Letter to the Editor

The immortal amoeba: a useful model to study cellular differentiation processes?

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Our inability to fully understand the process of differentiation or de-differentiation in real time, in the context of genes, proteins, and the environment has hindered diagnostic and therapeutic advances. For example, the ability of cancer stem cells to remain dormant or self-renewal and differentiation into various types of cancer cells,[1] or the ability of *Plasmodium* parasite to switch its phenotype from sporozoite to merozoite to target red blood cells, or the ability of cyst-forming parasites such as *Entamoeba* or *Giardia* to form cyst or re-emerge as active trophozoite has been puzzling scientists for centuries. In this regard, a whole organism approach is seen as relevant to understand the behavior and to study the molecular mechanisms in response to noxious stimuli and the environment. Although vertebrates are physiologically relevant, here we propose that the use of evolutionarily lower organisms such as *Acanthamoeba*, as they exhibit cellular differentiation and de-differentiation properties, offer advantages in studying the "how and why" of transition of a normal cell into a dormant cell, at least at the early stage of research discovery.

The ability of free-living amoebae such as *Acanthamoeba*, *Balamuthia,* and *Naegleria* to breed at a constant rate under favourable conditions, and differentiate into a dormant form under unfavorable conditions and *vice versa*, without "dying" is intriguing, yet incompletely understood. For example, the life cycle of *Acanthamoeba*[2,3] or *Balamuthia*[4,5] consists of at least two stages: a trophozoite stage, and a cyst stage. During the trophozoite stage, both amoebae are metabolically active and divide mitotically. During the cyst stage, they remain dormant with minimal metabolic activity. Upon returning to favorable conditions, the trophozoite emerges from the cyst, a process known as excystment and continues with its life cycle.[4] What is more puzzling are the properties of another protist, known as *Naegleria*. Like the other free-living amoebae such as *Acanthamoeba*, it has the ability to exhibit amoeboid (crawling)-like movement, during which it actively feeds and breeds mitotically.[6,7] Upon nutrient deprivation, it can differentiate into either (i) a flagellate form (non-feeding and non-replicating form), during which the amoeba can swim long distance in search of food, or (ii) a dormant cyst form (non-feeding and non-replicating form) during which there is little metabolic activity. The flagellate form is transient and lasts only for a few hours. The discovery of food reverses the flagellate form into the amoeboid form, but the unavailability of food for more than a few hours results in differentiation of the flagellate form into the long-lasting cyst form. The return of favorable conditions encourages cyst de-differentiation into the trophozoite form. The versatile nature of *Acanthamoeba*, *Balamuthia*, and *Naegleria* to increase their progeny during auspicious conditions, and endure callous conditions by switching their life form into morphological distinct phenotypes is a remarkable property. This property has been studied in the context of targeting cyst-forming parasites. For example, transformation of cyst-forming protozoa from so-called "living" (metabolically-active) organisms to "dead" (minimal metabolic activity) has been the subject of study.[8,9] This property has impeded successful treatment of many parasitic infections. This is due to the fact that the majority of available drugs are targeted against the functional aspect of parasites (e.g. protein synthesis, DNA/RNA synthesis, cell wall synthesis etc.), as it is easier to raze function than to demolish a structure. The exciting potential of cellular differentiation to induce cells such as stem cells to form different cell types or to understand cancer cell dormancy has been the focus of research for many investigators.[1]

It is believed that a complete understanding of the molecular mechanisms of cellular differentiation and de-differentiation will help us influence these processes that can offer tremendous opportunities in the advancement of stem cell therapy, cancer treatment, and control over parasitic infections. It is proposed that free-living amoebae such as *Acanthamoeba*, *Balamuthia,* and *Naegleria* provide useful models to study cellular differentiation processes and the underlying molecular mechanisms. The fact that encystment and excystment processes in

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DOI 10.1080/20477724.2015.1103504

differentiation of these amoebae are associated with structural and functional changes that are induced in response to environmental conditions, make them attractive models to study cellular differentiation processes in eukaryotic development.[8,9] This is further strengthened with the availability of large quantities of cells in axenic cultures, which may also be suited for biochemical studies. Previously, this unicellular amoeba, i.e. *Acanthamoeba* has been used in studies of the capture of prey by phagocytosis, growth, and encystment,[2,8,9] its ability to harbor pathogenic bacteria,[10] the molecular biology of motility,[11] its ability to cause serious human diseases.[3,4] Thus, it is an attractive model for studying various aspects of biochemical, cellular and molecular biology, as well as its pathogenicity. Findings arising from these eukaryotic microbes are translatable to other eukaryotic cells, including human cells.

Contributors and sources

NAK has a lifelong interest in the field of free-living amoebae and became interested in its role to model cellular differentiation processes. RS is a microbiology researcher. Both authors contributed equally to the manuscript and will act as guarantors.

Conflict of interest statement

The authors of this Letter to Editor declare (1) no conflicts of interests for the submitted work; (2) no financial relationships with commercial entities that might have an interest in the submitted work; (3) no spouses, partners, or children with relationships with commercial entities that might have an interest in the submitted work; and (4) no non-financial interests that may be relevant to the submitted work.

Acknowledgement

This work was supported by the Sunway University, Malaysia.

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