Effects of Transforming Growth Factor β on β1 Integrin Expression and Localization during Myogenesis in Chicken

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Abstract

Myoblast-extracellular environment interactions are mediated by the integrin family of cell adhesion receptors. Integrins have been shown to play a pivotal role in skeletal muscle development. The β1 integrin has been shown to play a critical role in muscle cell attachment, migration and the formation of multinucleated myotubes. Transforming growth factor β (TGF-β) is a potent inhibitor of both skeletal muscle myoblast proliferation and differentiation. The expression of TGF-β will affect cell β1 integrin expression and localization. The chicken genetic muscle weakness, Low Score Normal (LSN), exhibits modified myotube and sarcomere structure, and increased TGF-β and reduced β1 integrin expression during myogenesis. The current study used LSN satellite cells, myogenic precursor cells, as a model to further investigate the role of TGF-β and β1 integrin during myogenesis, compared to normal satellite cells. The LSN satellite cells have elevated expression of TGF-β and decreased β1 integrin during proliferation and differentiation. The β1 integrin was localized at areas of cell–cell contact in normal cells, whereas in LSN satellite cell cultures β1 integrin was observed within cells. The addition of the exogenous TGF-β in normal cell cultures decreased both β1 integrin mRNA and protein expression at 24 and 48 h of differentiation. The localization of β1 integrin was altered also, from areas of cell-cell contact to inside cells. These data suggest that TGF-β may play a pivotal role in myogenesis through modulation of the expression and localization of β1 integrin which is important in the control of cell migration and growth regulation.

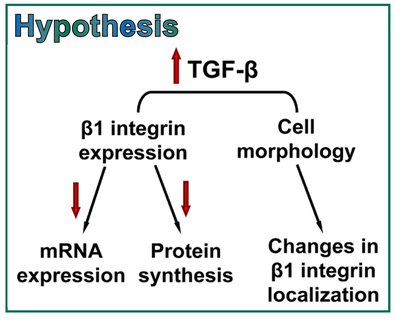
Introduction

- **During skeletal muscle development, growth factors are involved in regulating cell proliferation and differentiation. Transforming growth factor-β is a potent inhibitor of both muscle cell proliferation and differentiation (Allen and Boxhorn, 1987).**
- **Interaction of the muscle cells with the extracellular environment is accomplished through cell adhesion receptors, integrins. Each integrin consists of α- and β-subunits. The β1 integrin, the most ubiquitous β subunit, plays a critical role in the cell attachment, cell migration, proliferation, and differentiation (Berman et al., 2003).**
- **Transforming growth factor-β may, in part, regulate cell proliferation and differentiation through its effect on β1 integrin expression.**
- **The chicken genetic muscle weakness, Low Score Normal (LSN), and normal muscle cells were used to investigate how TGF-β signaling regulates β1 integrin during myogenesis. The LSN is a genetic muscle weakness condition, which is characterized by altered myotube formation and sarcomere structure (Velleman et al., 1997). The LSN chicken has altered TGF-β expression and decreased β1 integrin expression (Velleman and Coy, 1998; Velleman and McFarland, 2004).**
- **To address the relationship between TGF-β and β1 integrin, normal and LSN satellite cells were used as in vitro models. Satellite cells are quiescent myogenic cells and largely responsible for postnatal muscle growth and muscle regeneration. Satellite cell cultures allow the in vitro monitoring of satellite cell proliferation through the formation and differentiation of multinucleated myotubes.**

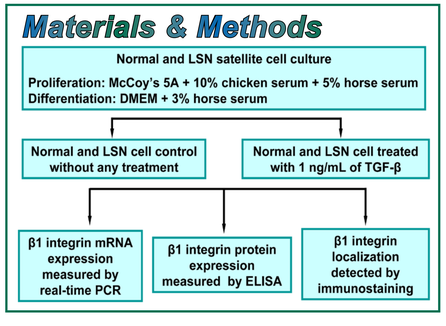
Objective

To address how TGF-β affects β1 integrin expression and localization during chicken satellite cell proliferation and differentiation.

Hypothesis



Materials & Methods



Results

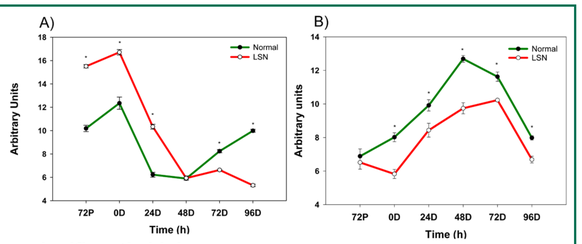


Fig. 1 Real-time PCR analysis of TGF-β and β1 integrin mRNA expression in chicken normal and LSN satellite cells during proliferation (P) and differentiation (D). A) TGF-β; B) β1 integrin. Error bars represent the standard error of the mean. * Indicates a significant difference (P < 0.05) between the lines at a sampling time. (Li et al., 2006)

- **Expression of TGF-β was significantly higher in the LSN cells from 72 h of proliferation through 24 h of differentiation than in the normal cells which expressed more TGF-β compared to the LSN cells at 72 and 96 h of differentiation (Fig. 1 A);**
- **Expression of β1 integrin was significantly higher at all times measured except 72 h of proliferation in the normal satellite cell cultures compared to the LSN satellite cells (Fig. 1 B);**
- **Expression of β1 integrin was reduced in the TGF-β treated cultures at 24 and 48 h of differentiation and by 96 h of differentiation was significantly increased in the TGF-β treated cultures (Fig. 2).**

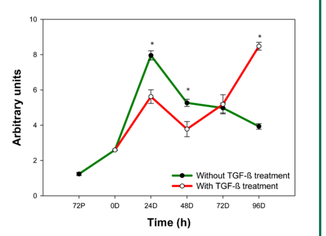


Fig. 2 Real-time PCR analysis of β1 integrin mRNA expression in TGF-β treated chicken normal satellite cells during differentiation (D). *Indicates a significant difference (P < 0.05) between the lines at a sampling time. (Li et al., 2006)

- **The β1 integrin protein synthesis was significantly higher at all times measured except 96 h of differentiation in the normal satellite cell cultures compared to the LSN satellite cells (Fig. 3 A).**
- **The addition of TGF-β decreased β1 integrin protein synthesis significantly at 24 and 48 h of differentiation and increased it by 96 h of differentiation (Fig. 3 B).**

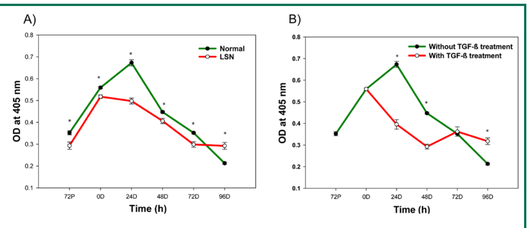


Fig. 3 ELISA analysis of β1 integrin protein expression in chicken normal and LSN satellite cells during proliferation (P) and differentiation (D). A) β1 integrin in normal and LSN; B) β1 integrin in TGF-β treated normal satellite cells. * Indicates a significant difference (P < 0.05) between the lines at a sampling time.

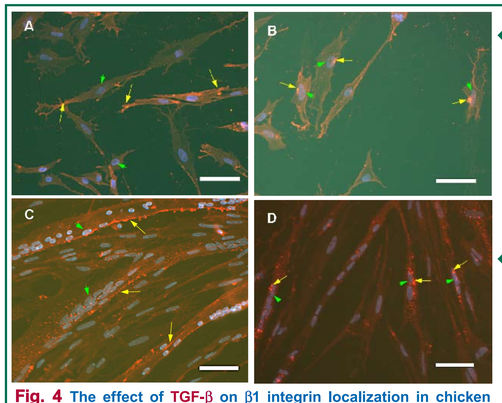


Fig. 4 The effect of TGF-β on β1 integrin localization in chicken normal satellite cells. A) Cells without TGF-β treatment at 48 h of proliferation; B) Cells with TGF-β treatment at 48 h of proliferation; C) Cells without TGF-β treatment at 48 h of differentiation; D) Cells with TGF-β treatment at 48 h of differentiation. Yellow arrows indicate β1 integrin; green arrows indicate nuclei; bar = 5μm.

- **The β1 integrin localization was detected by immunostaining. In normal satellite cells, β1 integrin formed clusters that localized at pseudopodia indicated by the arrows in Fig. 4A during proliferation. During differentiation, β1 integrin located at areas of cell–cell contact.**
- **After the normal satellite cells were treated with TGF-β, β1 integrin still formed clusters. Expression of β1 integrin was reduced at points of cell-cell contact and the pseudopodia, but β1 integrin clusters were mostly found within the cells during both proliferation and differentiation.**

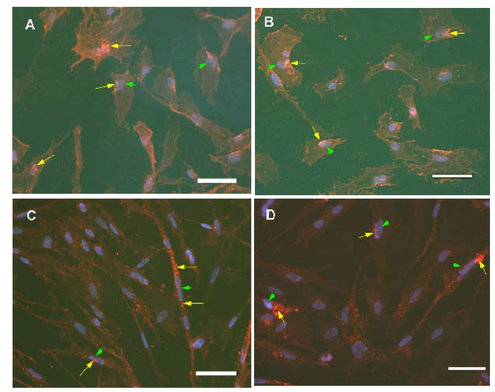


Fig. 5 The effect of TGF-β on β1 integrin localization in chicken LSN satellite cells. A) Cells without TGF-β treatment at 48 h of proliferation; B) Cells with TGF-β treatment at 48 h of proliferation; C) Cells without TGF-β treatment at 48 h of differentiation; D) Cells with TGF-β treatment at 48 h of differentiation. Yellow arrows indicate β1 integrin; green arrows indicate nuclei; bar = 5μm.

- **In LSN satellite cells, β1 integrin also formed clusters, but was generally localized within the cells during proliferation. During differentiation, the integrin clusters were not detected at points of cell-cell contact, although some β1 integrin clusters were distributed at the cell surface.**
- **In LSN satellite cells treated with TGF-β, the integrin clusters localized within cells during both proliferation and differentiation. No significant difference on integrin localization was observed between TGF-β untreated and treated LSN cells.**

Discussion

- **The addition of exogenous TGF-β in normal satellite cells decreased β1 integrin expression at both transcriptional and translational levels. Since the LSN satellite cells expressed higher concentration of TGF-β and lower amounts of β1 integrin, the changes in β1 integrin expression may result from altered TGF-β expression.**
- **Beta 1 integrin temporal and spatial localization were different between normal and LSN satellite cells during proliferation and differentiation. The β1 integrin clustering in normal satellite cells leads to the formation of focal adhesions and the formation of cytoskeletal actin filaments. However, the TGF-β treated normal satellite cells have altered focal adhesion formation in terms of changes in the β1 integrin cluster temporal localization, which was similar to what is observed in LSN satellite cells.**
- **The localization of integrins is associated with cell survival. Cells undergo apoptosis, programmed cell death, in the absence of appropriate integrin extracellar contacts (Zhang et al., 1995). The α5β1 integrin increases Bcl2 expression, an anti-apoptosis gene (Zhang et al., 1995). If the α5β1 integrin expression is reduced, the muscle cells will not properly adhere and likely undergo apoptosis. The TGF-β regulates the localization of β1 integrin during myogenesis. However, the apoptotic effect of TGF-β signaling on integrins involved in myogenesis is not well understood yet. Future research will need to address the apoptotic effect of TGF-β during myogenesis.**

Conclusions

- **Increased TGF-β expression in LSN satellite cells reduces β1 integrin expression at both the RNA and protein levels.**
- **The addition of TGF-β in normal satellite cell cultures decreases β1 integrin expression at both the RNA and protein levels.**
- **Beta 1 integrin is functionally involved in both satellite cell proliferation and differentiation.**
- **TGF-β regulates muscle cell proliferation and differentiation, in part, through regulating β1 integrin expression and localization.**

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Acknowledgments

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