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# Growth hormone facilitates 5′-azacytidine-induced myogenic but inhibits 5′-azacytidine-induced adipogenic commitment in C3H10T1/2 mesenchymal stem cells

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ABSTRACT

The C3H10T1/2 cells are considered mesenchymal stem cells (MSCs) because they can be induced to become the progenitor cells for myocytes, adipocytes, osteoblasts, and chondrocytes by the DNA methyltransferase inhibitor 5′-azacytidine. In this study, we determined the effect of growth hormone (GH) on the myogenic and adipogenic lineage commitment in C3H10T1/2 cells. The C3H10T1/2 cells were treated with recombinant bovine GH in the presence or absence of 5′-azacytidine for 4 days. The myogenic commitment in C3H10T1/2 cells was assessed by immunostaining them for MyoD, the marker for myoblasts, and by determining their capacity to differentiate into the multinucleated myotubes. The adipogenic commitment in C3H10T1/2 cells was assessed by determining their ability to differentiate into adipocytes. Myotubes and adipocyteswere identified by immunocytochemistry and Oil Red O staining, respectively. C3H10T1/2 cells treated with 5′-azacytidine and GH for 4 days contained a greater percentage of MyoD-positive cells than those treated with 5′-axacytidine alone ($P < 0.05$). The former generated more myotubes than the latter upon induced myoblast differentiation ($P < 0.05$). However, C3H10T1/2 cells treated with GH alone did not form any myotubes. C3H10T1/2 cells treated with 5′-azacytidine formed adipocytes upon adipocyte differentiation induction, whereas C3H10T1/2 cells treated with GH alone did not form any adipocytes. C3H10T1/2 cells treated with both 5′-azacytidine and GH formed fewer adipocytes than those treated with 5′-azacytidine alone ($P < 0.05$). Both GHR and IGF-I mRNA expression in C3H10T1/2 cells were increased by 5′-azacytidine ($P < 0.05$), but neither was affected by GH. Overall, this study showed that GH enhanced 5′-azacytidine-induced commitment in C3H10T1/2 cells to myoblasts but inhibited 5′-azacytidine-induced commitment to preadipocytes. These results support the possibility that GH stimulates skeletal muscle growth and inhibits adipose tissue growth in part by stimulating the myogenic commitment and inhibiting the adipogenic commitment, respectively, in mesenchymal stem cells.

## 1. Introduction

Growth hormone (GH) is a polypeptide hormone produced by the anterior pituitary under the stimulation of the hypothalamic hormone GH-releasing hormone (GHRH). Growth hormone is a major regulator of not only longitudinal body growth but also body composition. GH-deficient children have less muscle but more fat compared to children with normal GH production [1–4]. Similar abnormalities in body composition are displayed in GHRH receptor (GHRHR)-mutated lit/lit mice [5], GHRH knockout mice [6], and GH receptor (GHR) knockout mice [7]. These abnormalities in body composition reflect the fact that GH stimulates skeletal muscle growth while inhibiting fat deposition [8–10].

Skeletal muscle is composed of mainly myofibers. A myofiber is a multinucleated cell formed from fusion of mononuclear myoblasts during myoblast differentiation [11]. Adipose tissue is composed of mainly adipocytes, which are differentiated from preadipocytes [12,13]. Both myoblasts and preadipocytes are derived from mesenchymal stem cells (MSCs) [14,15]. Thus, there is a possibility that GH stimulates skeletal muscle growth and inhibits adipose tissue growth by stimulating the commitment of MSCs toward the myogenic lineage and inhibiting the commitment of MSCs toward the adipogenic lineage, respectively.

The C3H10T1/2 cells are multipotent cells derived from an early mouse embryo and are considered an appropriate model of MSCs because they have the potential to become the progenitors for myocytes,

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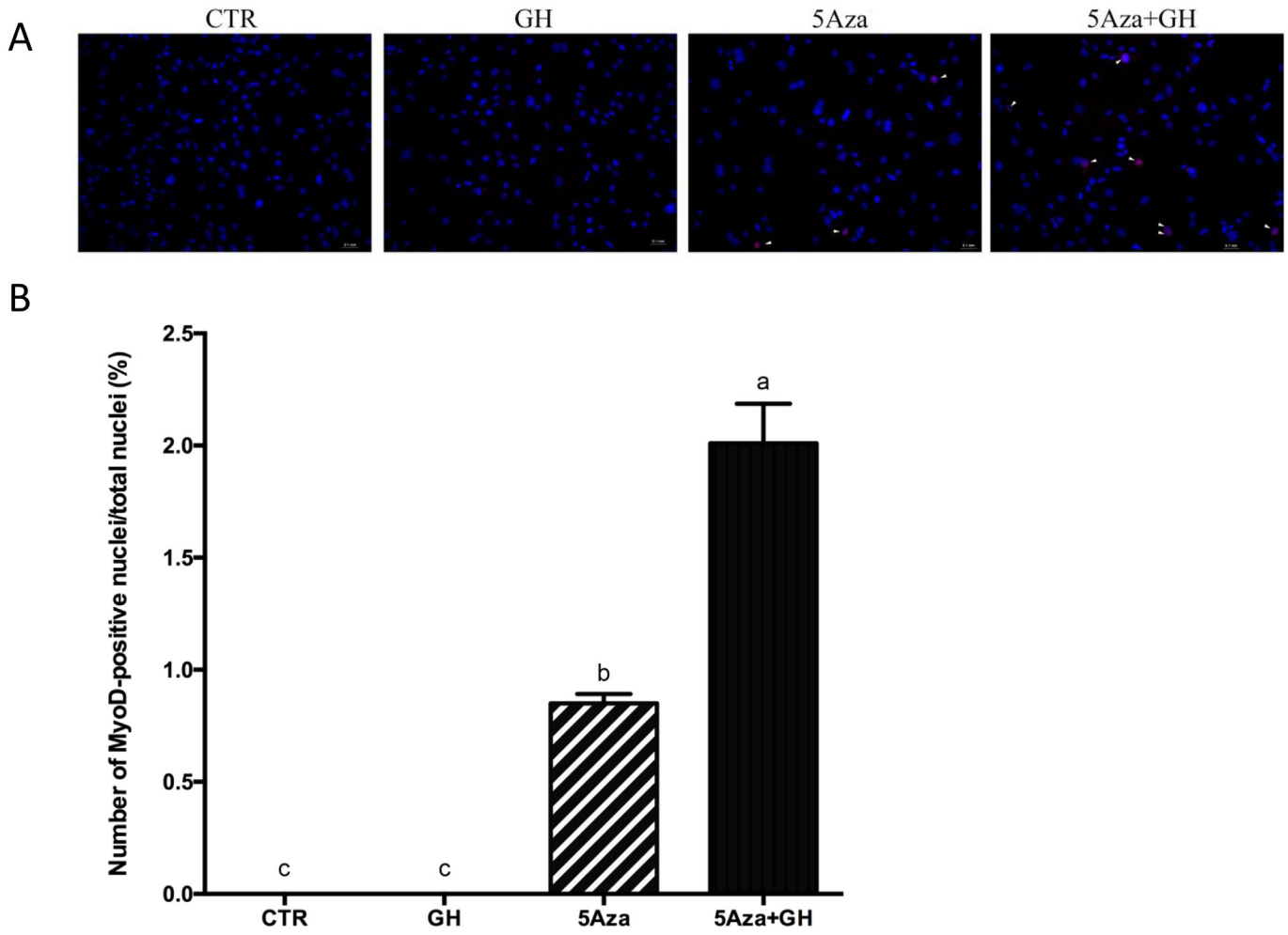


**Fig. 1.** Effects of GH on the generation of myogenic cells from C3H10T1/2 cells. C3H10T1/2 cells cultured in growth medium were treated with 100 ng/ml bGH, 3 μM 5′-azacytidine (Aza), or 100 ng/ml bGH plus 3 μM 5Aza for 4 days. Untreated cells were used as control (CTR). At the end of 4-day culture, cells were stained with the antibody for MyoD (red) and DAPI for nuclei (blue). A) Representative micrographs. MyoD-positive nuclei are shown in magenta and indicated by arrowheads. B) Quantification of MyoD-positive cells. MyoD-positive and total nuclei were counted from 4 separate cell cultures. Bars not sharing the same letter labels are different from each other ($P < 0.05$).

adipocytes, osteoblasts, and chondrocytes [16–19]. The C3H10T1/2 cells express the GH receptors [20]. In this study, we determined the effect of GH on the commitment of C3H10T1/2 cells to the myogenic and adipogenic lineages.

## 2. Materials and methods

### 2.1. Cell culture and treatments

C3H10T1/2 cells (ATCC, Manassas, VA) were expanded in medium consisting of Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA), 2 mM L-glutamine, and 1% 100× antibiotic-antimycotic (ABAM), which consisted of 10,000 units/ml of penicillin, 10,000 μg/ml of streptomycin, and 25 μg/ml of Amphotericin B (ThermoFisher Scientific, Waltham, MA), at 37 °C and 5% $CO_2$ with saturating humidity. 5′-Azacytidine (5Aza), a DNA methyltransferase inhibitor, was effective in inducing lineage commitment in C3H10T1/2 cells [16,21]. To determine the effect of GH on the myogenic and adipogenic commitments in C3H10T1/2 cells, C3H10T1/2 cells were treated with 3 μM 5Aza (Sigma-Aldrich, St. Louis, MO), 100 ng/ml recombinant bovine GH (bGH; The National Hormone and Peptide Program, Torrance, CA), or 3 μM 5Aza plus 100 ng/ml bGH, for 4 days. C3H10T1/2 cells treated with phosphate buffered saline (PBS), the vehicle for 5Aza and bGH, were used as control. Medium and treatment were refreshed every day. At the end of the 4-day culture, C3H10T1/2 cells were used for myogenic and adipogenic differentiation analyses described below.

### 2.2. Myogenic and adipogenic differentiation analyses

To induce myogenic differentiation, C3H10T1/2 cells treated with 5′Aza, bGH, or both for 4 days were reseeded at approximately 90% confluency in medium composed of DMEM, 2% horse serum (Atlanta Biologicals), 2 mM L-glutamine, and 1% 100× ABAM. C3H10T1/2 cells were cultured for 8 days, during which medium was refreshed every 2 days. To induce adipogenic differentiation, C3H10T1/2 cells treated with 5′Aza, bGH, or both for 4 days were reseeded at 100% confluency in medium composed of DMEM, 5% FBS, 1% 100× ABAM, 2 mM L-glutamine, 10 μg/ml insulin (Sigma-Aldrich), 0.1 μM dexamethasone (Sigma-Aldrich), and 250 μM 3-Isobutyl-1-methylxanthine (Sigma-Aldrich). C3H10T1/2 cells were cultured in this medium for 2 days and then in medium composed of DMEM, 5% FBS, 1% 100× ABAM, 2 mM L-glutamine, and 10 μg/ml insulin for 10 days, during which culture medium was refreshed every 2 days.

### 2.3. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 15 min and