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Detection of Human *Cytomegalovirus* by Clinical Metagenomics in a Non-Healing Wound of Recurrent Malignant Tenosynovial Giant Cell Tumor

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Abstract

Clinical metagenomic next-generation sequencing is an emerging approach for identifying pathogens of various infectious diseases. We present a case with a malignant tenosynovial giant cell tumor and chronic non-healing wound. Metagenomic next-generation sequencing of wound tissue biopsy identified *Cytomegalovirus*. Tumor recurrence over the previous non-healing wound was found three months later. The literature regarding CMV infection and tumorigenesis, the pathogenesis of malignant tenosynovial giant cell tumor, and the application of metagenomics in oncology are also reviewed herein.

Keywords: *Cytomegalovirus* infections; Metagenomics; High-throughput nucleotide sequencing; Giant cell tumor of tendon sheath

Introduction

The application of metagenomics has been increasing in infectious diseases for microorganisms' detection [1]. Metagenomic next-generation sequencing not only detects bacteria but also viruses, *Mycobacterium* and *Fungi* [2]. Moreover, applying metagenomic next-generation sequencing in tumors with viral content can be used for simultaneous pathogen detection [3-5].

A growing number of studies have applied this method for the detection of viral nucleic acid in tumors and have confirmed virus-tumor associations. It is estimated that viruses could be associated to carcinogenesis in 15% to 20% cancer cases [6]. The discovery of virus-tumor associations and the role of virus infection in human carcinogenesis may potentially lead to the discovery of viruses as a therapeutic target in various types of virus-positive cancers [3].

In this study, we present a case with a malignant tenosynovial giant cell tumor who had a postoperative non-healing wound following tumor resection. We used metagenomics analysis to identify the possible role of microbes in the non-healing wound.

Materials and Methods

A brief study flow was illustrated in Figure 1. We firstly grounded the tissue specimen and transfer it into a 2 ml tube which containing 1 g of 0.5 mm diameter glass beads and 600 u/L ddH_2O. Then the tube was placed on the FastPrep-24 5G instrument (MP Biomedicals, USA) for beating at the speed of 10 m/s for 30 min. TIANamp Micro DNA Kit (Cat No. DP316, Tiangen Biotech, Beijing, China), as per the manufacturer's instructions, was used for DNA extraction in 400 μ/L

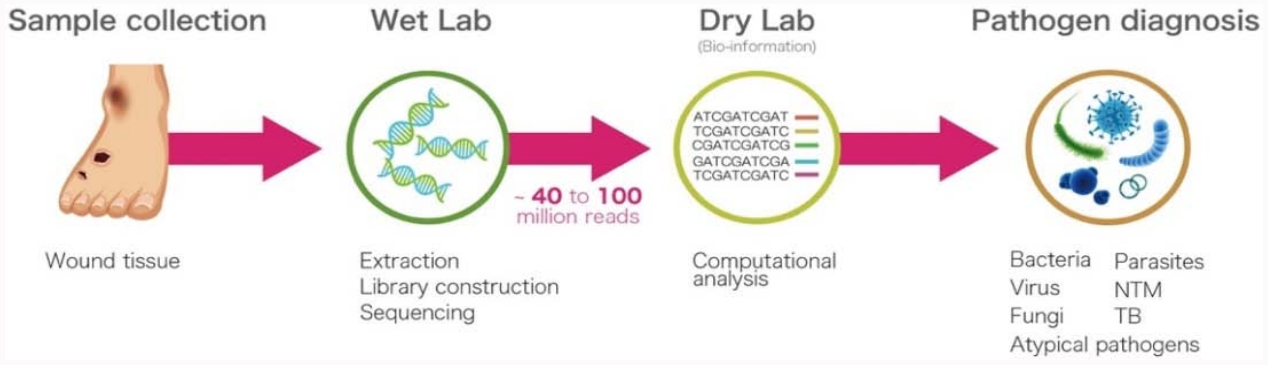

Figure 1: Tissue specimens were collected, subjected to DNA extraction, constructed by DNA libraries sequences, sequenced and aligned to a microbial reference database.

of each pretreated sample. The total genomic DNA was quantified by Qubit dsDNA HS Assay Kit (Thermo Scientific) and ~100 ng of the gDNA was used for the library construction based of MGIEasy Cell-free DNA Library Prep Kit (MGI, Shezhen, China) through the steps of DNA-fragmentation, end-repair, add A-tailing, adapter-ligation and PCR amplification. In brief, DNA fragmentation is based on enzymatic method by using the fragmentation enzyme provided by the kit under 32°C for 8 min to generate DNA fragments around 150 bp to 250 bp in size. Then, DNA purification beads in a ratio of 1.8x was used to purify the fragmented products followed by 2 times of 80% ethanol washing step and eluted in 45 u/L EB buffer. Purified DNA fragments were end-repaired and added A-tail carrying on in a PCR machine under the conditions: 37°C for 10 min followed by 65°C for 15 min. Adaptors/barcodes were ligated onto the DNA fragment from the end-repair and A-tailing step at 23°C for 20 min and followed by the beads-purification in a ratio of 0.5x. Next, the purified product was amplified under the PCR condition: 98°C for 2 min; 12 cycles of: 98°C for 15 sec, 56°C for 15 sec, 72°C for 30 sec; 72°C for 5 min and hold at 4°C, to generate the sequencing library. The library quality was assessed on the Qubit 4.0 Fluorometer (Thermo Scientific). Then, DNA library was denatured at 95°C for 6 min to become single-stranded DNA and circularized by ligase under the condition of 37°C for 30 min. Single-strand circularized DNA library was then transformed to DNA Nanoballs (DNBs) by the DNB polymerase I provided by the DNBSEQ-G400RS FCL SE50 sequencing kit (MGI, Shezhen, China) at 30°C for 25 min and sequenced by MGISEQ-2000 platform with the DNBSEQ-G400RS sequencing flow cell. High-quality sequencing data were generated by removing adapter contamination, discarding short (<35 bp), low-quality (Q5 ≤ 0.7 & N ≥ 10), and low-complexity reads. Next, a filtering of human sequences was performed by mapping to the human reference genome hg38 (GRCh38, December 2017) using the Burrows-Wheeler Aligner (match sites >45 nt and mismatch sites ≤ 2 nt was defined as the mapping quality cut-off) [7]. The remaining data were aligned to the Microbial Genome Database using Burrows-Wheeler Alignment tool (v0.7.10-r789). All organisms identified in one sample must be sorted according to the coverage rate of each organism. For further analysis, unique reads were defined as reads whose alignment length was higher than 80% and identity with reference sequence higher than 95% by samtools (v1.10). Specific reads were defined as reads that doesn't map to other microorganism with a same or a higher mapping score.

Results

Case presentation

A Seventy-Seven-year-old woman was admitted to this hospital due to a painless, slowly progressive soft tissue mass over her right elbow (Figure 2A). She first noticed the lesion 5 years before this hospitalization. She underwent en-bloc resection and reconstruction with a left anterolateral musculocutaneous free flap. The pathology report demonstrated malignant tenosynovial giant cell tumor with infiltration to adjacent muscle tissue. One month after the operation, the patient was treated with intensity-modulated radiation therapy.

Eighteen months after the operation, a fungated mass measuring 3 cm recurred over the right elbow (Figure 2B). The patient underwent wide excision and free flap reconstruction. The tissue was proven to be a malignant tenosynovial giant cell tumor. Poor healing of the surgical wound developed two weeks after the operation. The patient underwent surgical debridement on the second week and third week after wide excision and free flap reconstruction. The Kirschner wires were used for delayed primary closure of the surgical wounds (Figure 2C). Given the absence of an etiological diagnosis of the non-healing surgical wound, the metagenomic next-generation sequencing was used to detect the causative pathogen.

While planning for adjuvant radiation therapy at the outpatient department on the third month following the wide excision of the tumor, MRI study revealed a 5.2 cm enhanced mass infiltrating the distal triceps muscle at the olecranon fossa and distal upper arm. The patient underwent tumor excision for the third time, and the pathology finding revealed recurrence of a malignant tenosynovial giant cell tumor.

Metagenomic next-generation sequencing

The gDNA extracted from the wound tissue was subject to metagenomic shotgun Next-Generation Sequencing (mNGS) analysis. The results of metagenomic suggested that Serratia marcescens, *Staphylococcus aureus* and HCMV were detected from the surgical wound. To further confirm the reliability of the analysis, the reads of CMV were aligned with the CMV genome. This result indicates that the read was distributed across the entire CMV genome, and were not due to false amplification from a single viral DNA neither fragments nor alignment artifacts caused by repetitive.

Discussion

We presented a case with recurrent malignant tenosynovial giant cell tumor. Poor wound healing developed after tumor resection.

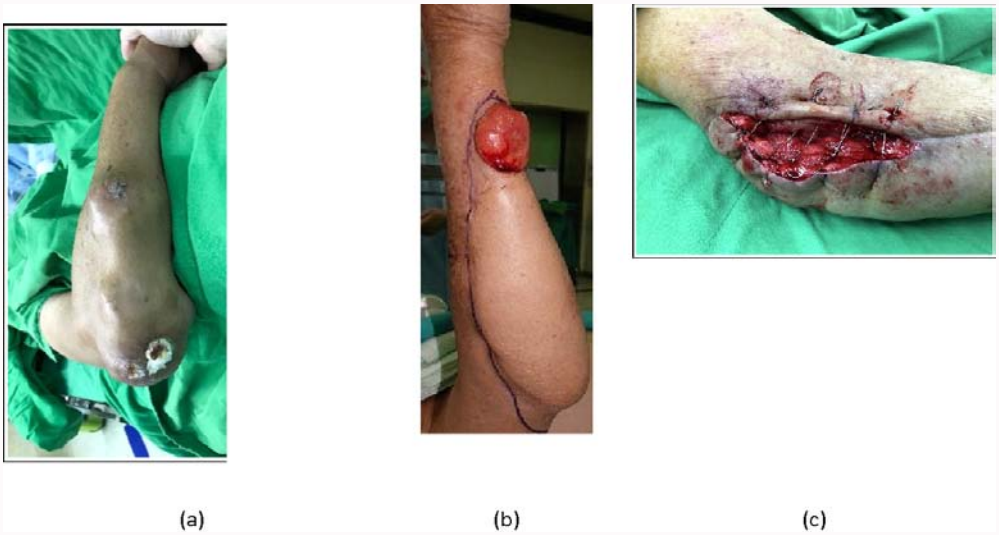

Figure 2: (a) The initial presentation of soft tissue mass over the right elbow of the patient; (b) A fungated mass measuring around 3 cm recurred over the right elbow; (c) Poor healing of the surgical wound three week after the wide excision and free flap reconstruction. The Kirschner wires were used for delayed primary closure of the surgical wounds.

Metagenomic sequencing identified the presence of CMV. MRI examination 3 months after the second tumor resection disclosed tumor recurrence.

Metagenomics shotgun sequences all of the nucleic acids present in a specimen. As for its application in oncology, sequencing of tumor tissue has been investigated for simultaneous microorganisms' detection. For instance, high-throughput genomics of Merkel cell carcinoma led to the discovery of the Merkel cell polyomavirus and mapping of viral genomic integration, which is now believed to be the cause of Merkel cell carcinoma [3]. In our case of recurrent malignant tenosynovial giant cell, using of metagenomics, we identified CMV in the non-healing surgical wound at the site of tumor recurrence four months later, which caught our attention and led to further to exploration of the relationships among malignant giant cell tumor, non-healing surgical wound, and CMV.

Once human infected with CMV, CMV establishes latent infection in CD34+ myeloid mononuclear cells. CMV later moves out from bone marrow to different tissue as macrophages. These macrophages carry reactivated virus and can infect other cell types, including fibroblasts, endothelial cells, smooth muscle cells, endothelial cells, and epithelial cells, and can be disseminated to organs [8-10].

Accumulating evidence suggests a link between CMV infection and cancer. Several studies have identified HCMV in tumors including colon, prostate cancer, breast, salivary gland tumors, hepatocellular cancer, rhabdomyosarcoma, neuroblastoma, and brain tumors (Medulloblastoma and Glioblastoma (GBM)) [11-17]. There is robust evidence that CMV exerts its oncomodulatory effect on tumor cells, and modulates the malignant properties. By interfering cellular mechanism of cell apoptosis, proliferation, metastasis, and angiogenesis, CMV makes tumor cells to be more malignant [18]. Other clues of the association between CMV infection and cancer are listed below. Firstly, the level of HCMV infection has a prognostic value, as proved in GBM [19]. Secondly, studies demonstrate that in HCMV positive cancer patients, using antiviral therapy improved prognosis [11,17]. A retrospective survival analysis of patients with secondary glioblastoma who were treated with valganciclovir demonstrated a potentially positive effect [20]. A randomized trial to explore the efficacy of antiviral treatment as an add-on to the standard therapy in glioblastoma patients is undergoing [21].

Malignant tenosynovial giant cell tumors are uncommon, with fewer than 50 cases reported in previous studies [22]. The etiology of malignant tenosynovial giant cell tumor is unclear. Translocation of the Colony-Stimulating Factor 1 (CSF-1) gene was discovered in tenosynovial giant cell tumors. The CSF-1 gene encodes CSF-1, and recruits and induces the proliferation of non-neoplastic CSF1 receptor-expressing cells of the monocyte-macrophage lineage [23]. The natural history of malignant tenosynovial giant cell tumor is aggressive with a high potential for metastasis to regional lymph nodes and distant locations [24]. Previous study has displayed that *Cytomegalovirus* in a tissue sample of peripheral giant cell granuloma by using real-time Polymerase Chain Reaction (PCR) and concluded CMV has the potential to induce multi-nucleated giant cells [25]. While peripheral giant cell granuloma is a reactive proliferation caused by chronic irritation of the gingival mucosa [26] and in consideration of pathophysiology, we consider peripheral giant cell granuloma is different from tenosynovial giant cell tumor. Nonetheless, this study gave us clue that *Cytomegalovirus* can impair the functions of polymorphonuclear leukocytes.

As mentioned above, CMV resides in macrophages, and macrophages carrying reactivated virus can infect other cell types, including fibroblasts and smooth muscle cells. We proposed that if CMV attribute to the pathogenesis of the malignant tenosynovial giant cell tumor, HCMV might be found in the macrophages of malignant tenosynovial giant cell tumor. Unfortunately, we were not able to identify HCMV by immunohistochemistry staining or *in situ* hybridization in the tissue specimens to complement the finding of metagenomics sequences and to identify which cell types that are infected with this virus. Despite immunohistochemistry is a common method to search for HCMV in tissue specimens, false negative results can occur due to several reasons, such as focal distribution of the virus, poor tissue fixation, antibodies not properly optimized, epitope retrieval method not optimized for individual antibodies, or degradation of the molecule [27-29].

Another explanation of our finding is CMV reactivated during radiotherapy, as demonstrated by previous study [30], which could be explained by radiotherapy induced a stress response and activates Cytomegalovirus-Immediate Early (CMV-IE) promoter [31].

In conclusion, we report the identification of CMV by metagenomic sequencing in a non-healing surgical wound of recurrent malignant tenosynovial giant cell tumor. Further studies are needed to investigate the association of CMV infection and malignant tenosynovial giant cell tumors.

Author Contributions

Conceptualization: Yang WT, Tseng CH and Liu PY; Data curation: Yang WT, Tseng CH and Lai KL; Formal analysis: Yang WT, Chiang I, Huang YT and Liu PY; Funding acquisition: Mao YC, Lai KL, Huang YT and Liu PY; Investigation: Lai CS and Mao YC; Methodology: Lai CS and Lai KL; Project administration: Mao YC; Resources: Mao YC; Validation: Tseng CH; Writing-original draft: Yang WT, Huang YT and Liu PY; Writing-review & editing: Yang WT, Huang YT and Liu PY.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (CE20004B).

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