Regulation of sexually dimorphic growth of murine skeletal muscle by Stat5a and Stat5b

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Introduction

• Growth hormone (GH) regulates insulin-like growth factor one (IGF-1) predominantly through the intracellular signalers Stat5a and Stat5b.1
• Inactivating mutations of Stat5b in humans results in severe growth retardation and low circulating concentrations of IGF-1 in both sexes.2
• Deletion of Stat5b in mice results in loss of sexually dimorphic growth with a reduction of growth and circulating concentrations of IGF-1 in males only.3,4
• The reasons for the discrepancy in the role of Stat5b between humans and mice is not known.
• No study has observed Stat5b−/− mice beyond 12 weeks of age or investigated any subsequent changes in the mass of skeletal muscles or expression of IGF-1.

Aims

• To determine the regulation of the sexually dimorphic growth of skeletal muscles in mouse by Stat5b.
• To determine the regulation of expression of IGF-1 mRNA in skeletal muscles of mice by Stat5a and Stat5b.

Study design

• Blood and hindlimb muscles of male and female Stat5b−/− mice and wild-type littermates were collected at 6, 12 and 24 weeks of age.
• n = 16 per age and sex.
• Concentrations of IGF-1 in plasma and skeletal muscle were determined by ELISA & muscle mass was normalised to bone length.
• C2C12 myoblast cell lines were treated with viral Stat5b and/or Stat5a siRNA or a scrambled vector (control), then differentiated and treated with GH 100 ng/mL for 24 hours (n=6). RNA was harvested for qPCR.
• The siRNA treated cell lines were also treated with GH 100 ng/mL for 96 hours and differentiation was assessed by immunochemistry.

Table 1: Mean length (±/− S.E.M.) of the tibia (mm) and concentrations of IGF-1 in plasma and skeletal muscle relative to male WT mice at 6 weeks of age

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Genotype</th>
<th>Tibia length</th>
<th>P&lt;</th>
<th>Plasma IGF-1</th>
<th>P&lt;</th>
<th>Muscle IGF-1</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks</td>
<td>M</td>
<td>WT</td>
<td>16.8 ±/−0.2</td>
<td>a</td>
<td>100 ±/−8.4</td>
<td>a</td>
<td>100 ±/−7.3</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Stat5b−/−</td>
<td>15.8 ±/−0.2</td>
<td>b</td>
<td>56.3 ±/−4.0</td>
<td>b</td>
<td>43.8 ±/−2.4</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>WT</td>
<td>16.4 ±/−0.2</td>
<td>a</td>
<td>107.6 ±/−6.3</td>
<td>a</td>
<td>79.9 ±/−7.8</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Stat5b−/−</td>
<td>15.9 ±/−0.1</td>
<td>b</td>
<td>79.9 ±/−3.9</td>
<td>c</td>
<td>64.2 ±/−5.5</td>
<td>d</td>
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<tr>
<td>12 weeks</td>
<td>M</td>
<td>WT</td>
<td>18.3 ±/−0.2</td>
<td>a</td>
<td>93.2 ±/−4.7</td>
<td>a</td>
<td>106.9 ±/−6.0</td>
<td>a</td>
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<tr>
<td></td>
<td>M</td>
<td>Stat5b−/−</td>
<td>16.6 ±/−0.2</td>
<td>b</td>
<td>57.2 ±/−4.6</td>
<td>b</td>
<td>48.3 ±/−4.6</td>
<td>b</td>
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<tr>
<td></td>
<td>F</td>
<td>WT</td>
<td>17.7 ±/−0.2</td>
<td>a</td>
<td>91.2 ±/−5.4</td>
<td>ac</td>
<td>67.6 ±/−6.6</td>
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<tr>
<td></td>
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<td>Stat5b−/−</td>
<td>16.7 ±/−0.2</td>
<td>b</td>
<td>80.8 ±/−2.1</td>
<td>c</td>
<td>63.6 ±/−5.1</td>
<td>c</td>
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<tr>
<td>24 weeks</td>
<td>M</td>
<td>WT</td>
<td>18.1 ±/−0.1</td>
<td>a</td>
<td>101.8 ±/−5.5</td>
<td>a</td>
<td>79.6 ±/−5.9</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>Stat5b−/−</td>
<td>17.2 ±/−0.1</td>
<td>b</td>
<td>77.6 ±/−4.3</td>
<td>b</td>
<td>47.9 ±/−2.2</td>
<td>b</td>
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<tr>
<td></td>
<td>F</td>
<td>WT</td>
<td>18.4 ±/−0.2</td>
<td>a</td>
<td>107.8 ±/−5.6</td>
<td>a</td>
<td>64.1 ±/−3.3</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>Stat5b−/−</td>
<td>17.4 ±/−0.1</td>
<td>b</td>
<td>99.9 ±/−7.5</td>
<td>a</td>
<td>58.3 ±/−4.6</td>
<td>bc</td>
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</tbody>
</table>

Results

• Nasal bone length, tibia length and the normalised mass of hindlimb muscles were reduced to a greater extent in male (23%) than in female (14%) Stat5b−/− mice at all ages (P < 0.001; Figure 1 and Table 1).
• Concentrations of IGF-1 in plasma and skeletal muscle were reduced in male Stat5b−/− mice at all ages and in female Stat5b−/− mice at only 6 weeks of age (P < 0.01 versus wild-type littermates of the same sex).
• Knockdown of Stat5a alone reduced the differentiation of myotubes (36%) to a lesser extent than knockdown of Stat5b or both Stat5a and Stat5b (80%; P < 0.001; Figure 2).
• Knockdown of either Stat5a or Stat5b prevented the GH-induced increase in concentrations of IGF-1 mRNA and myotube hypertrophy

Conclusions

• Stat5a and Stat5b are both required for signaling of GH in skeletal muscles of mice.
• Similar to humans, loss of function of Stat5b in mice is associated with retarded growth and reduced circulating concentrations of IGF-1 in both males and females.
• Sexually dimorphic growth of skeletal muscles was reduced, but persisted in Stat5b−/− mice.

References

2) Hwa et al. PBRCEM 2001; 25(1): 61-75.
4) Udy et al. PNSA 1997; 94(14): 7239-7244