Cancer Frequency in MuSK Myasthenia Gravis and Histological Evidence of Paraneoplastic Etiology

Silvia Falso, MD,[1] Marco Gessi, MD, PhD,[2] Sofia Marini, MD,[1] Roberta Benvenuto, BSc,[2] Eleonora Sabatelli, MD,[3] Antonio D'Amati, MD,[4] Martina Marini, MD,[1] Amelia Evoli, MD,[1,3] and Raffaele Iorio, MD, PhD[1,3]

Cancer frequency in muscle-specific kinase myasthenia gravis (MuSK-MG) has not yet been explored and the mechanisms leading to the formation of MuSK IgG remain elusive. We aimed to explore cancer frequency in MuSK-MG patients and to assess MuSK expression in cancer cells from 2 tumors occurred in this cohort. Immunohistochemistry on tumor specimens revealed the expression of MuSK in the cancer cells from primary mediastinal B cell lymphoma and endometrial carcinoma. Twenty-one males and 73 females were enrolled. Fifteen cancers occurred in 13 of 94 patients (13.8%). Patients with cancer were significantly older at time of MuSK-MG onset.

ANN NEUROL 2024;96:1020–1025

Myasthenia gravis (MG) is an autoimmune disorder characterized by muscle fatigability, caused by impaired synaptic transmission at neuromuscular junction. Approximately 5 to 7% of MG patients have autoantibodies (Abs) targeting the muscle-specific kinase (MuSK), with different pathogenesis and clinical features.[1]

MuSK-Abs belong to IgG4 subclasses that fail to engage complement and are functionally monovalent. Despite ongoing research, pathogenic mechanisms leading to autoimmunization against MuSK remain elusive.

IgG4 Abs typically emerge in response to sustained antigenic stimulation. Tumor lesions frequently display characteristics analogous to those seen in chronic inflammatory responses. IgG4 responses have been reported in different cancers such as melanoma,[2] lymphoma,[3] and glioblastoma.[4]

The relationship between neurological autoimmunity and cancer is bidirectional, with autoimmune diseases potentially increasing cancer risk through long-term immunosuppression and anti-tumor immune responses potentially leading to autoimmunity.

Assessing cancer frequency among MG patients is particularly relevant given the emergence of novel molecular therapies avoiding immune system suppression.[5]

Cancer prevalence among MuSK-MG patients remains unclear, and there are no documented cases of MuSK-MG as paraneoplastic syndrome. This study aimed to explore the frequency and timing of cancer in this population.

Methods

Ethics Approval

This study involves human participants and was conducted in conformation with Helsinki Declaration. It was approved by ethics committee of Università Cattolica del Sacro Cuore (protocol 23752/14). Participants gave informed consent to participate before taking part.

Study Subjects

In this retrospective study, we reviewed records of patients diagnosed with MuSK-MG between 2005 and 2022 at Fondazione Policlinico A. Gemelli (Rome, Italy). Patients with at least 1-year follow-up were included. We recorded associated cancers, timing of oncological diagnosis in relation to MuSK-MG onset, type and duration of immunosuppressive therapy. We performed immunohistochemistry to assess MuSK expression in cancer cells of tumor specimens from 2 patients with MuSK-MG onset after cancer diagnosis.

From the [1]Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy; [2]Neuropathology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [3]Neurology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; and [4]Gynecopathology and Breast Pathology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Address correspondence to Dr Raffaele Iorio, Neurology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy. E-mail: raffaele.iorio@policlinicogemelli.it

Received Feb 17, 2024, and in revised form Jun 4, 2024. Accepted for publication Jul 2, 2024.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.27033.

Immunohistochemistry of Tumor Specimens

Formalin-fixed paraffin embedded sections (3μm) were stained with hematoxylin and eosin and with polyclonal rabbit IgG specific to the extracellular MuSK domain (PA1-1741, Invitrogen). To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH 6.0) buffer for 30 minutes using a microwave. Following antigen retrieval, tissues were blocked in 3% bovine serum albumin-phosphate-buffered saline for 5 minutes and then probed with or without (negative control) a MuSK polyclonal antibody at dilution of 1:20 for 30 minutes at room temperature. Tissues were washed extensively with Wash Solution Buffer 1× and endogenous peroxidase activity quenched with Peroxidase Suppressor for 30 minutes at room temperature. Detection was performed using a goat anti-rabbit horseradish peroxidase secondary antibody (1:200) followed by colorimetric detection using metal enhancer 3-3'-diaminobenzidine tetrahydrochloride. Tissues were counterstained with hematoxylin and prepped for mounting.

Statistical Analysis

Descriptive statistics were used to summarize all variables. Qualitative variables were expressed as absolute counts and percentages, quantitative variables were presented using median and range. Shapiro–Wilk test was used to assess distribution of the data. Fisher's exact test was used for comparing qualitative variables. Mann–Whitney test was performed for analyzing quantitative differences. A multivariate logistic regression model was developed to identify factors associated with presence of neoplasm. Statistical analyses were performed using GraphPad Prism 10.1.0 and JMP 18.

Results

Case 1

A 42-year-old man was diagnosed with primary mediastinal large B cell lymphoma (PMBCL). Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone were performed followed by autologous stem cell transplantation and mediastinal radiotherapy, according to current guidelines.[6] This treatment achieved PMBCL remission. Three years later, he developed fluctuating diplopia, which was subsequently associated with dysarthria, dysphagia, facial weakness, and limb fatigability. Anti-MuSK Abs resulted positive by RIA (1.14nMol/L; normal value <0.05) and pathologic decrement (−27%) of compound muscle action potential on repetitive nerve stimulation with 3Hz was detected. MuSK-MG was diagnosed and prednisone was started at 0.5mg/kg daily, leading to clinical improvement. At last follow-up, minimal manifestation status (MMs) was achieved according to MG Foundation of America post-intervention status.[7]

Case 2

A 69-year-old woman was diagnosed with an endometrial clear cell carcinoma (ECCC). She underwent hysterectomy and bilateral salpingo-oophorectomy followed by chemotherapy achieving cancer remission. Three years later, she presented with fluctuating diplopia and ptosis; after 6 months, she was admitted to our emergency room because of dysarthria, dyspnea, and muscle fatigability. She developed acute respiratory failure requiring orotracheal intubation followed by tracheostomy. MG was suspected based on clinical grounds and MuSK-Abs were detected by RIA (2.40nMol/L). Prednisone was started at 0.5mg/kg daily and she underwent plasma-exchange with partial improvement, achieving discharge from intensive care unit. Subsequently, rituximab was administered (1.000 mg intravenously 2 weeks apart) leading to progressive clinical improvement and the tracheostomy was successfully closed. At the last follow-up, 6 months after discharge, the patient had reached MMs.

MuSK Expression in Tumor Specimens

Tissue specimens from these 2 patients were available and examined for MuSK expression. Immunohistochemistry detected MuSK expression in cancer cells from both the PMBCL and the ECCC. Additionally, immunohistochemical analysis was performed on tissue specimens (2 PMBCLs and 2 ECCCs) from patients without MuSK-MG, available at our pathology unit. MuSK expression was detected in both the PMBCLs and in 1 of 2 ECCCs. In all the positive cases there was a pronounced MuSK-specific nuclear reactivity, suggesting the nuclear translocation of MuSK in cancer cells. Notably, MuSK expression was not detected in normal endometrial tissue. (Fig. 1).

Cancer Frequency and Oncological Associations in MuSK-MG

We included 94 MuSK-MG patients, 73 (78%) females, with a median age at MG onset of 51 years (19–76). Ninety-two of 94 (97.9%) cases required corticosteroids and/or immunosuppressants (IS). Detailed characteristics are summarized in Table.

Fifteen cancers occurred in 13 of 94 patients (13.8%). Median age at cancer onset was 64 years (18–79). In terms of temporal relationship with MuSK-MG onset, cancer preceded MG diagnosis in 5 patients (median: 11 years, [3–17]), occurred concurrently in 2, and followed MG diagnosis in 8 (median: 11.5 years, [1–32]) (Fig. 2). Six patients were on long-term immunosuppression at cancer diagnosis (corticosteroids with azathioprine 1/6; mycophenolate mofetil 1/6; rituximab 1/6).

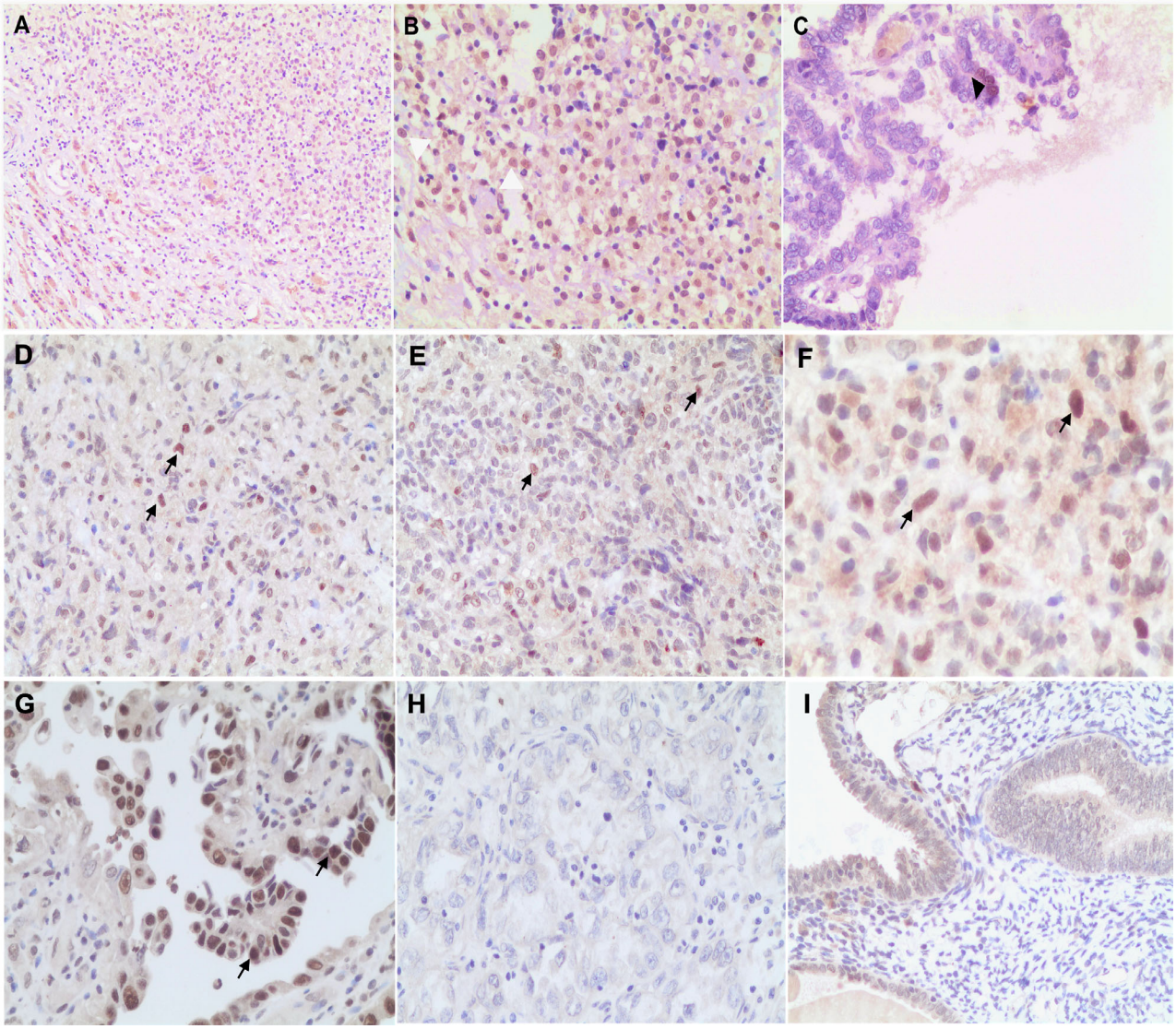

FIGURE 1: Expression of muscle-specific kinase (MuSK) by patients' tumors. Immunohistochemical analysis shows high level of MuSK expression in cancer cells of primary mediastinal large B cell lymphoma (PMBCL) and in muscle cells (A). Higher magnification shows mild MuSK expression in the cytoplasm and high expression in the nuclei of lymphoma cells (white arrows), but not in normal infiltrating lymphocytes (B). High level of MuSK expression is detected in the nuclei of tumor cells from endometrial clear cell carcinoma (ECCC) of a patient with MuSK myasthenia gravis (MuSK-MG) (C). Immunohistochemistry reveals high level of MuSK expression in the nuclei of cancer cells from PMBCLs of two patients without MuSK-MG (D–F). High level of expression of MuSK is detected in the nuclei of tumor cells from ECCC of a patient without MuSK-MG (G). Immunohistochemical analysis did not show MuSK expression in ECCC of another patient without MuSK-MG (H). MuSK expression was not detected in normal endometrial tissue (I). Tissues immunostained with the indicated avidin-biotin peroxidase method and mildly counterstained with hematoxylin. All panels ×400. [Color figure can be viewed at www.annalsofneurology.org]

Hematologic malignancies were the most frequent cancers (n = 5), all occurred before MG onset, 2 mediastinal Hodgkin's lymphomas and 1 of each PMBCL, orbital lymphoma, and myelodysplastic syndrome evolved to leukemia. Other cancer types are reported in Table.

According to median age at MuSK-MG onset, we divided patients in two categories (below and above 51 years) for multivariate logistic regression analysis. Gender, age at MuSK-MG onset, outcome (MMs-or-better), and severity of MuSK-MG were included in the multivariate logistic regression model. Immunosuppressive therapy was not included because few patients were untreated in our cohort. Age at MuSK-MG onset (odds rate [OR]: 3.584, 95% CI: 1.087–12.906, $p = 0.036$) was independently associated with cancer presence.

Discussion

We detected MuSK expression in tumor specimens of a PMBCL and ECCC, obtained from two patients who

TABLE. Number and type of tumors and immunosuppressive therapy in MuSK-MG patients with and without cancer

Variable	Cancer patients (n)	%	No cancer patients (n)	%	*p*-value
Total	13	13.8	81	86.2	
Median age at MG onset (range)	59 (22–76)		41 (19–75)		0.036[a]
Median age at cancer diagnosis (range)	64 (18–79)		–	–	
Cancer (n = 15)					
Cancer patient 1	11	84.6	–	–	
Cancer patient 2	2	15.4	–	–	
Type (n = 15)			–	–	
Hematological malignancies	5	33.3	–	–	
Breast	3	20.0	–	–	
Uterus	2	13.3	–	–	
Digestive organs	2	13.3	–	–	
Lung (neuroendocrine)	1	6.6	–	–	
Vocal cords	1	6.6	–	–	
Skin (squamous cell)	1	6.6	–	–	
Gender					
Men	3	23.1	18	22.2	
Women	10	76.9	63	77.8	
IST [b]	6/13	85.7	80/81	98.8	
None	7	14.3	1	1.2	
Only CS	3	42.8	27	33.3	
CS + 1 IS	1	14.3	25	30.9	
CS + 2 IS	1	14.3	13	16	
Rituximab	1	14.3	15	18.5	
MGFA-PIS					
MMs-or-better	9	69.2	44	54.3	

[a]The median age of MG onset was significantly higher in cancer group.
[b]In cancer group, only patients treated with IST before cancer onset are considered.
CS = corticosteroids; IS = immunosuppressant; IST = immunosuppressive therapy; MG = myasthenia gravis; MGFA = Myasthenia Gravis Foundation of America; MMs = minimal manifestation status; PIS = post-intervention status.

developed MuSK-MG after cancer diagnosis. The patient with PMBCL was treated with rituximab at the time of cancer diagnosis, which probably delayed the onset of MG symptoms. Interestingly, MuSK expression was detected also in cancer cells of two PMBCLs and one of two ECCCs from patients without MuSK-MG. Notably, normal endometrial tissue did not show MuSK expression.

Immunohistochemistry revealed strong nuclear expression of the MuSK protein in cancer cells of these tumors. MuSK is a receptor tyrosine-kinase (RTK) normally expressed on plasma membrane. Our data show nuclear

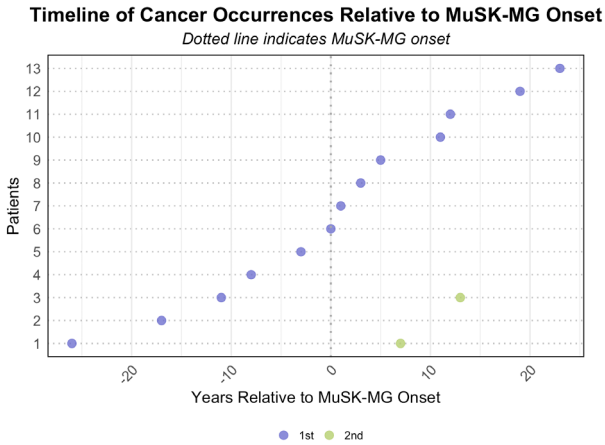

FIGURE 2: Interval (years) between myasthenia gravis (MG) onset and cancer diagnosis in patients 1–13. Cancer preceded MG diagnosis in five patients, occurred concurrently in two patients, and followed MG diagnosis in eight patients. Patient 1 and 3 had 2 cancers. [Color figure can be viewed at www.annalsofneurology.org]

translocation of the whole MuSK protein, or at least its extracellular region, and are in line with previous evidence on RTKs translocation in cancer cells in response to environmental stimuli.[8]

These findings, previously unexplored for MuSK, might indicate a novel pathway in tumorigenesis and a potential therapeutic target similar to other RTKs. RTKs are membrane receptors with high affinity for extracellular growth factors, cytokines, and hormones. Overexpression and mutation of RTKs as well as dysregulation of their signaling may lead to human diseases, including cancer.[9]

The demonstration of MuSK nuclear immunoreactivity in patients' tumors supports a possible paraneoplastic etiology for some MuSK-MG cases. MuSK expression in cancer cells may initiate MuSK autoantibody production as it has been demonstrated for classical paraneoplastic syndromes.[10,11] Likewise, CDR2 and CDR2L are expressed in ovarian cancers from patients with and without Yo Abs[12] as well as HuD protein is expressed in 100% of small–cell lung carcinoma of patients with and without paraneoplastic syndrome.[13,14] In our cohort, the cancer rate was 14%, suggesting a low cancer association, limited by the small number of patients.[15] The most frequent link was with onco-hematological malignancies (5/15, 33.3%); of these, 3 (60%) were mediastinal lymphomas. The association with PMBCL may support the administration of B-cell-depleting agents that could be effective both for MuSK-MG and PMBCL.

MuSK-MG generally shows a striking prevalence in women with a peak in the late 30s.[1] Multivariate logistic regression analysis predicted a higher likelihood of underlying cancer in patients with MG onset above 51 years. This finding may suggest the need for a tailored cancer screening protocol in this MuSK-MG population.

The mechanisms underlying MuSK's nuclear translocation in cancer cells and its potential role in tumorigenesis are unclear. Further studies are needed to explore a possible paraneoplastic origin of MuSK-MG.

Acknowledgments

The work was supported by the MGNet a member of the Rare Disease Clinical Research Network (RDCRN) NIH U54 NS115054. Open access funding provided by BIBLIOSAN.

Author Contributions

S.F. and R.I. contributed to the conception and design of the study; S.F., M.G., S.M., R.B., E.S., A.D., M.M., A.E., and R.I. contributed to the acquisition and analysis of data; S.F. and R.I. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

Data are available on reasonable request.

References

1. Evoli A, Alboini PE, Damato V, et al. Myasthenia gravis with antibodies to MuSK: an update. Ann N Y Acad Sci 2018;1412:82–89. https://doi.org/10.1111/nyas.13518.

2. Karagiannis P, Gilbert AE, Josephs DH, et al. IgG4 subclass antibodies impair antitumor immunity in melanoma. J Clin Invest 2013;123:1457–1474. https://doi.org/10.1172/JCI65579.

3. Takahashi N, Ghazale AH, Smyrk TC, et al. Possible association between IgG4-associated systemic disease with or without autoimmune pancreatitis and non-Hodgkin lymphoma. Pancreas 2009;38:523–526. https://doi.org/10.1097/MPA.0b013e31819d73ca.

4. Harshyne LA, Nasca BJ, Kenyon LC, et al. Serum exosomes and cytokines promote a T-helper cell type 2 environment in the peripheral blood of glioblastoma patients. Neuro Oncol 2016;18:206–215. https://doi.org/10.1093/neuonc/nov107.

5. Iorio R. Myasthenia gravis: the changing treatment landscape in the era of molecular therapies. Nat Rev Neurol 2024;20:84–98. https://doi.org/10.1038/s41582-023-00916-w.

6. Fakhri B, Ai W. Current and emerging treatment options in primary mediastinal B-cell lymphoma. Ther Adv Hematol 2021;12:20406207211048959. https://doi.org/10.1177/20406207211048959.

7. Jaretzki AIII, Barohn RJ, Ernstoff RM, et al. Myasthenia gravis. Neurology 2000;55:16–23. https://doi.org/10.1212/WNL.55.1.16.

8. Carpenter G, Liao HJ. Receptor tyrosine kinases in the nucleus. Cold Spring Harb Perspect Biol 2013;5:a008979. https://doi.org/10.1101/cshperspect.a008979.

9. Schlessinger J. Receptor tyrosine kinases: legacy of the first two decades. Cold Spring Harb Perspect Biol 2014;6:1–13. https://doi.org/10.1101/cshperspect.a008912.

10. Iorio R, Spagni G, Masi G. Paraneoplastic neurological syndromes. Semin Diagn Pathol 2019;36:279–292. https://doi.org/10.1053/j.semdp.2019.06.005.

11. Gilligan M, McGuigan C, McKeon A. Paraneoplastic neurologic disorders. Curr Neurol Neurosci Rep 2023;23:67–82. https://doi.org/10.1007/s11910-023-01250-w.

12. Raspotnig M, Haugen M, Thorsteinsdottir M, et al. Cerebellar degeneration-related proteins 2 and 2-like are present in ovarian cancer in patients with and without Yo antibodies. Cancer Immunol Immunother 2017;66:1463–1471. https://doi.org/10.1007/s00262-017-2041-8.

13. Manley GT, Smitt PS, Dalmau J, Posner JB. Hu antigens: reactivity with hu antibodies, tumor expression, and major immunogenic sites. Ann Neurol 1995;38:102–110. https://doi.org/10.1002/ana.410380117.

14. Ehrlich D, Wang B, Lu W, et al. Intratumoral anti-HuD immunotoxin therapy for small cell lung cancer and neuroblastoma. J Hematol Oncol 2014;7:91. https://doi.org/10.1186/s13045-014-0091-3.

15. Graus F, Vogrig A, Muñiz-Castrillo S, et al. Updated diagnostic criteria for paraneoplastic neurologic syndromes. Neurol Neuroimmunol Neuroinflamm 2021;8:e1014. https://doi.org/10.1212/NXI.0000000000001014.