Data in Brief

Genome-wide expression analysis comparing hypertrophic changes in normal and dysferlinopathy mice

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Abstract

Because myostatin normally limits skeletal muscle growth, there are extensive efforts to develop myostatin inhibitors for clinical use. One potential concern is that in muscle degenerative diseases, inducing hypertrophy may increase stress on dystrophic fibers. Our study shows that blocking this pathway in dysferlin deficient mice results in early improvement in histopathology but ultimately accelerates muscle degeneration. Hence, benefits of this approach should be weighed against these potential detrimental effects. Here, we present detailed experimental methods and analysis for the gene expression profiling described in our recently published study in Human Molecular Genetics (Lee et al., 2015). Our data sets have been deposited in the Gene Expression Omnibus (GEO) database (GSE62945) and are available athttp://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62945. Our data provide a resource for exploring molecular mechanisms that are related to hypertrophy-induced, accelerated muscular degeneration in dysferlinopathy.

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1. Direct link to deposited data

Raw and processed microarray data is available in GEO under accession GSE62945 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62945.
2. Experimental design, materials and methods

2.1. Study design

The identification of myostatin as a negative regulator of skeletal muscle mass raised the possibility that blocking the myostatin signaling could have important applications for treating patients with muscle degenerative diseases [1]. However, there is one theoretical concern that inducing muscle hypertrophy may cause additional membrane stress.

| Genotype   | ACVR2B/Fc treatment | Replicate | GEO accession URL |
|------------|---------------------|-----------|-------------------|
| F66; Dysf<sup>−/−</sup> | No      | 3         | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1536852 |
|            |         |           | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1536853 |
|            |         |           | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1536854 |
|            |         |           | http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1536855 |

Fig. 1. Microarray quality assessment of 18 chips for three biological replicates in 6 different groups; wt, Dysf<sup>−/−</sup>, F66, F66; Dysf<sup>−/−</sup>, and ACVR2B/Fc-injected wt and Dysf<sup>−/−</sup> mice. (a) Box plots of 18 chips show median-centered raw data distributions. (b) Line graphs of 18 chips also support that all the chips’ probes have signals of similar distribution and median value. (c) The magnitude of between class variation (myostatin inhibition by ACVR2B/Fc and F66, and dysferlin deficiency) is compared to that of within-class variation (Error), demonstrating biological variation to be far greater than experimental noise.
causing further damage to already fragile muscle fibers. To investigate this possibility, we used both genetic and pharmacological approaches to examine the effect of blocking myostatin in dysferin mutant (Dysf<sup>−/−</sup>) mice, which is a model for limb-girdle type 2B and Miyoshi muscular dystrophies. Our rationale was that if myostatin inhibition caused increased membrane damage, these effects would be enhanced in Dysf<sup>−/−</sup> mice, in which membrane repair is compromised [2]. For the genetic approach, we used transgenic mice expressing the myostatin inhibitor, follistatin, exclusively in skeletal muscle (F66<sup>−/−</sup>; Dysf<sup>−/−</sup>) mice, in which membrane repair is compromised [2]. For the pharmacological approach, we used a soluble form of the activin type IIB receptor (ACVR2B) in which the extracellular ligand binding domain was fused to an Fc domain

Table 1

| Top canonical pathways from Ingenuity Pathway Analysis (IPA). | p-Value | Ratio |
|---------------------------------------------------------------|---------|-------|
| wt versus ACVR2B/Fc-injected wt | 1.14E−03 | 10/84 (0.119) |
| Leptin signaling in obesity | 1.73E−03 | 14/158 (0.089) |
| Cardiac β-adrenergic signaling | 5.04E−03 | 20/275 (0.073) |
| G-protein coupled receptor signaling | 6.66E−03 | 13/169 (0.077) |
| AMPK signaling | 6.79E−03 | 6/46 (0.130) |
| ACVR2B/Fc-injected wt versus ACVR2B/Fc-injected Dysf<sup>−/−</sup> | 5.37E−03 | 10/144 (0.069) |
| 14-3-3-mediated signaling | 9.88E−03 | 9/121 (0.074) |
| Role of Oct4 in mammalian embryonic stem cell pluripotency | 1.1E−02 | 5/45 (0.111) |
| Assembly of RNA polymerase ii complex | 1.69E−02 | 5/56 (0.089) |
| Complement system | 1.84E−02 | 4/35 (0.114) |
| IL-10 signaling | 6.78E−04 | 9/78 (0.115) |
| Hepatic fibrosis/hepatic stellate cell activation | 8.53E−04 | 13/146 (0.089) |
| TREM1 signaling | 8.67E−04 | 8/71 (0.113) |
| Granulocyte adhesion and diapedesis | 8.78E−04 | 15/178 (0.084) |
| Altered T cell and B cell signaling in rheumatoid arthritis | 2.42E−03 | 9/92 (0.098) |
| wt versus F66 | 3.68E−07 | 22/146 (0.151) |
| Hepatic fibrosis/hepatic stellate cell activation | 1.22E−06 | 11/40 (0.275) |
| Inhibition of matrix metalloproteinases | 3.21E−04 | 10/61 (0.164) |
| Granulocyte adhesion and diapedesis | 4.49E−04 | 19/178 (0.107) |
| Coagulation system | 9.15E−04 | 7/38 (0.184) |
| Dysf<sup>−/−</sup> versus F66/Dysf<sup>−/−</sup> | 6.25E−16 | 36/146 (0.247) |
| Hepatic fibrosis/hepatic stellate cell activation | 9.52E−15 | 29/102 (0.284) |
| Fcγ receptor-mediated phagocytosis in macrophages and monocytes | 5.89E−12 | 35/178 (0.197) |
| Granulocyte adhesion and diapedesis | 6.81E−12 | 36/189 (0.190) |
| Leukocyte extravasation signaling | 5.06E−10 | 35/207 (0.169) |
| F66 versus F66/Dysf<sup>−/−</sup> | 2.4E−13 | 26/102 (0.255) |
| Fcγ receptor-mediated phagocytosis in macrophages and monocytes | 1.82E−10 | 31/209 (0.148) |
| Leukocyte extravasation signaling | 3.53E−10 | 33/207 (0.159) |
| Role of pattern recognition receptors in recognition of bacteria and viruses | 3.7E−10 | 22/106 (0.208) |
| Hepatic fibrosis/hepatic stellate cell activation | 1.09E−09 | 26/146 (0.178) |

Table 2

| Top upstream regulators from IPA. | p-Value of overlap | Predicted activation state |
|----------------------------------|--------------------|---------------------------|
| wt versus ACVR2B/Fc-injected wt | 4.60E−08 | TGFβ1 | 8.48E−25 | Activated |
| miR-141-3p (and other miRNAs w/seed AACACUG) | 3.41E−07 |
| Lipopolysaccharide | 5.11E−07 |
| Dexamethasone | 9.34E−07 |
| GW501516 | 1.14E−06 |
| Dysf<sup>−/−</sup> versus ACVR2B/Fc-injected Dysf<sup>−/−</sup> | 1.78E−06 | IL1B | 1.15E−17 | Activated |
| miR-27a-3p (and other miRNAs w/seed UCCACUG) | 1.99E−06 |
| miR-128-3p (and other miRNAs w/seed CACACUG) | 6.04E−06 |
| DYSF | 1.29E−05 |
| miR-844-3p (and other miRNAs w/seed AUUAUAC) | 3.15E−04 |
| ACVR2B/Fc-injected wt versus ACVR2B/Fc-injected Dysf<sup>−/−</sup> | 1.42E−22 |
| DYSF | 1.32E−20 | Activated |
| Lipopolysaccharide | 1.15E−17 |
| TGFβ1 | 2.06E−17 | Activated |
| IL6 | 1.47E−13 |
| DYSF | 8.48E−25 |
| IL1B | 3.48E−22 |
| TGFβ1 | 2.18E−20 |
| Lipopolysaccharide | 2.88E−20 |
| Dexamethasone | 7.91E−17 |
| Dysf<sup>−/−</sup> versus F66/Dysf<sup>−/−</sup> | 2.80E−58 | Lipopolysaccharide | 2.80E−58 | Activated |
| IL1B | 1.31E−41 |
| TGFβ1 | 1.32E−39 |
| TGFβ1 | 1.57E−39 | Activated |
| DYSF | 2.23E−32 |
| F66 versus F66/Dysf<sup>−/−</sup> | 1.12E−44 | Lipopolysaccharide | 7.23E−36 | Activated |
| IFNG | 8.94E−35 |
| DYSF | 3.02E−34 |
| TGFβ1 | 5.02E−34 | Activated |
To analyze the effect of F66 in Dysf−/− mice, F66 transgenic mice were mated with Dysf−/− mice. F66; Dysf+/- males from this cross were mated to Dysf−/− females to obtain F66; Dysf−/- and F66; Dysf+/- (F66) males. Because the F66 transgene is located on the Y chromosome, we focused all of our analysis on male mice. All mice were maintained on a C57BL/6 background. To analyze the effect of ACRVR2B/Fc administration in Dysf−/− mice, male C57BL/6 (wt) and Dysf−/− mice beginning at 6 weeks of age were given four weekly intraperitoneal (i.p.) injections of either ACRVR2B/Fc (10 mg kg−1, i.e., 200 μg per injection) or PBS.
the dramatic changes of gene expression profiling. Our data sets provide a resource for exploring molecular mechanisms that are related to hypertrophy-induced, accelerated muscular degeneration in dysferlinopathy.

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**Conflict of interest**

The authors declare that they have no conflicts of interest.

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