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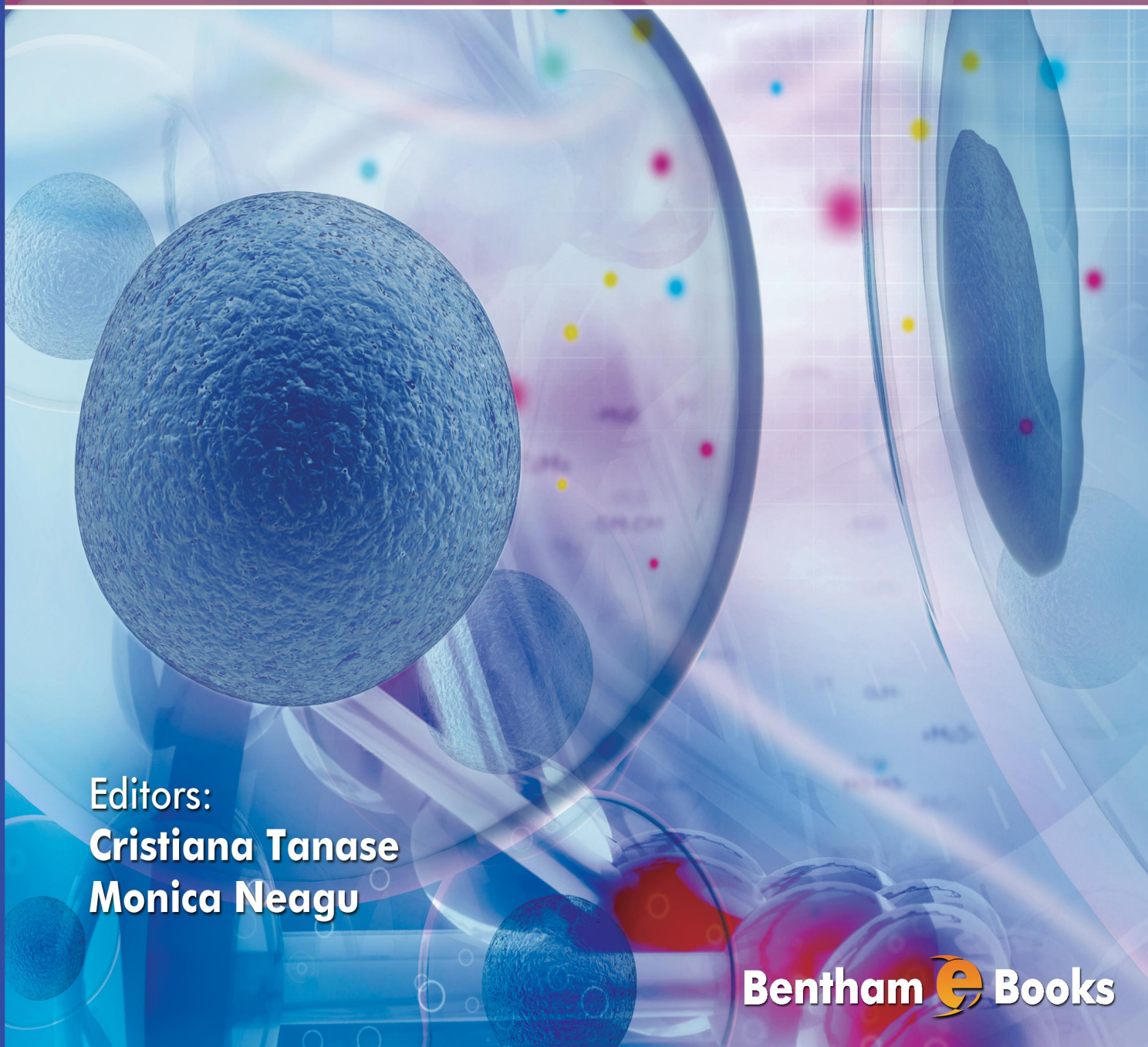
# STEM CELLS BETWEEN REGENERATION AND TUMORIGENESIS



Editors:
**Cristiana Tanase**
**Monica Neagu**

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# FOREWORD

Crucial findings in research and clinical application results have proved stem cell to be endowed with dual characteristics, on one hand exploited for beneficial regeneration processes and source of uncontrolled tumoral proliferation, on the other.

Statistics predicts that there will be over 20 million new cancer cases worldwide by the end of 2014, because the incidence of major cancers and population growth are steadily increasing. Hence we will be facing 56% more cancer cases diagnosed in 2030 in comparison to 2012 [1, 2].

In contrast to cancer world-wide statistics, where there are clear and accurate statistics, the figures for tissue regeneration domain are more on the economic side. Therefore the global tissue engineering and regeneration market is estimated to $20.8 billion in 2014 and will triple in 2019 [3].

In this global image of human diseases, stem cells are *walking a thin thread*, balancing two competing ideas: one is stem cell as main tumorigenesis player and the other is stem cells as the crucial regeneration trigger. Between these two sides of the *barricade* stands the book that I am kindly inviting you to decipher.

Almost all of the chapters are elaborating on issues that stem cells are endowed with. Preserving the genetic information and retaining the proliferative potential of the tissue in which these cells reside are sustained by their capacity to self-renew and the ability to differentiate into different cell types. Stem cells have a long life span, slow cycling, but their quiescence may turn into high proliferation ability when exposed to certain stimuli. Various factors, including the ones resident in their niche can influence the fate of stem cells.

You will discover the latest up-dates in hematopoietic stem cells, cancer stem cells in oral pathology, stem cells that are involved in skin's regeneration and tumorigenesis, in neurodegeneration and brain tumours, in endocrine pathology, how the immune system controls stem cells, the proteomics behind these cells and the latest technologies involved in stem cells identification.

The book raises its voice in the chorus of publications that would enhance the information regarding stem cells development as therapy approaches in precision medicine.

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[1] Ferlay J, Soerjomataram I, Ervik M, *et al.* GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed December 2013.

[2] Bray F, Jemal A, Grey N, *et al.* Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. Lancet Oncol 2012; 13:790-801

[3] Available at: http://www.reportlinker.com/p02313354-summary/Tissue-Engineering-and-Regeneration-Technologies-and-Global-Markets.html

*"Dream, then think, act, and pray."*

*Professor YAMAMURA*

# PREFACE

The proposed book will enrich the previous publications regarding stem cell, by promoting information in two research/clinical renewable areas, such as regeneration and tumorigenesis. Recent information gathered both from research and clinical application proved stem cell to have a “Janus face” characteristics, on one hand exploited for beneficial regeneration processes and source of uncontrolled tumoral proliferation, on the other. Therefore, these cells while used in experimental regeneration they can induce unwanted tumorigenesis pathways.

In this respect, signal transduction, stem cell markers, immune-related processes, “omics” technologies as up-dated identification, as well as pharmacological trends will emphasize the relationship between research and clinical behaviour of this controversy.

This book is emerging from the need to create a clear image regarding the complex mechanisms governing the involvement of stem cell in both regeneration and tumorigenesis.

The giants of cellular biology, have shown that the reawakening of pluripotency inherent in all cells have challenged forever our notions of cellular identity. The implications of the new reprogramming paradigm in biomedicine is steadily enhancing our understanding of cell differentiation and prospects for cellular therapies and *in vivo* regeneration.

The multi-authored book that we propose focuses specifically on various approaches in terms of organ and specific pathways that trigger the two opposite ways that a stem cell can follow. By definition, a stem cell has both self-renewal and multi-potentiality abilities. For this unique dual capacity, stem cells interpret signalling pathways in specialized ways. Adult stem cell for the treatment of damaged or diseased tissues relies on the ability of stem cells to produce paracrine factors that have a trophic effect on existing tissue cells, improving their functional capacity and develops the complex process of regeneration. On the other side of the *barricade*, the notion of cancer stem cells gained prominence in recent years but whether they are only the initiators and/or perpetuator of neoplasia, is still a matter of intense debate. Local factors from the microenvironment (niche) can sustain the self-renewal potential and possibly guide towards multiple stem cell populations.

The chapters will follow the balance between regeneration and tumorigenesis focusing several tissue and systems types: neuro-, haemato-, oro-, dermato-, digestive, endocrine domain. Chapters that focus on the immune processes that regulate and control the stem cell duality and state of the art identification *omics* technologies and pharmacological approaches. This viewpoint will have as central pillar the stem cell and its capacity to be main target for solving system medicine issues.

This e-book begins with the chapter elaborated by *Chen et al.* showing that the discovery of hematopoietic stem cells (HSCs) ushered in a new era in stem cell and life science research. The therapeutic benefits of HSCs have long been recognized; as bone marrow transplants have saved many lives, and with an increasing understanding of HSC biology and its translation to the clinic, studies of HSCs will continue benefit the health of humans and our curiosity of life in general.

The chapter elaborated by *Slootweg and Zurac* focuses on the epithelial stem cells in oral mucosa. Normal epithelial stem cells are characterized, their location, methods of identification and stemness markers are presented. The concept of cancer stem cells in oral cancer, their origin and markers are discussed along with cancer stem cells niche and the interference with treatment. The chapter discuss the role of oral stem cells in oral mucosa wound repair.

The chapter dedicated to skin stem cells elaborated by *Caruntu et al.* highlights the main characteristics of processes like regeneration and tumorigenesis in skin. The chapter describes the skin regeneration pathways and it elaborates on an interesting link regarding regeneration triggering tumorigenesis in cutaneous tissue. Stem cells that can trigger non-melanoma and melanoma skin cancers are described in separate sections.

The chapter focusing on adult pituitary stem cells elaborated by *Gheorghișan-Galateanu* shows the recent reports of potential populations of stem cells in the pituitary. The nature of pituitary stem cells remains a matter of debate. The variety of markers and approaches used to identify pituitary progenitors and stem cells makes it difficult to compare results and integrate the findings.

There are two chapters that focus on stem cells in brain. One chapter elaborated by *Enciu* shows the neuroregeneration of mammalian brain, where a functional stem cell niche and proper molecular cues is needed. Neural stem cells have been initially considered, as a putative source for lost neurons, but in terms of cognitive rescue, the results have been disappointing. The chapter discusses the regenerative potential of stem cells therapy in the modified cellular and molecular context of the aged brain.

The other chapter focusing on brain, developed by *Cruceru and Popa* presents a quick view of the entangled signaling pathways involved in the cancer stem cells in brain tumors with

aggressive behavior such as glioblastoma, presenting possible targets for future personalized therapies with improved outcome.

The chapter that brings data regarding the main issues triggered by the immune response in stem cell approaches is elaborated by *Neagu and Constantin*. The chapter characterizes the immunogenicity of stem cells, where major histocompatibility expression is the *immune mould* that can drive toward regeneration or tumorigenesis. The chapter shows the processes that are involved in stem cells modulating the immune system elements. Immune cells are important players for stem cell differentiation in both regeneration and tumorigenesis processes.

New proteomic insights in this domain are elaborated by *Tanase et al.* showing the studies on stem cells and protein interactions using proteomics approaches. Development of stem cell approaches has evolved in the post-genomic era and the implementation of proteomic applications represent the great challenge. Current proteomics studies of stem cell signaling pathway can lead to the discovery of molecular mechanisms that govern cell-cell interaction and/or with stem cell niche.

The last chapter elaborated by *Larisa-Emilia Cheran et al.* gives an overview of the new micro- and nano-technologies designed to monitor stem cell differentiation in the context of their potential applications in disease modeling, tissue engineering, regenerative medicine, as well as drug screening and toxicology.

The reader will gain a good insight on the complexity and controversy surrounding the "stem cell paradigms", namely the dual stem cell behaviour, regeneration *versus* tumorigenesis. Intimate processes like stem cells promoting the maintenance of other stem cells, background for optimization of tailored therapy intending the close collaboration between bench and bedside will be the crucial target in personalized medicine.

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CHAPTER 1

# Stem Cells in Hematopoietic Processes and Therapy Tools

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**Abstract:** The discovery of hematopoietic stem cells (HSCs) ushered in a new era in stem cell and life science research. Much of the technology and knowledge that has been gained through HSC studies is now applied in many fields of biology and has fundamentally changed our understanding of stem cells. For example, cell identification and purification using cell surface markers, which were developed and fine-tuned in HSC studies, are now routinely used to investigate the developmental stages of different cell populations not only in hematopoiesis but also in the development of other organs and in tumor biology. The therapeutic benefits of HSCs have long been recognized as bone marrow transplants have saved many lives, and with an increasing understanding of HSC biology and its translation to the clinic, studies of HSCs will continue to benefit the health of humans and our curiosity of life in general. In this chapter, we will briefly describe the discovery, regulation, and therapeutic application of HSCs. We apologize that many studies are not included here due to the nature of this review.

**Keywords:** Gene therapy, Hematopoietic niche, Hematopoietic stem cells, Transplant.

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# HEMATOPOIETIC STEM CELLS (HSCS)

## Hematopoiesis

Blood accounts for ~7% of the weight of the human body; the peripheral blood of an adult human averages five liters and is composed of different types of blood cells suspended in plasma. Erythrocytes (red blood cells) comprise the vast majority proportion of blood cells, representing 45% of whole blood by volume, whereas leukocytes (white blood cells) account for approximately 0.7%; and plasma accounts for approximately 50% of the blood volume. Different types of blood cells vary significantly in terms of life span, for example, a few hours for certain granulocytes, 120 days for red blood cells, and many years for certain lymphocytes. As a tissue, an adult human's blood contains $3x10^{13}$ cells, with approximately $10^{12}$ cells being replenished every day, making blood one of the most highly regenerative tissues. The process of regenerating new blood cells, or hematopoiesis, is one of the most actively studied topics and has led to knowledge of the detailed regulation of hematopoiesis and the discovery of hematopoietic stem cells.

## The Discovery of Hematopoietic Stem Cells (HSCs)

It is currently accepted that the regeneration of blood cells begins with the self-replication and differentiation of hematopoietic stem cells (HSCs). However, the actual discovery of HSCs did not occur until more than a hundred years after the concept of HSCs was introduced in the 19$^{th}$ century, when anatomists noticed a wide variety of cellular morphologies while examining human bone marrow. In the early 20$^{th}$ century, the Russian scientists A. Maximow *et al.* postulated that hematopoiesis in humans could be characterized as a cellular hierarchy that was derived from one precursor cell, the hematopoietic stem cell [1]. However, no direct evidence or substantial experiments supported the existence of the HSC until much later. Extensive research on the hematopoietic system did not begin until the end of World War II.

The atomic bombing on Hiroshima and Nagasaki caused numerous deaths due to the severe failure of hematopoietic function resulting from the lethal dosage of ionizing radiation. Scientists began studying the damage caused by ionizing

radiation, especially to the hematopoietic system, and how to repair such damage. Jacoboson *et al.* discovered that certain cells from the bone marrow (BM) and spleen of mice could completely reconstitute the hematopoietic system of recipient animals that had suffered a lethal dosage of ionizing radiation [2, 3], indicating the existence of cells with self-renewal capacity that were transplantable. Further evidence of hematopoietic cell self-renewal came in 1951 when Lorenz *et al.* discovered that after the injection of spleen cells or bone marrow cells from radiation-free donors into recipients who had received ionizing radiation, the patients' hematopoietic function improved, and none died [4]. In the same year, Brecher *et al.* confirmed that the transplantation of bone morrow from rats that had not received radiation could fully repair the severe bone morrow failure of rats that had suffered lethal radiation [5]. The existence of hematopoietic stem cells was demonstrated by these series of studies, which also confirmed that the failure of the hematopoietic system could be reestablished *via* cell transplantation, indicating the existence of hematopoietic stem cells and their transplant ability.

In the 1960s, the existence of a multi-lineage hematopoietic stem cell pool was confirmed by a series of *in vivo* clonal repopulation tests, which represented the regenerative ability of hematopoietic stem cells [6, 7]. The visible colony-forming unit-spleen (CFU-S) assay was also developed to identify the multipotent cells of different blood lineages [7]. Nonetheless, due to the poor understanding of the hematopoietic system, results obtained with the CFU-S were incorrectly interpreted as confirmation of the existence of hematopoietic stem cells in mice. Furthermore, it was not clear whether HSCs consisted of one type of cells that can differentiate into all other types of mature cells or two or more different cell types that have different functions. Nevertheless, the CFU-S and transplant assays were the foundation for later functional studies of HSCs and progenitor cells.

## The Identification of HSCs

In the late 20$^{th}$ century, the rapid advancement of molecular biology, immunology, flow cytometry, and other technologies aided in breakthrough discoveries in the areas of hematopoietic system and hematopoietic stem cells. Indeed, the general understanding of the development and function of hematopoietic system and its

CHAPTER 2

# Epithelial Oral Stem Cells

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**Abstract:** This chapter focuses on the epithelial stem cells in oral mucosa. It starts with the characterization of the normal epithelial stem cells (location, methods of identification, stemness markers). Next, it discusses the concept of cancer stem cells: origin, cancer stem cell markers, cancer stem cells niche and interference with treatment. Finally, the chapter discusses the role of oral stem cells in oral mucosa wound repair.

**Keywords:** Oral cancer stem cells, Oral mucosa regeneration, Oral stem cells, Stemness.

## INTRODUCTION

Epithelial stem cells within oral mucosa have features similar to other adult stem cells: capacity of self-renewal and the ability to differentiate into mature cell types [1]. Identification of oral stem cells follows the gold standards used for the identification of any adult stem cells: genetic labeling of a putative stem cell (with subsequent transmission of the label to that cell progenitors) and transplantation (with subsequent formation of the tissue in the new location) [2]. Since these techniques are difficult to use, efforts were made in order to identify oral stem cell markers.

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## Genetic Labeling

Stem cells are very slow cycling cells; they are capable of retaining a certain genetic label for a long time [1]. Several labeled DNA nucleotides were experimentally tested in order to identify the low turnover rate cells, thus tritiated-thymidine (3H-TdR), 5-bromo-29-deoxyuridine (BrdU) and 5-iodo-2'-deoxyuridine (IdU) were used to label the cells. 3H-TdR retaining cells were identified 240 days after labeling in palate and buccal mucosa of the mice [3] and 69 days after labeling in tongue mucosa of the hamsters [4]. BrdU studies revealed the presence of 3-7% of BrdU retaining cells in oral mucosa in rats after 10 weeks post-labeling [5] and 1.45-4.28% of BrdU retaining cells in oral mucosa in mice after 35 days post-labeling [6]. No studies of genetic label retaining cells in human oral mucosa are available yet but an elegant study of normal human esophagus and stomach identifies IdU-retaining cells (less than 0,1% of epithelial cells) with stem cells features (long living uncommitted cells located in stem cell niches) [7].

## Clone Formation

Stem cells have high *in vitro* proliferation potential. Older studies showed the capability of keratinocytes to form clones. The number and morphologic appearance of these clones depends on the size of founding cells: holoclones (cells smaller than 11 microns – most likely stem cells) form large round smooth edges colonies with reduced tendency towards terminal differentiation with small cells in the periphery; meroclones (larger cells with high proliferative potential and more differentiate phenotype - transit amplifying cells) form smaller colonies with wrinkled irregular edges with intermediate growing potential; paraclones (large terminally differentiating cells) form small highly irregular terminal colonies of large cells with short *in vitro* lifespan [8]. Further studies confirmed the presumption that holoclones are indeed stem cells. Magnetically separated oral human keratinocytes with $CD71^{-}$ phenotype are the smallest and most clonogenic cells, also expressing cytokeratin 19 and p63 keratinocyte stem cell markers but lacking cytokeratin 10 and involucrin (differentiation markers) [9]; Krt5-GFP(hi) cells in a line of transgenic mice also function as holoclones [10].

## Stem Cell Markers

Several putative oral stem cell markers were proposed. Nestin, nanog and c-kit (CD117) (otherwise stem cells markers for other tissues) were not reported in putative oral epithelial stem cells [11]. These markers were identified in dental pulp and periodontal ligament stem cells [12 - 14] and also in *lamina propria* of oral mucosa [15] and palate [16], nestin positive stem cells from these locations being able to differentiate into cell-types of cranial neural crest ontology [17]. Most of the oral stem cells markers were first identified in stem cell of the hair follicle and epidermis; in oral mucosa they are also expressed by almost all the basal cells, thus preventing the isolation of pure populations of stem cells [1]. On these issues, there is still no consensus, several studies identifying a plethora of putative stem cells markers in different areas of oral mucosa: masticatory mucosa -α6β4 integrin, β1 integrin, CD44, CD71, melanoma chondroitin sulphate proteoglycan (MCSP), p75, CK15, CK19, p63, Oct3/4; lining mucosa - β1 integrin, collagen IV, p75, CK14, ABCG2; specialized mucosa - β1 integrin, CK5, CK14, sox2. All cells positive for these markers proved either self-renewal capabilities by high colony forming properties and/or differentiation towards oral epithelial equivalents in cell culture [1, 9 - 11, 18 - 22].

## Location of Oral Epithelial Stem Cells

Most likely, oral epithelial stem cells are located within the basal layer of the squamous epithelium, representing 1-7% of the cells [5].

There are differences concerning their density between different areas of oral mucosa. BrdU retaining cells (putative stem cells) are more frequent in masticatory mucosa (most frequent in gingiva, followed by palate – thick keratinized mucosa with prominent rete ridges), less frequent in lining mucosa (alveolar and buccal mucosa and inferior surface of tongue – thin mostly non-keratinized mucosa almost devoid of rete ridges) and least frequent in specialized mucosa (*dorsum linguae* – mucosa with lingual papillas) [6]. Similar data concerning location were noted in humans based on *in vitro* studies using both 3H-TdR and BrdU in single or sequential double-labeling technique: buccal mucosa showing higher S phase labeling than mandibular gingiva [23].

CHAPTER 3

# Skin Stem Cells in Cutaneous Wound Healing and Tumorigenesis

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**Abstract:** This chapter will highlight the main characteristics of processes like regeneration and tumorigenesis in skin. The chapter describes the skin regeneration pathways and it elaborates on interesting link regarding regeneration triggering tumorigenesis in cutaneous tissue. Stem cells that are the key player in non-melanoma and melanoma skin cancers will be described in separate sections.

**Keywords:** Regeneration, Skin, Stem cells, Tumorigenesis, Wound healing.

## INTRODUCTION

Skin plays an essential protective role against physical, chemical or infectious, potentially harmful agents. It is also involved in thermoregulation, hemodynamic homeostasis, fluid and electrolyte balance, excretory function and has important metabolic activities. Another major role of skin is to provide information about the environment, functioning as a sense organ.

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Skin has a complex structure with various tissue types including epithelium, connective tissue, smooth muscle, fat, vessels and nerves. These are organized in separate layers with different embryonic origin, but an interdependent functionality.

Epidermis, the outer layer of the skin, is composed of stratified squamous epithelium of ectodermal origin, which functions as a protective coating. Beneath epidermis lies the dermis, a collagen-rich connective tissue of mesodermal origin, which is separated from epidermis by dermo-epidermal junction. It provides support, elasticity and nourishment for the skin. Below dermis is hypodermis, consisting of adipose tissue, is also derived from embryonic mesoderm, with important protective and metabolic functions. Hair follicles, sebaceous glands and sweat glands, known as appendages of the skin, are also components of cutaneous tissue [1, 2].

Due to its multiple functions and the permanent contact with aggressive environmental factors, skin undergoes continuous renewal and has a high regenerative potential [3], processes that depends on the numerous stem cell populations residing in various skin compartments [4]. Skin stem cells are the cells of origin for all terminally differentiated cells in cutaneous tissue [5] and are essential for preserving the genetic information and retaining the proliferative potential of the skin. They are relatively undifferentiated, have the capacity to self-renewal and the ability of differentiation into different cell types. Skin stem cells have a long life span, are slow cycling, but their quiescence may turn into a high proliferation ability when exposed to certain stimuli [6 - 14].

The skin stem cells divide asymmetrically, resulting in a daughter stem cell, and a committed progenitor cell, known as transit amplifying cell, with a limited proliferative potential. Transit amplifying cells are rapidly cycling, increasing the number of cells available for subsequent maturation. After a few division cycles, their daughter cells, called post-mitotic cells, no longer divide and undergo terminal differentiation [2, 9 - 12, 15 - 18]. Skin stem cells can also divide symmetrically, to generate two identical daughter cells [19, 20]. The proliferation and differentiation processes arising from stem cells maintain tissue homeostasis in all skin compartments [21].

Cutaneous tissue is rich in stem cells, it can be explored easily and research on skin stem cells has known a rapid development [12, 22, 23]. Skin stem cells are located in niches, which are distinct regions with specific microenvironment conditions that have a strong influence on their activities [24, 25].

In epidermis, stem cells are found in the basal layer, but their precise location varies from one region to another. In glabrous skin of palms and soles, epidermal stem cells are located in the deep part of the rete ridges, an area less exposed to aggressive environmental factors [13, 26]. In hairy skin epidermis, stem cells are found at the tips of dermal papillae; very close to the underlying blood vessel. This location favors their nourishment. [10]. Other skin stem cells populations reside in various regions such as the secretory segments of sweat glands, sebaceous glands, germinal matrix of hair follicles, and particularly the bulge region of hair follicles [10, 12 - 14, 18, 19, 27].

Dermis and hypodermis also contain multiple stem cells populations located mainly around hair follicles and blood vessels [21, 28, 29].

The heterogeneous skin stem cell pools accomplish multiple functions, being the cellular base for tissue development, renewal, and repair [3, 24]. Their activities show circadian oscillations and are controlled by complex regulatory interactions between intrinsic mechanisms, local signals from microenvironment and different extrinsic factors [30 - 32].

## Interfollicular Epidermal Stem Cells

Interfollicular epidermis is the most superficial layer of skin, located between hair follicles, and is composed of keratinized stratified squamous epithelium [14]. The vast majority of epidermal cells are keratinocytes. These cells are in a continuous transition from deep regions to the surface of the epidermis. They are arranged in layers and change their differentiation degree from one layer to the next. In epidermis, the differentiation process is mainly defined by keratinization. Cells from the inner basal layer pass through the spinous and then granular layers to reach the superficial layer as flat cornified cells, full of keratin, lacking nucleus, and eventually are discarded from the surface [10, 18, 19]. Epithelial homeostasis is maintained by the fine balance between cell production in deeper layers and cell

**CHAPTER 4**

# Stem Cells in Neurodegeneration

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**Abstract:** The neural stem cell niche in the adult brain is a complex environment formed by several types of cells, a particular type of extracellular matrix and specific molecular cues. Ageing is characterized by reduced neurogenesis, due to altered neurotrophin signaling, activation of senescence programmes within the niche, imbalanced growth factor signalling. A recent concept is that blood-born ageing factors signal or cross the blood brain barrier to negatively influence the neurogenesis. Neurodegenerative diseases are an appealing target for regenerative medicine and stem cell transplant was held for quite some time in high consideration and brought about high hopes for the replacement of lost cells and tissue function restoration. One of the major drawbacks is that most transplanted cells differentiate on glial line; therefore, new strategies were implemented. From transplantation of committed neuronal progenitors, to neurosphere transplant or autologous mesenchymal stem cells, these strategies are presented and some of the drawbacks highlighted in the present chapter.

**Keywords:** Alzheimer's disease, Hippocampus, Neural precursors, Neurodegeneration, Parkinson's disease, Stem cells, Subventricular zone.

## INTRODUCTION

Paradoxically, age is a risk factor for both neurodegenerative diseases and brain tumors. Taken separately, it makes sense that, on one hand, loss of neuronal stem cells (NSC), neurons, neuronal plasticity and synapses would lead to neuro-degeneration. On the other hand, an increase in oxidative stress, accumulation of

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mutations and deficiency of DNA repair mechanisms with age would accumulate in generation of malignancy. The paradox rises when both processes are asserted from the stem cell perspective: neurodegeneration is thought to be accompanied by low regenerative reserve, whereas brain tumors "benefit" from the proliferative advantage of cancer stem cells (CSCs). The transplant of stem cells for neurodegenerative disorders has been considered for the restoration of neural networks supporting the cognitive processes. The evaluation of the faith of transplanted cells, as well as the accumulation of knowledge regarding adult stem cells form other tissues, highlighted interesting behaviours of stem cells, such as migration out of transplanted site or the niche into chemotactive areas. Such areas could be stroke area, traumatic area or even a tumor. There is also a concern that transplanted stem cells could harbour a latent potential of malignant transformation.

## NEURAL STEM CELL NICHE ORGANIZATION IN ADULT MAMMALIAN BRAIN

Neural stem cell niche of mammalian brain is mainly found in the sub ventricular zone, responsible for the generation of new neurons for the olfactory bulb and hippocampus dentate gyrus. It generates small granular neurons that are included into neuronal networks involved in learning and memory [1]. Additional areas of adult neurogenesis have been described in hypothalamus [2], spinal central canal ependymal zone [3], substantia nigra, striatum, amygdala, neocortex [4] and leptomeninges [5]. Literature abounds in morphological and molecular characterization of mammalian neural stem cell niche, mostly in animal models. Same as most stem niches in the adult mammalian organisms, the neural niche has a cellular compartment with several subpopulations: quiescent stem cells, transit amplifying cells, precursor cells and adult cells. Each of those subpopulations is characterized by the expression of specific markers [4] and phenotypic features [6] (Fig **1**). From sub ventricular zone, newly formed neuroblasts migrate towards the olfactory bulb *via* the rostral migratory stream. But unlike other mammalian brains, human sub ventricular neurogenic zone is tri-layered: Layer I - the ependymal lining of lateral ventricle, Layer II - cellular elongations and rare neuroblasts and Layer III, or "astrocytic ribbon", - mitotic active cells [7]. The rostral migratory stream is prominent and populated with migrating neuroblasts in humans, mostly during foetal period. However, during adulthood, this olfactory

tract seems to contain a very low number of cells that double stain positive for doublecortin and polysialylated-neural cell adhesion molecule, indicating either a low generation rate of neuroblasts, or a very low migratory capacity [8].

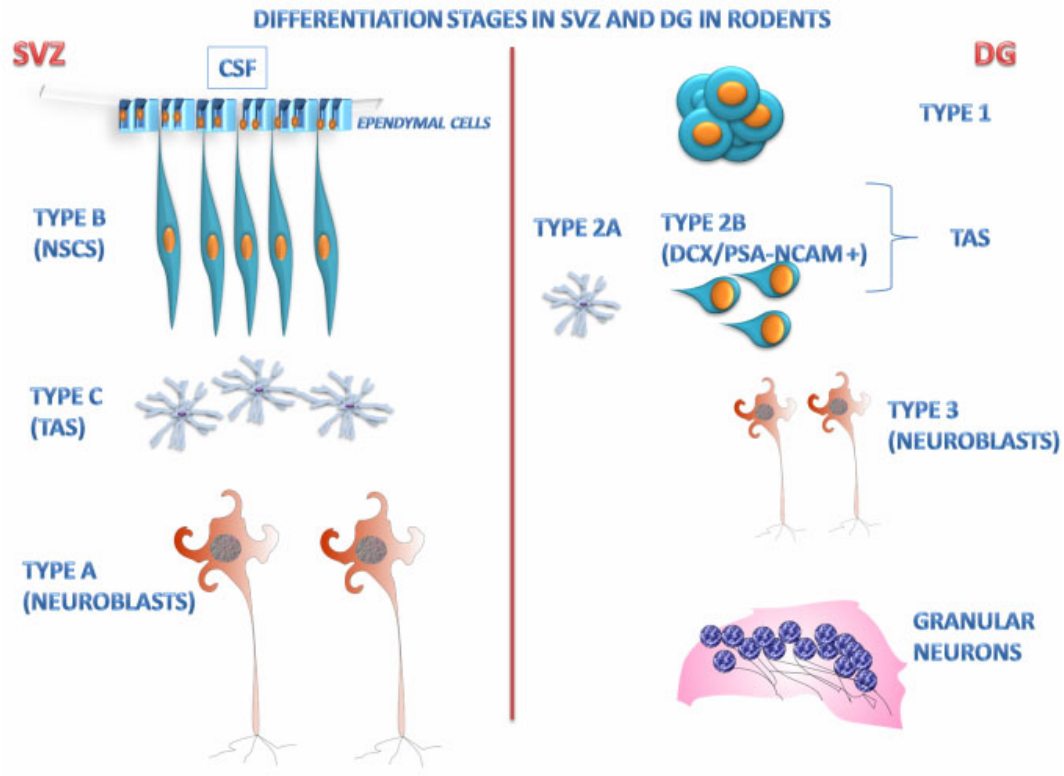


**Fig. (1).** Models of neuroregenerations. Abbreviations: SVZ – subventricular zone; NSCS – neural stem cells; TAS – transit amplifying cells; DG – dentate gyrus; DCX – doublecortin; PSA-NCAM - Polysialylated-neural cell adhesion molecule.

Increasing evidence had revealed the importance of brain endothelial cells of the vascular niche in the regulation of neurogenesis [9]. In adult rodent brain, neural progenitor cells are located next to endothelial cells in both SVZ and the dentate gyrus and express genes involved in angiogenesis [10]. Teng *et al.* showed that there is a dual relationship between NSC and angiogenic niche: activated NSC release angiogenic factors and in turn, activated endothelial cells enhance neural progenitor cells proliferation and differentiation [10].

Although acknowledged only recently, the extracellular matrix seems to play an

CHAPTER 5

# Cancer Stem Cells in Brain Tumorigenesis

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**Abstract:** The presence of cancer stem cells in brain tumors has been suggested in the recent years, extrapolating from other types of malignancies. The actual debate centers on the possibility that this small group of cells or a single cell can initiate and maintain such an aggressive malignancy status. Cancer stem cells are a major theme for aggressive brain tumors, their extensive study offering potential biomarkers for identification of therapy targets based on abnormal and connected signaling pathways. This chapter presents the phenomenology of cancer stem cells in their microenvironment, in its astonishing complexity. Identification markers, pathologic signaling profiles and cross-talk, microenvironment interactions and novel therapies targeting cancer stem cells are discussed. Continuous studies are researching genetic and epigenetic modifications associated with malignant evolution in glioma cancer stem cells. From the complex entangled signaling pathways presented, one must extract only the important molecules involved in oncogenesis onset and propagation, putting aside the coincidentally modified molecules that can be misleading. After removing the "smoke screen" of oncogenic irrelevant, but modified molecules, there remain the true therapy targets, to address by specific therapies in a combined manner to overcome the adapting processes governed by signaling pathway cross-talk.

**Keywords:** Anti cancer stem cell therapy, Brain tumors, Cancer stem cells, Signaling pathways.

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## INTRODUCTION

Potential cancer stem cells have been identified and characterized in several human solid tumors, including aggressive brain tumors [1 - 4].

During the last decade, intensive research is undergoing on various tumors associated with an aggressive course and many studies have been dedicated to the involvement of cancer stem cells in cancer tumorigenesis – between 2000 and 3000 on PubMed. Concerning brain tumors, recent papers have focused on the role of cancer stem cells in tumor initiation, progression and relapse, as well as on their biological and clinical characterization [5 - 9].

The high grade gliomas – anaplastic astrocytoma (WHO grade III) and glioblastoma (WHO grade IV) – have a very poor prognosis and, despite intensive conventional therapy, the median survival rate for patients with glioblastoma (GBM) is approximately 1 year [10].

The most recent discoveries regarding glioma cancer stem cells (GCSCs) have provided a valuable input on the evaluation of aggressiveness, recurrence and treatment resistance [11].

This chapter will highlight the features of cancer stem cells (CSCs) and the challenges that they raise, as well as conventional *versus* specific signaling pathways involved in the initiation, maintenance and propagation of brain tumor malignant phenotype.

## CANCER STEM CELLS AND THE TUMOR MALIGNANCY GRADE

Nowadays, the general consensus is that GCSCs and neural stem cells (NSCs) have some common features, despite their differences: both of them show pluripotency and self-renewal capacity and even the tendency to develop spheres when cultivated *in vitro* [9]. The GCSCs can present *in vitro* or *in vivo* heterogeneity, genetic alterations, abnormal proliferation and differentiation patterns [12 - 15].

Regarding the ability of the GCSCs to form spheres in culture, the more aggressive the tumor is (as shown by injection into immune-compromised mice),

the more spheres will appear *in vitro* [16].

In order to study the cancer stem cells in brain tumors, one must follow few generic steps:

1. To isolate the cancer stem cells from tumoral tissue (tissue identified macroscopically by a pathologist), using specific stemness markers;
2. To pursue the development of spheroid cell cultures;
3. To observe the ability of stem cells in the spheroid colonies to differentiate into neurons, astrocytes and oligodendrocytes [16, 17];
4. To verify the malignant character of the cells isolated in the spheroid cultures when injected into mice with compromised immune system.

Another feature observed in GCSCs when compared with NSCs is their resistance to aggressions (chemotherapy drugs). Also, differences may exist regarding proliferation pattern and the markers presented [9, 10]. Other types of tumors derived from nervous tissue, such as medulloblastoma, show high proliferation rates – up to 30% proliferation rate in cells expressing vimentin in the perivascular niche in contrast with 1% proliferation rate of NSCs from the subventricular zone [18].

Compared to NSCs, GCSCs show more resistance to treatment, being susceptible to develop new tumors and to promote recurrence [9].

In order to fully understand the GCSCs we must understand NSCs. Although a great quantity of information is available on NSCs, some aspects remain unclear and are theoreticized based on similarities with the hematopoietic stem cells (HSCs). For example the maintenance of quiescent phenotype of NSCs is supposed to imply epigenetic regulation, similar to HSCs. However, the exact signals are yet to be discovered.

In the same manner, it has been speculated that a cytokine could be the promoter of NSC quiescence, and also stimulate the proliferation of committed progenitors. Such a cytokine could belong to the IL-6 family, since they show strong activation of JAK-STAT signaling, especially of STAT3, similar to the hematopoietic system [10]. Due to genetic modifications in any downstream signaling molecule,

**CHAPTER 6**

# Adult Pituitary Stem Cells

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**Abstract:** Stem cells were first identified in adult organs with high regenerative capacity including skin, liver, intestine and bone marrow. The pituitary gland is an organ with low cell turnover, and while differentiated cells can re-enter the cell cycle, most hormone producing cells are not dividing. The adult pituitary gland has some capacity to regenerate after tissue injury. Recent studies have reported potential populations of stem cells in the pituitary. Stem cells population belongs to the chromophobe/folliculo-stellate population of adenohypophysis. Studies to date suggest that at least a part of the endocrine cells originates from marginal layer adjacent to Rathke's cleft. Different groups, using diverse approaches, have demonstrated the presence in the pituitary of cells with progenitor or stem cell capacities and have characterized the pituitary stem cells, progenitors, and transit amplifying cells. A large variety of markers used to identify pituitary progenitors and stem cells make the integrated view difficult over the results obtained. Until now the nature of pituitary stem cells remains a matter of debate, and additional studies are needed to define the progenitors and stem cells in adenohypophysis.

**Keywords:** Adenohypophysis, Folliculo-stellate cells, Markers of stem cells, Progenitor/stem.

## INTRODUCTION

The anterior pituitary (adenohypophysis) is composed of the anterior, intermediate

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and tuberal lobes and develops from the oral ectoderm by invagination in the early stage of the embryo. During embryonic organogenesis, five types of polypeptide hormone-producing cells (granular cells) are differentiated. These cells include: somatotrophs that produce GH, lactotrophs producing prolactin, gonadotrophs that secrete both LH and FSH, thyrotrophs producing TSH, and corticotrophs that express proopiomelanocortin (POMC) and cleave it to produce ACTH. These pituitary hormones steer essential physiological processes such as reproduction, lactation, growth, metabolism and immune system activity.

In addition to hormone-producing cells, the anterior pituitary gland also comprises of non-endocrine cells (agranular cells), such as folliculo-stellate cells. In hematoxylin and eosin-stained sections, three principal cell types are identified in the normal adenohypophysis: acidophils, basophils, and chromophobes. Basophilic cells can be strongly stained *via* PAS method and are adrenocorticotropin (ACTH) and glycoprotein hormones (LH, FSH, TSH) producing. Acidophilic cells are engaged in growth hormone (GH) or in prolactin (PRL) production. The chromophobe cells were identified as folliculo-stellate cells.

## THE FOLLICULO-STELLATE CELLS

The folliculo-stellate cells (FS cells) were first identified in 1953, by Rinehart JF and Farquhar MG, using electron microscopy. FS cells are located in the center of the anterior lobe and constitute about 5–10% of the whole cells population of the anterior pituitary lobe [1]. They are star-shaped with long cytoplasmic projections that envelop hormone- producing cells and extending to the blood capillaries [2]. FS cells are located in the parenchymal tissue mainly around the lumen of large follicles scattered throughout the lobe [3]. Occasionally, the follicles contain colloid, an electron dense material. Clusterin, the major protein of colloid, which is not present in the cytoplasm of FS cells, is related to the phagocytic activity of FS cells. FS cells are recognized by the lack of cytoplasmic granules, many microvilli on the apical pole facing the follicular lumen, numerous fibrous structures in their cytoplasmic processes, and primary cilia (central cilia) which face the follicular lumen [4].

The impact of aging on pituitary FS cells is not well-known. It seems that only

moderate changes in the endoplasmic reticulum were observed in old and senescent animals [5], and generally they increase with age [6, 7].

FS cells currently are considered as functionally and phenotypically heterogeneous and seem to have multifunctional properties. They are connected by gap junctions and form an extensive and complex three dimensional network. FS cells form numerous interconnections with neighboring FS cells in the presence of laminin, an extracellular matrix component of the basal lamina [8]. It was hypothesized that FS cells transmit signals *via* gap junctions so as to regulate hormone release from hormone-producing cells, in addition to the hypophyseal-portal system [9]. They also act as scavenger cells with phagocytic activity [1]. FS cells seems to be important in the paracrine regulation of anterior pituitary (particularly gonadotroph activity) by producing radical nitric oxide [10], various growth factors and peptides such as basic fibroblast growth factor (bFGF) [11], vascular endothelial cell growth factor (VEGF) and follistatin [12], as well as cytokines, such as interleukin-6 (IL-6) [13], leukemia inhibitory factor (LIF) [14], macrophage migration inhibitory factor (MIF) [15], and macrophage colony-stimulating factor (M-CSF) [16]. In addition, FS cells respond to β– adrenergic stimuli and enhance cAMP-accumulation upon β-adrenergic stimulation [17].

Over the past years, several studies have suggested the progenitor potential of a subpopulation of FS cells, on the basis of their ability to form colonies *in vitro* and to differentiate *in vivo*. Their role is supposed to be as the stem cell in the adult gland, involved in the normal cell turnover. A subset of the FS cells may be a source of pituitary stem cells, and another subset may be involved in creating a niche [18]. The possibility that FS cell be a type of stem cell with the potential to differentiate in endocrine cells or at least to be involved in their differentiation has been suggested by Yoshimura *et al.* [19].

Indirect evidence suggests that there is the possibility of retro-differentiation of endocrine cells into folliculo-stellate cells [20], or expansion of the folliculo-stellate compartment in parallel with gonadotrophs cells after castration [21].

It has been shown that FS cells can be detected by S100 calcium binding protein B (S100β) [22, 23], glial fibrillary acidic protein (GFAP) [24], and nestin. In

CHAPTER 7

# Cancer Stem Cells in Pancreatic and Hepatocellular Carcinoma: Similarities and Differences

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**Abstract:** The objective of the present chapter is to discuss the identification of cancer stem cells (CSC) in pancreatic ductal adenocarcinomas (PDAs) and hepatocellular carcinoma (HCC) and to briefly overview the current therapies targeting this cell population. The reason to restrict the discussion to these particular cancer types is that both are described by a poor prognosis and resistance to conventional therapies. Therefore, CSC may be an important hint in improving the therapeutic approach in both instances. Due to the capacity of CSC to escape chemo and radiotherapy, novel means of approaching these cells are necessary in order to improve outcome therapeutically.

**Keywords:** Cancer stem cell, Cell surface marker, Drug resistance, Hepatic cellular carcinoma, Liver cancer, Metastases, Pancreatic cancer.

## INTRODUCTION

In the last decades, tremendous progress has been made in "the war on cancer".

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**Cristiana Tanase & Monica Neagu (Eds.)**

Since 2007, when the National Cancer Act was signed, early detection and new therapies brought hope to patients whose life span had considerably improved. Nowadays, 5-years survival rate for prostate cancer is almost 100%, in Hodgkin's lymphoma and breast cancer about 80%; and for colon cancer and non-Hodgkin's lymphoma more than 60%. However, in strong contrast with these results, for two types of cancers -hepatocellular carcinoma and pancreatic adenocarcinoma-the improvements are still below expectations. For pancreatic adenocarcinoma (PA), 5 years survival rate is about 6% and around 16% for hepatocellular carcinoma (HCC) (https://www.baylorhealth.com/Site CollectionDocuments/BUMC%20Cancer%20Care/Spring_2013_Cancer-Update.pdf). The reason why these types of cancer are still undertreated is because patients are usually asymptomatic and the diagnosis is late. Therefore, metastatic dissemination and resistance to chemotherapy became the determinant factors in PA and HCC prognosis. Unfortunately, new cancer therapies are facing the same fate: surgical removal of tumours and cytotoxic drugs cannot completely eradicate the malignancy. This situation led to a new paradigm that sustains the idea of the heterogeneity of cancer cells designed in a specific microenvironment which makes the treatment extremely difficult.

In the last years, many studies focus on the description of cancer –stroma interactions, which imply a dynamic network between inflammation, epithelial to mesenchymal transition (EMT), specific mutations and cancer stem cells. In this chapter, we will bring an integrative view of the molecular, cellular and extracellular milieu of cancer stem cells in pancreatic and hepatocellular tumours, and discuss thoroughly in the perspective of personalized therapeutic approaches.

## Cancer Stem Cells: Definition and Origin

The cancer stem cells (CSC) concept indicates specific cell populations with particular characteristics, which may represent a promising tool in cancer therapy. In order to assign CSC as potential druggable targets, it is important to have a clear definition, that specific markers and unambiguous description of the processes that maintain a distinct lineage within tumours.

It has been recognized for a long time that tumours are formed by a heterogeneous mass of cells in different stages of development, but the first experiments showing that cells with specific growth and division characteristics coexist with other tumour cells as described in the studies of acute myelogenous leukemia (AML) [1]. Using xenotransplatation assays, these experiments provided the first

evidence of a distinct immature cell population able to produce postmytotic progeny. Furthermore, there are defined as "cells with the capacity of cell renewal and differentiation", this rather small pool of cells was identified and described in many other cancers. Cancer stem cells (CSC) share some common characteristics with normal stem cells in terms of controlling their self-renewal and proliferation, and residence in a physiological niche which manages these processes [2]. However, this definition created some uncertainties, when CSC are defined as cells derived from normal stem cells. Although, in many cancers CSC are derived from normal stem cells that bared several mutations and transform them into cancerous cells, in other cancers a progenitor acquires "stemness" properties by later mutations. It is therefore feasible that more differentiated cells that supported several mutagenic events may acquire self-renewal capacity and personify the CSC.

An even more interesting feature, similar to normal stem cells is the dormancy pattern of CSC. In tumours, these cells are mainly quiescent, preventing them to be targeted by chemotherapeutic drugs. The quiescent state, which is in fact a reversible cell cycle arrest maintained by many regulatory mechanisms, provides CSC with the fuel for triggering metastasis when the microenvironment is suited for it [3]. A similar model was described for hematopoietic stem cells that have stopped the entire DNA replication and moved to an almost eternal $G_0$ phase which allows them to preserve the energy for stress situation such as injury or toxic insult. Moreover, an intriguing fact is that tumour cells may, in certain situations, spontaneously convert to CSCs. This result is in comparison with a clear model of specific CSC and sustains the idea that in a tumour population any cell may acquire stemness properties at a certain moment [4].

The theory of CSC became very appealing because of the easy intuitive picture that only a part of tumour cells possesses the metastatic phenotype and these cells are resistant to chemotherapy. This would explain why removing the tumour mass maintains the capacity of proliferation and metastasis. With a slow rate of growing CSCs are less sensitive to treatments. An even more attractive theory was presented by Yachida *et al.* who gave a timing genetic evolution of PDA, indicating that a single clonal population of "parental" cells support mutations and that this event appears one decade after these cells are born [5].

**CHAPTER 8**

# Immunogenicity of Stem Cell in Tumorigenesis *Versus* Regeneration

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**Abstract:** This chapter will focus on the main immunological issues in stem cell approaches. The scientific world faces an important immunological dilemma when investigating stem cells immunogenicity. One is the need to have low stem cell's immunogenicity, property that provides modest inflammatory reaction microenvironment and hence lack of rejection of the transplanted stem cells. On the other side any neoplastic transformation can increase the natural immune evasive properties of stem cells linking immune escape and tumorigenicity. In this light oncogenes expression can directly orchestrate inflammation and immune escape to drive the multistep process of cancer progression, independent of any immuno-editing in the tumor microenvironment. The chapter starts with characterizing the immunogenicity of stem cells, where major histocompatibility expression is the immune mold that can drive toward generation or tumorigenesis. The chapter continues with the processes that are involved in stem cells modulating the immune system elements whether in *Regeneration or Tumorigenesis*. The chapter ends with the main immune hurdles in the most recent clinical trials using stem cells.

**Keywords:** Adaptive, Immunity, Innate, Stem cells, Regeneration, Tumorigenesis.

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## INTRODUCTION

As we are facing an escalating number of clinical trials using stem cell therapies, the immunological issues that emerged are complex. Therefore, the immunological properties of the administered stem cell can easily direct a perfectly designed therapy toward a clinical failure. Then, the relative immune tolerance of the site where stem cells are administered can favour the clinical outcome or not. Last, but not the least, the immune status of the patient can be the main issue of transforming a regenerative therapy into an unwanted tumorigenesis process [1].

Stem cell-based therapy stands on the exploitation of pluripotent stem cells (PSCs) that are manipulated to repair and/or replace tissues or organs. The main immunological advantage in this field is that functional PSC are not tumorigenic and moreover they do not trigger any immune response that can induce host *versus* graft rejection (GVHD). GVHD is an inflammatory syndrome initiated by alloreactive donor T cells [2].

In transplant and tissue regeneration immune rejection is a crucial issue, thus induced PSC-based therapy, due to its decreased immunogenicity can represent the future solution for an unmet medical problem. Characterizing the phenotype of stem cells furnishes the investigator with the boundaries in which immune response triggering can be kept under control. Thus only in the last years, PSCs entered the cell-therapy armamentarium, therapies applied for patient-tailored advanced therapy. The main issue arisen within the domain was the fact that during the reprogramming methodology, several genetic and epigenetic aberrations can appear. Reprogramming process represents the removal and the remodelling of epigenetic markers, such as DNA methylation, a process that takes place during mammalian development [3]. If the medical community is planning the large clinical utilization of these cells, it is essential to comprehend the effects of these abnormalities inflicted upon the stem cells' immunogenicity, further ensuring the survival of PSC grafts [4].

There are several types of stem cells foreseen in this domain and an overview of these cell populations along with their phenotype is *depicted below*.

***Embryonic Stem Cells.*** Although considered great promising tool for cell-based therapies, embryonic stem cells (ESCs) could be eliminated by the immune effector cells of an immunocompetent allogeneic host [5, 6]. The major drawback resides in the fact that ESCs can trigger an immune response in recipient even though they have reduced immunomodulatory potential. This potential resides in the low expression of MHC I and II molecules (major histocompatibility complex), the absence of co-stimulatory molecules such as CD80 and CD86 and in the TGF (transforming growth factor) expression that can inhibit T cell proliferation [7].

***Induced Pluripotent Stem Cells.*** Keeping the typical stem cells abilities related to indefinitely proliferation and differentiation, induced pluripotent stem cells (iPSC) are very promising for therapeutical approaches. There are four transcription factors overexpressed in ESCs which turned on to iPSC phenotype when they are retrovirally induced. Thus, octamer-binding transcription factor 4 (Oct4), sex determining region Y-box 2 (Sox2), Krueppel-like factor 4 (Klf4) and c-myelocytomatosis viral oncogene homolog (c-Myc) are the main responsible for iPSCs derived from ESC [8].

Established as an alternative for stem cells therapeutics, iPSC are designed to solve the ethical issues associated with exploitation of human ESCs. Balancing similarities (morphology, proliferation capacity or cell surface antigens) with differences (global gene expression, genomic imprinting and somatic mutations) between ESC and iPSC, there is a continuous search for the best clinically used formula of iPSCs. Very recently a study with iPSCs derived neurons *via* fibroblast isolated from familial and sporadic Alzheimer's disease patients [9] allowed expression levels evaluation for some key pathological biomarkers such as Aβ, phospho-tau and active glycogen synthase kinase-3β (GSK-3β). This study opened the opportunity for related studies in generating neural progenitor like cells for neurodegenerative diseases treatment [10].

The iPSC regenerative potential could be turned unfortunately to a tumorigenic one by genomic alterations onset during differentiation step. Thus, in a study of Mullally *et al.*, it was emphasized that aneuploidy and hence the tumorigenic potential of iPSC is triggered in some circumstances, by accumulating cell cycle

CHAPTER 9

# Current Proteomic Studies for New Concept in Stem Cell Biology

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**Abstract:** Recent studies on stem cells and protein interactions using proteomics approaches have yielded novel perception on processes regulating development and stem cell biology. The development of stem cell approaches has evolved in the post-genomic era and the implementation of proteomic applications represents a great challenge.

Understanding the mechanism that controls stem cell pluripotency, self-renewal and differentiation will also improve the ability to develop stem cell-based therapies.

Current proteomics studies of stem cell signaling pathway can lead to the discovery of molecular mechanisms that govern cell-cell interaction and/or with stem cell niche.

Stem cells represent an important potential therapeutical approach, both in regenerative medicine and tumor pathology, allowing the understanding of the mechanisms that underlie the biology of these cells.

**Keywords:** Cancer stem cells, Interactomics, Proteomics, Regenerative medicine, Secretome, Signaling pathways, Stem cell biology, Stem cells therapy.

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## INTRODUCTION

The domain of stem cells is of outstanding importance in life sciences; a great amount of resources is invested in the development of stem cell based technologies for regenerative medicine. However, there is a “dark” counter-part, represented by cancer stem cells, which are increasingly demonstrated in tumorigenesis and tumor progression.

Regenerative medicine holds the promise of engineering damaged tissues and organs by means of stem cells of both embryonic and adult origins, due to their unique properties of unlimited self-renewal and differentiation once they receive appropriate signals. Proteomics rely on state-of-the-art technology platforms interacting with advancements in mass spectrometry and bioinformatics to develop a large-scale comprehensive study of a specific proteome, including information on protein abundances, their variations and modifications and studies of post-translational modifications and protein-protein interactions. Development of stem cell approaches has evolved in the post-genomic era and the implementation of proteomic applications represent the great challenge. Recent studies on stem cells and protein interactions using proteomics approaches have yielded novel perception on processes regulating development and stem cell pluripotency [1].

Transcriptome alone provides an incomplete and unfairly interpretation of stem cell biology [2, 3]; however, transcriptome mapping using DNA microarray technology is commonplace in the current post-genomic era [4, 5]. Transcriptional profiling approach has some limitations regarding the yet unidentified "stemness" genes that are not present on the microarray and the mRNA level that may not be properly correlated with changes in protein expression [6]. Furthermore, protein complex formation and degradation, post-translational modificationsα (PTMs) highly influence protein-protein and protein-DNA interactions; therefore, it is rather difficult to predict the functional outcome of these systems based only on gene expression and/or genomic data.

The term “proteome” was used for the first time by Wilkins *et al.* [7] and refers to the entire complement of proteins expressed in a given population. Proteomics

encompasses a wide variety of advanced techniques capable to provide invaluable insights into the proteome at a genome wide level: from yeast two-hybrid screens for identifying protein-protein interactions [8], antibody-based protein chips for identifying proteins [9] and high throughput crystallography screens [10]; the most widely utilized group of techniques center around mass spectrometry (MS).

Both embryonic stem cells (ESCs) and adult stem cells, the two main groups of stem cells, have been intensively and widely explored to understand the molecular mechanisms such as self-renewal, proliferation, differentiation and the promising results were of great use in many clinical applications [11]. The overall understanding of biological features of stem cells is the great goal of the so-called "omics" technologies, which comprise an increasingly wide range of biology fields to denote studies undertaken on a large or genome-wide scale. The "omics" range from genomics, transcriptomics, proteomics to epigenomics and are based on advanced technologies responsible for the great opportunities and advances in the postgenomic biology and medicine [12, 13], bringing new insights in the field of stem cell biology. The investigation of stem cells using high-throughput screening techniques provided large amounts of information facilitating systemic understanding of relationship between molecular components [14].

It is assumed that the cancer stem cells (CSCs) are the underlying cells which can cause the heterogeneous lineage of cancer cells, also responsible for tumor progression: metastasis, recurrence and chemoresistance [15 - 18]. CSCs were first discovered in acute myeloid leukemia [19], but there are studies that report their existence in a wide variety of cancers, including gastric cancer [20]. The discovery of CSCs markers, which selectively target this specific small population of cells, may arise novel therapeutic opportunities [21, 22]. Some of these CSCs markers: aldehyde dehydrogenase 1 (ALDH1) [23], CD44 [24, 25] and CD133 [26] have been recently reported, even if they are not unique to CSCs. Quantitative protein expression profiling currently gives the most accurate and reproducible differential expression values for proteins [27]. A powerful proteomic instrument useful for tumor biomarkers is iTRAQ (isobaric tags for relative and absolute quantitation) combined with multidimensional liquid chromatography (LC) and tandem mass spectrometry (LC-MS/MS) analysis [28].

CHAPTER 10

# Nano and Microtechnology for Monitoring Stem Cell Differentiation

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**Abstract:** This chapter gives an overview of the new micro- and nano-technologies designed to monitor SC differentiation in the context of their potential applications in disease modeling, tissue engineering, regenerative medicine, as well as drug screening and toxicology. Representative examples of such applications are presented and the crucial importance of the differentiation processes for the safety of SC therapies is discussed. The roles of the main factors that influence SC differentiation are briefly summarized and the vital need to control and monitor the differentiation process using non-invasive methods is emphasized. The basic principles of new micro- and nano-technologies for monitoring SC differentiation are presented, with special focus on the use of acoustic vibrational fields to characterize SC. Literature studies on SC differentiation, involving methods based on Impedance Sensing (IS), Surface Enhanced Raman Spectroscopy (SERS), Fourier Transformed Infrared (FTIR) spectroscopy, Microelectrode Array (MEA) sensors, Light-addressable Potentiometric Sensors (LAPS), Field-effect Transistors (FET), Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance (QCM), are briefly described.

**Keywords:** Differentiation, Monitoring, Nano and microtechnology, Stem cells.

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**Cristiana Tanase & Monica Neagu (Eds.)**

## INTRODUCTION

The potential uses of stem cells (SC) in treating a series of neurological, hepatic, hematopoietic, diabetic and skin diseases [1 - 5] rely on their capacities to self-renew and differentiate into multiple cell lineages.

These characteristics promote SC as basic ingredients in the design of efficient tissue repair and vital organ regeneration therapies [3, 6].

Since SC evolution strongly depends on their microenvironment [6 - 8], the safety of SC therapies can only be ensured by studying SC under relevant conditions, mimicking their *in vivo* environments. However, standard cell culture methods are inappropriate for SC culture. SC need sequential delivery of specific growth factors and other soluble molecules, an extracellular scaffold allowing proper cell-cell interactions, lower oxygen concentration than those provided by the majority of cell culture incubators *etc.* [7, 9, 10].

To avoid such inconveniences, efforts have been made to develop nano and micro-technologies and devices capable to replicate the *in vivo* SC microenvironments, as well as to monitor SC behavior and differentiation *in vitro* or to track their location and distribution following their transplantation to the human body. Such multifunctional tools allow for a better understanding of SC biology and a more meaningful extrapolation of results from *in vitro* studies to *in vivo* situations.

Regarding the monitoring methods, they should ideally be based on physical principles that allow them not to interfere with cellular processes.

While the advances regarding SC tracking have been presented in several comprehensive review articles and books [1, 11 - 16], the monitoring of SC differentiation by means of advanced nano and micro-technologies has been poorly summarized.

This chapter gives an overview of the present technologies used to monitor SC differentiation, classified with respect to their underlying detection/monitoring principles, with special focus on the use of electro-acoustic vibrational fields in sensing and monitoring the differentiation process.

## THE NEED TO MONITOR SC DIFFERENTIATION IN THE CONTEXT OF THEIR RESEARCH AND THERAPEUTIC APPLICATIONS

### SC – Research

Due to their remarkable properties, SC represent excellent experimental models for both basic as well as applied research.

### Basic Research

SC offer valuable information concerning cell biology and tissue formation in embryogenesis and postembryonic development, allowing in-depth studies regarding normal growth and development and/or identification of major causes of birth defects [17].

### Applied Research

#### *a) Disease Modeling*

Induced pluripotent SC (iPSC)-based research offers both clinical translation opportunities and new approaches to clarify the molecular basis of human diseases [18, 19]. Depending on the desired application, iPS cells can be used to generate disease specific somatic cells or patient specific cells. By linking disease, patient specificity and drug discovery and design, SC-based research allows patients to benefit from the newly emerging personalized medicine [2, 20, 21].

Due to specific similarities between SC and cancer cells (*e.g.* high telomerase activity and unlimited cell division capacity), SC represent key models for the study of abnormal cell division that leads to cancer [5, 22].

#### *b) Tissue Engineering and Regenerative Medicine*

Transplanted SC are expected to differentiate in response to tissue specific stimuli, leading to tissue regeneration and repair [23]. Like any other therapy, SC transplantation needs to provide maximum therapeutic benefits to patient, with minimum side effects [4].

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