Parasite movement: crucial anchor revealed

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A novel protein implicated in the movement and survival of the parasite *Toxoplasma gondii* has been revealed in a recent issue of *Journal of Cell Biology* [1]. The two collaborating research groups based in the USA, one led by Gary Ward at the University of Vermont (http://www.uvm.edu/) and the other by Con Beckers at University of North Carolina at Chapel Hill (http://www.unc.edu/), set out to determine the molecular mechanism responsible for movement in the parasite *T. gondii,* which is the causative agent of toxoplasmosis – a disease that causes severe complications in fetuses, encephalitis in immunocompromized individuals and, in some cases, can lead to blindness. As Beckers explained: 'The way these organisms move and the way they are controlled is absolutely critical to their ability to cause disease.'

Muscling in

After a series of immunofluorescence and immunoprecipitation experiments, the authors found a protein, designated TgGAP50, located in the inner membrane complex of *T. gondii*. Further experiments revealed that this protein was anchoring another significant protein involved in locomotion – *T. gondii* myosin A (TgMyoA). Indeed, myosin, a ubiquitous protein, is the main component of thick filaments in human muscle. The interaction of myosin with actin, a major constituent of thin filaments in human muscle, is responsible for muscle contraction in humans. The authors, excited by their results, described TgGAP50 as 'specific membrane receptor – a myosin motor' and its significant discovery cannot be understated for the parasite research community. '*Toxoplasma* motility may be a result of the myosin moving along the length of actin filaments in the parasite. Alternatively, it may be caused by the myosin holding onto the end of a growing actin filament' explained Beckers. 'Either way, the myosin molecule needs to be anchored in the parasite for movement to occur.'

T. gondii belongs to the phylum Apicomplexa, which contains several clinically important parasites, including *Plasmodium falciparum*, the causative agent of human malaria, and *Cryptosporidium parvum*, associated with severe diarrhea in immunocompromised individuals. Because of the increase in the prevalence of immunocompromized patients, particularly those with AIDS and HIV, infections with *Toxoplasma* and *C. parvum* are a cause for concern. All of these apicomplexan parasites are characterized by an absence of locomotory organelles, such as cilia and flagella, and there is no apparent change in cell shape during locomotion. So, how do these parasites move from one host cell to the next, or even escape from a host cell? It was these questions that the two research groups wanted to answer.

Gliding along

T. gondii moves by a substrate-dependent gliding motion in the form of 'circular and forward twisting movements', also described as a 'bizarre spinning and corkscrewing motility' by another researcher [2]. This type of locomotion has been linked to an actin–myosin-based motor, whereby the actin filaments embed in the plasma membrane of the parasite interacts with a myosin motor. In order to explain the significance of the group's findings, Beckers said, 'If the myosin is not anchored anywhere, its movement with respect to an actin filament will not result in parasite motility…As an analogy, if you're sitting in a small boat and throw a rope out to the dock and someone's there to hold it, you can pull yourself toward that person. But if no one is there, all you'll do is pull the rope and no net movement will occur'.

Pivotal role

Gaskins *et al.* found two novel proteins, TgGAP45 and TgGAP50, which were linked to the major myosin heavy and light chains of *Toxoplasma.* At this stage, it is not clear what is the function of TgGAP45, but the inability to generate transgenic parasites with a disrupted TgGAP45 gene indicates that this protein is essential. Previous studies have demonstrated the existence of a 'glideosome', a complex comprising TgMyoA and *T. gondii* myosin light chain (TgMLC1) [3]. Based on their new data, Gaskins *et al.* proposed that TgGAP50, a transmembrane glycoprotein, is also a member of this glideosome complex and has a 'pivotal role' in parasite motility by behaving like an 'anchor'.

'The discovery of two proteins, TgGAP45 and TgGAP50, bridging the myosin to the inner membrane complex has unravelled the missing link between the motor responsible for

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gliding and the structural chassis of the protozoa' commented Jean-Francois Dubremetz (University of Montpellier, France; http://www.univ-montp2.fr).

In conclusion, Joseph Schwartzman from the Dartmouth-Hitchcock Medical Center, NH, USA (http://www.hitchcock.org/) told *Drug Discovery Today* that 'This work opens up the possibility of finding a way to pharmacologically interfere with an essential function in the parasite's life cycle'. He continued, 'Motility is a novel drug target, but the unique mechanism of the glideosome makes it likely that it can be disrupted selectively from host cell functions'.

References

1 Gaskins, E. *et al*. (2004) Identification of the membrane receptor of class XIV myosin in *Toxoplasma gondii*. *J. Cell Biol*. 165, 383–393

2 Wells, W. (2004) An anchor for motoring. *J. Cell Biol*. 165, 294

3 Opitz C, and Soldati D. (2002) 'The glideosome': a dynamic complex powering gliding motion and host cell invasion by *Toxoplasma gondii*. *Mol. Microbiol*. 45, 597–604

New ray of hope for cancer patients

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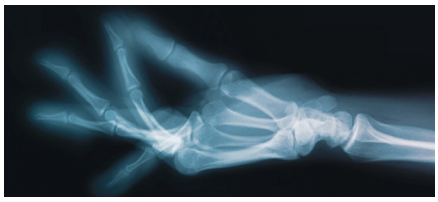
By using radiation-induced gene therapy to increase the amount of nitric oxide in tumour cells, researchers at the University of Ulster (http://www.ulst.ac.uk) have doubled the efficacy of radiotherapy in mice [1]. Their research has been seminal to a lot of work that other gene therapy groups are now doing with the radiation-inducible WAF1 promoter.

Nitric oxide synthase

Nitric oxide, a signaling molecule with important regulatory functions in most tissues of the body, has enormous potential as an anticancer agent because of its cytotoxic effect at higher doses. In addition, nitric oxide sensitizes cells to the effects of radiation and chemotherapy. Various research groups are therefore working on gene-therapy approaches to deliver the inducible nitric oxide synthase (iNOS, the enzyme responsible for generating nitric oxide in the body) to tumour cells. However, many of these approaches suffer from a lack of specificity for the target cells.

In the *Journal of Gene Medicine*, David Hirst and colleagues recently reported a strategy to overcome this problem. 'What they come up with here is a neat trick, because they have used the promoter to WAF1 or p21, which we know is […] a radiosensitive promoter,' says Lawrence Young, who develops novel gene therapy approaches for the



treatment of cancer at the University of Birmingham (http://www.bham.ac.uk). The promoter can be switched on by an external X-ray beam at radiation doses within the therapeutic range (2–4 Gy); the X-ray beam is targeted to cancer cells, thus limiting nitric oxide production to these cells. Then, the investigators can come in with higher-level doses of radiation (10–20 Gy), and that, combined with the cytotoxic effects of nitric oxide, results in a significant antitumour effect.

iNOS gene therapy

In mice carrying murine RIF1 fibrosarcoma cells or human HT29 colon carcinoma cells, radiation-induced iNOS gene therapy increased the effectiveness of radiotherapy by the factor 2.0 and 1.3, respectively. This means that 'you could either reduce the radiation dose by 50% and reduce the severity of normal tissue complications, or you could keep the radiation dose the same and you'll have more damage to the tumour cells, equivalent to giving 50% more radiation,' concludes Hirst.

Young finds the philosophy behind the approach attractive, but adds that 'transferring this into patients without some increased efficiency of delivery of the gene would be very difficult.' In the present study, Hirst and colleagues have used liposomes to deliver the iNOS gene. But 'liposomes are very inefficient,' says Young. 'I think you could build this type of system into one of the modern, second- or third-generation viral vectors and evaluate its use then.'

Systemic delivery

Hirst and colleagues are already busy refining their strategy. 'We are doing systemic delivery studies as well as using some other approaches,' hints Hirst. Once they have tweaked the system to satisfaction, this concept will be combined with a whole variety of other things, believes Marie Boyd at the Department of Radiation Oncology, University of Glasgow (http://www.gla.ac.uk). She is currently looking into combining Hirst's system with chemotherapy; other gene therapy groups are now also working with the WAF1 promoter. 'I'm convinced that some aspect of this will be in patients within the next five years,' predicts Boyd.

Reference

1 Worthington, J. *et al*. (2004) Use of the radiation-inducible WAF1 promoter to drive iNOS gene therapy as a novel anti-cancer treatment. *J. Gene Med*. 6, 673–680

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