MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

Capstone for Impact Submission | GY2019

Project Title: Genomic analysis and molecular biomarkers of 10 uterine smooth muscle tumors: emphasis on problematic cases

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Advisor Names(s): Dr. Aaron Udager

Branch: Diagnostics & Therapeutics

Path of Excellence: Scientific Discovery

Handover/Transition:
If this project can be continued by another UMMS student, you may contact them at the following email address/phone number (N/A if project cannot be handed over): **N/A**

Summary:

Histologic classification of uterine smooth muscle tumors (USMTs) can be difficult for tumors with unusual combinations of increased mitotic rate, nuclear atypia, and geographic tumor necrosis. Typically, usual leiomyomas (ULM) have none of these features, and leiomyosarcomas (LMS) are defined by the presence of 2/3 of these features. However, these are open to interpretation by the pathologist and many benign leiomyoma variants have been found to share some of these features.

Here, we study a challenging case of a USMT that I encountered on a pathology elective with a drastically increased mitotic rate and questionable presence of geographic tumor necrosis, without nuclear atypia or architectural distortion, making a definitive assessment of this tumor's malignant potential difficult.

We then reviewed the morphologic patterns of various USMTs, by examining a total of 10 tumors (12 samples) from 8 separate patients, including our original case, and performed targeted next generation sequencing (NGS) to identify somatic alterations in formalin fixed paraffin embedded (FFPE) patient specimens. NGS revealed mutations in mediator complex subunit 12 (MED12) in ULM cases and deleterious mutations in the tumor suppressors tumor protein p53 (TP53) and retinoblastoma (RB1) in LMS cases.

In addition, high-level copy number alterations (CNAs) were much more common in LMS cases, compared to ULM and leiomyoma variants, with the exception of one leiomyoma variant harboring a deep deletion of the fumarate dehydrogenase (FH) gene. Our original challenging case demonstrated a MED12 mutation without any additional CNAs, implicating a diagnosis of mitotically active leiomyoma and likely benign behavior. Given these findings, we propose a diagnostic schematic demonstrating utility for an integrated DNA/RNA profile to subtype diagnostically challenging USMTs.

Methodology:

The methodology of this project is outlined in detail in the manuscript draft uploaded below. In short, 12 FFPE uterine smooth muscle tumor specimens from the UofM Pathology Tissue Archive were obtained. For each specimen, 3-6 punches of FFPE sections were obtained from a single representative block per case, by a board-certified pathologist (S.A.T.) to obtain minimal estimated tumor purity of 70%. DNA and RNA were coisolated using the Qiagen Allprep FFPE DNA/RNA kit (Qiagen) and the Qiagen QIAcube (Qiagen), according to the manufacturer's instructions and were quantified using the Qubit 2.0 flurorometer (Life Technologies, Foster City, CA. Targeted multiplexed PCR based NGS was performed using a custom panel targeting 116 pan-cancer genes. Data analysis was was performed using in-house-developed, previously validated pipelines using Torrent suite 4.0.2, with alignment by TMAP and variant calling using the Torrent Variant Caller. To identify copy number alterations, normalized, GC-content corrected read counts per amplicon for each sample were divided by those from a pool of normal male genomic DNA samples (FFPE and frozen tissue, individual, and pooled samples), yielding a copy number ratio for each amplicon. Targeted mxPCR-based NGS of RNA was performed on coisolated RNA from all 12 UT samples (UT201-212) using a custom Ion Ampliseq panel assessing 155 target genes and 8 housekeeping genes (previously 103 target genes and 8 housekeeping) relevant for urothelial carcinoma.

Results/Conclusion:

Results and discussion are further detailed in the attached manuscript. Briefly, NGS revealed mutations in mediator complex subunit 12 (MED12) in ULM cases and deleterious mutations in the tumor suppressors tumor protein p53 (TP53) and retinoblastoma (RB1) in LMS cases.

In addition, high-level copy number alterations (CNAs) were much more common in LMS cases, compared to ULM and leiomyoma variants, with the exception of one leiomyoma variant harboring a deep deletion of the fumarate dehydrogenase (FH) gene. Our original challenging case demonstrated a MED12 mutation without any additional CNAs, implicating a diagnosis of mitotically active leiomyoma and likely benign behavior. Given these findings, we also proposed a diagnostic schematic demonstrating utility for an integrated DNA/RNA profile to subtype diagnostically challenging USMTs.

Reflection/Lessons Learned:

In general, completing a CFI project forced me to take complete ownership of a project for the first time. I appreciated the process of creating my own research question and figuring out how to approach the investigating this question. Additionally, I learned how to interpret genomic data and how to write a manuscript. I learned how to understand the current level of research on a specific topic and how to use that to inform my research question and the writing of conclusions. I learned a lot about molecular pathology and the relationship between histologic pattern and the molecular landscape of a given tumor. This project made me more excited for my future in pathology and academic medicine. It definitely helped define my career goals and served as a great topic for residency interviews!