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Quiescence in Adult Stem Cells: Biological Significance and Relevance to Tissue Regeneration

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ABSTRACT

Adult stem cells (ASCs) are tissue resident stem cells responsible for tissue homeostasis and regeneration following injury. In uninjured tissues, ASCs exist in a non-proliferating, reversibly cell cycle-arrested state known as quiescence or G0. A key function of the quiescent state is to preserve stemness in ASCs by preventing precocious differentiation, and thus maintaining a pool of undifferentiated ASCs. Recent evidences suggest that quiescence is an actively maintained state and that excessive or defective quiescence may lead to compromised tissue regeneration or tumorigenesis. The aim of this review is to provide an update regarding the biological mechanisms of ASC quiescence and their role in tissue regeneration.

SIGNIFICANCE STATEMENT

Stem cell quiescence is a novel area of research within the stem cell field. The traditional view of cell quiescence is an inactive cell state. However, this view is changing into a more complex picture. Cellular quiescence is active cellular state where myriad of molecular changes are taking place within the cells and that are relevant to stem cell functions and integrity. In the present review, we provide an updated information regarding the biological significance of cellular quiescence and the molecular mechanisms underlying this phenomenon as relevant to stem cell biology. Particular emphasis has been given to hematopoietic stem cells, muscle satellite cells and mesenchymal (stromal) stem cells. Stem Cells 2014; 00:000–000

INTRODUCTION

Quiescence is a reversible cell cycle arrested state characterized by the absence of cell proliferation but unlike terminally differentiated cells, quiescent cells maintain the ability to re-enter cell cycle and resume proliferation. As with the cell division cycle, much of our knowledge about quiescence has been derived from extensive studies in cultured cells- from yeast to mammalian cells and recently from *in vivo* studies in animal models.

In baker's yeast, *Saccharomyces cerevisiae,* quiescence is often described as a "*Sleeping Beauty*" state, and is induced by nutrient limitation [1]. Thus, quiescence represents a survival mechanism that promotes viability in adverse environmental conditions [2]. In multicellular organisms, postnatal tissue homeostasis and regeneration following injury are mediated by a

small population of cells known as adult (or tissue specific) stem cells (ASCs), with the capacity to proliferate and subsequently differentiate into lineage specific cell types. ASCs are maintained within uninjured tissues in a quiescent and undifferentiated state [3]. Quiescent ASCs are activated upon tissue injury via soluble and mechanical signals emanating from the site of injury, leading to the production of transit amplifying progenitors that in turn differentiate into functional mature cells capable of tissue regeneration [3]. A small population of transit amplifying ASCs exits the cell cycle and re-enters quiescence to maintain a reserve of quiescent ASCs that can respond to future demands [4]. From an evolutionary perspective, quiescence may help to ensure a steady state number of ASCs available for tissue regeneration, and act as a protective mechanism against genotoxic stresses [5, 6].

In the present review, we will focus on quiescence studies conducted in ASCs, notably mesoderm-derived ASCs (hematopoietic stem cells, muscle satellite cells and skeletal (mesenchymal) stem cells) that are clinically relevant for enhancing tissue regeneration. We will discuss current methodologies employed to study the quiescent state and the molecular mechanisms regulating quiescence. In addition, we will evaluate evidences suggesting that quiescence is important for optimal functioning of ASCs, and that the inability of ASCs to maintain the quiescent state during ageing and under pathological conditions contributes to compromised tissue homeostasis and regeneration.

I. Defining cellular quiescence

Cellular quiescence or G0 is defined as a transient state where cells exit the cell cycle in response to either growth-inhibiting signals or absence of growth-promoting signals. Cellular quiescence is characterized by an un-replicated genome or G1 DNA content, an altered cellular metabolism, increased autophagy and distinct morphological changes such as decreased cell size and increased nucleus to cytoplasm ratio [7, 8]. Cell cycle arrest observed during quiescence is reversible, and thus distinct from permanent growth arrest observed in terminally differentiated or senescent cells [9, 10]

At the molecular level, ASCs entering the quiescent state *in vitro* exhibit altered expression of cell cycle regulatory genes, with down-regulation of positive regulators of cell proliferation such as cyclins and cyclin dependent kinases (CDKs), and up-regulation of negative regulators of cell cycle such as cyclin dependent kinase inhibitors (CDKIs) [10, 11]. While similar changes are observed during ASC differentiation, differences have been reported in the type of negative cell cycle regulators associated with cell differentiation compared to quiescence-related cell cycle arrest [10]. Quiescence in ASCs is also associated with reversible suppression of global RNA and protein synthesis [8, 10].

Entry and exit from cell cycle

The cell cycle proceeds through a sequence of coordinated events that are divided into phases (G1, S, G2 and M) based on landmark events of DNA Synthesis (S) and mitosis (M). Phase-specific cyclin-CDK complexes phosphorylate key targets in order to facilitate cell cycle progression. The G1 cyclins include *cyclin D (CCND)* that partners with *cyclin dependent kinase-4 (CDK4)* or *cyclin dependent kinase-6 (CDK6)* and *cyclin E (CCNE)* that partners with *cyclin dependent kinase-2* (*CDK2*). G1 cyclin-CDK complexes drive cell cycle progression through the G1 phase and play a role in G1-S transition by phosphorylating and inhibiting *retinoblastoma* protein *(Rb)*. In S phase, *CDK2* partners with *cyclin A (CCNA)* and promotes initiation of DNA replication whereas *CCNA-cyclin dependent kinase-1 (CDK1*) complex regulates S-phase to G2-phase transition. *Cyclin B (CCNB)-CDK1* complex which is active in M-phase phosphorylates key molecules mediating chromosomal condensation, spindle formation, nuclear envelope disintegration and centriole separation ***(Figure 1a).***

The transition from one phase of cell cycle to the next is regulated by cell cycle checkpoint proteins that act as brakes when conditions are not favourable for cell proliferation [12]. For instance, in order for cells to transit from G1 to S-phase and initiate DNA replication, cells must assess the availability of nutrients and enzymes needed for DNA replication, availability of growth factors as well as the absence of DNA damage. The G1 check point proteins (*ataxia telangiectasia mutated* (*ATM*) and *ataxia telangiectasia and rad3 related* (*ATR*)) ensure that these criteria are fulfilled. Chemical agents or irradiation that cause DNA damage, result in G1 cell cycle arrest. Cell proliferation resumes after DNA damage is repaired. Similarly, in order to pass the G2 checkpoint, which is temporally located before the onset of mitosis, cells must ensure that DNA replication has been completed without any errors [13]. Loss of checkpoint control proteins can lead to genomic instability, as observed frequently in a variety of cancers [14].

While the molecular mechanisms regulating cell cycle progression have been extensively studied, little is known about the mechanisms of entry into the quiescent state. Earlier studies suggested that a “restriction point” (R) temporally located in late G1 phase, governs the decision to enter S-phase [15]. Extended periods in G1 arrest lead to quiescence or G0. The elucidation of *Rb*-mediated negative control of S-phase entry provides a molecular understanding of the restriction point, and supported the concept that integration of extrinsic cues with intrinsic parameters leads to quiescence. While this framework has adequately explained the behavior of synchronized cultured cell models, recent studies using asynchronous populations of cells indicate that the quiescence decision point may differ from the *Rb*-regulated restriction point.

A landmark study using a sensor of *CDK2* activity coupled with quantitative live cell imaging and auto-

mated tracking of successive cell divisions in culture has generated a new view of the mechanisms regulating entry into G0 [16]. This study identifies a quiescence decision point temporally located in late G2/M phase. In a mitogen-activated cell population, the cells that fail to achieve a threshold activity of *CDK2,* are committed to enter G0 arrest. This subset of cells is marked by higher levels of *p21.* Loss of *p21* expression causes continuous cell proliferation even in absence of mitogenic signals [16]. A detailed understanding of the regulation of the *p21-CDK2* axis at G2/M should reveal new players in the quiescence decision point and the extent of its conservation *in vivo*.

Until recently, quiescence in ASCs *in vivo* has been considered to be a dormant cellular state with little metabolic activity. However, a recent study by Rodgers et al. [17] suggests that quiescent mammalian ASCs cycle between two molecularly distinct states: a sleeping or deeply quiescent (G0) state and a primed but still non-dividing state (G(Alert)), induced in response to tissue injury [17] ***(Figure 1b)***. Damage to skeletal muscle has long been known to activate resident stem cells to leave G0 and enter G1 [18]. The study by Rodgers et al., showed that even quiescent muscle satellite cells (MuSCs) in non-injured tissues respond to distant tissue injury by transiting to an alert or primed state (G(Alert)). Interestingly, not only MuSCs but also fibro-adipogenic progenitors (FAPs) and HSCs enter a G(Alert) state in response to muscle injury. Once the muscle regeneration process is complete, the primed cells slowly revert to the deeply quiescent (G0) state. In keeping with the original observations in yeast [1], this finding provides support for the notion of a "*Quiescence Cycle*" (by analogy to the cell division cycle). Furthermore, Rodgers et al., have demonstrated that the G(Alert) state is maintained through the TOR pathway, first identified in yeast as a central regulator of cell growth. The *mammalian target of rapamycin complex 1* (*mTORC1)* is known to be sensitive to environmental and nutritional stimuli. In damaged muscle, *mTORC1* is activated via signaling from *hepatocyte growth factor*/*scatter factor* (*HGF*/*SF*) that is stored in the extracellular matrix and released in an active form by the action of serum protease during tissue injury. Once activated, *HGF* induces a signaling cascade through the *PI3K-Akt* pathway, resulting in activation of *mTORC1*. Interestingly, the primed (G(Alert)) MuSCs exhibit enhanced muscle regenerative capacity [17], strongly supporting the notion that the quiescence cycle contributes to stem cell function.

II. Identification of quiescent cells

Due to the paucity of information on quiescence-specific events, quiescent cells have traditionally been identified by the absence of markers associated with proliferation. Several techniques are available to identify quiescent cells *in vivo* and *in vitro*. Proliferating cells are identifiable by labeling of newly synthesized DNA using nucleotide analogues such as tritiated thymidine (^{3}H-TdR), 5-Bromo 2'-deoxyuridine (BrdU) and 5-Ethynyl-2'-deoxyuridine (EdU). Once incorporated in proliferating cells, the labeled DNA can be detected by autoradiography (^{3}H-TdR) or immuno-cytochemistry/immuno-histochemistry (BrdU and EdU). Endogenous markers of proliferating cells such as *proliferating cell nuclear antigen* (*PCNA,* a DNA polymerase accessory protein which is expressed in S-phase), Ki67 (a protein associated with ribosomal RNA transcription and expressed in all phases except G0), *minichromosome maintenance-2* (*MCM-2*, a protein that functions in replication origins and expressed in S phase) and phospho-histone H3 (an M-phase-specific histone modification) are extensively used to distinguish between proliferating and quiescent cells [19, 20]. Cell cycle status can also be identified on the basis of DNA and RNA content, using DNA binding dyes such as propidium iodide (PI), DRAQ-5 and DAPI and RNA binding dyes such as pyronin Y and SYTO dyes. Although cells in either G1 or G0 phase possess an un-replicated genome (2N complement of DNA), quiescent (G0) cells are transcriptionally less active and possess lower total RNA content [21], which together readily distinguishes G0 cells from G1 cells in flow cytometry.

In experimental organisms, the identification of quiescent cells *in vivo* is based on their 'label retaining' characteristics i.e., quiescent cells retain the incorporated DNA label due to infrequent cell division [22]. BrdU is most commonly used label, taken up by cells during a period of BrdU exposure sufficient for the cell to cycle at least once. The label is retained within quiescent cells, but is diluted below detectable limits in proliferating cells due frequent cell divisions. Label-retaining cells (LRCs) have been detected in most adult mammalian tissues and have been shown to participate in homeostatic and regenerative repair [23]. More recently, lineage-tracing techniques that enable fluorescent tagging of a particular cell type have been used to identify quiescent cells in transgenic mice [24]. Histones are commonly tagged proteins as their incorporation is replication-dependent and the fusion proteins exhibit nuclear localization [25, 26].

Quiescent stem cells can also been identified by eliminating the proliferating cell population. Studies in mice showed that intravenous administration of 5-FU eliminates cycling HSCs while sparing a small population of quiescent HSCs that can repopulate the bone marrow in serial transplantation studies [27]. 5-FU is a pyrimidine analogue that irreversibly inhibits *thymidylate synthase*, the enzyme required for synthesis of the nucleotide thymidine monophosphate (dTMP), essential for DNA synthesis. Once taken up by cells, 5-FU induces apoptosis selectively in proliferating cells, while sparing quiescent cells. Similarly, quiescent MuSCs are less sensitive to a lethal dose of radiation which eliminates the proliferating cells in skeletal muscle [28].

Two newly developed fluorescent protein-based sensors have been used to identify quiescent cells directly. A *CDK2* based sensor consisting of a fluorescent protein tagged to the C-terminal fragment of human

DNA helicase B (*DHB*) can distinguish quiescent cells from actively proliferating cells [16]. The sub-cellular localization of the sensor is cell cycle dependent. In quiescent cells, the sensor is primarily localized in the nucleus due to low *CDK2* activity, whereas in proliferating cells, the sensor is progressively translocated out of the nucleus to the cytoplasm due to phosphorylation by *CCNE-CDK2* and *CCNA-CDK2* complexes. Thus, quiescent cells can be distinguished from proliferating cells by determining the relative distribution of the fluorescent sensor between nucleus and cytoplasm. Similarly, Okai et al., developed a fusion protein consisting of a defective mutant of *p27* fused to a fluorescent tag [29]. The fluorescent probe intensity is high in quiescent cells due to *p27* accumulation and is rapidly lost due to degradation as quiescent cells enter the cell cycle. The tagged *p27* probe has also been demonstrated to detect and isolate quiescent cells from various adult tissues in mice when expressed as transgene [29].

III. Ex vivo induction of quiescence

Cellular quiescence can be modeled in *ex vivo* cell cultures using a variety of approaches. Mammalian cells can be induced to enter a quiescent state by manipulating a number of culture conditions including anchorage deprivation, growth to confluence and contact inhibition, mitogen deprivation and nutrient/amino acid limitation [30-32] ***(Figure 2a)***. A key consideration concerns the type of growth arrest attained by the abrogation of mitogenic signaling in different cell types. While contact inhibition, mitogen deprivation and anchorage deprivation all induce reversible quiescence in fibroblasts, ASCs may respond differently to these culture conditions and enter different states of permanent arrest. For example, mitogen deprivation leads to differentiation in skeletal myoblasts [33] ***(Figure 2b)***, and non-adherent culture leads to anoikis (cell death) in epithelial cells [34] which are both irreversible. Thus, alternative approaches are required to establish reversible quiescence in ASCs. In mesoderm-derived cells such as myoblasts and MSCs, where attachment to a substrate is essential for cell growth, cells are effectively triggered into quiescence by suspension culture or culture on soft substrates in the presence of mitogens [35, 36] or by inhibiting adhesion-dependent signals using small molecule inhibitors of cellular contractility [37]. Importantly, restoration of surface attachment/contractility leads to a synchronous return to the cell cycle.

IV. Analysis of quiescence in adult stem cells (ASCs)

Cellular quiescence has been studied in several tissue-specific ASCs and a growing body of evidence suggests that quiescence is an intrinsic property of ASCs *in vivo,* permitting these cells to persist in an undifferentiated state for prolonged periods of time. Defects in maintaining quiescence can lead to stem cell exhaustion and degenerative diseases [25, 38]. Studies analyzing the quiescent state in different types of ASCs are presented below.

Quiescence in hematopoietic stem cells (HSCs)

Hematopoietic stem cells (HSCs) reside within the bone marrow and are responsible for renewal of mature blood cells throughout life. The quiescent nature of HSCs was first reported during studies of resistance of HSCs towards 5-FU-induced apoptosis [27]. In adult mice, direct evidence for the presence of quiescent HSCs has been provided in studies showing that continuous *in vivo* administration of BrdU for more than 12 weeks is required for labelling HSCs [39] indicating their slow cycling nature. On the other hand, it is possible to isolate quiescent adult HSCs based on combinations of cell surface markers that select primitive undifferentiated HSCs. Examples include *c-Kit$^+$Sca-1$^+$Lin$^-$Tie2$^+$* [5], *c-Kit$^+$Sca-1$^+$Lin$^-$CD150$^+$CD48$^-$CD34$^-$* [40] and *c-Kit$^+$Sca-1$^+$Lin$^-$CD48$^-$CD150$^+$* [26]. Although these marker-defined populations may differ subtly in their stem cell function, they are all quiescent.

Quiescence in muscle satellite cells (MuSCs)

Muscle satellite cells (MuSCs) are stem cells located between the plasma membrane of the muscle fibers and the surrounding basal lamina. MuSCs are undifferentiated progenitors that express the lineage determinant transcription factor, *Pax7* [41] but do not express transcription factors *MyoD* and *Myogenin* [42] which drive myogenic determination and differentiation respectively. MuSCs are key mediators of muscle repair and regeneration following muscle injury [43]. MuSCs are quiescent in uninjured adult muscles, as demonstrated by studies where continuous administration of ^{3}H-thymidine in mice for 9 days failed to label the majority of MuSCs [3]. Also in mice, MuSCs identified on the basis of cell surface markers such as *SMC2.6$^+$CD45$^-$* [11], *M-cadherin$^+$* [44] and *c-met$^+$* [45] exhibit a quiescence phenotype as evidenced by 2N DNA content, low RNA content, no BrdU incorporation and absence of *MyoD*/*Myogenin* expression. Quiescent MuSCs are resistant to sub-lethal doses of irradiation that eliminate the majority of proliferating cells [46]. Molecular profiling of quiescent MuSCs revealed up-regulation of cell cycle inhibitory genes such as *Gas3*, *CDKN1C*, *CDKN1B* and *Spry1* as well as negative regulators of myogenic differentiation such as *BMP2*, *BMP4*, *BMP6*, *HEY1* and *Notch3* [11]. Interestingly, novel surface markers of freshly isolated satellite cells including *Caveolin-1*, *Calcitonin receptor* and *integrin alpha 7* [47] can be used to localize and purify undifferentiated MuSCs.

Quiescence in skeletal stem cells

Skeletal stem cells or mesenchymal stem cells (MSCs) were originally isolated from bone marrow [48], but MSC-like cells have been isolated from the stromal compartment of several tissues including fat, lung, mus-

cle, kidney and skin [49]. Although some studies suggest that MSCs are quiescent in their niche, limited information is available regarding their quiescence phenotype, primarily because specific markers of quiescent MSCs have not been identified [50, 51]. Early studies of bone regeneration identified the presence of non-hematopoietic, label-retaining cells within bone marrow, suggesting the presence of quiescent MSCs capable of responding to bone injury and contributing to bone regeneration. For example, label-retaining MSCs were detected within bone marrow after injecting ^{3}H-TdR label in rat, from day 9 of pregnancy till birth [52]. In addition, label-retaining MSCs within murine bone marrow could be enriched by 5-FU treatment [53]. MSCs purified from humans as well as from rodent bone marrow by employing a combination of cell surface markers: *PDGFRα^{+}Sca-1^{+}CD45^{-}TER119^{-}* [50] and *STRO1^{+}VCAM1^{+}* [51], contain a non-cycling quiescent subpopulation as assessed by DNA and RNA content analysis. A recent lineage-tracing study in mice reports that MSC-like cells in the intestine are slow cycling, but quiescent bone marrow MSCs have not been described [54].

V. Molecular mechanisms of adult stem cell quiescence

Given that quiescent ASCs display a distinct transcriptome and surface marker profile, it is evident that the quiescent state is not merely a consequence of reduced metabolism, but is actively achieved and maintained. Both cell intrinsic and cell extrinsic factors have been demonstrated to play a role in regulating ASCs quiescence [6, 41, 55]. Mechanisms regulating entry, maintenance and exit from the quiescent state are best described in yeast [56]. In mammalian cells, the molecular mechanisms of quiescence have been studied extensively in *ex vivo* cell culture models of quiescence. Recent studies in genetically modified animals have demonstrated the relevance of these mechanisms for tissue regeneration under physiological conditions.

(A) Cell intrinsic mechanisms regulating quiescence

Tumor suppressor genes (TSGs) inhibit cell division and loss of function of TSGs leads to uncontrolled cell proliferation. TSGs function either directly or indirectly by suppressing genes required for cell cycle progression [57]. In ASCs, quiescence is associated with induction of TSGs and transgenic animal models deficient for TSGs exhibit impaired self-renewal of ASCs compartment and compromised tissue regeneration [38, 58]. Evidence for TSG association with quiescent ASCs is discussed below.

Retinoblastoma Family

The Rb or pocket protein family consists of three members: *Rb* (*retinoblastoma* protein), *p107* (*retinoblastoma like protein-1*) and *p130* (*retinoblastoma like protein-2*). The founding member of this family, *Rb* acts as the gate keeper of the G1/S transition and is a key target for the *CCND-CDK4/6* pathway [59]. *Rb* restrains cell cycle progression via control of S-phase transcriptional activators-particularly the *E2F* transcription factor family [59].

In HSCs, all three *Rb* family proteins are expressed and loss of an individual *Rb* family protein does not perturb quiescence in these cells [60]. However in mice, deficient for all three *Rb* family proteins, the HSC compartment is severely defective and mutant HSCs exhibit enhanced proliferation and impaired marrow repopulation ability upon serial transplantation [61, 62]. This phenotype is consistent with the loss of ability of pocket protein-deficient HSCs to maintain quiescence. By contrast, mice deficient for *Rb* alone exhibit an increased number of myoblasts within uninjured muscles as compared to wild type animals. When cultured *in vitro*, myoblasts from *Rb*-deficient mice exhibit accelerated cell cycle entry, loss of myogenic differentiation as well as increased cell death and autophagy [63, 64]. Thus, *Rb* is essential for maintaining MuSC quiescence *in vivo* as well as cell survival during myogenic differentiation. Interestingly, *p130* is highly expressed in quiescent MuSCs and over-expression of *p130* in proliferating myoblasts leads to cell cycle arrest. Also, *p130* inhibits myogenic differentiation by suppressing myogenic genes [65]. Thus, *p130* maintains MuSCs quiescence by a dual mechanism: blocking cell cycle progression and suppressing the myogenic differentiation program.

Cyclin Dependent Kinase Inhibitors (CKIs)

CKIs regulate quiescence through inhibition of cyclin dependent kinases. *p21^{Cip1}*, *p27^{Kip1}* and *p57^{Kip2}* inhibit *CDK2*, *CDK4* and *CDK6* blocking cell cycle progression [66]. Loss of CKIs abolishes the ability of ASCs to maintain cellular quiescence. Targeted ablation of *p21* in mice is associated with increased HSC proliferation, greater susceptibility to cell cycle-specific myelotoxic injury and poor bone marrow reconstitution ability during serial transplantation, suggesting that increased proliferation is associated with compromised stemness [38]. Similarly, *p57*-deficient mice exhibit reduced numbers of quiescent HSCs within bone marrow as well as reduced HSC self-renewal [58]. Furthermore, HSCs deficient for both, *p57* and *p27* exhibit higher proliferative capacity and lower bone marrow reconstitution ability [67]. Similarly, MuSCs deficient for both *p21* and *p57,* display increased proliferation *in vivo* and fail to undergo myogenic differentiation [68]. While CKIs would be expected to participate in slowing the cell cycle, it appears that distinct constellations of cell cycle inhibitors distinguish reversibly quiescent myoblasts from permanently arrested myotubes [10]. Thus, the precise networks governed by the individual CKIs in different quiescent cell types remain to be uncovered.

(B) Cell extrinsic factors regulating quiescence

The local microenvironment of ASCs known as the stem cell *'niche'* plays an important role in regulating quiescence. The dynamic interaction between ASCs and their niche which includes different cell types, blood vessels, extracellular matrix (ECM) proteins and nerve fibers, is critical for optimal stem cell function [5, 69, 70]. Communication between cells within the niche is mediated through secreted paracrine factors and their cognate receptors, cell-cell and cell-extracellular matrix (ECM) interactions that stimulate key signaling pathways in ASCs, particularly *Wnt*, *Notch* and *FGF* pathways. ***(Table 1)***

Wnt signaling is known to play a context-dependent role in ASCs. In HSCs, inhibition of Wnt signaling by ectopic expression of the secreted Wnt inhibitor *Dkk1* results in loss of HSC quiescence and self-renewal ability, whereas over-expression of a constitutively active form of *β-catenin* (the transcriptional mediator of canonical Wnt signaling) increases self-renewal of HSCs while blocking their differentiation [69, 71]. Wnt signaling appears to regulate HSC fate through regulation of *p21* [71]. In the HSC niche, the Wnt pathway is regulated by osteoblastic cells that are in close contact with HSCs [69]. In MuSCs, levels of Wnt signaling mark different cellular states: moderate levels of Wnt signaling is required for the quiescent state [10], as either enhancing or inhibiting Wnt signaling alters the quiescence program.

The Notch pathway plays a critical role in cell fate decision and stem cell homeostasis [72]. Notch signaling is commonly associated with inhibition of differentiation and maintenance of a self-renewing state. In adult murine HSCs, ectopic expression of activated Notch (*Notch Intracellular Domain -NICD*) enhances self-renewal and proliferation *in vitro* [73]. Conversely, loss of Notch signaling leads to increased HSCs differentiation *in vitro* and HSC depletion *in vivo* [74]. Thus, Notch signaling induces expansion of HSCs but preserves self-renewal ability. Unlike the soluble Wnt ligands, Notch ligands are often cell-surface molecules and mediate signaling via direct cell-cell interaction. Interestingly, like Wnt signaling, Notch signaling in HSCs is also regulated by osteoblastic cells [75, 76]. In MuSCs, Notch signaling maintains cellular quiescence [55, 77]. Over-expression of *NICD* in mouse MuSCs, suppresses proliferation and preserves self-renewal ability while inhibiting myogenic differentiation *in vitro* [78], whereas loss of *RBP-J*, a downstream transcriptional effector of Notch, leads to loss of quiescence and induction of precocious differentiation [6, 79].

Extracellular signal regulated kinase (ERK) signaling is involved in regulating quiescence via multiple receptors. Receptor tyrosine kinases (RTKs) are cell surface receptors which upon extracellular ligand binding induce kinase activity in their cytoplasmic domains. Secreted growth factors such as *HGF*, *IGF*, *PDGF* and *FGF* are potent activators of receptor tyrosine kinases [80]. Quiescent MuSCs express tyrosine kinase receptors for such ligands. However, several intracellular inhibitors that bind to and inactivate specific growth factors signaling molecules are highly expressed in quiescent MuSCs. Quiescent MuSCs express *Sprouty1 (Spry1)*, an inhibitor of RTKs, which binds and inhibits the kinase activity of these receptors even in the presence of their cognate ligands. *Spry1* expression is lost in activated MuSCs, and re-expressed in their progeny re-entering quiescence. Targeted deletion of *Spry1* in mice leads to enhanced ERK signaling and failure of MuSCs to re-enter quiescence after repair of injury [4]. Similarly, sustained *FGF2* signaling is shown to disrupt MuSCs quiescence *in vivo* [25].

The downstream effectors of ERK pathways are *mitogen-activated protein kinases* (*MAPK*) such as *p38α/β MAPK*. In MuSCs, *p38α/β MAPK* induces myogenic determination factor *MyoD* and subsequent proliferation. In transgenic mice, constitutively active *p38α/β MAPK* leads to precocious myoblast differentiation [81, 82]. Some evidence exists for asymmetric division as a contributor to quiescence in MuSCs, wherein one daughter cell commits to proliferation and eventual differentiation while the other daughter cell retains the stem cell characteristics [83]. During activation of MuSCs, p38*α/β MAPK* is shown to localize asymmetrically in one of the daughter cell that becomes the transit amplifying progenitor whereas the other daughter cell contributes toward replenishment of quiescent cells [84]. The upstream regulators of this asymmetric distribution of *p38α/β MAPK* are *Partitioning Defective 3* (*PAR-3*) and *Protein Kinase C* (*PKC*) [84].

VI. Role of quiescence in ASC function

Loss of quiescence is associated with defective ASC function. Quiescence in HSCs preserves the stem cell compartment and a balance between proliferation, quiescence and differentiation ensures persistence of regenerative cells throughout life [5, 69]. Reconstitution of bone marrow after lethal irradiation or other myelotoxic injury is dependent on stem cell quiescence, as this process is impaired when quiescence in HSCs is abolished through ablation of *Rb* proteins [61, 70]. As mentioned above, disruption of quiescence regulators such as *p21* or *p57* also decreases bone marrow reconstitution ability of HSC [38, 85]. Targeted deletion of the chromatin remodeler *Satb1* leads to loss of quiescence in HSCs *in vivo,* causing over-proliferation and precocious differentiation resulting in gradual depletion of functional stem cells [86]. Thus in HSCs, quiescence helps maintaining the stem cell phenotype and preserves self-renewal ability.

Disruption of quiescence in MuSCs leads to impaired muscle regeneration and repair. In mice MuSCs, loss of quiescence regulators such as *Spry1* (downstream of RTKs) and *RBPJ* (downstream of Notch) leads to depletion of MuSCs due to over-proliferation and precocious differentiation. Age-dependent changes in the muscle micro-environment affect MuSCs function by disrupting quiescence. For example, increased *FGF2*

expression in aged muscles leads to the persistent activation of MuSCs and prevents the restoration of the quiescent compartment leading to impaired muscle regeneration [25]. Victor et al., found that continuous expression of *p16* in aged MuSCs causes a shift from quiescence-associated to senescence-associated cell cycle arrest. MuSCs from aged mice thus undergo senescence upon injury induced activation signals [87]. The translational machinery also regulates quiescence in MuSCs. Compromising the RNAi pathway by targeted inactivation of *Dicer* in mice disrupts quiescence in myoblasts, and leads to loss of muscle regeneration [88]. As a corollary, transplantation of quiescent MuSCs have an enhanced muscle regeneration capacity as compared to culture expanded MuSCs [89]

Finally, cellular quiescence appears to protect ASCs from oxidative stress. Reactive oxygen species (ROS) encompass a variety of partially reduced metabolites of oxygen (e.g., superoxide anions, hydrogen peroxide, and hydroxyl radicals) that are generated intracellularly. At high and/or sustained levels, ROS can cause severe damage to DNA, protein, and lipids. Recent studies suggest that quiescent ASCs are protected from ROS, through upregulation of genes that mitigate the toxic effect of free radicals. For example, genes implicated in response to oxidative stress such as *glutathione peroxidase 3* (*GPX3*), *sulfiredoxin* (*SRXN*) and *thioredoxin reductase 1* (*TXNRD1*) are highly expressed in quiescent ASCs [90]. Also, cell surface transporters *Abcb1a*, *Abca5* and *Abcc9* that mediate efflux of toxic substances from the cell, are upregulated in quiescent stem cells [90]. Quiescent HSCs also possess mechanisms enhancing cell survival under adverse conditions such as hypoxia. Under hypoxic conditions, *HIF1α* is up-regulated in quiescent HSCs, translocates to the nucleus where it binds with *HIF1β* and transcriptionally regulates pro-survival genes [91].

VII. Concluding Remarks and Future Prospects

Cellular quiescence is emerging as an actively maintained state playing an important role in regulating ASC functions. The quiescent state protects ASCs from proliferation-associated genotoxic stresses as well as from damaging environmental conditions. Thus, quiescent ASCs exhibit better survival ability under adverse conditions of tissue injury. In addition, there is accumulating evidence that loss of quiescence in ASCs leads to compromised tissue regeneration. It is plausible that stem cell therapies using quiescent ASCs might prove beneficial over current approaches that focus on transplanting proliferating cells following *ex-vivo* expansion. With the establishment of new quiescence culture models as well as the introduction of valuable new reagents based on quiescence sensors *in vivo*, a more detailed understanding of the biological role of cellular quiescence will be uncovered. Integrating global studies of quiescence-specific transcriptomes, epigenomes, proteomes and secretomes will establish a detailed description of the biology of quiescent state, with the hope of specifically targeting quiescent stem cells *in vivo* to enhance tissue regeneration.

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Author Contributions

J.D. and M.K.: conception and design; M.R., J.D. and M.K.: manuscript writing; J.D. and M.K.: final approval of manuscript.

References

1 Gray JV, Petsko GA, Johnston GC et al. "Sleeping beauty": quiescence in Saccharomyces cerevisiae. Microbiology and molecular biology reviews : MMBR. 2004;68:187-206.

2 Herman PK. Stationary phase in yeast. Current opinion in microbiology. 2002;5:602-607.

3 Schultz E, Gibson MC, Champion T. Satellite cells are mitotically quiescent in mature mouse muscle: an EM and radioautographic study. The Journal of experimental zoology. 1978;206:451-456.

4 Shea KL, Xiang W, LaPorta VS et al. Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell stem cell. 2010;6:117-129.

5 Arai F, Hirao A, Ohmura M et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. 2004;118:149-161.

6 Mourikis P, Sambasivan R, Castel D et al. A critical requirement for notch signaling in maintenance of the quiescent skeletal muscle stem cell state. Stem cells. 2012;30:243-252.

7 Coller HA, Sang L, Roberts JM. A new description of cellular quiescence. PLoS biology. 2006;4:e83.

8 Lemons JM, Feng XJ, Bennett BD et al. Quiescent fibroblasts exhibit high metabolic activity. PLoS biology. 2010;8:e1000514.

9 Sachidanandan C, Sambasivan R, Dhawan J. Tristetraprolin and LPS-inducible CXC chemokine are rapidly induced in presumptive satellite cells in response to skeletal muscle injury. Journal of cell science. 2002;115:2701-2712.

10 Subramaniam S, Sreenivas P, Cheedipudi S et al. Distinct transcriptional networks in quiescent myoblasts: a role for Wnt signaling in reversible vs. irreversible arrest. PloS one. 2013;8:e65097.

11 Fukada S, Uezumi A, Ikemoto M et al. Molecular signature of quiescent satellite cells in adult skeletal muscle. Stem cells. 2007;25:2448-2459.

12 Murray AW. Recycling the cell cycle: cyclins revisited. Cell. 2004;116:221-234.

13 Johnson A, Skotheim JM. Start and the restriction point. Current opinion in cell biology. 2013;25:717-723.

14 Hartwell L. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. Cell. 1992;71:543-546.

15 Pardee AB. A restriction point for control of normal animal cell proliferation. Proceed-

ings of the National Academy of Sciences of the United States of America. 1974;71:1286-1290.

16 Spencer SL, Cappell SD, Tsai FC et al. The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit. Cell. 2013;155:369-383.

17 Rodgers JT, King KY, Brett JO et al. mTORC1 controls the adaptive transition of quiescent stem cells from G0 to G(Alert). Nature. 2014;510:393-396.

18 Bischoff R. Cell cycle commitment of rat muscle satellite cells. The Journal of cell biology. 1990;111:201-207.

19 Bravo R, Macdonald-Bravo H. Induction of the nuclear protein 'cyclin' in quiescent mouse 3T3 cells stimulated by serum and growth factors. Correlation with DNA synthesis. The EMBO journal. 1984;3:3177-3181.

20 Gerdes J, Li L, Schlueter C et al. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. The American journal of pathology. 1991;138:867-873.

21 Rhee WJ, Bao G. Simultaneous detection of mRNA and protein stem cell markers in live cells. BMC biotechnology. 2009;9:30.

22 Kubota Y, Takubo K, Suda T. Bone marrow long label-retaining cells reside in the sinusoidal hypoxic niche. Biochemical and biophysical research communications. 2008;366:335-339.

23 Duvillie B, Attali M, Aiello V et al. Label-retaining cells in the rat pancreas: location and differentiation potential in vitro. Diabetes. 2003;52:2035-2042.

24 Giachino C, Taylor V. Lineage analysis of quiescent regenerative stem cells in the adult brain by genetic labelling reveals spatially restricted neurogenic niches in the olfactory bulb. The European journal of neuroscience. 2009;30:9-24.

25 Chakkalakal JV, Jones KM, Basson MA et al. The aged niche disrupts muscle stem cell quiescence. Nature. 2012;490:355-360.

26 Foudi A, Hochedlinger K, Van Buren D et al. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nature biotechnology. 2009;27:84-90.

27 Van Zant G. Studies of hematopoietic stem cells spared by 5-fluorouracil. The Journal of experimental medicine. 1984;159:679-690.

28 Heslop L, Morgan JE, Partridge TA. Evidence for a myogenic stem cell that is exhausted in dystrophic muscle. Journal of cell science. 2000;113 (Pt 12):2299-2308.

29 Oki T, Nishimura K, Kitaura J et al. A novel cell-cycle-indicator, mVenus-p27K-, identifies quiescent cells and visualizes G0-G1 transition. Scientific reports. 2014;4:4012.

30 Macpherson I, Montagnier L. Agar Suspension Culture for the Selective Assay of Cells Transformed by Polyoma Virus. Virology. 1964;23:291-294.

31 Benecke BJ, Ben-Ze'ev A, Penman S. The control of mRNA production, translation and turnover in suspended and reattached anchorage-dependent fibroblasts. Cell. 1978;14:931-939.

32 Dike LE, Farmer SR. Cell adhesion induces expression of growth-associated genes in suspension-arrested fibroblasts. Proceedings of the National Academy of Sciences of the United States of America. 1988;85:6792-6796.

33 Yaffe D, Saxel O. A myogenic cell line with altered serum requirements for differentiation. Differentiation; research in biological diversity. 1977;7:159-166.

34 Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. The Journal of cell biology. 1994;124:619-626.

35 Milasincic DJ, Dhawan J, Farmer SR. Anchorage-dependent control of muscle-specific gene expression in C2C12 mouse myoblasts. In vitro cellular & developmental biology Animal. 1996;32:90-99.

36 Winer JP, Janmey PA, McCormick ME et al. Bone marrow-derived human mesenchymal stem cells become quiescent on soft substrates but remain responsive to chemical or mechanical stimuli. Tissue engineering Part A. 2009;15:147-154.

37 Dhawan J, Helfman DM. Modulation of acto-myosin contractility in skeletal muscle myoblasts uncouples growth arrest from differentiation. Journal of cell science. 2004;117:3735-3748.

38 Cheng T, Rodrigues N, Shen H et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000;287:1804-1808.

39 Bradford GB, Williams B, Rossi R et al. Quiescence, cycling, and turnover in the primitive hematopoietic stem cell compartment. Experimental hematology. 1997;25:445-453.

40 Wilson A, Laurenti E, Oser G et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;135:1118-1129.

41 Seale P, Sabourin LA, Girgis-Gabardo A et al. Pax7 is required for the specification of myogenic satellite cells. Cell. 2000;102:777-786.

42 Grounds MD, Garrett KL, Lai MC et al. Identification of skeletal muscle precursor cells in vivo by use of MyoD1 and myogenin probes. Cell and tissue research. 1992;267:99-104.

43 Mauro A. Satellite cell of skeletal muscle fibers. The Journal of biophysical and biochemical cytology. 1961;9:493-495.

44 Irintchev A, Zeschnigk M, Starzinski-Powitz A et al. Expression pattern of M-cadherin in normal, denervated, and regenerating mouse muscles. Developmental dynamics : an official publication of the American Association of Anatomists. 1994;199:326-337.

45 Cornelison DD, Wold BJ. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Developmental biology. 1997;191:270-283.

46 Wakeford S, Watt DJ, Partridge TA. X-irradiation improves mdx mouse muscle as a model of myofiber loss in DMD. Muscle & nerve. 1991;14:42-50.

47 Gnocchi VF, White RB, Ono Y et al. Further characterisation of the molecular signature of quiescent and activated mouse muscle satellite cells. PloS one. 2009;4:e5205.

48 Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Experimental hematology. 1976;4:267-274.

49 da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. Journal of cell science. 2006;119:2204-2213.

50 Morikawa S, Mabuchi Y, Kubota Y et al. Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. The Journal of experimental medicine. 2009;206:2483-2496.

51 Gronthos S, Zannettino AC, Hay SJ et al. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. Journal of cell science. 2003;116:1827-1835.

52 Haas RJ, Bohne F, Fliedner TM. On the development of slowly-turning-over cell types in neonatal rat bone marrow (studies utilizing the complete tritiated thymidine labelling method complemented by C-14 thymidine administration). Blood. 1969;34:791-805.

53 Falla N, Van V, Bierkens J et al. Characterization of a 5-fluorouracil-enriched osteoprogenitor population of the murine bone marrow. Blood. 1993;82:3580-3591.

54 Worthley DL, Churchill M, Compton JT et al. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell. 2015;160:269-284.

55 Fukada S. Molecular regulation of muscle stem cells by 'quiescence genes'. Yakugaku zasshi : Journal of the Pharmaceutical Society of Japan. 2011;131:1329-1332.

56 Crespo JL, Hall MN. Elucidating TOR signaling and rapamycin action: lessons from Saccharomyces cerevisiae. Microbiology and molecular biology reviews : MMBR. 2002;66:579-591, table of contents.

57 Voorhoeve PM, Agami R. Unraveling human tumor suppressor pathways: a tale of the INK4A locus. Cell cycle. 2004;3:616-620.

58 Matsumoto A, Takeishi S, Kanie T et al. p57 is required for quiescence and maintenance of adult hematopoietic stem cells. Cell stem cell. 2011;9:262-271.

59 Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995;81:323-330.

60 LeCouter JE, Kablar B, Hardy WR et al. Strain-dependent myeloid hyperplasia, growth deficiency, and accelerated cell cycle in mice lacking the Rb-related p107 gene. Molecular and cellular biology. 1998;18:7455-7465.

61 Viatour P, Somervaille TC, Venkatasubrahmanyam S et al. Hematopoietic stem cell quiescence is maintained by compound contributions of the retinoblastoma gene family. Cell stem cell. 2008;3:416-428.

62 Sankaran VG, Orkin SH, Walkley CR. Rb intrinsically promotes erythropoiesis by coupling cell cycle exit with mitochondrial biogenesis. Genes & development. 2008;22:463-475.

63 Hosoyama T, Nishijo K, Prajapati SI et al. Rb1 gene inactivation expands satellite cell and postnatal myoblast pools. The Journal of biological chemistry. 2011;286:19556-19564.

64 Huh MS, Parker MH, Scime A et al. Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation. The Journal of cell biology. 2004;166:865-876.

6**5** Carnac G, Fajas L, L'Honore A et al. The retinoblastoma-like protein p130 is involved in the determination of reserve cells in differentiating myoblasts. Current biology : CB. 2000;10:543-546.

6**6** Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes & development. 1999;13:1501-1512.

6**7** Zou P, Yoshihara H, Hosokawa K et al. p57(Kip2) and p27(Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70. Cell stem cell. 2011;9:247-261.

6**8** Zhang P, Wong C, Liu D et al. p21(CIP1) and p57(KIP2) control muscle differentiation at the myogenin step. Genes & development. 1999;13:213-224.

6**9** Fleming HE, Janzen V, Lo Celso C et al. Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. Cell stem cell. 2008;2:274-283.

7**0** Walkley CR, Olsen GH, Dworkin S et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. Cell. 2007;129:1097-1110.

7**1** Reya T, Duncan AW, Ailles L et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature. 2003;423:409-414.

7**2** Liu J, Sato C, Cerletti M et al. Notch signaling in the regulation of stem cell self-renewal and differentiation. Current topics in developmental biology. 2010;92:367-409.

7**3** Varnum-Finney B, Xu L, Brashem-Stein C et al. Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling. Nature medicine. 2000;6:1278-1281.

7**4** Duncan AW, Rattis FM, DiMascio LN et al. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nature immunology. 2005;6:314-322.

7**5** Li L, Milner LA, Deng Y et al. The human homolog of rat Jagged1 expressed by marrow stroma inhibits differentiation of 32D cells through interaction with Notch1. Immunity. 1998;8:43-55.

7**6** Calvi LM, Adams GB, Weibrecht KW et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003;425:841-846.

7**7** Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Developmental cell. 2002;3:397-409.

7**8** Wen Y, Bi P, Liu W et al. Constitutive Notch activation upregulates Pax7 and promotes the self-renewal of skeletal muscle satellite cells. Molecular and cellular biology. 2012;32:2300-2311.

7**9** Bjornson CR, Cheung TH, Liu L et al. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem cells. 2012;30:232-242.

8**0** Pouyssegur J, Volmat V, Lenormand P. Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. Biochemical pharmacology. 2002;64:755-763.

8**1** Shi H, Boadu E, Mercan F et al. MAP kinase phosphatase-1 deficiency impairs skeletal muscle regeneration and exacerbates muscular dystrophy. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2010;24:2985-2997.

8**2** Baeza-Raja B, Munoz-Canoves P. p38 MAPK-induced nuclear factor-kappaB activity is required for skeletal muscle differentiation: role of interleukin-6. Molecular biology of the cell. 2004;15:2013-2026.

8**3** Lin H. Cell biology of stem cells: an enigma of asymmetry and self-renewal. The Journal of cell biology. 2008;180:257-260.

8**4** Troy A, Cadwallader AB, Fedorov Y et al. Coordination of satellite cell activation and self-renewal by Par-complex-dependent asymmetric activation of p38alpha/beta MAPK. Cell stem cell. 2012;11:541-553.

8**5** Wilson A, Murphy MJ, Oskarsson T et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes & development. 2004;18:2747-2763.

8**6** Will B, Vogler TO, Bartholdy B et al. Satb1 regulates the self-renewal of hematopoietic stem cells by promoting quiescence and repressing differentiation commitment. Nature immunology. 2013;14:437-445.

8**7** Sousa-Victor P, Perdiguero E, Munoz-Canoves P. Geroconversion of aged muscle stem cells under regenerative pressure. Cell cycle. 2014;13:3183-3190.

8**8** Cheung TH, Quach NL, Charville GW et al. Maintenance of muscle stem-cell quiescence by microRNA-489. Nature. 2012;482:524-528.

8**9** Ikemoto M, Fukada S, Uezumi A et al. Autologous transplantation of SM/C-2.6(+) satellite cells transduced with microdystrophin CS1 cDNA by lentiviral vector into mdx mice. Molecular therapy : the journal of the American Society of Gene Therapy. 2007;15:2178-2185.

9**0** Pallafacchina G, Francois S, Regnault B et al. An adult tissue-specific stem cell in its niche: a gene profiling analysis of in vivo quiescent and activated muscle satellite cells. Stem cell research. 2010;4:77-91.

9**1** Takubo K, Goda N, Yamada W et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. Cell stem cell. 2010;7:391-402.

9**2** Abou-Khalil R, Le Grand F, Pallafacchina G et al. Autocrine and paracrine angiopoietin 1/Tie-2 signaling promotes muscle satellite cell self-renewal. Cell stem cell. 2009;5:298-309.

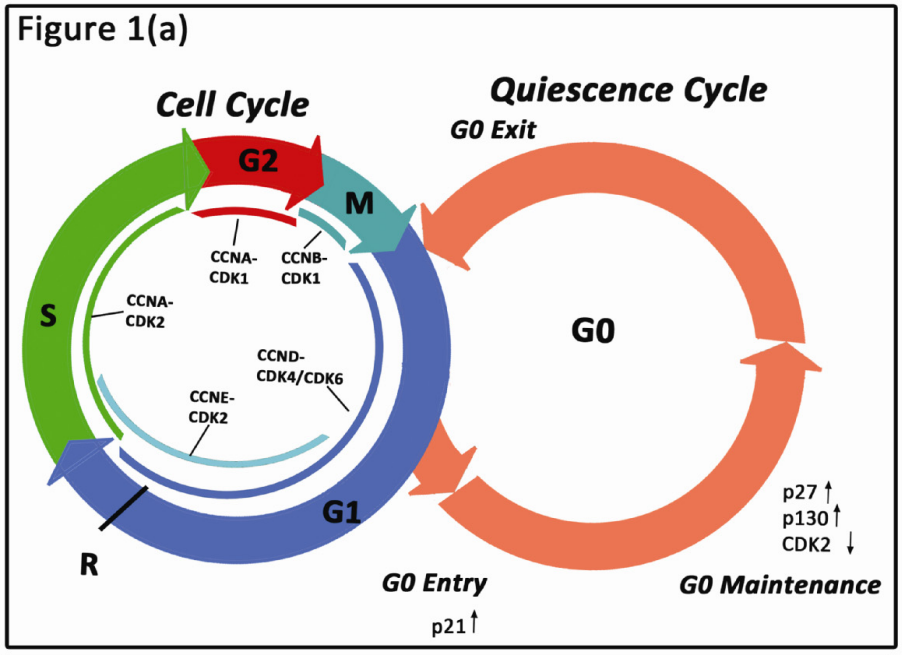
9**3** Grenier G, Scime A, Le Grand F et al. Resident endothelial precursors in muscle, adipose, and dermis contribute to postnatal vasculogenesis. Stem cells. 2007;25:3101-3110.

9**4** Trensz F, Haroun S, Cloutier A et al. A muscle resident cell population promotes fibrosis in hindlimb skeletal muscles of mdx mice through the Wnt canonical pathway. American journal of physiology Cell physiology. 2010;299:C939-947.

9**5** Brack AS, Conboy MJ, Roy S et al. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007;317:807-810.

9**6** Miller KJ, Thaloor D, Matteson S et al. Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. American journal of physiology Cell physiology. 2000;278:C174-181.

Figure 1(a). The cell cycle and the quiescence cycle.
The cell cycle is divided into specific phases- G1, S, G2 and M phase. Each phase is regulated by a specific *cyclin* (CCN)-*cyclin dependent kinase* (CDK) complex (*CCNA- Cyclin A; CCNB- Cyclin B; CCND- Cyclin D; CCNE- Cyclin E*). The window of particular CCN-CDK activity is depicted as colored arcs within cell cycle. The restriction point (R) in late G1 phase depicts the point of commitment; once cell crosses the restriction point, mitogenic signals are no more required by the cell to complete the cell cycle. The "*quiescence cycle*" is a current working model. Molecular mechanisms regulating entry into quiescence (G0 entry) and exit from quiescence (G0 exit) are poorly described. *p27*, *p130* and *CDK2* can be used as markers of quiescent state and based on recent data *p21* regulates G0 entry.
1(b). Quiescence cycle in adult stem cells (ASCs).
ASCs oscillates between "deep" quiescent (G0) and quiescent "alert" (G(Alert)) states. Quiescent ASCs within uninjured tissues respond to distant tissue injury by transiting to a quiescent "alert" state with increased propensity for cell proliferation and tissue regeneration. The transition from G0 to G(Alert) state is under the control of *mTORC1* (*mammalian target of rapamcin complex 1*) signaling. Downstream effectors of *mTORC1* signaling in G(Alert) state is shown to be group of genes regulating mitochondrial metabolism.



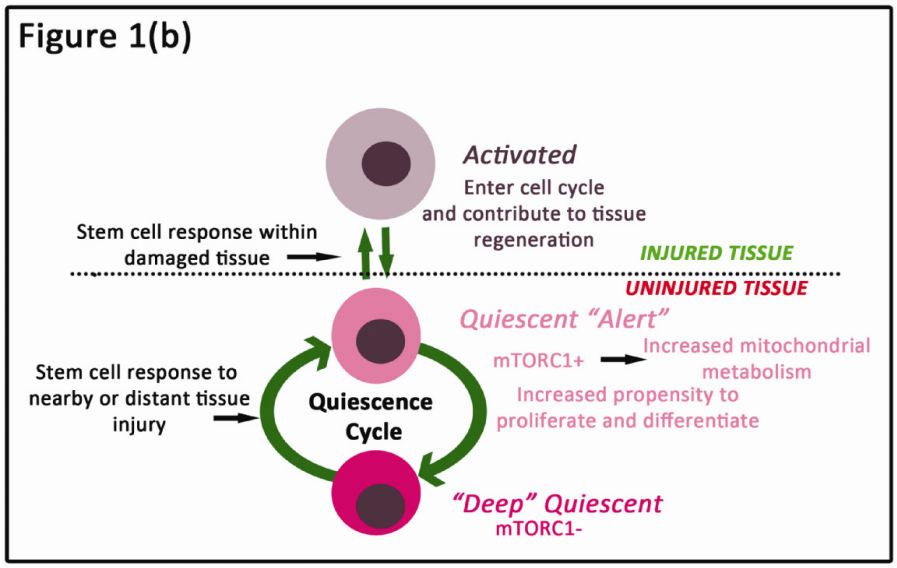


Figure 2(a). *In vitro* methods to induce quiescence (G0 state) in cultured mammalian cells.
An illustration of current methods for inducing cellular quiescence in *ex vivo* cell cultures. The effector proteins and signaling pathways are mentioned. *FAK-Focal adhesion kinase*; *ERK- Extracellular signal regulated kinases; PTPR- Protein tyrosine phosphatase, receptor type; MAPK- Mitogen activated protein kinases; IP3- Inositol triphosphate.*
2(b). Different types of cell cycle arrest identified in mammalian cells.
Different types of cell cycle arrests known in mammalian cells: quiescence, differentiation, senescence and apoptosis. The effector molecules and signaling pathways mentioned refer to studies conducted mainly in myoblast cell cultures.

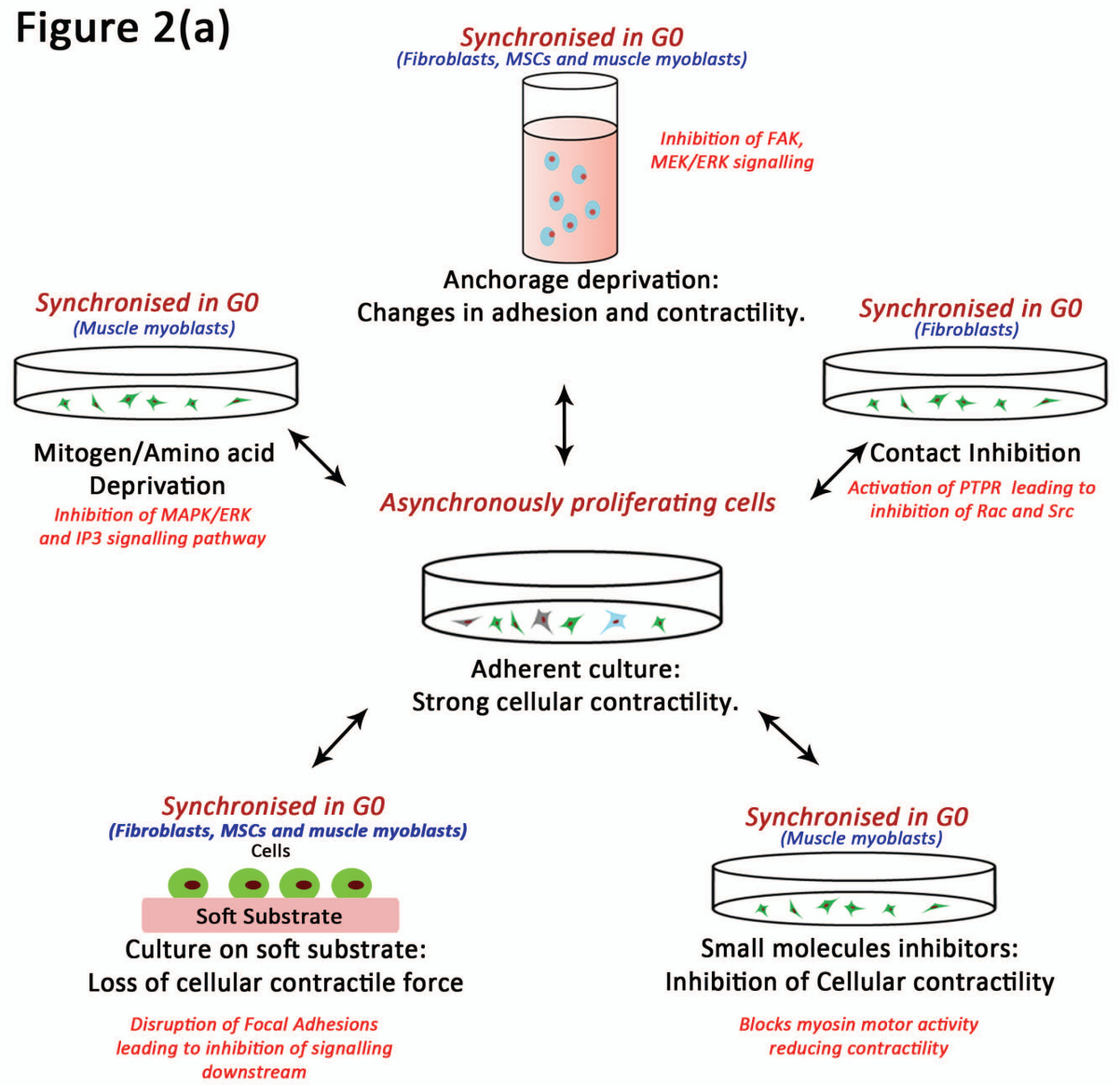


Figure 2(b)

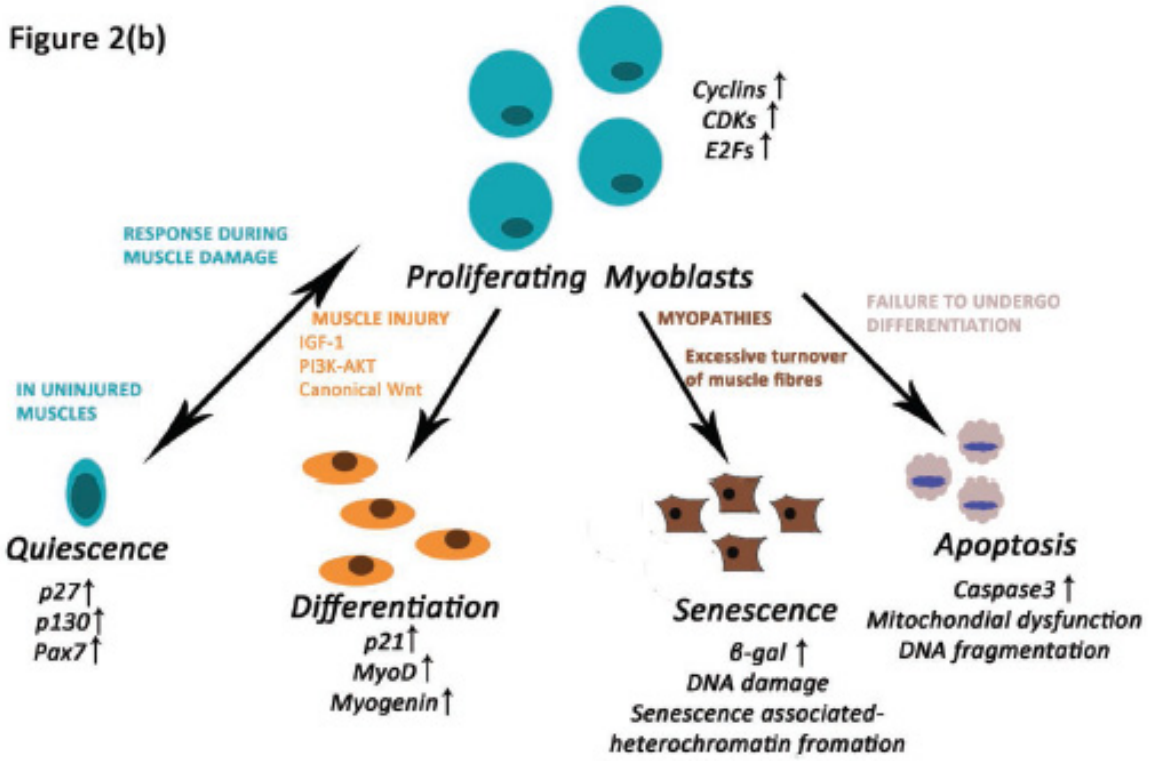


Table 1: Niche components involved in regulating quiescence associated signaling pathways in ASCs.

Signaling Pathways associated with ASC quiescence	Niche components that affect signaling in ASCs	References
ERK signaling	Osteoblastic cells in bone marrow niche and perivascular and interstitial cells within muscles regulate ERK signaling in HSCs and MuSCs respectively through secreted ligands.	[5, 92]
Wnt signaling	Osteoblastic cells in bone marrow niche and endothelial cells within muscles regulate Wnt signaling in HSCs and MuSCs respectively.	[69, 93-95]
Notch signaling	Jagged 1 expressing osteoblastic cells that are in close contact with HSCs regulate Notch signaling in HSCs.	[76]
FGF signaling	Over expression of FGF2 in aged muscle induces proliferation in mouse MuSCs and prevents establishment of quiescent MuSCs.	[25]
HGF signaling	HGF present in the extracellular matrix of various tissues is shown to regulate quiescence in ASCs.	[17, 96]