Health & Medicine | Dr Lukasz Kozubowski

**Combating drug resistance:**

From yeasts to cancer

The fungus Cryptococcus neoformans is a leading cause of death in HIV/AIDS patients and rapidly develops resistance to standard antifungal treatments. Dr Lukasz Kozubowski, of Clemson University in South Carolina, USA, believes this resistance stems from the organism’s method of reproduction, which is prone to changes in genetic composition when under stress. His research may revolutionise treatments for Cryptococcus and could help us understand how cancer cells develop similar mechanisms of drug resistance.

You probably come into contact with Cryptococcus neoformans on a daily basis without even realising it. This tiny fungus is found in soils throughout the world, and its spores can be present in the air we breathe. In healthy people, although it can cause respiratory infections, Cryptococcus rarely causes serious illness. In the immunocompromised, however, the fungus can not only lead to a serious pulmonary infection but also spread from the lungs to colonise other parts of the body, triggering life-threatening diseases.

If it spreads to the brain, for instance, Cryptococcus can cause a form of meningitis, which has become one of the leading causes of death among HIV/AIDS patients. Worldwide, over 200,000 cases of cryptococcal meningitis occur each year, more than 80% of which are fatal. In developing countries where HIV/AIDS treatment lags behind the rest of the world, such as in much of sub-Saharan Africa, cryptococcal meningitis now kills more HIV/AIDS patients than tuberculosis. Even in developed countries like the USA, Cryptococcus species cause occasional meningitis outbreaks.

**IT’S A YEAST, JIM, BUT NOT AS WE KNOW IT**

Cryptococcus neoformans usually takes the form of a yeast (a single-celled fungus), but studies have shown it is only distantly related to commonly known yeasts, such as the familiar and well-studied brewer’s yeast (Saccharomyces cerevisiae). In fact, while Saccharomyces and other well-researched yeasts belong to a group of fungi known as the ascomycetes, Cryptococcus is ascribed to a completely different group, the basidiomycetes, which diverged from the ascomycetes as long as 400 million years ago. Dr Kozubowski hopes that studying Cryptococcus neoformans will shed light on the biology of this little-known group.

As he observes, “The biology of C. neoformans appears different from what we know about yeast so far.” In fact, he believes Cryptococcus may have evolved unique characteristics, such as modified pathways of cell division, which help it to survive within the human body.

One key focus of Dr Kozubowski’s work is how Cryptococcus cells reproduce by dividing in two, a process known as budding. The mechanism of budding in Cryptococcus appears to be distinctly different from Saccharomyces and other ascomycetes yeasts and, in fact, in some respects more similar to cell division in multicellular animals like ourselves. Dr Kozubowski believes that this fact may underpin crucial features of the pathogen, such as how it develops resistance to commonly used antifungal drugs. The model of how drug resistance may develop that is emerging for Cryptococcus also shows striking parallels to the development of drug resistance in certain cancers.

**ONE BECOMES TWO**

Like animals and plants, fungi, including Cryptococcus, are eukaryotes (organisms in which the genetic material is contained in a membrane-bound nucleus). In most eukaryotes, growth occurs by the division of single cells into two daughter cells. The process of cell division involves the duplication of the genetic material, held on DNA-based structures known as ‘chromosomes,’ followed by partition of the mother cell into two daughters, each containing a full copy of the genes of the organism. This final step, generating two separate daughter cells, is known as ‘cytokinesis.’ The process needs to be precisely coordinated and strictly regulated to ensure that each daughter cell gets a full set of chromosomes providing a complete set of genes to ensure a fully-functioning cell.

Mechanisms of cytokinesis vary between organisms. In fungi and animals, a ring of proteins known as an ‘actomyosin ring’ develops around the division plane in the original cell, then contracts to allow final partition of the cell content into two. Uniquely in fungal cells, contraction of the actomyosin ring is accompanied by building a septum (wall) between what will become the daughter cells, which eventually splits in two as they separate. However, this model – and most of what we know about fungal cytokinesis – comes mostly from studies of ascomycetes such as Saccharomyces and another classic model yeast Schizosaccharomyces pombe. Dr Kozubowski believes that Cryptococcus and other basidiomycetes may display significant differences in the processes of cytokinesis. Understanding these variations may be important in building a fuller picture of the evolution and mechanisms of this essential element of cell division across eukaryotes as a whole.

In particular, Dr Kozubowski’s lab are focusing on questions such as: how do key proteins involved in cytokinesis allow growth and cell division in Cryptococcus under stressful conditions such as the high temperature of the human body, or treatment with antifungal drugs? And how is cell division, and in particular chromosome segregation, affected by such drugs?

In collaboration with a graduate student Vikas Yadav, and his mentor Dr Kaustuv Sanjay, Dr Kozubowski, at a time a postdoctoral fellow in Dr Joseph Heitman’s laboratory, initiated research that focused on an enigmatic structure called the ‘kinetochore’, a complex of proteins associated with each chromosome, which attaches to a cellular structure called the spindle, ensuring that the chromosomes are correctly distributed between the daughter cells during cell division. Errors in the interaction between the kinetochore and the spindle can cause unequal chromosome distribution and are a key feature of certain types of cancer cell.

Using high-resolution time-lapse microscopy, Dr Kozubowski and his collaborators have been able to localise the proteins making up the kinetochore during cell division in Cryptococcus. They have found that the kinetochore assembles in an ordered manner and that the position of the kinetochores – and therefore the chromosomes – during cell division in Cryptococcus is very different from the location found in the ascomycete fungi and in fact more similar to that seen in animals.

Cryptococcus may have evolved unique characteristics, such as pathways of cell division, which help it to survive within the human body.
Inhibition of growth of the daughter cell may affect all chromosomes and the genes held upon them — is that some daughter cells by chance inherit an extra copy of genes conferring properties of resistance to fluconazole. In the presence of fluconazole, these resistant Cryptococcus cells are able to survive and reproduce whilst other cells are inhibited. This leads to an overall increase in the proportion of resistant cells in the population, ultimately resulting in drug-resistant cases of cryptococcal meningitis. Drug resistance is now one of the major challenges in fighting infections such as cryptococcal meningitis. Dr Kozubowski’s work on Cryptococcus highlights this new basidiomycete ‘model organism’, which in addition to the ascomycete yeasts can provide insights into cellular processes in eukaryotes, how fungal pathogens adapt, and can point at potential new targets for drug therapy. This also offers the intriguing possibility of applying our knowledge of Cryptococcus to better understand how cancer cells respond to stress and develop drug resistance. The tools that could be developed from a growing understanding of this human pathogen may have roles far beyond the immediate disease that it causes.

Of critical importance is the link between kinetochore-mediated chromosome segregation and the development of resistance to common antifungal drugs. Antifungal drugs, such as the ‘azoles’, which inhibit fungal growth and reproduction, are commonly used against Cryptococcus, has been associated with the growth of cell populations with abnormal chromosome numbers in C. neoformans. Dr Kozubowski’s studies have now uncovered the mechanisms behind this effect which include defects in cytokinesis and governing cytokinesis in C. neoformans.

Dr Kozubowski’s research team focuses on the mechanisms of cell division in Cryptococcus neoformans and how those mechanisms support survival of this pathogen in the environment of the human host.

References

How do you see your research into Cryptococcus being translated into actual medical advances for the treatment of fungal infections and even cancer?

Our goal is two-fold: in addition to a conceptual understanding of cytokinesis in fungal pathogens, defining the mechanisms of cell division in fungi would enable the development of novel therapeutic approaches to fight the increasing incidence of human fungal infections. Like fungal cells that must cope with stressful environment in the human host, cancer cells experience genomic instability, which in turn promotes uncontrolled proliferation within normal tissue. Potentially similar strategies allow fungal and cancer cells to escape drug therapies, and so we hope our studies may also facilitate better anticancer treatments.