Asian Journal of Biological Sciences
AJBS
OPEN ACCESS

Myostatin Expression in MDS Patients with Wasting Syndrome

[1]K. Garlemou, [2]M. Alexandrakis, [1]G. Karras, [1]D. Kyriakou and [1]T.V. Kourelis
[1]Department of Hematology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Greece
[2]School of Health Sciences, Faculty of Medicine, University of Crete, Greece

ABSTRACT

Background and Objective: Myostatin (Myo) regulates the growth of skeletal muscles and inactivating Myo leads to excessive muscle growth. This study aim is to investigate a possible role of myostatin in the wasting syndrome of MDS patients. **Materials and Methods:** Eighty-one patients with MDS and 17 healthy controls were involved in this study. Muscle tissue was obtained from the biceps muscle. Myo expression was estimated using real-time PCR (Myo/actin gene ratio was used). TNF-α, IL-1, IL-2, Il-6, TGF-β and leptin was measured in the serum using ELISA. Myo RNA μg^{-1} of whole muscle RNA was calculated. **Results:** Mutations or polymorphisms in the myostatin gene were not detected suggesting different regulations of the gene. Higher expression Myo gene was observed in RA, RAEB and RAEB-T patients compared to CMML, RARS patients and healthy individuals. Higher serum TNF-α, IL-1, IL-2, Il-6, TGF-β and leptin were observed in patients with wasting syndrome in comparison to those without and healthy. Conclusion: TNF-α levels >500 ng mL^{-1} is associated with increased expression of the Myo gene in muscle cell cultures and therefore may contribute to wasting. Other mechanisms cannot be excluded of course.

KEYWORDS

Cytokines, myostatin, myelodysplastic syndromes, wasting syndrome, growth factors

INTRODUCTION

Wasting syndrome is observed in many cancer patients especially during terminal stages. These patients lose the quality of life unrelated to disease progression with increased morbidities and need for hospitalization[1]. The mechanism of body mass loss is not well understood in patients with wasting syndrome. Many factors have been correlated with wasting syndrome in chronic diseases. Serum TNF-α, IL-1, IL-2, IL-6, TGF-β and leptin are at higher levels in patients with wasting syndrome[2,3].

Myostatin is a chalone with the main regulatory effect on skeletal muscles. It belongs to Transforming Growth Factor–β superfamily[4,5] and its first name were GDF-8[6,7]. In mice, myostatin begins to express at embryonic somites and subsequently in developing skeletal muscles[8,9]. Myostatin in adults is expressed in skeletal muscle almost exclusively, although it is detected in adipose tissue[10]. Gene targeting studies in mice elucidated the function of myostatin[6,7]. At an early stage of development, the myostatin null mice presented increased muscle mass retained for their whole life[6,11]. Increased muscle weight at about 25% has been described in heterozygous mice compared to the wild type mice suggesting dose dependency[2]. So, GDF-8 regulates negatively muscle growth[7] and this observation gave GDF-8 the name myostatin[7,4].

ISSN: 1996-3351 (Print)
https://doi.org/10.17311/ajbs.2022.40.48

Received: 02 Sep. 2021
Accepted: 05 Nov. 2021
Published: 01 Jan. 2022

Myostatin is a ligand of activin receptor IIB that activates the Smad pathway to regulate gene expression[5,10]. Wasting syndrome in mice with increased levels of myostatin has similarities with human wasting syndrome[12].

Myostatin is implicated in muscle atrophy in humans such as disuse muscle atrophy[13], myopathies[14], obesity[15], cardiac cachexia[16], cancer cachexia[17]. Increased myostatin levels have been identified in patients with HIV infection and body mass loss[18].

We examined the expression of the GDF-8 gene in patients with MDS to study mechanisms involved in the development of the cachexia syndrome in these patients[19]. We also searched for possible agents influencing myostatin expression in these patients.

The objective of this study was to investigate whether myostatin is involved in the mechanisms of wasting syndrome. Future studies in activating the myostatin gene might provide benefit to MDS patients.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Hematology and Transfusion Medicine of the University Hospital of Larissa, Greece from September, 2009-December, 2015.

Patients: Eighty-one consecutive patients suffering from MDS were included in the study. Fifty-one were males with a median age of 71 years (range 33-85) and 30 females with a median age of 68 (range 54-80). Seventeen healthy controls (matched in age and gender) were included. Forty-nine patients were classified according to FAB[20] as refractory anaemia, 6 as refractory anaemia with ringed sideroblasts, 12 as refractory anaemia with excess of blasts, 9 as refractory anaemia with excess of blasts in transformation and 5 as chronic myelomonocytic leukaemia. Patients and healthy individuals characteristics are shown in Table 1 and 2. The study has the approval of the Ethical Committee of our Institution.

Isolation of muscle tissue: Muscle tissue was obtained through a biopsy of the biceps muscle. In detail, 500 mg of muscle was homogenized in 3.75 mL TRIZOL LS (Life Technologies) with a tissue homogenizer. RNA was extracted from the homogenate by adding 1 mL chloroform, incubating at 25°C for 15 min, centrifugation at 12000 g for 15 min and collecting the upper aqueous phase[19]. RNA was precipitated using isopropyl alcohol according to standard protocol[19,21-23].

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): The RT-PCR was performed using 1 μg of RNA from patients and healthy individuals using the SuperScript™ One-Step RT-PCR kit (Invitrogen, Carlsbad, USA) as described earlier[19].

The β-actin amplification was done as described elsewhere and a product of 290 bp in the 3'-UTR of the mRNA was obtained[19].

For myostatin cDNA PCR we used the following primers: GDF-8-F: 5'-CCG/GGA/ACT/GAT/TGA/TCA/GTA/TGA-3' and GDF-8-R: 5'-GGG-TTT-TCC-ATC-CAC-TTG-CAT-TAG-3' and we obtained a product of 146 bp in the untranslated (3-UTR) region of mRNA to avoid false-positive products from possible DNA contamination. cDNA production and initial denaturation were done at 50 for 30 min and at 94 for 2 min for 1 cycle. Then 35 cycles with denaturation at 94 for 15 sec, annealing at 51 for 30 sec, extension at 68 for 30 sec were followed for 35 cycles. A final extension cycle was made 10 min at 72°C. The DNA Engine Peltier Gradient Thermal Cycler (Bio-Rad Ltd. Corston Bath UK) was used[19].

Quantitative real-time PCR (Q-PCR): THERotorgene RG-3000 (RCorbett Research) and Invitrogen CYBR® GreenUniversal kit Carlsbad, USA were used as previously described[19] using the above primers. Five ng of cDNA in a volume of 25 μL and 40 cycles were done.

Table 1: Clinical and laboratory features of patients with MDS

Clinical features	RA	RARS	RAEB	RAEB-T	CMML	Total
M	31	5	7	5	3	51
F	18	1	5	4	2	30
Age median (range)	69 (33-85)	65 (56-75)	72 (52-80)	69 (55-80)	75 (61-82)	71 (33-85)
Duration of disease in months before analysis (Median±SD)	34±2.8	30±8.4	24±16.9	11.5±9.1	11±4.24	28±7.8
Hepatomegaly	12	0	10	9	5	36
Splenomegaly	23	0	12	9	5	49
Lymphadenopathy	2	0	2	1	2	7
Laboratory findings						
Blood (Mean±SD)						
Ht (%)	33.5±6.36	28±2.82	29.8±4.94	29.5±5	25±9.89	28±5
Hb (g dL^{-1})	11±1.41	11±1.41	9.6±1.41	11±1.41	8.5±3.53	10.5±1.5
MCV (fL)	99.5±0.7	83.4±14.84	80.5±3.53	92.3±2.1	92.5±7.77	94±6
MCH (pg)	29±4.24	26.15±5.44	27.75±1.41	30±0	30±2.82	29±1.3
MCHC (g dL^{-1})	31.5±0.707	32±0.2	32±0.1	31.33±0.7	33±0.1	31.8±0.2
WBC (µL)	3350±212.1	4120±265	3420±735.4	3066±667	7850±1020	3450±520
Neutrophils (µL)	860±58.8	3548±33.8	2235±135.5	1073±342	1812±586	912±433.5
Lymphocytes (µL)	2072±426	1520±520	1023±265	1520±423	1566±450	1962±632
Monocytes (µL)	268±85	206±189	179±98	674±256	4562±1250	524±325
Blasts (µL)	0	0	342±125	995±235	425±125	482±123
PLT×10^3 (µL)	103±62.25	291±98.94	135.6±23.7	122±82.8	26±42.42	103±88
Bone marrow (Mean±SD)						
Blasts (%)	0	0	6	22.5±3.5	32.5±3.5	
Hypercellular	45	6	10	6	5	72
Hypocellular	4	0	1	3	0	8
Fibrosis	6	0	1	2	2	11
Chromosomal abnormalities	21	0	9	4	0	34
Autoimmune phenomena	12	1	3	3	1	20
Treatment						
Blood transfusion	12	2	5	9	2	30
Epo only	9	3	0	0	2	14
Epo+GCSF	40	0	5	0	0	45
Chemotherapy	0	0	2	8	2	12
Steroids	12	0	4	2	1	19

RA: Refractory anaemia, RARS: Refractory anaemia with ringed sideroblasts, RAEB: Refractory anaemia with excess of blasts, RAEB-T: Refractory anaemia with excess of blasts in transformation, CMML: Chronic myelomonocytic leukemia, Ht: Haematocrit, Hb: Haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cells, Epo: Human recombinant erythropoietin, subcutaneously, GCSF: Granulocyte colony stimulating factor

Table 2: Clinical and laboratory features in healthy individuals

Clinical features	Healthy individuals
M	9
F	8
Age median (range)	71 (33-85)
Laboratory findings (Mean±SD)	
Ht (%)	40±4.23
Hb (g dL^{-1})	14±1.41
MCV (fL)	96±8.5
MCH (pg)	33.5±3.5
MCHC (g dL^{-1})	33.5±0.7
WBC (µL)	9200±707.10
Neutrophils (µL)	6072±605
Lymphocytes (µL)	2024±356
Monocytes (µL)	864±235
PLT×10^3 (µL)	354.5±17.68

Ht: Haematocrit, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cells, PLT: Platelets

DNA sequencing: Flanking primers to amplify the 3 exons of the GDF-8 gene were used as follow: Exon 1: F1: (5'-AGA-TTC-ACT-GGT-GTG-GCA-AG-3'), R1: (5'-ATA-GGA-CTA-CTT-ACA-CTC-3') exon 2: F2: (5'-ATA-GCT-GAT-TTT-CTA-ATG-CG-3') R2: (5'-GTT-ATC-ACT-TAC-CAG-CCC-AT-3'), exon 3: F3: F3(5'-AGT-GTT-CCA-GGC-CTA-TTG-ATA-T-3') R3: (5'-AAA-CAC-TTT-AAT-ATA-ACT-TAT-AC-3'). The product was processed for direct sequencing on ABI PRISM (Applied Biosystems).

Cytokines levels in the blood: We measured the serum Tumour Necrosis Factor-α, Transforming Growth Factor-β, leptin, Interleukin-1, Interleukin-2, Interleukin-6 and myostatin by using a sensitive sandwich ELISA (kit R&D, MN 55413, USA, BIOVENDOR for myostatin and Quantikine for leptin), to investigate the role of these cytokines in myostatin levels.

Statistical analysis: SPSS v15 was used. Shapiro-Wilk test was used for normality check and Mann-Whitney test (with Bonferroni's correction for multiple comparisons) for non-parametric variables was used. Student's t-test was used for parametric comparisons and correlation coefficient was used to detect significant correlations between parametric variables.

RESULTS

Analysis of the DNA sequence of GDF-8 gene: Direct sequencing was applied for mutation analysis in GDF-8 and no mutation or polymorphism was detected in these patients suggesting that the myostatin gene is well conserved. That also means that variations in expression may be due to different regulatory effects.

Expression analysis of GDF-8: Myostatin levels in blood serum were 14.7±2.25 ng mL^{-1} in MDS patients with wasting syndrome, 3.70±0.95 ng mL^{-1} in MDS without wasting and 4.76±1.25 ng mL^{-1} in normal individuals.

Some RT-PCR results are shown in Fig. 1. The ratio of myostatin/β-actin RNA in patients divided by the ratio of myostatin/β-actin RNA in the normal individuals is presented in Table 3. The myostatin RNA in MDS patients was higher compared to the controls ($p<0.05$). CMML patients expressed lower myostatin than the controls ($p<0.05$). RARS patients did not present any difference from controls (NS), while RA, RAEB and RAEB-T expressed significantly higher myostatin than the controls ($p<0.05$ for all). RAEB patients presented the highest levels of expression (250±30%).

We measured also the RNA copies of myostatin in various groups of patients. In patients with wasting syndrome, the mean value of RNA copies were 46997.45±8934.09 and in patients without wasting it was 10702.97±3885.59 and in normal individuals, it was 12657.12±3231.873 (Table 4, Fig. 2).

Cytokine analysis: TNF-α levels in blood serum were 719.88±126.22 pg dL^{-1} in patients with wasting syndrome, 394.52±60.93 pg dL^{-1} in MDS without wasting and 249.84±64.95 pg dL^{-1} in normal individuals. IL-1 levels in blood serum were 3713±892.50 pg dL^{-1} in MDS patients with wasting syndrome, 1965.5±314.20 pg dL^{-1} in MDS without wasting and 827.23±125.27 pg dL^{-1} in normal individuals.

IL-6 levels in blood serum were 773.96±124.11 pg dL^{-1} in MDS patients with wasting syndrome, 478.12±64.44 pg dL^{-1} in MDS without wasting and 271±66 pg dL^{-1} in normal individuals. IL-2 levels in blood serum were 2725.03±406.28 pg dL^{-1} in MDS patients with wasting syndrome, 1477±247.42 pg dL^{-1} in MDS without wasting and 534.53±75.39 pg dL^{-1} in normal individuals.

Leptin levels in blood serum were 53.83±23.16 pg mL^{-1} in MDS patients with wasting syndrome, 9.22±3.26 pg mL^{-1} in MDS without wasting and 6.83±3.28 pg mL^{-1} in normal individuals.

TGF-β levels in blood serum were 928.64±107.52 pg dL^{-1} in MDS patients with wasting syndrome, 343.18±139.06 pg dL^{-1} in MDS without wasting and 75±25.70 pg dL^{-1} in normal individuals (Table 5).

Table 3: Expression of the GDF-8 and β-actin in patients with MDS and healthy individuals

MDS category	GDF-8 expression (%) Average±SD
CMML	
M	
F	
Total	25±18% (p<0.05)
RARS	
M	
F	
Total	56±35% NS
RA	
M	
F	
Total	127±50% (p<0.05)
RAEB	
M	
F	
Total	250±30% (p<0.05)
RAEB-T	
M	
F	
Total	104±25% (p<0.05)
Healthy	
M	
F	
Total	59±18%

GDF-8 transcript number was measured by quantitative real-time PCR and is expressed as a percentage of β-actin transcript number performed at the same time in the same tube, RA: Refractory anaemia, RARS: Refractory anaemia with ringed sideroblasts, RAEB: Refractory anaemia with an excess of blasts, RAEB-T: Refractory anaemia with an excess of blasts in transformation, CMML: Chronic myelomonocytic leukemia

Table 4: Myostatin mRNA copies and body mass index in MDS patients and controls

Parameters	Emaciated MDS patients	Non-emaciated MDS patients	Normal controls
Myostatin RNA copies	46997.45±8934.09	10702.97±3885.59	12657.12±3231.873
BMI	20.414±1.801	27.825±2.097	26.692±1.748

Myostatin copies were significantly higher in emaciated MDS patients compared to non-emaciated (p<0.001) and normal controls (p<0.001), Body Mass Index (BMI) was significantly lower in emaciated patients compared to non-emaciated (p<0.001) and normal controls (p<0.001)

Table 5: Cytokines levels in the blood of the individuals tested

Levels	MDS emaciated	Emaciated/ non-emaciated (p_1)	MDS non-emaciated	Emaciated/ normal (p_2)	Normal	Non-emaciated/ normal (p_3)
TNF-α	719.88	0.004771	394.5294	0.019112	249.8462	0.80264
TNF-α SD	126.2222		60.93381		64.95363	
IL-1	3713	0.00011442	1965.5	1.7546×10^{-8}	827.230769	0.00271554
IL-1SD	892.5012		314.2093		125.2751	
IL-6	773.9643	0.010309	478.125	0.026785	271.9231	0.895411
IL-6 SD	124.1124		64.44313		66.46736	
TGF-β	928.6428571	0.237452256	343.1875	1.09182×10^{-5}	75	7.73511×10^{-7}
TGF-β SD	107.5244		139.069		25.70019	
IL-2	2725.035714	0.047812082	1477	4.48915×10^{-7}	534.5384615	0.000186739
IL-2 SD	406.2815556		247.4254096		75.39077683	
Myo	14.68571	0.001178	3.70625	0.037314	4.769231	0.324053
Myo-SD	2.248739		0.958797		1.253917	
Leptin	53.83	$3.378\cdot10^{-10}$	9.22	$1.97\cdot10^{-8}$	6.83	0.96
Leptin SD	23.16		3.26		3.28	

Mean values, standard deviations and mean comparisons (t-test, p-value) among the groups

Fig. 1: RT-PCR from patients with MDS (samples: 2-49)

Sample 1 and 50: 100 bp DNA ladder, the PCR product of GDF-8 is 146 bp and the PCR product of β-actin is 290 bp

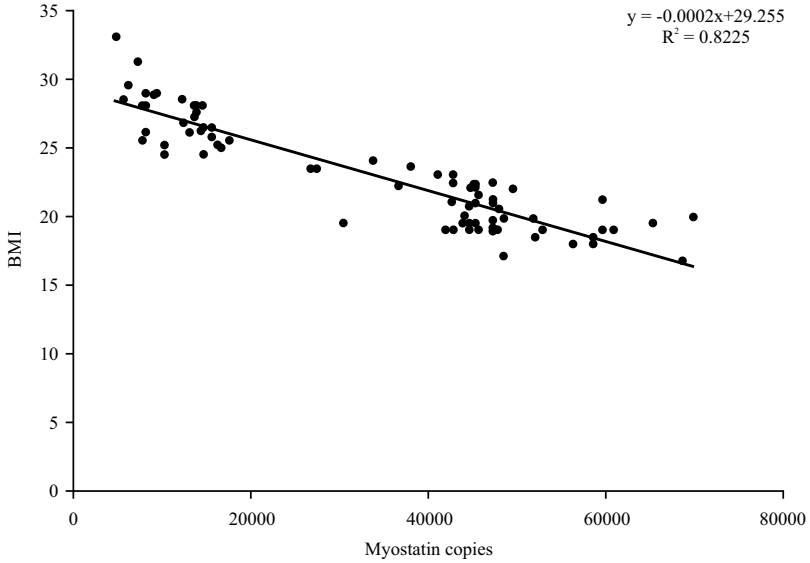

Fig. 2: Correlation between body mass index and myostatin mRNA copies

Myostatin mRNA copies were measured using 1 μg of RNA from muscle tissue obtained from the biceps muscle after biopsy and RNA extraction as described in the text

DISCUSSION

This is the first study investigating the possible role of myostatin in MDS patients with wasting syndrome. The role of myostatin in muscle atrophy has been extensively studied in various cancer patients with wasting syndrome and in many cases, myostatin was found overexpressed[24,25] although other mechanisms and underlying conditions exist in different patients[26-30]. We found that MDS patients with wasting syndrome expressed higher levels of myostatin compared to those without wasting syndrome and normal controls and this is inconsistent with previous studies in animals and other cancer patients[24-31].We did not measure other growth and differentiation factors such as GDF-11 and GDF-15 that are known to have homology with myostatin (GDF-8) and similar function[1] and this is a limitation of this study.

Previous studies have reported that inflammatory and pro-inflammatory proteins (TNF-α, TGF-β, IL-1, IL-2, IL-6, leptin) enhance myostatin expression leading to skeletal muscle atrophy[27]. In this study, we found significantly increased levels of serum TNF-α, IL-1, IL-2, IL-6 and leptin in emaciated MDS patients vs. non-emaciated and emaciated vs. normal controls. Serum TGF-β was found increased in emaciated and non-emaciated without significant difference between them, but TGF- serum levels were significantly increased between all MDS patients (emaciated or not) vs. normals. This is not well clarified since it is known that TGF-β has been shown to promote muscle loss in other cancer patients[27]. We do not know if they act through myostatin regulation or have any direct effect on muscle growth and/or degradation.

Although TNF-α, IL-1β and IL-6 inhibit via the NF-κB pathway muscle cell differentiation by down regulation of MyoD and augmenting muscle cell degradation via UPP (Ubiquitin-dependent Proteasome Pathway), NF-κB pathway is not usually activated in MDS patients as reported previously[27]. We did not study the NF-κB pathway in our patients.

TNF-α has been reported to have a pleiotropic effect on protein, lipid and carbohydrate metabolism as well as on myosin heavy chain degradation[27]. Consequently, TNF-α may act through myostatin activation but also other mechanisms. We did not investigate the exact mechanism of TNF-α action in our patients.

Although systemic inflammatory response with increase levels of inflammatory cytokines is well known[27,29,30] antibodies against these inflammatory molecules have not been extensively used (probably due to their serious side effects) and where they were used the response was not satisfactory especially in cachexia[27,30]. Antibodies against IL-6 have been used to treat wasting syndrome in advanced cancer patients with no effect on the syndrome although there was an improvement in anorexia[30]. On the contrary antibodies against myostatin have been used in animals and cell lines showing improvement of muscle wasting in animals and muscle growth in human cell lines[24,25,28] while antibodies against GDF-11 have been introduced in early trials in humans with unknown results[31].

We found that myostatin (GDF-8) is overexpressed in MDS patients with wasting syndrome. Independently to other factors involved in muscle wasting this group of patients could be a target group for a possible study of the effects of myostatin blockers and regulators on preventing or reversing muscle atrophy. It is well known that wasting syndrome in cancer patients is an independent factor for unfavourable outcome[1,27].

The limitations of our study are the absence of measurements of GDF-11 (another myostatin homologous with muscle growth inhibition) and the absence of investigation of other factors leading to muscle wasting in these patients. To have a maximum effect on muscle atrophy probably the inhibition of all parameters contributing to muscle wasting should be essential.

The implication of our findings is the possible use of myostatin antagonists in treating muscle wasting in cancer patients. Since the mechanism of muscle atrophy may be different in various cancer patients we found that this group of patients is a target group for these antagonists. Since monoclonal antibodies have been developed against myostatin MDS patients could be enrolled in future trials using these drugs and this could be recommended.

CONCLUSION

Myostatin seems to be involved in the development of wasting syndrome in MDS patients Other inflammatory cytokines may regulate myostatin levels or may act synergistically. Inactivating myostatin could lead to improvement of the syndrome.

SIGNIFICANCE STATEMENT

This is the first study showing the possible role of myostatin in the development of wasting syndrome in MDS patients. The role of other inflammatory and pro-inflammatory cytokines in this syndrome was also studied. Further studies are needed to find out methods to control serum myostatin levels expecting to improve the syndrome in these patients.

ACKNOWLEDGMENT

We would like to thank E. Rouka for the measurement of cytokines' levels.

REFERENCES

1. Bruggeman, A.R., A.H. Kamal, T.W. LeBlanc, J.D. Ma, V.E. Baracos and E.J. Roeland, 2016. Cancer cachexia: Beyond weight loss. J. Oncol. Pract., 12: 1163-1171.
2. Fearon, K.C.H., D.J. Glass and D.C. Guttridge, 2012. Cancer cachexia: Mediators, signaling and metabolic pathways. Cell Metab., 16: 153-166.
3. Tomasin, R., A.C.B.M. Martin and M.R. Cominetti, 2019. Metastasis and cachexia: Alongside in clinics, but not so in animal models. J. Cachexia, Sarcopenia Muscle, 10: 1183-1194.

4. Walsh, F.S. and A.J. Celeste, 2005. Myostatin: A modulator of skeletal-muscle stem cells. Biochem. Soc. Trans., 33: 1513-1517.
5. McNally, E.M., 2004. Powerful genes-myostatin regulation of human muscle mass. New Engl. J. Med., 350: 2642-2644.
6. Lee, S.J., 2004. Regulation of muscle mass by myostatin. Annu. Rev. Cell Dev. Biol., 20: 61-86.
7. McPherron, A.C., A.M. Lawler and S.J. Lee, 1997. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature, 387: 83-90.
8. Yang, W., Y. Zhang, G. Ma, X. Zhao, Y. Chen and D. Zhu, 2005. Identification of gene expression modifications in myostatin-stimulated myoblasts. Biochem. Biophys. Res. Commun., 326: 660-666.
9. Yao, C., W. Wei, X. Li and K. Kosoi, 2005. Acute phase protein induction by experimental inflammation in the salivary gland. J. Oral. Pathol. Med., 34: 364-367.
10. Tsuchida, K., 2004. Activins, myostatin and related TGF-β family members as novel therapeutic targets for endocrine, metabolic and immune disorders. Curr. Drug Targets Immune, Endocr. Metab. Disord., 4: 157-166.
11. McPherron, A.C. and S.J. Lee, 2002. Suppression of body fat accumulation in myostatin-deficient mice. J. Clin. Investigat., 109: 595-601.
12. Zimmers, T.A., M.V. Davies, L.G. Koniaris, P. Haynes and A.F. Esquela *et al*., 2002. Induction of cachexia in mice by systemically administered myostatin. Science, 296: 1486-1488.
13. Reardon, K.A., J. Davis, R.M.I. Kapsa, P. Choong and E. Byrne, 2001. Myostatin, insulin-like growth factor-1 and leukemia inhibitory factor mrnas are upregulated in chronic human disuse muscle atrophy. Muscle Nerve, 24: 893-899.
14. Bogdanovich, S., T.O. Krag, E.R. Barton, L.D. Morris, L.A. Whittemore, R.S. Ahima and T.S. Khurana, 2002. Functional improvement of dystrophic muscle by myostatin blockade. Nature, 420: 418-421.
15. Milan, G., E.D. Nora, C. Pilon, C. Pagano and M. Granzotto *et al*., 2004. Changes in muscle myostatin expression in obese subjects after weight loss. J. Clin. Endocrinol. Metab., 89: 2724-2727.
16. Kung, T., T. Szabó, J. Springer, W. Doehner, S.D. Anker and S. von Haehling, 2011. Cachexia in heart disease: Highlights from the ESC 2010. J. Cachexia, Sarcopenia Muscle, 2: 63-69.
17. Argilés, J.M., M. Orpí, S. Busquets and F.J. López-Soriano, 2012. Myostatin: More than just a regulator of muscle mass. Drug Discovery Today, 17: 702-709.
18. Gonzalez-Cadavid, N.F., W.E. Taylor, K. Yarasheski, I. Sinha-Hikim and K. Ma *et al*., 1998. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. Proc. Natl. Acad. Sci., 95: 14938-14943.
19. Karadonta, A-V., M.G. Alexandrakis, T.V. Kourelis, A. Manola, K. Garlemou, N.E. Stathakis and D. Kyriakou, 2009. Abnormal expression of C3ORF9 gene in patients with myelodysplastic syndromes. Int. J. Immunopathol. Pharmacol., 22: 787-793.
20. Vardiman, J., 2012. The classification of MDS: From FAB to WHO and beyond. Leukemia Res., 36: 1453-1458.
21. Zhao, Q., S. Xie, Y. Sun, Y. Chen and J. Gao *et al*., 2015. Development and evaluation of a SYBR Green real-time RT-PCR assay for detection of avian hepatitis E virus. BMC Vet. Res., Vol. 11. 10.1186/s12917-015-0507-5.
22. Fischer, M., M. Skowron and F. Berthold, 2011. Reliable transcript quantification by real-time reverse transcriptasepolymerase chain reaction in primary neuroblastoma using normalization to averaged expression levels of the control genes HPRT1 and SDHA. J. Mol. Diagn., 7: 89-96.
23. Hummon, A.B., S.R. Lim, M.J. Difilippantonio and T. Ried, 2007. Isolation and solubilization of proteins after TRIzol® extraction of RNA and DNA from patient material following prolonged storage. BioTech., 42: 467-472.
24. Pirruccello-Straub, M., J. Jackson, S. Wawersik, M.T. Webster and L. Salta *et al*., 2018. Blocking extracellular activation of myostatin as a strategy for treating muscle wasting. Sci. Rep., Vol. 8. 10.1038/s41598-018-20524-9.

25. Liu, D., X. Qiao, Z. Ge, Y. Shang and Y. Li *et al*., 2019. IMB0901 inhibits muscle atrophy induced by cancer cachexia through MSTN signaling pathway. Skeletal Muscle, Vol. 9. 10.1186/s13395-019-0193-2.
26. Batsis, J.A. and D.T. Villareal, 2018. Sarcopenic obesity in older adults: Aetiology, epidemiology and treatment strategies. Nat. Rev. Endocrinol., 14: 513-537.
27. Siddiqui, J.A., R. Pothuraju, M. Jain, S.K. Batra and M.W. Nasser, 2020. Advances in cancer cachexia: Intersection between affected organs, mediators and pharmacological interventions. Biochim. Biophys. Acta Rev. Cancer, Vol. 1873. 10.1016/j.bbcan.2020.188359.
28. Farhang-Sardroodi, S. and K.P. Wilkie, 2020. Mathematical model of muscle wasting in cancer cachexia. J. Clin. Med., Vol. 9. 10.3390/jcm9072029.
29. Argilés, J.M., F.J. López-Soriano and S. Busquets, 2019. Mediators of cachexia in cancer patients. Nutrition, 66: 11-15.
30. Yakovenko, A., M. Cameron and J.G. Trevino, 2018. Molecular therapeutic strategies targeting pancreatic cancer induced cachexia World J. Gastrointest. Surg., 10: 95-106.
31. Smith, R.C. and B.K. Lin, 2013. Myostatin inhibitors as therapies for muscle wasting associated with cancer and other disorders. Curr. Opin. Support Palliat Care, 7: 352-360.