DIFFERENTIATION OF ADULT MUSCLE-DERIVED STEM CELLS INDUCES TRANSIENT DNA DAMAGE - A LINK BETWEEN DIFFERENTIATION AND TRANSFORMATION

ABSTRACT INTRODUCTION: It is established that multiple signaling pathways cooperate to maintain stem cell identity and, therefore, dysregulation of the tight control of these pathways will likely lead to changes in cell homeostasis. These changes can result in aberrant behavior and potentially transformation. Our lab, as well as others, has shown transformation of stem cells following their implantation for the purposes of tissue regeneration. Previously, we reported that a population of muscle-derived stem cells (MDSCs) with a strong myogenic predilection can undergo myogenic, osteogenic, and adipoigenic differentiation in vitro, as well as regenerate dystrophic skeletal muscle in mdx mice. Furthermore, these same cells, when transduced to express bone morphogenetic protein 4 (BMP4), can heal critical-sized bone defects in the long bone and skull, with no deleterious effects. However, they undergo microenvironment-specific transformation and generate tumors when implanted in the skeletal muscle of mice. Herein, we report a putative mechanism to explain our previously observed spontaneous microenvironment-specific transformation of stem cells. Preliminary experiments using a DNA integration method that induces GFP expression have revealed that DNA integration events increase when stem cells are subjected to differentiation signals. When adult MDSCs were treated to undergo either myogenic or osteogenic differentiation we found that 1% and 3.5% of MDSCs that underwent osteogenic and myogenic differentiation, respectively, had integrated the reporter plasmids (over controls) after 7 days of culture. The concept of differentiation-induced DNA damage is not new. It was reported over 20 years ago that differentiation induces DNA nicking and strand breaks in a variety of cell types. To further investigate this, we focused primarily on myogenic differentiation. When we used a more refined fluorescence-activated cell sorting-based technique to explore DNA nicking, we found that, within 24 hours of myogenic differentiation, 12% of the cells had DNA damage. This trend increased over time, with the amount of damage peaking at 56% at 96 hours. By day 7, however, the damage was completely repaired. Furthermore, though we observed significant amounts of DNA damage, we saw no decrease in cell number, and no increase in the amount of dead/apoptotic cells over our controls. This data, though preliminary, provides evidence of transient DNA damage due to the differentiation of adult stem cells, and can help explain the spontaneous transformation of stem cells used for regenerative medicine.

METHODS:

Cell Culture. MDSCs were cultured under standard stem cell conditions (250 cells/cm² in proliferation medium (DMEM containing 10% horse serum (HS), 10% fetal bovine serum (FBS), 0.5% chick embryo extract, and 1% penicillin–streptomycin)). To induce myogenesis or osteogenesis, cells were plated at a density of 1500 cells/cm² and incubated either in myogenic medium (DMEM + 2% HS) or proliferation medium supplemented with BMP4 (200 ng/ml), respectively.

DNA Integration. To investigate integration of a foreign plasmid, MDSCs were grown at 1500 cells/cm² and cultured in normal proliferation medium or under myogenic or osteogenic conditions in the presence of a linearized GFP plasmid (Retro-X IRES-GFP from Clontech). Integration of the GFP plasmid was assessed 7 days post-induction by FACS.

TUNEL Assay. To explore DNA damage, Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were performed. TUNEL is a method for detecting DNA damage by labeling the terminal ends of nucleic acids. To do this, cells were harvested under standard differentiating conditions (250 cells/cm² in proliferation medium), under confluent conditions (1500 cells/cm² in proliferation medium), or under myogenic conditions (1500 cells/cm² in myogenic medium), and underwent TUNEL analysis at various time-points post-induction. The assays were performed as per manufacturer’s instructions (R&D) and read by FACS.

RESULTS SECTION:

Differentiation of adult stem cells allows for DNA integration. When MDSCs were exposed to osteogenic or myogenic differentiation conditions and a linearized GFP plasmid, we found that the percent of DNA integration events significantly increased compared to cells grown in their normal proliferation medium. MDSCs under normal growth conditions had almost no detectable integration events. However, when they were exposed to 200ng/mL BMP4 or myogenic medium, the percent integration events increased to 1 and 3.5%, respectively (Figure 1). This increase in integrative events potentially signifies differentiation-induced DNA damage that allows the linearized plasmid to integrate into the host’s genome.

Myogenic differentiation induces rapid DNA damage. Using TUNEL assays, we subjected our cells to myogenic differentiation for 24 hours and then assayed the amount of DNA damage. Surprisingly, within 24 hours, close to 12% of the cells induced to undergo myogenic differentiation had detectable DNA damage, while untreated stem cells at the same confluency, and cells growing under standard stem cell conditions, had minimal or no DNA damage (Figure 2).

Differentiation-induced DNA damage increases over time, but is eventually repaired. When we increased the timeline for our studies we found that the amount of DNA damage increased over time. Similar to our initial experiments, we found that approximately 13% of cells undergoing myogenic differentiation had DNA damage within 24 hours, 15% by 48 hours and 56% of the cells had detectable levels of DNA damage within 96 hours (4 days post plating). However, by 168 hours post-induction (7 days) there were only minimal levels of DNA damage within the cells (Figure 3). Furthermore, within the time-frame of this experiment, there was no increase in cell death and the cells grew at the same rate as the control cells kept at the same confluency (data not shown).

DISCUSSION: There is increasing evidence that activation of the apoptotic protein caspase-3 (a member of the cysteine-aspartic acid protease (caspase) family) plays a role in differentiation. In 2002 and 2005, it was demonstrated that activation of caspase-3 is essential for the differentiation of skeletal muscle and neural stem cells, respectively. Furthermore, caspase-3 does not act as a sculpting molecule by causing apoptosis, but rather induces differentiation through initiating a signaling cascade that is necessary for progenitor cell differentiation. This data, coupled with our preliminary findings (presented here), provides a putative model for how stem cells undergo transformation. Hence, we posit that during differentiation, caspase-3 cleaves inhibitor of CAD (ICAD), releasing caspase-activated DNase (CAD). CAD then causes DNA breaks within differentiating cells. This DNA nicking induces rapid and sudden transcriptional regulation. Hence, concomitant signaling may not only induce activation of pathways necessary for transformation, but may induce DNA breaks in key proteins (which under “normal” circumstances would be easily repaired), that in time may lead to misrepair and eventual damage/mutation.