From Eye to Insight

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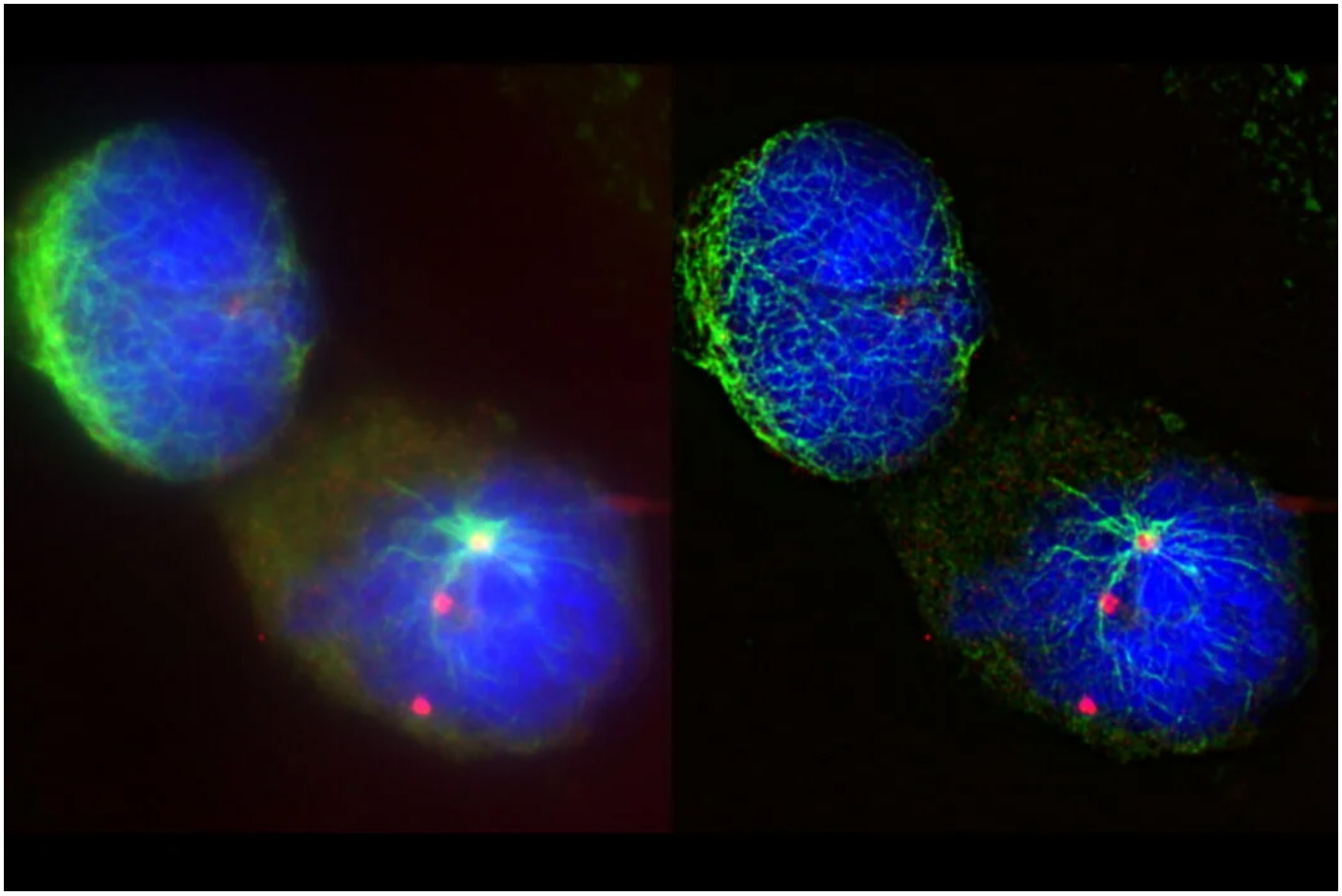
VISUALIZING THE MITOTIC SPINDLE IN CANCER CELLS

High-Contrast Imaging of Ewing Sarcoma Cells



Authors

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Abstract

This article demonstrates how this research is aided by visualizing more details of mitotic spindles in Ewing Sarcoma cells using the THUNDER Imager Tissue and Large Volume Computational Clearing (LVCC). Techniques like live-cell imaging are crucial for cancer research in terms of understanding progression and metastasis. The mitotic spindle is composed of hollow microtubules in eukaryotic cells. It plays a large role during mitosis in separating duplicated chromosomes within a cell and building the cytoskeletal structure of divided cells. In cancer cells like sarcomas the triggers of mitotic catastrophe are identified by examining dysfunctions of the mitotic spindle.

Introduction

Fluorescence microscopy allows the study of changes occurring in tissue and cells during cancer development and progression. Techniques like live-cell imaging are important for a better understanding of cancer progression and metastasis.

In eukaryotic cells, the mitotic spindle, composed of hollow microtubules, is instrumental to building the cytoskeletal structure of a duplicate cell and separating duplicated chromosomes from the original cell during mitosis. In cancerous cells, such as sarcomas, the triggers of mitotic catastrophe are identified by examining dysfunctions of the mitotic spindle [1].

Sarcomas are a family of cancers that develop in connective tissues such as the muscle or bone. Ewing's sarcoma and rhabdomyosarcoma, which develop in bone and muscle respectively, are pediatric cancers with a tendency to occur adjacent to areas of active bone growth. In addition to surgery and chemotherapy, ionizing radiation is used to treat these cancers, but this may result in permanent damage to growing bone, including asymmetric growth arrest, angular deformity, and increased susceptibility to fracture. The severity of bone injury is largely proportional to the dose of radiation that the bone receives. So, it is reasoned that strategies which selectively sensitize tumor tissue to radiation could lower the dose of radiation needed to achieve local control and minimize collateral injury of adjacent healthy tissue.

The hypothesis that pretreatment with mRNA synthesis inhibitor Mithramycin A could selectively radiosensitize EWS:Fli1+ tumor cells by altering the transcriptional response to radiation injury was tested by in vitro work and in murine xenograft model systems [2]. The results showed that Mithramycin A can significantly radiosensitize EWS:Fli1+ cells in vitro and in vivo by inhibiting transcription of genes involved in DNA damage repair, leading to tumor cell death by apoptosis [2].

Revealing more details of mitotic spindles in Sarcoma cells using the THUNDER Imager Tissue and Large Volume Computational Clearing (LVCC) can help cancer researchers gain useful insights.

Challenges

When imaging mitotic spindles, a solution that can quickly image them and achieve sharp, high-contrast 3D imaging, where important details are clearly resolved, is most practical. Conventional widefield microscopy is fast and offers detection sensitivity, but unfortunately images of thick specimens often show an out-of-focus blur or haze which reduces the contrast [3]. Unravelling the role of mitotic instability in a complex disease like cancer requires correlation of multiple biological markers in the same sample.

Methods

Ewing Sarcoma cells (SK-ES-1) were used for the study. The cells were stained for α-tubulin (Clone YL1/2 Thermo-Fisher Scientific # MA1-80017, 1:500 dilution/ Dylight 488 conjugated Donkey anti-rat Thermo-Fisher Scientific #SA5-10026), γ-tubulin (Clone TU-30, AbCam #ab27074, 1:200/Dylight 550 conjugated Donkey anti-mouse Thermo-Fisher Scientific #SA5-10167), and DNA (Hoechst 33342 blue). After staining, slides were cover slipped using ProLong Glass Antifade media (Thermo-Fisher Scientific #P36981) and imaged with a THUNDER Imager Tissue using a 63×/1.4 NA (numerical aperture) oil immersion objective. Large volume computational clearing (LVCC) [3] was applied to the image data set and a maximum intensity projection was generated.

Results

The α-tubulin (green) forms the mitotic spindles to which chromatids (blue) attach during mitosis, while γ-tubulin (red) localizes to the spindle poles in dividing cells. The THUNDER technology enables visualization of more details of mitotic spindles in Sarcoma cells. The sharp images reveal clear structures which can easily be segmented and used for further analysis.

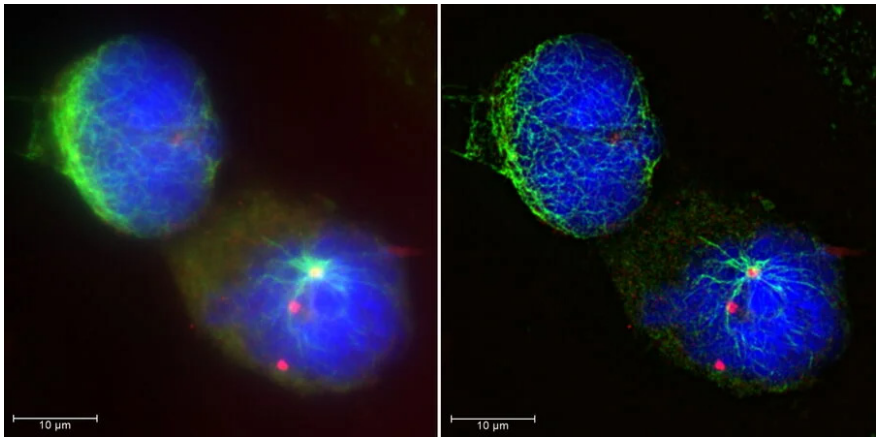

Fig. 1: Maximum intensity projection of Ewing Sarcoma cells (SK-ES-1) stained for α-tubulin (green), γ-tubulin (red) and DNA (blue): raw image data (left) and THUNDER image with LVCC (right).

Conclusions

The THUNDER technology Large Volume Computational Clearing (LVCC) [3] significantly enhances the contrast when imaging mitotic spindles in Ewing Sarcoma cells. More details of the mitotic spindles in the cells were resolved when compared to conventional widefield imaging.

References

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