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The Spindle Assembly Checkpoint Safeguards Genomic Integrity of Skeletal Muscle Satellite Cells

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SUMMARY

To ensure accurate genomic segregation, cells evolved the spindle assembly checkpoint (SAC), whose role in adult stem cells remains unknown. Inducible perturbation of a SAC kinase, *Mps1*, and its downstream effector, *Mad2*, in skeletal muscle stem cells shows the SAC to be critical for normal muscle growth, repair, and self-renewal of the stem cell pool. SAC-deficient muscle stem cells arrest in G1 phase of the cell cycle with elevated aneuploidy, resisting differentiation even under inductive conditions. $p21^{CIP1}$ is responsible for these SAC-deficient phenotypes. Despite aneuploidy's correlation with aging, we find that aged proliferating muscle stem cells display robust SAC activity without elevated aneuploidy. Thus, muscle stem cells have a two-step mechanism to safeguard their genomic integrity. The SAC prevents chromosome missegregation and, if it fails, $p21^{CIP1}$-dependent G1 arrest limits cellular propagation and tissue integration. These mechanisms ensure that muscle stem cells with compromised genomes do not contribute to tissue homeostasis.

INTRODUCTION

Stem cells are essential for tissue formation, maintenance, and repair by achieving a timely balance between error-free replicative expansion and differentiation. This balance is frequently jeopardized in cancer and aging, leading to tissue pathology and functional decline (Blanpain et al., 2011; Liu and Rando, 2011; Ricke and van Deursen, 2013). To ensure accurate segregation of chromosomes during mitosis, the spindle assembly checkpoint (SAC) prevents anaphase onset until each chromosome has attached properly to mitotic spindle microtubules via its kinetochore (Foley and Kapoor, 2013; Lara-Gonzalez et al., 2012; Musacchio and Salmon, 2007). Therefore, an active SAC will delay mitosis until all chromosomes have been properly attached and aligned.

Many molecular players participate in a spatiotemporally concerted manner to actuate the SAC. These include MAD2 (mitotic arrest deficient 2), MPS1 (monopolar spindle 1), BUB1 (budding uninhibited by benomyl 1), and BUBR1 (Foley and Kapoor, 2013; Lara-Gonzalez et al., 2012; Musacchio and Salmon, 2007; Suijkerbuijk et al., 2012). In the presence of improperly attached kinetochores, the SAC arrests cells in mitosis by inhibiting the ability of CDC20 to activate APC/C-mediated polyubiquitination and subsequent proteasomal degradation of securin and cyclin B1 (Hwang et al., 1998; Kim et al., 1998).

SAC disruption in different cellular contexts reveals distinct outcomes. Data from immortalized cell lines and single-cell organisms demonstrate that the consequences of SAC failure include premature onset of anaphase, mitotic slippage, chromosome missegregation, and promotion of aneuploidy (Jelluma et al., 2008; Kops et al., 2004). In vivo studies in vertebrates and invertebrates show the consequence of SAC failure to be context dependent. During development, defective SAC activity can be tolerated early during embryogenesis but leads to an eventual loss of viability (Dobles et al., 2000; Fischer et al., 2004). Similarly, a reduction in MAD2 and BUBR1 leads to a SAC defect that can be tolerated but accelerates tumor production (Dai et al., 2004; Michel et al., 2001). In contrast, the SAC is essential during development and adult tissue regeneration in zebrafish (Poss et al., 2002a, 2002b, 2004). Studies on the role of the SAC in stem cells are more limited. Using either germline mutants or developmentally induced *Cre* drivers to delete SAC genes, both hematopoietic stem cells and epidermal stem cells and their respective committed progeny display differential sensitivity to SAC disruption (Foijer et al., 2013; Ito et al., 2007). Sensitivity to SAC dysfunction in epidermal stem cells was associated with increased aneuploidy and apoptosis (Foijer et al., 2013).

To date, the role of the SAC exclusively in adult mammalian stem cells has not been addressed. *Pax7*-expressing satellite cells (SCs) possess the function of stem cells and are critical for postnatal growth and repair of adult skeletal muscle (Lepper et al., 2009; Murphy et al., 2011; Sambasivan et al., 2011; Seale et al., 2000). Using inducible *Pax7*-specific SAC perturbation models, we show that the SAC is essential for normal mammalian SC function, during both early postnatal growth and adult tissue regeneration.

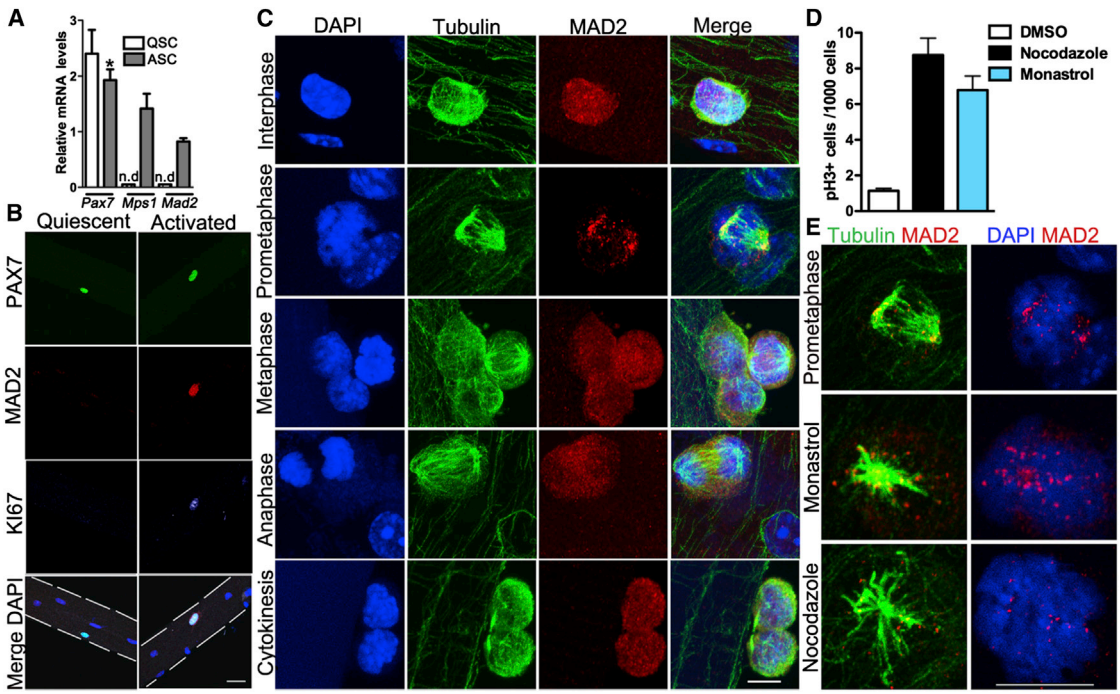

Figure 1. Activated Satellite Cells Have a Functional SAC

(A) Transcript levels of the satellite cell marker *Pax7* and SAC genes *Mps1* and *Mad2* from FACS-purified SCs immediately after isolation (quiescent; QSC) or cultured in high-serum media (activated; ASC) for 3 days. Data were collected from three mice per time point.

(B) Representative MAD2 and KI67 immunostaining pattern in PAX7^{+} SCs on single muscle fibers either fixed immediately or after 2 days in culture. 4′,6-diamidino-2-phenylindole (DAPI) stains nuclei. n = 3 mice.

(C) MAD2 (red) and tubulin (green) immunostaining at key stages of the cell cycle on single muscle fibers after 2 days in culture. DAPI stains nuclei blue.

(D) Number of cells that arrest in mitosis (pH3^{+} cells) after exposure to mitotic poisons. Triplicate data from n = 3 mice were pooled, with 800–2,000 cells per condition.

(E) Representative images of MAD2 localization in prometaphase (characterized by the tubulin staining pattern) and in the presence of mitotic poisons.

The scale bars represent 10 μm (B) and 5 μm (C and E). Data are presented as mean ± SEM. *p < 0.05. n.d., not detectable. See also Figure S1.

Deregulation of the SAC in SC progeny leads to a rapid G1 arrest and missegregation of chromosomes. p21^{CIP1} is critical for the cellular arrest, and its reduction in SAC-defective progenitors permits the expansion of SCs with faulty genomes. Furthermore, we show that SAC activity and the level of aneuploidy are not altered in cycling satellite cells as a function of physiological aging.

RESULTS

Muscle SCs Have a Functional Spindle Assembly Checkpoint

To investigate whether SCs have a functional spindle assembly checkpoint, we first examined expression of SAC components in quiescent and proliferating SCs. In quiescent SCs isolated by cell-surface markers (lin^{-}, Int-a7^{+}, Vcam^{+}, PI^{-}), *Mad2* and *Mps1* transcripts were barely detectable. After 3 days in culture in growth media, their levels were significantly upregulated (Figure 1A). To characterize the SAC component MAD2 in SCs residing within their niche, we examined single adult muscle fibers (Zammit et al., 2004). MAD2 protein as detected by immunohistochemistry was not detectable in SCs at 0 hr but was present in activated (KI67^{+}) SCs after 48 hr in culture (Figure 1B). To analyze MAD2 localization during progression through the cell cycle, we costained single fibers for tubulin and MAD2. In interphase, MAD2 localizes to the nuclear envelope and cytoplasm of activated SCs, but not to the nuclei of the postmitotic muscle fiber (Figures 1B and 1C; Figure S1). During prometaphase, MAD2 localizes to improperly attached kinetochores and, by late metaphase, when all the chromosomes are aligned, MAD2 redistributes from the kinetochores to the cytoplasm (presumably due to