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Interplay between Cell Proliferation and Cellular Differentiation: A mutually exclusive paradigm

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Abstract

The developmental processes in living organisms are tightly regulated by series of gene expression (gene switch off and switch on concept) driving both cell proliferation and cellular differentiation. However, the link between cell proliferation and differentiation is poorly understood. Thus, an insight on the mechanistic processes linking cell proliferation and differentiation at the molecular level will drastically increase the knowing of the unknown in developmental biology. Over the years, Cell proliferation (a process that produces two cells from one) and cellular differentiation (the transition of a cell from one cell type to another) processes have been viewed as a separate prodigy in developmental biology. However, it is now clear that in vast majority of cells, cell proliferation and cellular differentiation exhibit a significant inverse relationship occurring in a mutually exclusive paradigm. Cells after birth continues to proliferate before acquiring a differentiated state, thus, the initiation of cellular differentiation is associated with proliferation arrest and permanent exit of a cell from the cell division cycle. The relationship existing between cell proliferation and cellular differentiation are pivotal to a successful biochemical events regulating the development of a multicellular organism from a single fertilized egg to a complex organism. Evidence justifying the inverse relationship between cell proliferation and cellular differentiation have been reported from studies of cells in culture and in vivo models. The mechanistic events regulating cell proliferation and cellular differentiation must be closely coordinated to develop a functional organism, thus, dysregulation in any of the two processes are said to be one of the hallmarks of developmental abnormalities and carcinogenesis. This work reviewed the linked between cell proliferation and cellular differentiation at the molecular level as both processes plays a key role in a successful development of a complete functional living organism.

Keywords: *Mitogen, Cell proliferation, Cellular differentiation, Cyclin dependent kinase, Quiescent, Carcinogenesis, Cell cycle, Genome.*

1. Introduction

One of the central anchor point to cell and molecular biologist is to understand how the information encoded in the cell's genome is used to direct the complex repertoire of biochemical events leading to a successful development of a functional organism and how the genome of a cell is been propagated to a new generation, i.e. the transfer of the genome from the parental cell to its progeny [1]. Multicellular organisms constitute multiple cells that has an inherent ability to reproduce (proliferate), grow, respond to stimuli, process information, differentiation and carry out myriads of biochemical reactions that sustains and ensures longevity of an organism [2], [3], [4], [5] [6]. The ability of these individual cells to exhibit these bio-potentials that are embedded in the subcellular organelles whose collective integration following normal instructions and signals enhances the development of a functional organism and brings about life, but independently cannot, remains "The Molecular Logic of Life".

During the evolution of multicellular organism, new mechanisms arose to coordinate their production, to diversify cell types, to regulate their size and number, to organize them into functioning tissues, and to eliminate damaged and aged cells. Specific patterns of mitotic cell division in part are central in the formation of working tissues and organs during development of multicellular organisms [1], [4], [5], [7], [8]. The development of a complete functional organism devoid of abnormalities begins with a single fertilized egg called zygote [9], [10]. This processes employs the integration of complex biochemical and physiological events which are encoded in the genomic blue print of the fertilized egg. The fertilized egg begins to proliferate and after several cycles of division to produce appropriate number of cells which are unspecialized, at specific time depending on the expressed information encoded in the genome, the unspecialized cells acquires specialized functions (becomes differentiated) which changes the cell size, shape, membrane potential, metabolic activity and responsiveness to signals [5], [7], [10]. These changes are largely due to the modifications in gene expression (gene switch on/off) concept [4], [10]. However, the linkage between cell proliferation and cellular differentiation plays a central role in the development of a functional organism as dysregulation and imbalance between these two processes will halt the development of an organism [3], [5]. Cell proliferation gives birth to two cells from one which requires a biochemical sequence of event involving cell growth, replication (i.e. DNA replication), mitosis followed by cell division (cytokinesis) [7, 10, 11]. The cell cycle entails an ordered series of macromolecular events that lead to cell division and the production of two daughter cells each containing chromosomes identical to those of the potential cell [4, 7, 10].

It is imperative to understand that cell proliferation processes in living organisms have to take place in a coordinated way to ensure correct division and formation of progeny cells containing intact genomes. Dysregulation or uncontrolled cell proliferation is a hallmark of cancer. The mechanisms that maintain the balance between cell proliferation and differentiation are often compromised in cancer cells, leading to unperturbed

proliferation and a failure to differentiate. Cancer is as a group of diseases characterized by the uncontrolled proliferation of cells and spread of abnormal cells [12]. Cancer cells are characterized by the fact that they keep replicating when they are actually supposed to be differentiated [13]. The unsafe nature of our environment due to the presence of xenobiotics also plays a critical role in influencing gene expression regulating cell proliferation and differential. The case of environmental contamination with metals, have been reported to influence gene expression mainly because of their toxicity, mutagenicity and carcinogenic nature even at low concentration [14]. Heavy metals are among the most common environmental pollutants and their occurrence in waters and biota indicate the presence of natural or anthropogenic sources [15], [16]. Reactive oxygen species (ROS) are implicated in various pathological conditions [17]. Biochemically, imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system is the hallmark of oxidative stress [18]. Oxidative stress has been suggested in both experimental and clinical studies to play a major role in gene expression and pathogenesis of so many diseases such as cardiovascular disease and cancer [18].

To normal tissues, cell proliferation occurs in cells that replenish the tissue. Most tissues are known to contain stem cells that have this replenishment potential. Stem cells are self-renewing cells that can divide asymmetrically to yield a new stem cell and a progenitor cell. Progenitor cells may or may not undergo further divisions, ultimately leading to terminal differentiation. Once cells have terminally differentiated, they have a specialized function and are no longer dividing [4], [10]. Most tissues are made up of such non-dividing cells. Thus proliferation is normally tightly controlled so that only particular cells in the body are dividing.

Cell differentiation is a transition of a cell from one cell type to another involving a switch from one pattern of gene expression to another [1], [3], [5]. It is a process by which unspecialized cell becomes a more specialized cell type with specialized morphology, metabolism and physiology from the cells of the same origin. Cellular differentiation can be categorized into three state based on their ability to revert differentiation upon stimulation by growth signaling factor (Mitogen); Primary (Pre-differentiation State), Intermediate (Non-terminal differentiation state), and Terminal differentiation [1], [4], [5]. Cells at primary and intermediate state can reverts differentiation when stimulated by mitogen whereas cells at terminal differentiation state cannot revert differentiation upon stimulation with mitogens. Cell proliferation and cellular differentiation occurs numerous times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types [1], [3], [4].

How living organisms coordinate vast arrays of biochemical processes regulating cell proliferation and cellular differentiation has been a ‘holy grail’ and one of the most important fundamental question in developmental biology, cell biology and cancer biology until recently. Studies using cells in culture and genetic animal models have now revealed an insight on how the biochemical machineries of cells encoded in the genome directly

influence and link cell proliferation and cellular differentiation to a successful development of a functional organism [1], [3], [4].

The fundamental processes about the molecular event connecting cell proliferation and cellular differentiation in the development of a functional multicellular organism have been one of the focus point in other to understanding several developmental abnormalities over the years, yet little have been done to unravel the molecular interplay between them. However, this review presents a compelling reports geared toward addressing a fundamental question regarding the inverse relationship between cell proliferation and cellular differential, thus, providing a clear understanding of the molecular event connecting cell proliferation and cellular differentiation.

2. The Link between Cell Proliferation and Cellular Differentiation

The linkages in the control of cell proliferation and cellular differentiation are most evident at the Pre-differentiation Growth Arrest (PGA) state and at the Nonterminal Differentiation (NTD) state; cells at these two states are quiescent (Table 1) [19]. Cells at the PGA state can either differentiate or exit the PGA state and return to the cell division cycle and proliferate. The NTD cell state also serves to link the control of proliferation and differentiation because at this state, cells can either undergo terminal differentiation which is associated with the irreversible loss of proliferative potential or return to the cell cycle and proliferate in association with loss of the differentiation phenotype i.e. dedifferentiation (Table 1) [19].

Table 1: The Linkages of Cell Proliferation and Differentiation in 3T3T Mesenchymal Stem Cells.

Multistep Differentiation Process	Highly Differentiated Phenotype	Proliferation Potential	Examples of in vivo Counterparts
Rapid Growth	-ve	+ve	Regenerating Hepatocytes
↓↑			
Reversible PGA	-ve	+ve	Quiescent Stem Cell
↓↑			
Reversible NTD	+ve	+ve	Lymphocyte and Hepatocyte
↓			
Irreversible Terminal Differentiation (TD)	+ve	-ve	Neurons and Muscle cells.

Cells at a PGA-like state in vivo include a variety of quiescent stem cells and cells at NTD-like state in vivo include lymphocytes and hepatocytes that are highly differentiated cells that still retain their proliferative potential. Finally, cells at the Terminal Differentiation (TD) state in vivo are typified by striated muscle and neuronal cells, which are highly differentiated cells lacking proliferative potential (Table 1) [19]. NTD cells can be induced to differentiation and proliferation, whereas TD cells cannot. That is although cells at the NTD states are not as mitogenically responsive to growth factors as quiescent undifferentiated cells (PGA), they can be stimulated to proliferate [20]. This is illustrated by the fact that the high concentration on fetal bovine serum (30%

FBS) and insulin (50µg/ml) is required to induce mitogenesis in NTD cells whereas undifferentiated quiescent 3T3T cells can be induced to proliferate by a serum concentration as low as 5% FBS [20]. However, it has been shown that growth arrest in general precedes differentiation and that differentiation can ultimately result in the irreversible loss of proliferative potentials (i.e. terminal differentiation). Credible furtherance has been made in the last decade and have revealed multi-directional interactions between the molecular machinery regulating the processes of cell proliferation and cellular differentiation [21].

3.0. Cell Cycle Exit and Differentiation

Recent studies has revealed that differentiation and cell proliferation are regulated simultaneously but independently. Also, cells often start differentiating long before they stop dividing, and that the initiation of differentiation is not restricted to any particular segment of the cell cycle. The responses of cells to treatment with differentiating agents suggested that exit from cell cycle into G1/G0 occurs quite quickly, with functional differentiated characteristics acquired later, and so promoted the notion that cyclin-dependent kinase inhibitors (CDKIs) might be important initiators of normal differentiation [22].

The molecular ties between the cellular differentiation and cell proliferation are driven by; CDK activity complex, CDKI (Cyclin Dependent Kinase Inhibitors), Retinoblastoma (Rb) protein. Given the purported linked between cell cycle exit and differentiation, the identification of CDKIs seems to offer a useful starting point for identifying initiators of differentiation [23]. Cell cycle functions with the help of certain regulators. The primary regulators of the cell cycle includes; cyclin-dependent protein kinases (CDKs), their regulatory cyclins, and CDK inhibitors (CDKIs) [24]. Given the link between cell cycle exit and proliferation, the identification of CDKIs seemed to offer a useful starting point for identifying initiators of differentiation [23].

CDKIs are of two types: INK4 proteins ($p16^{INK4A}$, $p15^{INK4B}$, and $p19^{INK4C}$) interfere with cyclin D binding to CDK4 and CDK6 and so inhibit CDK activity [25] ; and KIP family members ($p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$) primarily inhibit CDK2 in vivo [26]. In terminal somatic cell culture models, inhibition of the cell cycle is almost always a requisite for differentiation. Forced inhibition of the cell cycle very often induces terminal differentiation and vice versa [27].

4.0. Cell cycle lengthening as a key model linking cell proliferation and cellular differentiation

The correlation between cell cycle lengthening and differentiation has been reported across several types of cell lineage and from diverse model organisms, both in vivo and in vitro. Furthermore, different cell fates might be determined during different phases of the preceding cell cycle, indicating direct cell cycle influences on both early lineage commitment and terminal cell fate decisions [28], [29], [30]. Cell cycle lengthening by the down-regulation of CDK activity is necessary and sufficient for neuronal differentiation, both in vitro in PC12 cells [28] and in vivo in whole embryo mouse culture [29]. Correlation between cell cycle length and differentiation have

been observed in other species. For instance, the lengthening (but not necessarily arresting) of the cell cycle by overexpression of the CDK inhibitor (CDKI) p27Xic1 can be enough to trigger precocious neuronal differentiation in developing Xenopus embryos [30].

Advances in gemonic manipulations with a number of studies seeking to manipulate cell cycle length and determining the its consequences, the mechanistic links between cell cycle length particularly the length of G1 and the decision to differentiate terminally are becoming increasingly clear . Acute knockdown of cyclin-D/CDK4 by RNA interference lengthens G1 by 20 % and increases the number of differentiated neurons by 40 % at 48 hours but depletes the basal progenitor population for long-term neuronal output [31]. Treatment of adult NSCs with a cdk4 inhibitor promotes differentiation under both self-renewing and induced differentiation culture conditions [32].

4.1. Cell-Cycle Lengthening Hypothesis

Cell cycle length hypothesis postulates that the length of G1 is a critical determinant of differentiation; a G1 phase beyond a certain threshold length is required for the sufficient accumulation and action of fate-determining factors that will then drive differentiation. However, if G1 phase is shorter than this threshold, differentiation will not occur and passage into S and G2 is not permissive for the differentiation signal to be executed [29]. It is interesting to view this model in the light of the recent data indicating that hESCs (human embryonic stem cells) show differential susceptibility to lineage specification signals depending on cell cycle phase whereas ESCs (embryonic stem cells) show changes in global epigenetic marks depending on their position in the cell cycle [33], [34]. Thus, the relative importance of the respective phases of the cell cycle might vary depending on the cell type and the nature of the exogenous determination signals. This is also consistent with recent work in chick spinal cord progenitor cells [35].

p27Xic1 over expression in the developing Xenopus embryo result in cell cycle arrest and massive cell death. However, lower level expression of p27Xic1 results in the precocious differentiation of neural plate progenitors into primary neurons [30]. In addition, p27Xic1 have been reported to cell fate within the developing Xenopus retina, and p27Xic1 overexpression leads to both premature cell cycle exit and conversion of retinal progenitor cells into Müller glial cells [36]. The inhibitory effects of vitamin D compound against the progression of cells from G1 to S have been reported [23], the D-type cyclins complex with CDK4 or CDK6 to form an active kinase that partially phosphorylate retinoblastoma family of proteins. This initial phosphorylation of retinoblastoma (Rb) partially inactivates Rb, resulting in the release of histones deacetylases and induction of transcription of certain genes, including the E-type cyclins. These cyclins bind to and activates CDK2, which further phosphorylates Rb and other substrates [23].

The timely accumulations of p27Kip1 in oligodendrocyte progenitors and in the development of a component of both the timer and effector mechanisms that determine a limited number of cell divisions before terminal differentiation have been reported in previous studies [37]. In addition, the involvement of CDK inhibitors in the differentiation of glial cells in the nervous system, and p27Kip1 and p21Cip1 might serve functionally separate and non-redundant roles during oligodendrocyte differentiation [37].

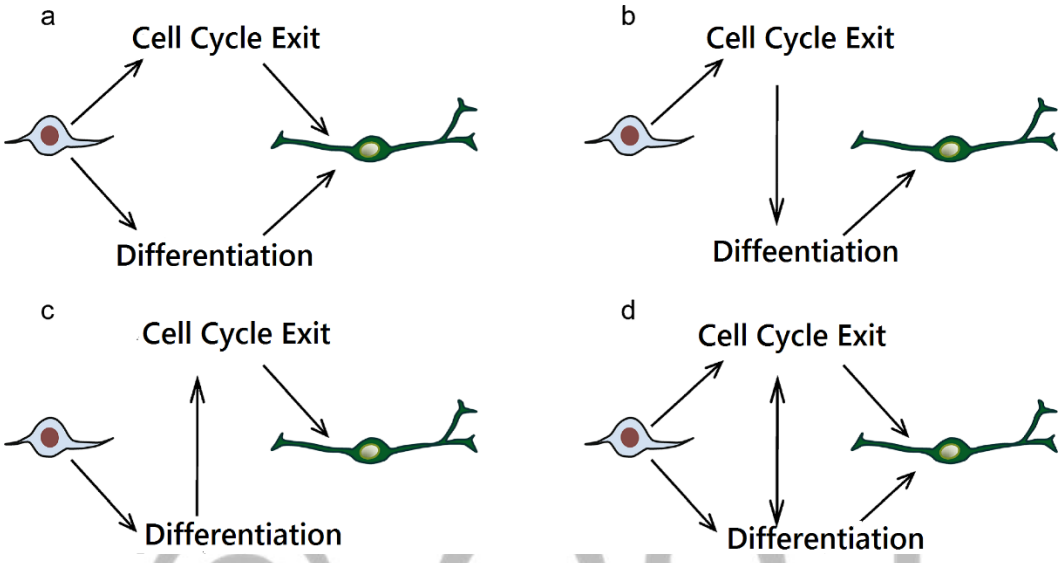

Figure 1: Linkages between cell cycle lengthening /exit and differentiation in nervous system [21]. ***(a)*** Cell cycle and differentiation are independently regulated [38]. **(b)** Induction of cell cycle lengthening and exit promotes differentiation [29]. **(c)** Initiation of differentiation results in cell cycle exit [39]. **(d)** Cell cycle lengthening and exit are coordinated with differentiation by dual actions of core components of the cell cycle and differentiation machinery [30, 40, 41].

Conclusion

Cell proliferation begets cellular differentiation processes, thus, differentiation brings about proliferation arrest and permanent exit of a cell from the division cycle. However, the events are mutually exclusive in a time-dependent manner as delay in the G1 phase of the cell cycle beyond a certain threshold length will induce a fate-determining factors to initiate cellular differentiation. The inverse relationship interlinking cell proliferation and cellular differentiation depends totally on the information expressed in the genome at a particular time required for a successful development, growth and tissue repairs in multicellular organisms.

Significance Statement: This work elucidate the molecular mechanisms interlinking cell proliferation and cellular differentiation in the development of a multicellular organism that can be beneficial in understanding variations in cell functions despite having the same copy of genetic materials. In addition, this review will help the researcher to uncover the critical areas of mitigating certain developmental abnormalities and diseases such as cancer that many researchers were not able to explore.

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