also stimulated by exosomes secreted by stromal fibroblasts which prime CSCs (Hu et al. 2015). Exosomes are defined as small homogeneous membrane particles of endocytic origin ranging in size from 40 to 100 nm (Lee et al. 2012). In esophageal squamous cell carcinoma, Zhang et al. found that CAFs conferred chemo-resistance of cancer cells via secretion of TGF- $\beta 1$  and that TGF- $\beta 1$  was associated with poor prognosis of patients treated with chemoradiotherapy (Zhang et al. 2016). This result highlights the importance of TME components as potential new targets of cancers therapy.

### **b. Mesenchymal Stem Cells**

Mesenchymal stem cells (MSCs) are undifferentiated, multipotent stromal cells which exert stimulatory effects on tumor growth (Beckermann et al. 2008), invasion (Nomoto-Kojima et al. 2011) and metastasis (Karnoub et al. 2007) via direct and indirect interactions with tumor cells. The common identification and characterization of MSCs is based on specific cell surface markers although they are not cell type specific (Dominici et al. 2006). Therefore, a minimal criteria to define human MSCs has proposed by the International Society for Cellular Therapy

factors then triggers the activation of  $\beta$ -catenin signaling in cancer cells leading to the generation of CSCs (Li et al. 2012). Moreover, it was suggested that MSCs can influence tumor progression by secreting Gremlin 1 as a mesenchymal bone morphogenetic protein (BMP) antagonist to promote the undifferentiated state (Davis et al. 2015).

Alternatively, exosomes which are released by MSCs can provide tumor cells with CSC properties by transferring e.g. micro-RNAs (miRs) (Vallabhaneni et al. 2015). Cuiffo et al. reported that MSCs can cause aberrant expression of miR199a in breast cancer cells leading to repressed expression of Forkhead box protein P2 (FOXP2) and providing enhanced CSC features. These elevated miR199a levels and depressed FOXP2 expression levels are known as outstanding properties of malignant breast cancer and are correlated with poor survival (Cuiffo et al. 2014). Further, the MSC-derived exosomes have been shown to induce EMT in tumor cells and promote malignant cell proliferation and migration (Shi et al. 2016).

All in all, MSCs can regulate cancer cell behavior through their effect on CSCs. However, it was also shown that MSCs could inhibit cancer proliferation. Although these molecular mechanisms are not fully known, for example DKK-1 (dickkopf-1) secreted by MSCs was shown to act as a negative regulator of WNT signaling pathway leading to the inhibitory effect (Zhu et al. 2009).

### **c. Inflammatory Cells**

The immunosuppressive TME restricts the role of the immune system which is the limitation of tumor spread. Thus, the role of inflammatory cells in the CSC niche is of great interest.

In general, the immunosuppression in the TME of the cytotoxic function and infiltration of natural killer cells (NK) and CD8<sup>+</sup> T cells is important for the evasion of immune surveillance (Kitamura et al. 2015). A suggested mechanisms is that human leukocyte antigen (HLA) class I antigens and NK-cell-activating ligands are released by tumor cells and may interfere with the function of cytotoxic cells (Campoli, Ferrone 2008). Further, many cancer-cell-intrinsic characteristics were reported to influence the immune response like the down-regulated expression of HLA class I enabling the escape of immune attacks (Zitvogel et al. 2006).

The secretion of cytokines and chemokines of cancer cells within the TME lead to recruitment of tumor-associated macrophages (TAMs), tumor-associated neutrophils (TANs), and a population functionally identified as myeloid-derived suppressor cells (MDSCs). TAMs and TANs are derived from polarized macrophages and neutrophils respectively and are pro-tumor phenotypes facilitating tumor growth and affecting angiogenesis (Kim, Bae 2016). Within established tumors, TANs can recruit immunosuppressive regulatory T cells (T<sub>reg</sub>s) into the

including the ability to adhere to plastic, the overexpression and lack of specific cell surface markers and their differentiation capacity into adipocytes, osteoblasts and chondrocytes (Dominici et al. 2006). Other unique characteristics of MSCs are their ability to migrate to sites of injury, inflammation and tumors and their immunosuppressive properties (Poggi, Giuliani 2016; Chamberlain et al. 2007). MSCs seems to migrate specially towards growth factors like vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and PDGF which are present, e.g. in pancreatic tumors. Moreover, they are suggested to have a supportive role in angiogenesis (Beckermann et al. 2008).

In terms of the TME, the term MSC and CAF are sometimes used interchangeable (Kalluri 2016) whereas other reviews clearly differentiate between them (Plaks et al. 2015; Carnero et al. 2016; Borovski et al. 2011). Kalluri hypothesized that due to similar properties of ‘activated’ fibroblasts and MSCs, like synthesizing ECM, generating cytokines and chemokines, recruiting immune cells, all or only a subset of activated fibroblasts are MSCs (Kalluri 2016). A study of inflammation-induced gastric cancer in mouse models revealed that 20% of CAFs originate from bone marrow and derive from MSCs (Quante et al. 2011). However, many different cells including resident tissue fibroblasts, bone marrow-derived MSCs, hematopoietic stem cells, epithelial cells and endothelial cells are considered possible predecessors of CAFs (Shiga et al. 2015).

Generally, MSCs play an important role during tumorigenesis featuring different types of intercellular communication (reviewed by (Melzer et al. 2016)) and promote cancer niche formation sustaining cancer progression (Quante et al. 2011). It is widely accepted that these different types of indirect and direct interactions of MSCs and cancer cells are always multidirectional, therefore influencing tumor cells as well as MSCs or other cellular partners (Melzer et al. 2016).

In breast cancer, aldehyde dehydrogenase expressing MSCs regulate CSCs through cytokine loops involving interleukin 6 (IL6) and CXCL7. IL6 secreted by cancer cells triggers a signaling-mediated chemotaxis of MSCs facilitating further MSC recruitment to the TME and induces CXCL7 production by MSCs. CXCL 7 stimulates the cancer cells to secrete additional cytokines, IL6 and interleukin 8 (IL8), to generate a positive feedback loop (Liu et al. 2011). Other studies showed that IL8 promotes self-renewal and invasive properties of CSCs (Ginestier et al. 2010; Charafe-Jauffret et al. 2009). Moreover, MSC-derived CXCL12 and IL6/8 also facilitate stemness in part through the NF- $\kappa$ B pathway (Cabarcas et al. 2011).

Another carcinoma cell-derived cytokine influencing the CSC pool is interleukin 1 (IL1). IL1 induces the secretion of prostaglandin E 2 (PGE<sub>2</sub>) and other cytokines by MSCs. These secreted

TME by secreting CCL17 and CCL22. T<sub>reg</sub>s then lead to suppression of an anti-tumor immune response (Mishalian et al. 2014). TAMs and MDSCs contribute to tumor progression via different mechanisms including promotion of immune dysfunction like T<sub>reg</sub> induction and non-immune related mechanisms as they induce angiogenesis, promote metastasis and stemness (reviewed by (Ugel et al. 2015; Kumar et al. 2016; Marvel, Gabrilovich 2015)). For example, CCL22 secreted by TAMs and IL6 and TGF- $\beta$  produced by MDSCs stimulate the recruitment of T<sub>reg</sub> and T helper 17 (Th17) cells. Th17 cells then secrete IL17 promoting the further recruitment of MDSCs and the production of granulocyte colony stimulating factor (G-CSF) from CAFs. This stimuli further promotes the immunosuppressive function (Kitamura et al. 2015). The complexity of these exemplary cell interactions highlights the extent of cross talks within the TME.

Due to the fact that TAMs can constitute up to 50 % of a tumor mass being a major component of immune cell infiltrate in the TME (Chanmee et al. 2014) the interaction between CSCs and TAMs will be discussed in more detail in the following paragraphs.

TAMs have been found to be present at all stages of tumor, promoting stem-like features of CSCs, tumor progression, metastasis, and CSC chemoresistance (Sainz, JR et al. 2016). Their potential to activate the CSC compartment most likely explains the correlation between high number of TAMs within a tumor and rapid disease progression and poor patients outcome (Bingle et al. 2002; Qian, Pollard 2010).

Macrophages are a very plastic population assuming multiple phenotypes in response to changing environmental cues (Mosser, Edwards 2008). Within a tumor, different TAMs subtypes can be identified which are driven by their interaction with TME-secreted factors. This means that angiogenesis promoting, metastasis-promoting, immunosuppressive and CSC-promoting TAMs show specific gene signatures. However, due to their plasticity TAMs can shift between subtypes based on stimuli (Sainz, JR et al. 2016).

In general, TAMs are considered to interact with CSCs within the TME to regulate CSC renewal and maintenance. An increase of a CSC population was shown if CSC were co-incubated with TAMs (Huang et al. 2016) indicating its correlation. Signal transducer and activator of transcription 3 (STAT3) and NF- $\kappa$ B are key regulators of CSCs and TAMs interaction (Sainz, JR et al. 2016). Yang et al. found that TAMs promote CSC-like phenotypes in murine breast cancer cells by increasing their expression of Sox-2 via the epidermal growth factor receptor (EGFR)/ STAT3/Sox-2 paracrine signaling pathway (Yang et al. 2013). Moreover, TAMs secrete various inflammatory cytokines like IL6 and IL8. The activation of STAT3 by IL6 was shown to directly stimulate the self-renewal of CSCs in breast cancer xenografts (Iliopoulos et

al. 2009). It was also shown that milk fat globulin epidermal growth factor-8 (MFGE-8) and IL6 secreted by TAMs induced tumor potential and CSC chemo-resistance through STAT3 and Hedgehog signaling (Jinushi et al. 2011).

Another proinflammatory cytokine produced by TAMs and Th17 cells is IL17 that has been demonstrated to promote the self-renewal of ovarian CD133+ cancer stem-like cells through a mechanism involving NF- $\kappa$ B and p38 mitogen-activated protein kinase (MAPK) (Xiang et al. 2015).

Many studies have shown that crosstalk between TAMs and CSCs affects CSC properties. In turn, there are also evidences suggesting that CSCs can stimulate macrophage recruitment and enhance its pro-tumorigenic phenotype (Sainz, JR et al. 2016). Zhou et al. found in glioblastoma multiforma that high levels of periostin secreted by CSCs correlated with increased TAM density. This results showed that CSC can recruit TAMs from the peripheral blood (Zhou et al. 2015). Further, the deletion of periostin in CSCs resulted in reduced M2 TAM density, reduced tumor growth and increased survival in xenografts indicating that this recruitment pathway may be an interesting target for therapy.

TAMs have been reported to promote an EMT phenotype in non-CSCs (Bonde et al. 2012) and recently also in CSCs (Sainz, JR et al. 2015; Sainz, JR et al. 2014). Hallmarks of EMT are the de-differentiation, acquisition of a migratory phenotype including the loss of cell adhesion. TAMs among others secrete tumor necrosis factor alpha (TNF- $\alpha$ ) upregulating NF- $\kappa$ B signaling pathways to induce Snail and Twist. This leads to the increased cross-talk with TGF- $\beta$  signaling pathways stimulating self-renewal (Cabarcas et al. 2011) and inducing EMT. The overexpression of the IFN-stimulated factor ISG15 in pancreatic ductal adenocarcinoma by TAMs in response to IFN $\beta$  secretion of CSCs enhanced the self-renewal of CSCs (Sainz, JR et al. 2014). Another factor, the immunomodulatory cationic antimicrobial peptide 18/leucine leucine-37 (hCAP-18/LL-37), which was also observed to be overexpressed by TAMs in pancreatic ductal adenocarcinomas was also found to potentiate the inherent properties of CSCs (Sainz, JR et al. 2015).

Macrophages are also known to play a major role in tumor invasion and metastasis (Noy, Pollard 2014; Kitamura et al. 2015) which will be discussed in the next chapter of this seminar paper.

**d. Hypoxia and Angiogenesis**

Hypoxia is known to be a major characteristic of the TME as it develops within a solid tumor due to impaired vascularization (Harris 2002). It is considered to promote the CSC phenotype, tumor initiation, progression and resistance to therapy and therefore may contribute to cancer development through several independent pathways which are most likely interconnected (Carnero, Leonart 2016).

Hypoxia-inducible factors (HIFs) enables the cellular adaptation to the hypoxic environment as they control the regulation of genes involved in angiogenesis, EMT, tumor invasion, metastasis and cellular metabolism (reviewed by (Garcia-Heredia et al. 2015; Ratcliffe 2013) for animal cancer models). A correlation between the expression HIF-1 $\alpha$  and HIF-2 $\beta$  involving hypoxic transcriptional signatures, aggressive cancer and metastasis have been found in breast cancer models (Semenza 2016) indicating the clinical importance of the hypoxic environment.

Hypoxia and the HIFs were demonstrated to contribute to increased CSC fractions and dedifferentiation to a CSC-like phenotype (Heddleston et al. 2010; Jogi et al. 2002). In glioblastoma, Heddleston et al. showed that hypoxia triggered a more-stem like phenotype with upregulated important stem cell factors as Oct4, Nanog and c-Myc (Heddleston et al. 2009). Interestingly, they showed that HIF-2 $\alpha$  was essential only in CSCs whereas HIF-1 $\alpha$  functioned in proliferation and survival of all cancer cells. This finding indicates the clinical importance of HIF-2 $\alpha$  as it may be used for selective eradication of CSCs. However, HIF-1 $\alpha$  was also reported to inhibit cell proliferation and enhances stemness as it antagonizes c-Myc inducing cell cycle arrest (Koshiji et al. 2004). Another interesting effect of HIF-1 was demonstrated in cervical cancer cells. Yatabe et al. found that hypoxia activates the telomerase via transcriptional activation of human telomerase reverse transcriptase gene and that HIF-1 is involved in this process as a transcription factor (Yatabe et al. 2004). Thus, this study suggested a novel mechanism of telomerase activation in cervical cancer.

Moreover, hypoxia increases reactive oxygen species (ROS) which promotes CSC survival and stemness by upregulating stress signaling pathways (Liu et al. 2008). In cell culture or xenograft models, an activation of stem cell regulatory pathways such as Akt/ $\beta$ -catenin or Notch were observed due to exposure to hypoxia (Conley et al. 2012; Irshad et al. 2015). These studies suggested different signaling pathways stimulating CSC maintenance in a hypoxic TME.

An increase of the CSC fraction within a tumor can be facilitated by dedifferentiation of cancer cells through EMT. Several studies have verified that hypoxia and high levels of HIF-1 $\alpha$  can promote the EMT phenotype. In breast cancer, hypoxia-mediated Notch signaling induced expression of the transcription factors Slug and Snail to promote EMT. Another hallmark of

EMT, the decreased expression of E-cadherin was also observed within this study (Chen et al. 2010).

Additionally, the hypoxic environment has been also demonstrated to contribute to chemo- and radiotherapy resistance. For example, hypoxia stimulates the quiescence of cancer cells and reduces radiation efficacy which depends on oxygen levels (Muz et al. 2015).

The immune resistance and immune suppression is also affected by a hypoxic microenvironment stimulating tumor cells to evade immune surveillance (reviewed by (Noman et al. 2015)). These immune-suppressive effects include among others shedding of immune-recognition molecules by tumor hypoxia resulting in decreased sensitivity to T- and NK-mediated killing (Siemens et al. 2008). Further, T<sub>reg</sub>S are promoted by HIFs to block immune effector cells (Sitkovsky 2009). The hypoxic environment inhibits also immune surveillance by inhibiting NK and CD8<sup>+</sup> T cells proliferation and activation. Further, macrophage phagocytosis was also inhibited (Wei et al. 2011).

Another important feature of a tumor is angiogenesis which is triggered by the activation of HIF genes of stromal cells and CSCs. In contrast to differentiated cancer cells, CSCs produce higher levels of pro-angiogenic factors like VEGF, IL8 (CXCL8) and stromal cell-derived factor 1 (SDF-1, CXCL12) that stimulates the proliferation, migration of endothelial cells forming new blood vessels (Ping et al. 2016; Bao et al. 2006b; Folkens et al. 2009). Further, VEGF-A then recruits monocytes and macrophages promoting angiogenesis (Kitamura et al. 2015). Moreover, CSC-derived microvesicles were found to stimulate angiogenesis as well (Grange et al. 2011).

#### **e. ECM-Cell Interactions and Cell-cell Contact**

The extracellular matrix (ECM) has been highlighted as an important noncellular component of the stem cell niche (Gattazzo et al. 2014) and the CSC niche (Lu et al. 2012). Interestingly, the deregulated ECM dynamics in cancer have been stated as a hallmark of cancer as the altered biochemical ECM properties potentiate the oncogenic effects of various growth factor signaling pathways and deregulate cell behavior (Lu et al. 2012; Multhaupt et al. 2016) .

The matrix consists of numerous proteins like collagens, fibronectin, laminins, glycosaminoglycans, e.g. hyaluronan, proteoglycans, matricellular proteins and ECM remodeling enzymes (Insua-Rodriguez, Oskarsson 2016). This diverse and dynamic network provides cells with biochemical and biomechanical cues influencing cell behavior. But on the other hand, changes of the ECM are also regulated by cellular activities demonstrating the regulatory feedback mechanism between cells and the ECM (Samuel et al. 2011). For example,

the role of hyaluronan in creating a specific TME which stimulates angiogenesis and tumor invasion was reviewed recently (Chanmee et al. 2016).

In contrast to normal tissue, fibrotic tumor tissues showed a 20-50 fold increase in tissue rigidity (Wei, Yang 2016). This increased ECM stiffness plays an important role in cancer progression and metastasis. Wei and Yang reviewed the role of tissue rigidity in tissue dissemination and in EMT regulation. Various factors including CAF-derived lysyl oxidase (LOX), which increases cross-links of collagen fibers, and caveolin-1 stimulate matrix stiffness and release of growth factors like TGF- $\beta$  that both promote EMT of cancer cells (Pickup et al. 2013). Stem-cell like cancer cells can then regulate ECM composition and modifications (Wei, Yang 2016). Further, this physical barrier can influence effectiveness of drugs and therefore protect CSCs from chemotherapeutics (Wong, Rustgi 2013).

Another abnormality observed in the ECM of tumors is the overexpression of matrix metalloproteinases (MMPs) that degrade ECM components, release various factors such as cytokines and growth factors and facilitate angiogenesis and metastasis (Noel et al. 2012; Kessenbrock et al. 2010). Generally, it is suggested that MMPs are produced by cells which are commonly found in the TME including inflammatory cells, ECs and CAFs (Kessenbrock et al. 2010). MMP9 is known as a regulator of angiogenesis because it regulates the bioavailability of sequestered VEGF that stimulates an angiogenic switch during cancer (Bergers et al. 2000). In fibroblasts and tumor cells, a membrane-tethered MMP, MT1-MMP, was identified that regulates invasion through the ECM and therefore drives tumor cell traffic (Sabeh et al. 2004). Additionally, it has been demonstrated that MMPs are also involved in EMT (Lochter et al. 1997) and increased Wnt signaling and stemness (Kessenbrock et al. 2015).

In normal stem cell niches, direct cell-cell contact of stem cells to stromal cells within the niche is very crucial as it shields them from differentiation stimuli and promotes their stemness (Borovski et al. 2011; Calabrese et al. 2007). CSCs are also dependent on cell-cell contact to maintain their typical state and features. The direct contact of non-small cell lung cancer cells to CAFs has been shown to induce EMT and to activate the hedgehog signaling pathway which increased the metastatic potential of the cancer cells (Choe et al. 2013). Further, Notch signaling pathways also require cell interactions as the Notch receptor is expressed on a cell surface and most of the Notch ligands are also membrane proteins (Guruharsha et al. 2012).

**Regulation of Stem Cell Self-Renewal and Oncogenesis by RNA Binding  
Proteins**

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**by Carina Huber**

**edited by Uwe Knippschild**

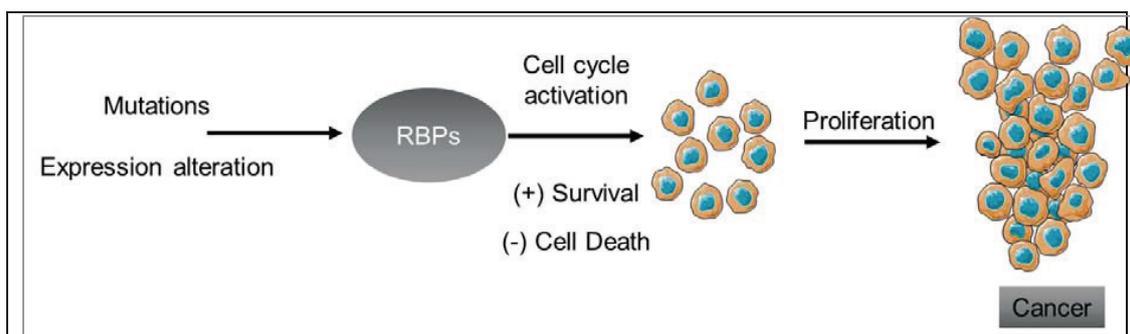
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## 1 Introduction

Stem cells in tissues and organs are responsible for maintaining their functionality under homeostasis. They promote the regeneration of tissues after damage or injury throughout life. Stem cells are distinguished from other cells because they are able to undergo self-renewal divisions (Suling Liu, Gabriela Dontu, Max S. Wicha 2005). Due to the mechanism of asymmetric division, stem cells can divide indefinitely while retaining the potential to differentiate into various cell types of the human body (Hattori et al. 2016). In normal tissues and organs the mechanism of asymmetric division is tightly regulated. Variations in the regulation are one of the key events in carcinogenesis and can promote tumor development and progression. The enhanced expression of distinct proteins can lead to development of cancer cell survival, increased proliferation and the feature to colonize other tissue (Hanahan D. 2000). Cancer-specific protein expression pattern were found in cancer cells, which indicates that the genes itself are not mutated, but aberrantly expressed (Sager R. 1997).

The regulation of RNA metabolism is mediated by a group of proteins called RNA-binding proteins (RBPs) (Campos-Melo et al. 2014). They modulate the metabolism of mRNAs in every step of RNA biology, from transcription, maturation/splicing, transport, stability, degradation and translation (Diana Guallar 2014). The post-transcriptional regulation of mRNAs by RBPs is essential for the determination of the cell fate (Lederer et al. 2014) due to the influence of expression levels of cancer related genes which can influence cell proliferation, invasion, metastasis, apoptosis of cancer (Kim et al. 2009b; Abdelmohsen, Gorospe 2010). A schematic overview about the influence of RBPs related to the development of cancer is shown in **Figure 1.1**.



**Figure 1.1:** Mutations or expression alteration of RBPs can lead to cell cycle activation, an enhanced survival and a reduced cell death which in turn can end up with cancer. Modification at the central point of RNA metabolism can impact the determination of cell fate towards an increased proliferation and cancer (Modified: Campos-Melo et al. 2014).

## Introduction

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RBPs consist of different RNA-binding domains (RBDs) which can associate with mRNAs to form ribonucleoproteins (RNPs) complexes (Shigunov, Dallagiovanna 2015). The RBDs are present in single or multiple copies through which they can interact with their target mRNA in sequence- and structure-dependent manner (Lukong K. E., Chang K. W., Khandjan, E. W. and Richard S. 2008; Lunde BM, Moore C, Varani G 2007). RBPs have multiple RBDs so that they can regulate multiple mRNAs. This results in a complex genetic network which characterization is essential to understand stem cell self-renewal and cancer development.

In this review the current understanding how RBPs can contribute oncogenesis due to its expression regulation of different oncoproteins and tumor suppressor proteins will be discussed.

### 1.1 Self-renewal

Stem cells can divide for indefinite periods asymmetrically or symmetrically. Symmetrically division leads to the generation of one daughter stem cell that has the same developmental potential similar to the mother cell (He et al. 2009). This process is called self-renewal and essential to maintain the stem cell pool in adult tissue (Gomez-Lopez et al. 2014) and to restore the stem cell pool after injury. The products of a symmetric division are two stem cells. Hence, the stem cell pool can be expanded (Suling Liu, Gabriela Dontu, Max S. Wicha 2005). The asymmetric division leads to one cell with the identical properties of the original cell, and a progenitor cell which undergo cellular differentiation (**Figure 2.1.**).

To maintain the undifferentiated state cell cycle control and a balance between proto-oncogenes (promoting self-renewal) and gate-keeping tumor suppressors (limiting self-renewal) is required. The regulation of self-renewal of normal stem cells is controlled by intrinsic factors (signaling pathways) and extrinsic cues from the niche and the microenvironment. Core intrinsic factors controlling the balance between self-renewal and differentiation are Wnt, Notch and Hedgehog pathways (Orkin SH & Zon LI 2008).

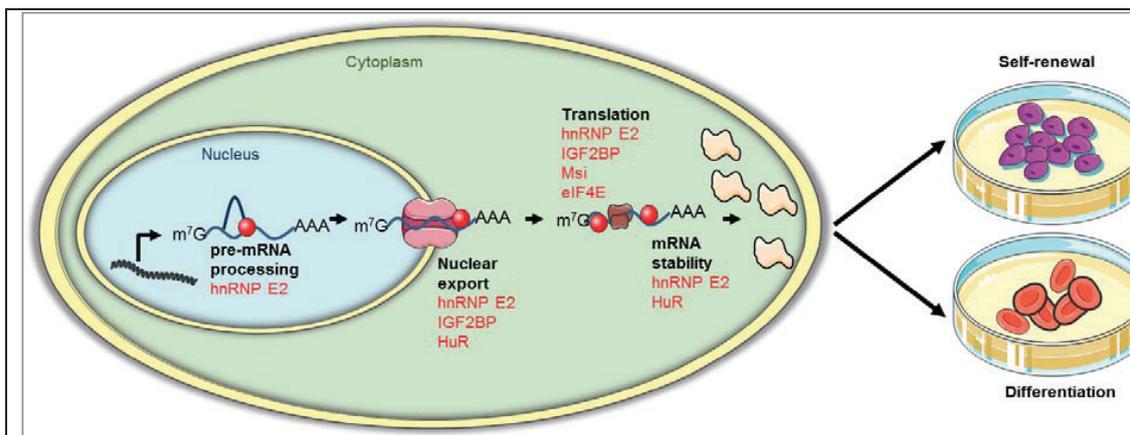
Defects in the regulation of self-renewal can lead to abnormal differentiation and the development of cancer. The presence of tumorigenic cancer cells with stem cell properties, the cancer stem cells (CSCs), supposes that the mechanism of self-renewal and their tight regulation play a crucial role in tumor maintenance. Therefore it is essential to understand the regulation mechanisms of self-renewal. The overexpression of RBPs in different cancer types suggests that the posttranscriptional regulation by RBPs is a key element in defects of the self-renewal mechanism which leads to cancer.

## Introduction

## 1.2 Post-transcriptional mechanisms

Post-transcriptional events like splicing, nuclear export, stabilization, localization and translation initiation are carefully regulated to reach a precise and tissue-specific control of gene expression (Keene 2007). Hence, post-transcriptional regulation seems to be an additional possibility of gene regulation to activation and repression at multiple transcription promoters.

RNA binding proteins play an essential role in every stem of mRNA maturation and processing and to determine the fate of the mRNA (**Figure 1.2**). RBPs regulate the splicing process, as well as alternative splicing, such as the RBP hnRNP E2. RBPs are also able to induce and to enhance the nuclear export of mRNAs like hnRNP E2, IGF2BP or HuR. In the cytoplasm, binding of RBPs to the 3'UTR of the target mRNA regulate mRNA stability and the level of translation. RBP that bind to the 3'UTR are hnRNP E2, IGF2bP, Musashi and eIF4E. The translation is also regulated by RBPs interacting with the 5'UTR of the transcript such as hnRNP2 E2 and HuR.

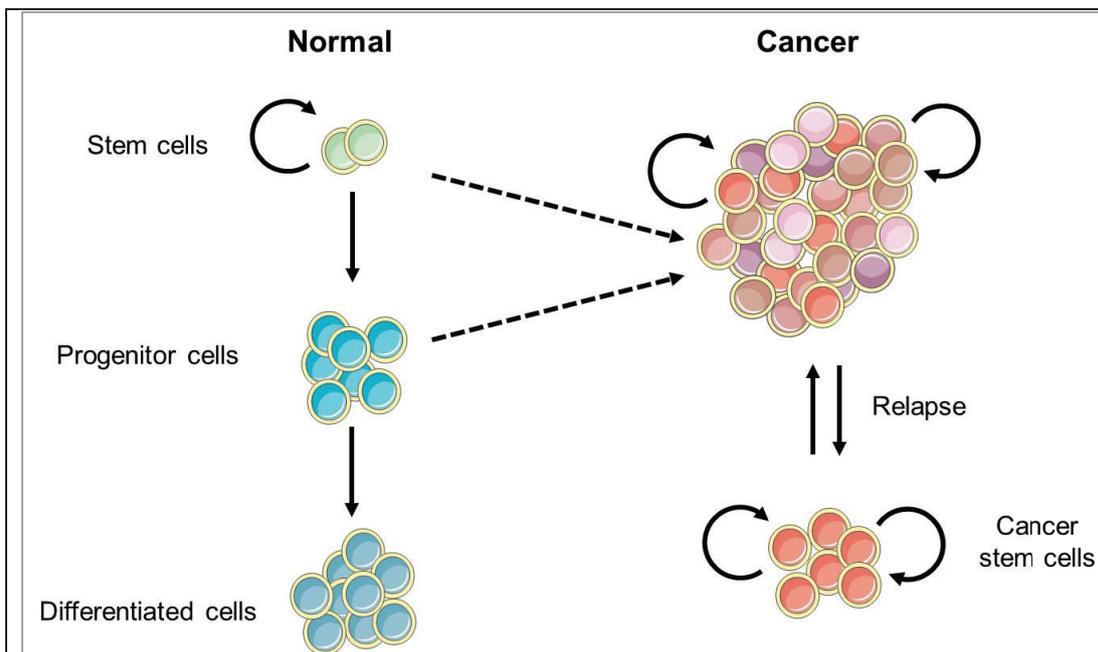


**Figure 1.2:** RBPs (red circles) involved in different steps of mRNA processing: RBPs regulate splicing and the export of the transcripts. RBPs can influence the mRNA stability and translation initiation by binding to the 3'UTR or 5'UTR of the transcript (Modified: Julia Ye, Robert Blelloch).

## 2 Stem Cell system in Tissue Homeostasis and Oncogenesis

Stem cell biology gives an insight into the cellular processes from development to aging and oncogenesis. Different types of stem cells are described based on their potency: Pluripotent stem cells are able to form any cell lineages in the body (Takashinas S., Hirose M., Ogonuki N., Ebisuya M., Inoue K., Kanatsu-Shinohara M., Tanaka T., Nishida E., Ogura A., Shinohara T. 2013). Types of pluripotent stem cells are embryonic stem cells, induced pluripotent stem cells, and germline stem cells. Adult stem cells are multipotent, which means that these cells can generate cell types of a given lineage.

Adult stem cells occur at low frequency in several tissues such as blood, intestine, brain, skin and skeletal muscle. These stem cells are responsible for tissue maturation, repair and regeneration and balancing the cellular turnover (Tuna, I. 2011). The stem cell themselves are slowly dividing, but their progenitor cells derived from the stem cell after asymmetric division are highly proliferative (Sherley JL 2002). The progenitor cells have the ability to differentiate into adult.....?



**Figure 2.1:** A stem cell system exist in normal tissue as well as in cancer. The adult tissue stem cell have the ability of self-renewal through asymmetric division. Thus generate a stem cell and a daughter cell that undergo differentiation processes while losing the self-renewal potential. Hence, stem cells can sustain the stem cell pool and maintain functional tissue like the hematopoietic system and intestinal tissue. The cancer tissue is distinguished by their functional and morphological heterogeneity. It often shows analog hierarchical structure as normal tissues with stem cells at the apex. The so called tumor stem cells are capable of self-renewal as well and can generate metastasis or regenerate the cancer after radiotherapy or chemotherapy. The cancer stem cells often use the same, but apparent, self-renewal program than normal stem cells (Modified: Hattori et al. 2016).

## Stem Cell system in Tissue Homeostasis and Oncogenesis

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In the following section the concept of stem cells and their ability to self-renew and differentiate is discussed on the basis of hematopoietic and intestinal stem cell system. Later on the hypothesis of transformation of stem cells leading to cancer stem cells will be pictured.

### 2.1 Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) reside in their niche in the bone marrow and can generate erythrocytes, megakaryocytes, monocytes, granulocytes, mast cell, T-and B-lymphocytes and natural killer cells. They exist at a very low frequency in the body (0.01% of the total nucleated cells) (Morrison 1994). In the hematopoietic system, three different populations of multipotent progenitors exist, which form a lineage to generate and regenerate the blood-forming and immune system (Reya et al. 2001). The long-term HSCs generate the short-term HSCs which in turn then generate the multipotent progenitors. They gradually lose their potential to self-renew, but therefore become more mitotically active. The multipotent progenitors have no more potential for self-renewal. During this process, the stem cells move inward in the central bone marrow and erase from their niche.

### 2.2 Intestinal stem cells

Intestinal stem cells (ISCs) are responsible for the renewal of the epithelium of the small and large intestine which is completely renewed every 4-5 days). The intestine consists of villi and crypts, where the stem cells of the small intestine reside. ISCs can generate enterocytes, goblet cells, Paneth cells and enteroendocrine cells. Two distinct types of ISCs pools exist in the intestine crypt. The active cycling crypt based cells which are positioned at the crypt bottom between the Paneth cells. The quiescent stem cells are at the +4 position located (Lopez-Garcia et al. 2010). Whereas the crypt based cells are responsible for maintaining the intestinal homeostasis are the +4 located cells injury-inducible reserve stem cells (Hattori et al. 2016).

The differentiated epithelial cells, the enteroendocrine cells and goblet cells occupy the intestinal villi whereas the undifferentiated cells reside in the crypt. There is an incremental upwards movement during differentiation of the enterocytes until they detach from the villus.

### 2.3 Cancer Stem Cells

Tumors are composed of various cell types thus resulting in heterogeneity in terms of morphology, cell surface marker, genetic lesions, cell proliferative kinetics and the response to therapy (Dick 2008) (**Figure 2.1**). The heterogeneity can also be seen within a tumor that has a clonal origin, initiated from a single cell. Currently there are two possible explanations for

## Stem Cell system in Tissue Homeostasis and Oncogenesis

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the generation of cellular heterogeneity. In the stochastic model are all tumor cells biological equivalent. Diverse extrinsic factors e.g. host factors, microenvironment and immune response or intrinsic factors e.g. levels of transcription factors and signaling pathways have different influences to the cells which lead to a random stochastic response (Dick 2008). The influences to the cells are unpredictable and random which give rise to the heterogeneous expression of cell surface markers or other markers of maturation. The cells differ also in their entry of cell cycle or tumor initiating capacity. Finally, through this process, tumor cell which can initiate tumor growth are selected. The hierarchy model implied that the tumor is a caricature of the normal tissue development. Within the tumor is a hierarchical organization with the cancer stem cells (CSCs) at the apex (Wang, Dick 2005). Like in other stem cell systems, for example blood or colon the cancer stem cells maintain the tumor by self-renewal followed by maturation and differentiation. These CSCs under analogue processes self-renew and differentiate like normal stem cells.

The essential difference between these two models is, that CSCs arise randomly within the tumor, since every cell has the potential given the right influences, which is in contrast to the hierarchical model where only a subset of the cells has the potential to propagate tumor growth. If the hierarchy model is correct, it would be possible, to divide the tumor in fractions with CSCs in one fraction and fractions with the absence of CSCs. If the stochastic model is correct it is not feasible to isolate CSCs because they can be found in every fraction (Dick 2008).

CSCs have distinct cell surface markers and a single cell can generate a heterogeneous population after transplantation thus coining the term tumor initiating cells. The properties of the CSCs subpopulation within the tumor are reflected by the heterogeneity of the tumor. CSCs exist in several tumors e.g. acute leukemia and solid tumors including brain, breast and colon (Ailles, Weissman 2007). Their ability to unlimited self-renewal capacity result in resistance to conventional tumor therapy like radiation or chemotherapy and the tumor relapse after therapy cessation or formation of secondary tumors, respectively (Hattori et al. 2016). The CSCs do not automatically originate from stem cells of normal conforming tissue (Jordan 2009) but can arise independent from tissue stem cells.

Furthermore, it is still not clear whether the ability of self-renewal is generated by a genomically advantaged stem cell or by a more differentiated cell which has reacquired the self-renewal capacity or if both events are possible (Verga Falzacappa et al. 2012). To establish effective targets therapies it is critical to understand the molecular regulation of self-renewal and the cellular properties of CSCs. Furthermore, it is crucial which mutations and events are responsible for the dysregulation of the self-renewal mechanism resulting in the formation of cancer stem cells with unlimited self-renewal capacity.

## **Ansprechpartner**

Dr. Gabriele Gröger  
Albert-Einstein-Allee 45  
89081 Ulm



Tel 0049 731 – 5 03 24 00  
Fax 0049 731 – 5 03 24 09

[gabriele.groeger@uni-ulm.de](mailto:gabriele.groeger@uni-ulm.de)  
[www.uni-ulm.de/saps](http://www.uni-ulm.de/saps)

Wiss. Leiter der SAPS: Prof. Dr.-Ing. Hermann Schumacher

## **Postanschrift**

Universität Ulm  
School of Advanced Professional Studies  
Albert-Einstein-Allee 45  
89081 Ulm

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