Transcriptional Analysis of *Articularis Genu* Sarcopenia in Osteoarthritis

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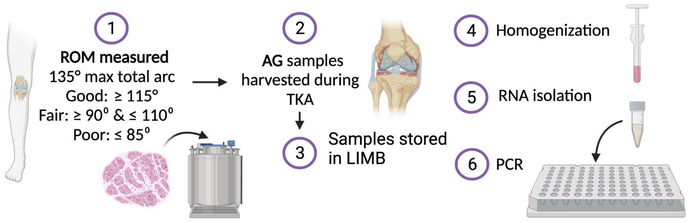
Introduction

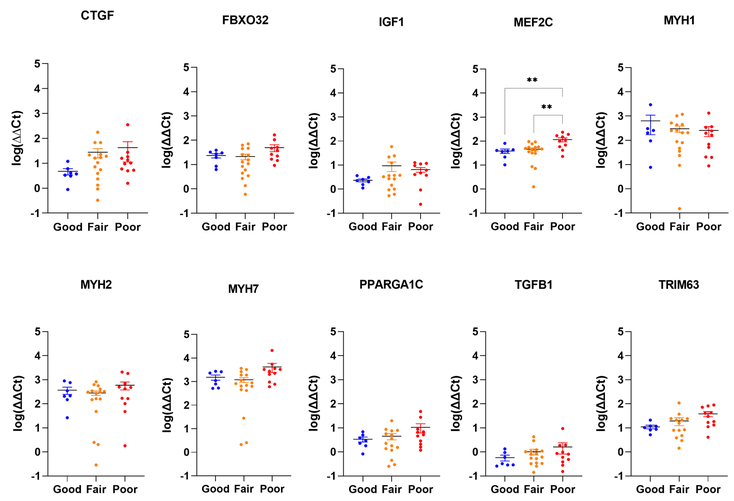
- Disuse of the musculature during knee osteoarthritis (kOA) involves atrophy, fibrosis, and changes in myofiber distribution involving a decrease in type (T)1 slow oxidative fibers, concurrent with an increase in T2a fast glycolytic, T2x super-fast twitch, and T2a/x hybrid fibers.
- Myofiber type can be measured by expression of myosin heavy chain (MHC) types using immunohistochemistry. Previous studies on the *Vastus lateralis* during kOA link a greater occurrence of T2x (MHC1) and T2a/x (co-expression of MHC2 and MHC1) compared to T1 (MHC7) or T2a (MHC2) to sedentary behavior.
- We have previously measured a higher incidence of T2a/x hybrids in the *Articularis genu* muscle from kOA patients with poor active range of motion (ROM) compared to cohorts with better ROM.
- Furthermore, we have measured the most elevated collagen deposition in the AG endomysium in patients with poor ROM and that collagen deposition in the AG is associated with severity of fibrosis in the neighboring synovium.
- The AG can be easily sampled during total knee arthroplasty (TKA).
- We predict the transcription of genes specific to muscular atrophy (*Fbxo32, Trim63*), myofiber type switching (*Pparga1c*), hypertrophy (*Igf1, Mef2c*), and fibrosis (*Tgfb1, Ctgf*) to be dysregulated in the kOA AG in agreement with the significant histological changes that we have previously measured in a ROM-dependent manner.
- We further predict that the myofiber types present in the AG, as measured by MHC expression, will show ROM-dependent dysregulation of MHC gene transcripts in samples with a high expression of MHC2 (Myh2; T2a), MHC 1 (Myh1; T2x), or their co-expression (T2a/x).

Methodology



Gene	Involved processes	Predicted
Myh1	Sarcomere of T2x super-fast twitch and T2a/x hybrid (+ MYH2) fibers	Increased in poor ROM group, corresponding to increase in T2a/x hybrid
Myh2	Sarcomere of T2a fast glycolytic and T2a/x hybrid (+ MYH1) fibers	Increased in poor ROM group, corresponding to increase in T2a/x hybrid fibers
Myh7	Sarcomere of T1 slow oxidative fibers Increased with age	Decrease in kOA patients with decreased ROM, corresponding to a decrease in T1 slow oxidative fibers
Ctgf	Fibrogenesis	Increased in poor ROM group
Tgfβ1	Fibrogenesis Chondrogenesis	Increased in poor ROM group
Igf1	Bone remodeling Cartilage degradation	Increased in with poor ROM group
Mef2c	Hypertrophy Muscle repair	Increase corresponding to myofiber hypertrophy
Ppargc	Adaptation to aerobic training Biogenesis/angiogenesis Metabolism of carbs and fats	Decreased in poor ROM group corresponding to change from type T1 fibers → T2a, T2x, and hybrid fibers
Fbxo32	Degradation of myosin heavy chain Skeletal muscle atrophy	Increased in poor ROM corresponding to atrophy of knee musculature
Trim63	Degradation of myosin heavy chain Skeletal muscle atrophy	Increased in poor ROM corresponding to atrophy of knee musculature
Hprt1	Housekeeping	

QPCR Results



Mean expression of gene transcripts tested by one way ANOVA between and within ROM groups. *p<0.05 between groups and #p<0.05 within groups; **p<0.01; ***p<0.001; and ****p<0.0001.

MHC vs Gene Expression

MEF2C MYH7	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	-29.27	22.85	0.582	-91.41	32.87
1st - 3rd Q	-63.12	22.17	0.037*	-123.4	-2.841
1st - 4th Q	-71.94	22.85	0.018*	-134.1	-9.804
2nd - 3rd Q	-33.85	22.85	0.461	-95.99	28.29
2nd - 4th Q	-42.67	23.51	0.286	-106.6	21.27
3rd - 4th Q	-8.819	22.85	0.980	-70.96	53.32

MEF2C MYH1	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	-74.53	23.60	0.018*	-138.8	-10.22
1st - 3rd Q	-30.78	23.60	0.568	-95.09	33.53
1st - 4th Q	-66.26	22.94	0.035*	-128.8	-3.765
2nd - 3rd Q	43.75	23.60	0.270	-20.56	108.1
2nd - 4th Q	8.270	22.94	0.984	-54.23	70.77
3rd - 4th Q	-35.48	22.94	0.424	-97.98	27.02

MEF2C MYH2	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	5.196	21.11	0.995	-52.20	62.60
1st - 3rd Q	-19.93	21.11	0.781	-77.34	37.47
1st - 4th Q	-76.47	21.11	0.006**	-133.9	-19.07
2nd - 3rd Q	-25.13	22.25	0.675	-85.64	35.37
2nd - 4th Q	-81.67	22.25	0.005**	-142.2	-21.16
3rd - 4th Q	-56.53	22.25	0.073	-117.0	3.971

PPARGC MYH7	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	-0.4111	3.896	0.999	-11.01	10.18
1st - 3rd Q	-2.157	3.780	0.940	-12.44	8.122
1st - 4th Q	-12.82	3.896	0.013*	-23.42	-2.227
2nd - 3rd Q	-1.746	3.896	0.970	-12.34	8.849
2nd - 4th Q	-12.41	4.009	0.021*	-23.31	-1.508
3rd - 4th Q	-10.66	3.896	0.048*	-21.26	-0.070

FBXO32 MYH2	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	5.280	13.65	0.980	-31.83	42.39
1st - 3rd Q	-3.820	13.65	0.992	-40.93	33.29
1st - 4th Q	-44.52	13.65	0.014*	-81.63	-7.405
2nd - 3rd Q	-9.101	14.39	0.921	-48.22	30.02
2nd - 4th Q	-49.80	14.39	0.008**	-88.91	-10.68
3rd - 4th Q	-40.70	14.39	0.039*	-79.81	-1.577

TRIM63 MYH7	Mean Diff	SE	p	95% CI Lower	95% CI Upper
1st - 2nd Q	3.921	11.12	0.985	-26.38	34.22
1st - 3rd Q	-10.82	10.81	0.750	-40.26	18.63
1st - 4th Q	-39.60	11.12	0.007**	-69.89	-9.298
2nd - 3rd Q	-14.74	10.81	0.531	-44.18	14.70
2nd - 4th Q	-43.52	10.12	0.003**	-73.81	-13.22
3rd - 4th Q	-28.78	10.81	0.057	-58.22	0.667

MHC expression separated by quartile. Mean expression of gene transcripts tested by one way ANOVA between and within MHC groups. Results of ANOVA that were significant included. *p<0.05 between groups and #p<0.05 within groups; **p<0.01; ***p<0.001; and ****p<0.0001.

Conclusions

- *Mef2c* is a transcription factor involved in the physiological changes in kOA musculature similar to those in cardiac hypertrophy, and fibrosis. Expression was significantly different in the Good vs Poor ROM comparison and the Fair vs Poor ROM comparison, indicating that *Mef2c* may be an indicator of remodeling that contributes to functional limitations in kOA. Furthermore, expression of MEF2C was significantly greater in the 3rd and 4th quartiles of *Myh7* expression when compared to the 1st quartile, meaning it is highly expressed in T1 myofibers. The 2nd and 4th quartiles of *Myh1* expression displayed significantly greater *Mef2c* expression compared to the 1st. MEF2C expression in the 4th quartile of *Myh2* expression was significantly greater than the 1st and 2nd quartiles. This indicates *Mef2c* may be involved in hypertrophic processes leading to T2a, T2x, and hybrid fiber accumulation.
- *Pparga1c* is a transcriptional activator highly expressed following acute exercise and modulates the expression of genes involved in tissue repair, angiogenesis, and fat and carbohydrate metabolism. The levels of *Pparga1c* are significantly greater in samples with elevated *Myh7* expression in the 4th quartile when compared to the 1st or 2nd quartiles. This implies that in tissues with high *Myh7* levels, and therefore, the amount of T1 fibers, *Pparga1c* plays a significant part in myofiber switching to T2 in kOA and the loss of ROM in patients. We predicted *Pparga1c* levels would decrease as fibers switched from T1 to T2x or T2a, but our findings indicate *Pparga1c* levels increase as fibers switch to T1. While this does not oppose our hypothesis, it shows support for a process in the inverse direction. T1 fibers increase with age, so it is possible the effect we see in *Pparga1c* levels are the result of an aging process independent of kOA.
- *Trim63* encodes E3 ubiquitin-protein ligase upregulated in skeletal muscle atrophy and is involved in the degradation of MHC. The expression of *Trim63* was also significantly greater in the 4th quartile of *Myh7* expression when compared to the 1st and 2nd quartiles. These results indicate that it is important indicator of T1 myofiber atrophy.
- *Fbxo32*, also involved in degradation of MHC, is expressed at significantly higher levels in the 4th quartile of *Myh1* expression in comparison to the 1st, 2nd, and 3rd quartiles. This indicates it is more likely involved in atrophy of T2x and T2a/x myofibers, which highly express *Myh1*.
- Although not significant using the current sample size, expression of pro-fibrotic genes *Tgfβ1* and *Ctgf* suggest a potential trend in increasing levels with worsening ROM, which would be consistent with increasing histological measures of AG fibrosis.

Limitations

- Limited patient sample size can affect the significance between ROM groups.
- We did not control for patient characteristics such as age, BMI, etc.
- We were unable to measure expression of NOS2, which can indicate switching from T1 to T2 fibers, potentially due to poor primer design.
- Limited to using MYH1/2/7 expression as a measurement of myofiber types found in sample.
- No control tissue from donors with healthy knees.

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