PREFACE

This new book presents reserch developments from around the globe in the field of cellular differentiation which is a concept from developmental biology describing the process by which cells acquire a "type". The morphology of a cell may change dramatically during differentiation, but the genetic material remains the same, with few exceptions. A cell that is able to differentiate into many cell types is known as pluripotent. These cells are called stem cells in animals and meristematic cells in higher plants. A cell that is able to differentiate into all cell types is known as totipotent. In mammals, only the zygote and early embryonic cells are totipotent, while in plants, many differentiated cells can become totipotent with simple laboratory techniques.

Chapter I - Differentiation is a complex multistep process that affects many biological pathways that regulate the expression of specific genes, and cell proliferation. All adult tissues are made up of lineages of cells consisting of tissue stem cells and their progeny (transit-amplifying cells and terminally differentiated cells). Recent data suggest that cancers arise from rare self-renewing stem cells that are biologically distinct from their more numerous differentiated progeny. Growing evidence suggests that pathways regulating normal stem cell self-renewal and differentiation are also present in cancer cells and cancer stem cells (CSCs). Malignant tumors can be viewed as an abnormal organ in which a small population of tumorigenic CSCs have escaped the normal limits of self-renewal giving rise to abnormally differentiated cancer cells that contribute to tumour progression and growth. This new model has important implications for the study and treatment of cancer. Understanding the molecular circuitry which contributes to the maintenance of stem cells may provide an insight into the molecular mechanisms of cancer and thus new approaches for elimination or differentiation therapy. The unique properties of stem cells are opening the door to the development of new therapeutic approaches notably in oncology and regenerative medicine. Adult mesenchymal stem cells (MSCs) possess particularly attractive properties, as they are easily expanded in vitro and possess the potential to differentiate into multiple cell lineages. Recent investigations show both the fundamental properties of MSCs and applicable therapies derived from their use, particularly in cardiology but also in cancer and immunomodulation. Moreover, circulating endothelial progenitor cells (EPC) isolated from bone marrow or peripheral blood of cancer patients are capable of contributing to the neovascularisation of tumors participating in angiogenesis process. Classical agents used in

differentiation therapy of cancer such as retinoic and butyric acid mixed as esters with hyaluronan primed the expression of cardiogenic genes and elicited a remarkable increase in cardiomyocyte yield in mouse embryonic stem cells. The authors have recently reported the synthesis and anticancer properties of several 5-fluorouracil derivatives. These compounds increased microtubule stability and HLA class I expression in rhabdomyosarcoma cells. The authors results suggest that there may be significant potential advantages in the use of these new differentiating agents for the treatment of cancer. Treating cancer cells with these compounds may provide a non-toxic therapeutic modality when the options for chemotherapy have been exhausted. This review compares cancer cells to embryonic stem cells and to adult tissue stem cells. Moreover, the authors review the current status of the CSC research and propose the targets for CSC cell-surface molecules, signal transduction pathways, the stem cell niche, the stem cell differentiation and drug resistance caused by designed and synthesized molecules.

Chapter II - The therapy of leukaemia based on the use of compounds able to reinitiate the differentiation program has been extensively studied. The use of calcitriol ($1,25D_3$) or trans-retinoic acid (ATRA) in myeloid or monocytic leukemic cell differentiation has been also clinically taken into consideration. Both are natural compounds accepted at relatively high doses by the organism, however, several other secondary effects take place because their other respective main functions different than regulating the differentiation program in cancerous cells. To avoid the secondary effects due to overloading of both, ATRA or $1,25D_3$, therapies based on the co-administration of other natural compounds such as vitamin C, vitamin E, polyphenols, carotenoids or other antioxidants acting as anti-cancer active compounds have been taking into consideration. In several cases, these natural compounds do not affect by themselves the differentiation program in leukemic cells but they are able to enhance the effect of the differentiation-active compounds such as ATRA or $1,25D_3$. The present chapter reviews the effect of the combination of these natural antioxidant compounds on the treatment of leukemic cells and will try to define the probable common mechanism of action for this plethora of substances.

Chapter III - Bone marrow-derived stem cells (BMDCs) gather considerable attention for their ability to trans-differentiate into various dedicated cell types, e.g. liver, heart and skeletal muscle, and brain. Thereby, BMDCs trans-differentiate in accordance to the specific tissue blueprint, which could be the local milieu (soluble factors, cell adhesion molecules and/or nanotubes), a single cell (cell fusion) or a combination of both. The local tissue blueprint has to guide the changes from a stem cell, by definition endowed with the ability for self-renewal, to a differentiated, dedicated cell exhibiting specific tissue functions. A similar process has to occur during tissue stem cell differentiation. Again, the local tissue milieu is responsible for providing a correct blueprint guaranteeing an accurate differentiation process.

The question arises what will be the result of BMDC trans- and tissue stem cell differentiation if stem cells will receive a faulty set of instructions, which might be provided by chronic inflamed tissue or tumor tissue. Both environments have lost many of the characteristics of the tissue of origin. Tumor tissue *per se* is heterogeneous and resembles chronic inflamed tissue. Chronic inflamed tissue itself is characterized by a chaotic interplay of tissue cells, immune competent cells, cytokines, chemokines, growth factors and reactive oxygen species.

An incomplete set of instructions concomitant with the combination of proliferative stimuli and DNA damaging agents represents an explosive mixture with a fatal outcome for (trans-) differentiating stem cells, especially for those stem cells adopting tissue function by cell fusion. Aneuploidy is frequently found in tumor cells and has been postulated to be the driving force in the pathogenesis of cancer. Fusion of stem cells and tumor cells will result in an increased aneuploidy in hybrid cells. Conjointly, the proliferative and DNA damaging environment will lead to the evolution of hybrid cells exhibiting new properties as e.g. a higher proliferation rate, the ability to metastasize and/ or to be drug resistant. In particular the latter property has been associated with so-called cancer stem cells, which apparently play a crucial role in metastasis formation and tumor relapse. Thus, it might be hypothesized that cancer stem cells might be the product of cell fusion between stem cells and tumor cells. The understanding of cell fusion and its consequences for the pathogenesis of cancer, possibly driving the evolution of cancer stem cells, is therefore crucial for the development of novel anti-cancer strategies.

Chapter IV - Studies exploring the potential neural transdifferentiation of bone marrow-derived stem cells (BMSCs) are of increasing value in stem cell research. New research demonstrates that in addition to providing a good model for studying adult stem cell plasticity, BMSCs have considerable potential for promoting neural repair. Use of these cells as a therapeutic model is facilitated by their easy accessibility for autologous transplantation. Methods for inducing neural differentiation include adding simple chemicals, cytokines, growth factors, and brain tissue extracts to cultured BMSCs, as well as co-culturing BMSCs with organotypic brain slices or neural cells. Induced marrow-derived stem cells resemble mature neurons in morphology, expression of neural marker genes, and electrophysiological properties, but whether these changes represent true neural differentiation merits further investigation. Notably, naïve BMSCs express several neural marker genes and exhibit small voltage-dependent membrane currents even before neural induction. This suggests a couple of possibilities. First, these properties may be related to the multidifferentiation potential of BMSCs and facilitate neural differentiation. Alternatively, the shared neural marker genes may not be as specific to neural cells as previously thought.

Chapter V - This review focuses on the development and technique of three dimensional (3D) cultures to study epithelial cell differentiation and tissue architecture. 3D cultures employ human basal epithelial cells, stem cells and heterotypic cultures to faithfully reproduce tissue complexity. These models have arisen from a requirement to model human cells in a tissue-like structure. They are more accessible than animal based models and have a more relevant complexity than 2D cell culture. A 3D environment is important to properly integrate chemical and biomechanical signals in the establishment of epithelial differentiation and architecture. Epithelial cell function is engineered by combining the influences of matrix, neighbouring cells and soluble growth factors.

In the last 10 years there has been a great expansion in the use of 3D model systems for cell research. These can be divided into two broad categories: 1) 3D spheres generated by cell aggregation using techniques such as rotary wall vessel culture, agar, pellet culture and liquid media and 2) 3D models which produce tissue-like architecture through culturing within gel or synthetic scaffolds. More recently 3D models are being employed to define the stem cell niche, allowing the control of self renewal and commitment to differentiation to be explored.

Chapter VI - SCF and c-*kit* signaling is essential for both human and murine mast cell development, while IL-3 is required for murine mast cell hyperplasia that occurs in response to various stimuli. Relatively stable numbers of mast cells within connective and mucosal tissues are maintained by the constitutive expression of SCF in its membrane-bound and soluble forms by stromal cells (such as fibroblasts, epithelial cells, endothelial cells, bronchial smooth muscle cells) and by the expression of c-*kit* on mast cells at all stages of differentiation. Besides SCF and IL-3, the Th2 cytokines IL-4, IL-9, IL-10 and IL-13, also play critical roles in mast cell development by synergistically promoting the actions of SCF and IL-3 on mast cell proliferation and differentiation. However, the Th2 cytokines alone are unable to support neither the proliferation nor survival of mast cells. In contrast, the Th1 cytokine IFN-γ suppresses mast cell development from various murine and human sources. Interestingly, the above generally-accepted roles of the Th1 and Th2 cytokines on mast cell development are currently being challenged by controversial findings showing that mast cell development is up-regulated by IFN-γ and down-regulated by IL-4, IL-10 and IL-13. This review summarizes the recent progress in our understanding of how the Th1 and Th2 cytokines regulate mast cell development either in a positive or negative manner, with a particular focus on IL-4-induced mast cell apoptosis.

Chapter VII - Cellular differentiation is the commitment of cells in response to internal and external signals, and is often accompanied by morphological changes. Hematopoiesis is a well-understood differentiation system that leads to the formation of the various blood cell types, which are all derived from the hematopoietic stem cell (HSC). Research about this process has exploded in the last few decades with the discovery of cell surface antigen-based isolation methods and cytokine response characterizations. However, the kinetics of these responses and the specific fates of individual cells remain unclear. This is because the data are based on the average of cell populations and/or conditions that observe cell behaviour outside of their normal environment. Therefore, new technologies must be developed and utilized, following single cells, to dissect the exact nature of this differentiation process.

It is becoming increasingly clear that the bone marrow has supporting cells that influence and control blood differentiation. Research has only begun to understand what cells types and factors are involved. The data so far have recognized that some "niche cells" allow HSC to self-renew and differentiate in a controlled manner that, in turn, leads to the mature adult hematopoietic system.

Although a few components of this complex niche are now recognized, the exact nature of the HSC-niche relationship is still unclear, as technical obstacles have yet to be overcome. Much of the data are based on fixed tissues that show the localization and behaviour of a HSC at a single point in time. This neglects the temporal component to this dynamic relationship between the HSC and its niche. Furthermore, researchers have had to measure the presence of HSC by indirect functional readouts for its progeny, thus ignoring the individual characteristics and behaviours of these cells. So far, HSC have not been directly observed as live single cells, with the individual characterization of their behaviour and differentiation within their normal niche.

To understand the temporal and spatial relationships the HSC has with the various supporting cell types, HSC must be visualized within their niche over time, under constant video surveillance. Only then will we be able to understand the HSC-microenvironment

relationship. This review will discuss the need for new technologies that will allow for a greater understanding of the HSC-niche relationship that influences normal blood differentiation.

Chapter VIII - Complex human tissues harbour stem cells and/or precursor cells, which are responsible for tissues' development or tissues' repair. These cells provide new insights into dental development and are important for characterizations of cellular differentiation processes under in vitro conditions. In recent times undifferentiated cells were isolated from dental tissues like the periodontal ligament, the dental papilla or the dental follicle. These cells are realistic sources for the construction for example of an engineered periodontium, which submits the connection between implanted tooth and jaw. Therefore, dental precursor cells are interesting for novel treatments of periodontitis or dental implantation. However, the authors need more information about their differentiation mechanisms for the evaluation of dental treatments. Dental follicle precursor cells (DFPCs) are derived from an embryonic dental tissue of extracted wisdom teeth. These cells are easy accessible and ideal for testing cell differentiation mechanisms during dental development. However, there are many publications about dental precursor cells or stem cells derived from dental ectomesenchymal tissues. This review gives also a short overview about known ectomesenchymal cells of dental origin and classifies these cells.

Chapter IX - In Podarcis, undifferentiated, pre-differentiated and differentiated follicle cells can be recognised by shape, size, position and function. Undifferentiated (stromal) and pre-differentiated (small) cells are stem cells destined to proliferate and differentiate while differentiated cells (pyriforms) are nurse cells programmed to sustain oocyte growth and then degenerate at the end of previtellogenesis.

The present work clarifies whether competence to undergo apoptosis in pyriforms is acquired at the time they originate from the small cells or later during aging. For this purpose the expression of Fas, Fas-L, caspase 3 and DNase have been investigated by immunocytochemistry in small and pyriform cells. Results indicate that all these cell death factors are present exclusively in pyriforms. Their synthesis starts as soon as cells differentiate and that while aging, Fas and Fas-L are down and up regulated respectively. The authors conclude that pyriforms are committed for apoptosis since differentiation and that death occurs via mechanisms alternative to receptor or mitochondrial mediated pathways.

Chapter X - Schwann cells (SCs) contribute to nerve regeneration by providing regeneration strands, many kinds of nerve growth factors and tight junctions to stabilize cell contact with injured nerve cells, as well as gap junctions to facilitate traffic of substances between the cells. It is recognized that Schwann cells play a crucial role in the process of peripheral nerve development and regeneration, even in the construction of tissue-engineered nerve grafts. However, the difficulty is to obtain sufficient numbers of autologous SCs in a short time. Use of allogeneic cells are involved in immunological rejection. Bone marrow stromal cells (MSCs) are nonhematopoietic multipotent stem cells that differentiate into connective tissue lineages including osteoblasts, chondrocytes, and adipocytes under special experimental conditions. In particular, MSCs showed potential to be differentiated to SCs in vitro and in vivo, which enhance peripheral nerve regeneration. In this charpter, the authors will summarize the various strategies that can be used to differentiate MSCs into Schwann cells in vitro and in vivo. Many different assays and techniques may be used to define and

characterize MSCs which were differentiated along SCs lineage. Functional assays that may be used to further define the differentiated MSCs and determine if they can promote peripheral nerve regeneration are described.