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International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Comparative Study of Spindle Assembly for Cancer Therapy

General information

Call	2024
Reference	2024-14-TRAN
Keyword(s)	Spindle; Mitosis; Kinetochore; Cancer; Chromosome segregation

Director(s) and team

Thesis director(s)	Phong Tran
Research team	Cytoskeletal Architecture and Cell Morphogenesis
Research department	UMR 144 – Cell Biology and Cancer

Description of the PhD thesis project

Abstract

Cancer is a multifaceted disease, but one cause is chromosome segregation errors resulting in aneuploidy, where daughter cells have abnormal number of chromosomes. Proper chromosome segregation requires two interacting processes: 1) the assembly of kinetochores on chromosomes – structures that allow spindle microtubule attachment and subsequent separation of sister chromatids, and 2) the assembly of the mitotic spindle – a microtubule-based structure organized by two centrosomes and other microtubule-associated proteins. A normal human cell has one kinetochore per chromosome, or "monocentric", and has an oval-shaped spindle. In contrast, a silkworm cell has multiple kinetochores per chromosome, or "holocentric", and has a square-shaped spindle. Interestingly, some cancer cells have multiple centrosomes, or "supernumerary" centrosomes, and/or di- or tricentric chromosomes. In this context, some cancer cells have chromosome and spindle features that resemble a silkworm cell. We hypothesize that understanding how the silkworm organizes its chromosome and spindle may help us understand how a cancer cell organizes its chromosome and spindle. Furthermore, treatments which kill silkworm cells, but not normal human cells, may selectively kill some cancer cells. In proof-of-concept experiments, we have shown that depletion of the kinesin-14 motor and/or the microtubule-nucleator Augmin, selectively killed silkworm cells and ovarian cancer cells, but importantly not normal human cells. This project aims at revealing targets that would kill silkworm cells and cancer cells, but not normal human cells.

Background & Objectives

We have developed silkworm cells as a model system to study cancer (Vanpoperinghe et al, 2021). Some cancer cells have been reported to be di- or tricentric, a phenomenon where there are more than one kinetochore per chromosome; also some cancer cells have supernumerary centrosomes, a phenomenon where there are more than one-pair of centrosomes per cell (Fig. 1C). In this context, these cancer cells may be very similar to the silkworm cells, which naturally have multiple kinetochores per chromosome (Fig. 1B), and a square-shaped spindle suggesting multiple centrosomes (Fig. 1A, 1C). There currently are ~200 genes

whose protein products control kinetochore and spindle assembly in human cells. These genes are conserved in evolution, and therefore also exist in the silkworm genome. Our starting hypothesis, based on the differences in kinetochore number and spindle shape between normal human and silkworm, was that some of these genes may act slightly differently depending on the type of cells. In preliminary experiments, we preformed RNA-interference on ~20 genes. Many of these showed similar phenotypes. For example, depletion of the motor kinesin-5, which have been shown to be essential for spindle formation, results in monopolar spindle and cell death in both normal human and silkworm cells (Fig. 1A). This indicates that kinesin-5 is essential for both normal human and silkworm cells. Interestingly, we found the depletion of two genes – kinesin-14, a motor involved in focusing the spindle poles, and Augmin, a protein complex involved in spindle microtubule amplification – killed silkworm cells but not human cells (Fig. 1A). The results indicate that kinesin-14 and Augmin are essential for silkworm, but not essential for normal human cells. Our next hypothesis is that what kills silkworm cells, but not normal human cells, may kill certain cancer cells. We thus preformed RNAi of kinesin-14 and Augmin in ovarian cancer cells. Ovarian OVCAR8 cancer cells are known to have supernumerary centrosomes. The results indicate that depletion of kinesin-14 and/or Augmin selectively kill OVCAR8 cancer cells (Fig. 1D). Thus, kinesin-14 and Augmin are good targets for ovarian cancer therapy. This is exciting! The next phase of the work will be to test the remaining ~180 genes!

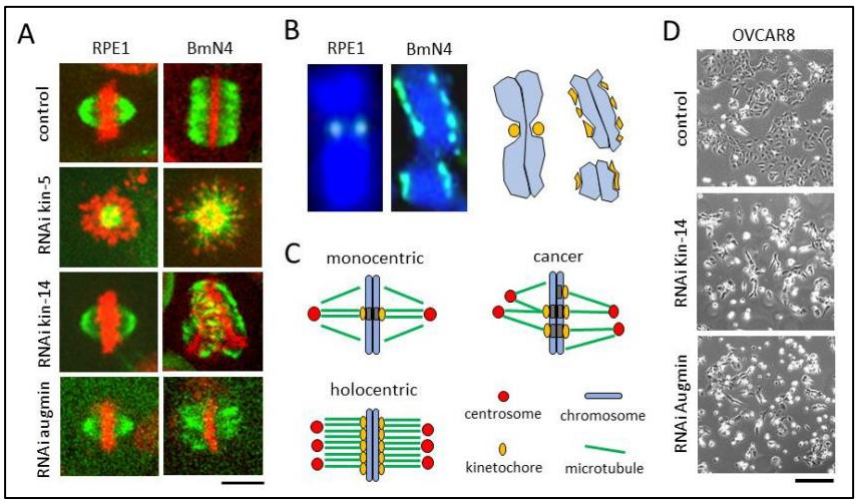

Figure 1. Silkworm *B. mori* spindle assembly and chromosome segregation provides insight into cancer cell proliferation.

(A) Metaphase spindle phenotypes of human RPE1 and silkworm BmN4 cells RNAi-mediated depletion of kinesin-5, kinesin-14, and Augmin. Note that the normal control metaphase spindle of RPE1 is oval- shaped and of BmN4 is square-shaped. Note that depletion of kinesin-5 results in monopolar spindles in both cells; but depletion of kinesin-14 and Augmin affected only the spindles of BmN4 cells, resulting in cell death. Scale bar, 10µm.

(B) Images of monocentric (RPE1) and holocentric (BmN4) mitotic chromatids (DAPI = blue) and kinetochores (CENP- T = green). Note that RPE1 cells have 1-pair of kinetochores per 1-pair of sister chromatids. In contrast, BmN4 cells have multiple kinetochores per chromatid. Cartoon shows chromosomes (blue) and kinetochores (yellow).

(C) Cartoon model of normal monocentric, normal holocentric, and di- or tricentric and/or supernumerary cancer cell metaphase spindles. We note that the *B. mori* chromosome and spindle architecture are reminiscent of cancer cells with are di- or tricentric, and cancer cells which have supernumerary centrosomes.

(D) Phase contrast images of OVCAR8 cancer cells treated for 48h with RNAi Control, RNAi Kinesin-14, and RNAi Augmin. Note that Control cells are confluent, with single layer of cells adhering to the surface. In contrast, RNAi Kinesin-14 and RNAi Augmin show much less adherent cells and much more round floating cell debris indicative of cell death. Scale bar, 100µm.

For the PhD training objective, the candidate will perform experiments to: 1) Identify genes essential for spindle assembly and chromosome segregation in insect cells, 2) Test these genes for potential cancer therapy, and 3) Understand mechanisms of how the genes affect normal and cancer cells.

Experimental approaches
The PhD candidate will learn : 1) cell culture techniques to maintain insect and human cell lines; 2) bioinformatics to reveal conserved genes involve in spindle assembly and chromosome segregation in the two species; 3) molecular biology techniques such as RNAi-mediated protein depletion, immunofluorescent localization of proteins in cells, and GFP-tagging; 4) confocal live-cell imaging to visualize spindle and chromosome dynamics; and 5) image analysis to quantify chromosome segregation errors.

International, interdisciplinary & intersectoral aspects of the project

There is a potential for international collaboration. While the cancer relevancy of this project is exciting, we currently do not have a mechanistic understanding of how diverse cell types organize their diverse spindle shapes and kinetochore number for proper chromosome segregation. This knowledge would help predict important interactions, and therefore important gene functions, in spindle assembly and chromosome segregation. Here, mathematical modelling and computer simulation will help. We are currently collaborating with Dr. Raja Paul of the Indian Association for the Cultivation of Sciences (IACS), who is a mathematical physicist. His team has developed mathematical models and computer simulations to arrive at a physical mechanism of how oval-shaped bipolar spindles such as found in monocentric cells are formed (Chatterjee et al, 2020). The next phase of the work will be to extend the potential interactions to include multiple centrosomes and multiple kinetochores, and through computer simulation, predicts interactions that would realistically lead to square-shaped spindles such as found in holocentric cells, or “weird” spindles which may be found in cancer cells.

Recent publications

1. Jain I, Rao M, **Tran PT** (2023). Reliable and robust control of nucleus centering is contingent on nonequilibrium force patterns. *iScience* 26(5):106665.

2. Lera-Ramirez M, Nedelec FJ, **Tran PT** (2022). Microtubule rescue at midzone edges promotes overlap stability and prevents spindle collapse during anaphase B. *Elife* 11:e72630.

3. Krüger LK, Gélin M, Ji L, Kikuti C, Houdusse A, Théry M, Blanchoin L, **Tran PT** (2021). Kinesin-6 Klp9 orchestrates spindle elongation by regulating microtubule sliding and growth. *Elife* 10:e67489.

4. Loncar A, Rincon SA, Lera Ramirez M, Paoletti A, **Tran PT** (2020). Kinesin-14 family proteins and microtubule dynamics define S. pombe mitotic and meiotic spindle assembly, and elongation. *J Cell Sci* 133(11):jcs240234.

5. Krüger LK, Sanchez JL, Paoletti A, **Tran PT** (2019). Kinesin-6 regulates cell-size-dependent spindle elongation velocity to keep mitosis duration constant in fission yeast. *Elife* 8:e42182.

Expected profile of the candidate

- 3i – intelligence, informative, integrity
- Strong research experience
- Strong academic record
- Strong interest for project
- Strong work ethics