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Original Contribution

Next generation sequencing for *GNAS* uncovers CD34 as a sensitive marker for intramuscular myxoma☆,☆☆

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ABSTRACT

Background: Intramuscular myxoma is a soft tissue myxoid tumor with a broad morphological differential diagnosis and recent developments have led to the identification of markers that can exclude some, but not all, differential diagnostic entities. However, a sensitive confirmatory marker for intramuscular myxoma has not been clearly identified. Since there is some evidence that mutations in the *GNAS* gene could be such a marker, we evaluated our results of next-generation sequencing testing for *GNAS* mutations performed in recent years on our series of intramuscular myxoma.

Materials and methods: Next-generation sequencing was performed on 10 cases of intramuscular myxoma diagnosed between 2015 and 2019, using either the TruSight Tumor 26 panel or an in-house developed 97 cancer gene panel. Additionally, immunohistochemistry for CD34 was performed on all cases.

Results: All intramuscular myxomas showed a diffuse and strong expression of CD34 and a *GNAS* mutation was found in 88% of cases, making this a very sensitive positive test for the diagnosis of intramuscular myxoma.

Conclusions: Under the condition that contemporary next-generation sequencing is applied as testing method, searching for *GNAS* mutations is a very sensitive confirmatory test for the diagnosis of intramuscular myxoma, obviating the necessity to perform tests that exclude other entities by the virtue of their negative result. The molecular tests results also identified strong and diffuse CD34 expression as a sensitive, albeit non-specific, marker for intramuscular myxoma.

1. Introduction

Intramuscular myxoma is a soft tissue myxoid tumor with a broad differential diagnosis at the morphological level. Recent advancements have led to the identification of specific and sensitive markers for some differential diagnostic entities, such as MUC4 immunohistochemistry for low-grade fibromyxoid sarcoma [1]. Nevertheless, intramuscular myxoma frequently remains a challenging diagnosis, especially on small biopsy specimens, and the distinction from low-grade myxofibrosarcoma can be very difficult since there exists no sensitive or specific marker for the latter.

Therefore, the identification of new positive markers for intramuscular myxoma would be of diagnostic relevance. Mutations in the *GNAS* gene, mainly gain-of-function mutations R201H and R201C, have been described in intramuscular myxoma and not in its morphological mimickers, but the percentage of positive cases ranges between 36 and 61%, depending on the study [2-4]. This observation suggests that the presence of a *GNAS* hotspot mutation is a specific but rather insensitive marker for intramuscular myxoma. However, none of these studies used next generation sequencing (NGS) and in a very recent study by Sunitsch et al. [5], the authors found that using NGS 92% (12/13) of their cases showed a *GNAS* hotspot mutation. Their results suggest that the *GNAS* mutation status can be used as a sensitive and specific test in the condition that NGS is used.

In view of these findings, we decided to review the results of our GNAS mutation analysis performed on the intramuscular myxoma cases that we have diagnosed at our institution in recent years. We also performed CD34 immunohistochemistry on these cases, given that there are some recent reports on expression of CD34 in a proportion of intramuscular myxomas [5-7].

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2. Materials and methods

In the period 2015–2019, 10 patients presenting at the orthopedic department of University Hospitals Saint-Luc were diagnosed with intramuscular myxoma by pathologists with specific interest in soft tissue pathology (LL, CG, RF). Biopsies (4 cases) or resection specimens (6 cases) were submitted for NGS, either in routine diagnostics (8 most recent cases) or NGS was performed retrospectively at the time this test became available in routine diagnostics (2 oldest cases). Until October 2018, targeted resequencing was performed using the TruSight Tumor 26 panel (Illumina NextSeq 500, 2 × 150 bp paired-end). Afterwards, an in-house developed 97 cancer gene panel was used (hybrid capture, KAPA HyperPlus Library Preparation kit, Kapa Biosystems, and XGen lockdown probes, IDT on an Illumina NextSeq 500, 2 × 150 bp paired-end).

3. Results

Our series consists of 5 men and 5 women, with a mean age of 62. Only 2 patients presented with a tumor in the upper extremity; 7 patients had a tumor in the upper part of a lower extremity and the tumor was localised in the calf in 1 patient. All cases had a minimum of 10% tumor cell fraction. NGS analysis was successful in 9 out of 10 cases. In 8 cases, a GNAS mutation could be detected, with mutation allele frequencies ranging between 5 and 28%, underscoring the importance of using a sensitive mutation detection assay. In 3 cases, the p.(R201C) mutation was found and in 5 cases the p.(R201H) mutation was detected. For one case, DNA quality was too low for NGS analysis and in another case, no mutation could be found and the initial diagnosis of an intramuscular myxoma was maintained after pathological review. Therefore, the test sensitivity was determined to be 88%.

Next, we decided to take advantage of this very-well characterised series by performing CD34 immunohistochemistry on all cases. A consistently positive immunohistochemical marker for intramuscular myxoma has hitherto not been identified, but there are some reports of CD34 positivity in intramuscular myxoma [5-7], which motivated us to perform this staining and we surprisingly found a diffuse and strong CD34 expression in all our cases (Figs. 1 and 2), so the sensitivity of CD34 expression for intramuscular myxoma.

4. Discussion

Our findings show the usefulness of NGS *GNAS* mutation analysis in the diagnosis of intramuscular myxoma. Mutation of *GNAS* was present in the very large majority of our cases, making this an almost universally present positive marker for intramuscular myxoma. Hitherto, the diagnosis of intramuscular myxoma is usually reached by performing several tests to exclude entities that bear morphological similarities, especially in difficult settings such as small biopsies and in cases of cellular variants. For example, in case of a suspected intramuscular myxoma, a negative MUC4 immunohistochemistry and a negative NGS analysis for CTNNB1 will exclude the possibility of a low-grade fibromyxoid sarcoma [1] and a myxoid desmoid tumor [8] mimicking an intramuscular myxoma, respectively, but these are “negative” tests excluding other entities, without conforming unequivocally the diagnosis of an intramuscular myxoma. In contrast, NGS *GNAS* testing is a confirmatory test for a morphologically suspect intramuscular myxoma, making it unnecessary to perform several “negative” immunohistochemical and molecular tests that will use up considerable tissue, which needs especially to be avoided when little biopsy material is received.

Taking our results together with those of previous studies [2-5], it becomes clear that applying contemporary NGS testing is a prerequisite to use *GNAS* mutation a sensitive marker for intramuscular myxoma. Similarly, it has been shown that NGS has a higher sensitivity for the detection of *CTNNB1* mutation in desmoid tumors compared to other types of molecular testing for CTNNB1 mutations [8,9]. This illustrates the general principle in soft tissue molecular pathology, and molecular pathology in general, that one should not only consider carefully which gene to test, but that one also has to pay attention to the choice of the testing method.

Furthermore, we also found that diffuse and strong CD34 expression is a very sensitive marker for intramuscular myxoma. Of course, CD34 is expressed by a variety of tumors and is not specific, but the absence of CD34 virtually excludes the possibility of an intramuscular myxoma, which can serve as an ancillary diagnostic tool. One wonders why CD34 has not been identified as a sensitive marker before and it is tempting to suggest that this is due to the fact that previous series and cases were «contaminated» by tumors that mimicked intramuscular myxoma but

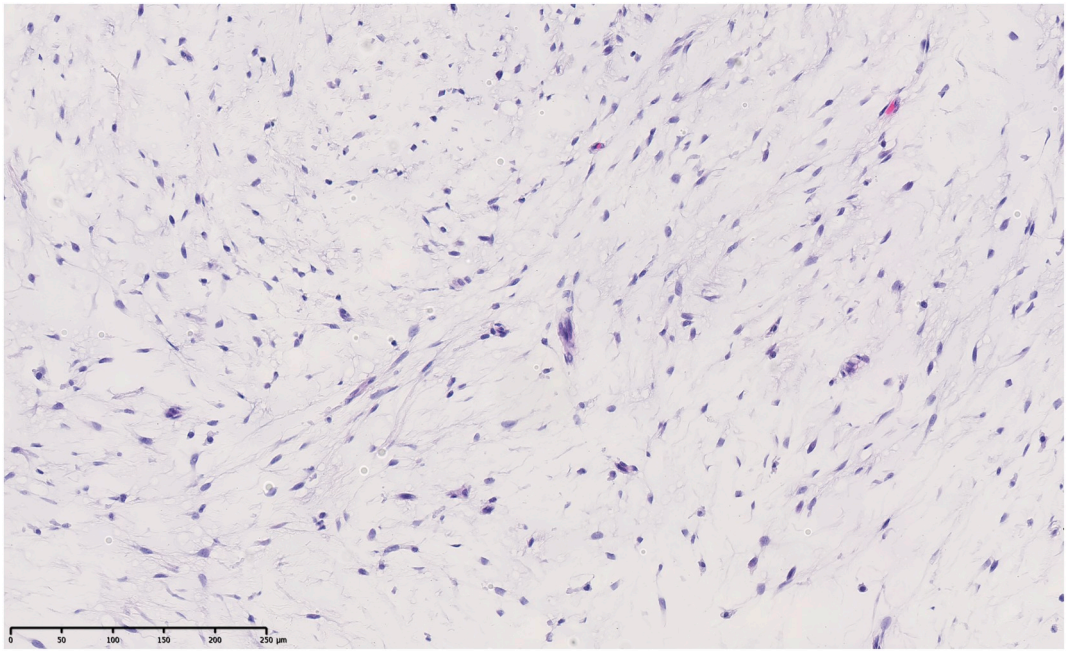

Fig. 1. Hematoxylin and eosin-stained section of an intramuscular myxoma showing bland spindle cells in a myxoid stroma.

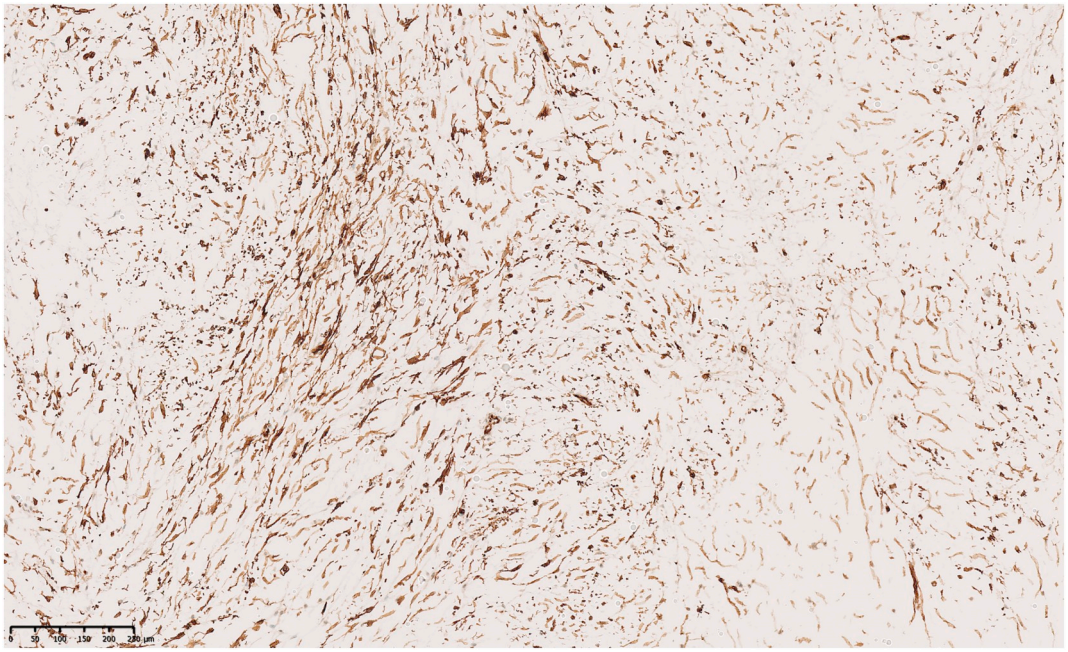

Fig. 2. the corresponding CD34-stained section shows a diffuse and intense staining of the tumoral spindle cells.

actually represented another entity, thereby «diluting» the results of potential positive tests performed on the series. Thanks to the high accuracy of NGS for detecting *GNAS* mutations, a «clean» series was ensured, leading to the uncovering of CD34 as a sensitive marker. Given the recent widespread application of NGS testing, our study rationale is applicable for several other tumor types.

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LL designed the study and participated to the writing of the manuscript. LL, RS and CG performed the pathological analyses. IVDB performed the NGS analyses. RS, TS, CG and IVDB participated in writing of the manuscript.

References

[1] Doyle LA, Moller E, Dal Cin P, Fletcher C, Mertens F, Hornick JL. MUC4 is a highly sensitive and specific marker for low-grade fibromyxoid sarcoma. Am J Surg Pathol 2011;35:733–41.
[2] Willems SM, Mohseny AB, Balog C, et al. Cellular/intramuscular myxoma and grade I myxofibrosarcoma are characterized by distinct genetic alterations and specific composition of their extracellular matrix. J Cell Mol Med 2009;13:1291–301.
[3] Delaney D, Diss TC, Presneau N, et al. GNAS1 mutations occur more commonly tha previously thought in intramuscular myxoma. Mod Pathol 2009;22:718–24.
[4] Walther I, Walther BM, Chen Y, Petersen I. Analysis of GNAS1 mutations in myxoid soft tissue and bone tumors. Pathol Res Pract 2014;210:1–4.
[5] Sunitsch S, Gilg MM, Kashofer K, Gollowitsch F, Leithner A, Liegl-Atzwanger B. Detection of GNAs mutations in intramuscular/cellular myxomas as a diagnostic tool in the classification of myxoid soft tissue tumors. Diagn Pathol 2018;13(52).
[6] Kernah B, Soylemez MS, Ceyran B, Senol S, Mutlu S, Ozkan K. A case of intramuscular myxoma presenting as a swollen shoulder: a case report. J Med Case Reports 2014;8(441).
[7] Costamagna D, Erra S, Durando R. BMJ Case Rep 2009. [pii: bcr08.2008.0678].
[8] Aitken SJ, Presneau N, Kalimuthu S, et al. Next-generation sequencing is highly sensitive for the detection of beta-catenin mutations in desmoid-type fibromatosis. Virchows Arch 2015;467:203–10.
[9] Crago AM, Chmielecki J, Rosenberg M, et al. Near universal detection of alterations in CTNNB1 and Wnt pathway regulators in desmoid-type fibromatosis by whole-exome sequencing and genomic analysis. Genes Chromosomes Cancer 2015;54:606–15.