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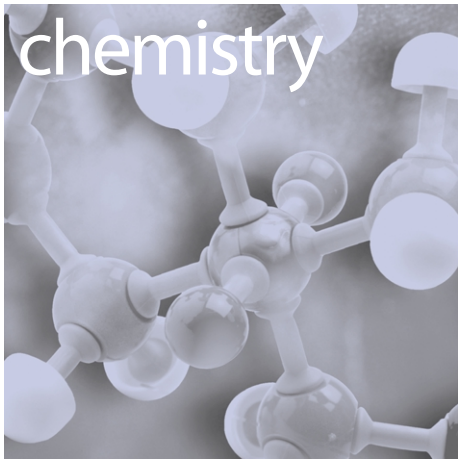
monitor



MOLECULES

Novel antitumour small molecule Inhibitors of hypoxia-inducible factor-1

Developing solid tumours are characterized by areas of hypoxia (low oxygen tension) that are commonly associated with an aggressive and metastatic cancer phenotype frequently linked to a poor response to radio- or chemo-therapy. Areas of low oxygen tension act as a stimulus for genes involved in proliferation, glycolysis and angiogenesis. A particularly important transcription factor involved in the regulation of hypoxia-activated genes is hypoxia-inducible factor-1 (HIF-1); and the HIF-1 subunit HIF-1α has emerged in recent years as an important anticancer drug target [1, 2].

Chau and co-workers (Institute of Cancer Research, Sutton, UK) have reported the development of U2OS human osteosarcoma cells that stably express a luciferase reporter construct under the control of a hypoxia response element (U2OS-HRE-luc) [3]. The group have used this construct in a high-throughput cellular screening assay to identify HIF-1 inhibitory compounds following HIF-1 induction with a hypoxia mimetic (deferoxamine mesylate). A pilot screen of the National Cancer Institute Diversity Set of 2,000 compounds led to the identification of two novel hit compounds, **(i)** and **(ii)**, which had not previously been found in previous hypoxia screens. Interestingly, the two molecules differentially blocked HIF-1 activity and HIF-1α induction in response to hypoxic stress and insulin-like growth factor-1.

1 Semenza, G.L. (2003) Targeting HIF-1 for cancer therapy. *Nat. Rev. Cancer* 3, 721–732
2 Hewitson, K.S and Schofield, C.J. (2004) The HIF pathway as a therapeutic target. *Drug Disc. Today* 9, 704-711
3 Chau, N-M. *et al.* (2005) Identification of novel small molecule inhibitors of hypoxia-inducible factor-1 that differentially block hypoxia-inducible factor-1 activity and hypoxia-inducible factor-1α induction in response to hypoxic stress and growth factors. *Cancer Res.* 65, 4918–4928

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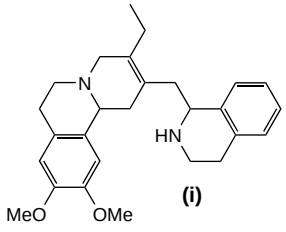
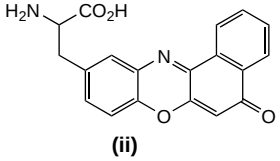


(i)



(ii)

MICROBIOLOGY

Universal vaccine and novel surface structure identified by Group B *Streptococcus* genomics

Group B *Streptococcus* (GBS) is a multiserotype bacterium that colonizes the anogenital mucosa of healthy women. GBS is the major cause of serious infections in newborns despite antibiotic prophylaxis. Because maternal antibodies against GBS reduce the risk of neonatal infection, vaccination of the mother has the potential to reduce the incidence of infection.

Maione *et al.* [2] mined for potential vaccine candidates by comparing the whole genomes of eight GBS strains of five different serotypes. A 'core' of ≈80% of the genes were conserved between strains whereas the remainder was variable. 312 proteins predicted to be extracellular were successfully expressed in *Escherichia coli* and used to immunize female mice. Mice were mated and the offspring was challenged with GBS. This screening identified one protein from the core genome (GBS322) and three from the variable (GBS67, GBS80, and GBS104) that significantly increased the survival of the mice. Immunization with each antigen gave protection against more than

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MOLECULAR BIOLOGY

Clustering centromeres

In the cell nucleus chromosomal DNA exists as decondensed, 'open' euchromatin and more-densely packed, inaccessible heterochromatin. Centromeres are a particularly striking example of the latter, forming large blocks of heterochromatin. Moreover, centromeres can cluster together into even bigger heterochromatic domains called chromocentres. The observation that silent genes can be recruited to centromeric heterochromatin has led to the suggestion that these domains are involved in gene repression and, more specifically, in maintaining the repressed state through multiple rounds of cell division during differentiation (heritable silencing).

A new study by Brero and colleagues now shows that the clustering of centromeres occurs as a process of cell differentiation and furthermore, identifies MeCP2 protein as a key component of the clustering mechanism [1]. MeCP2 binds methylated DNA (a mark of heterochromatin) and the group found that, upon differentiation of myoblasts into muscle cells *in vitro*, levels of MeCP2 at centromeres increased concurrent with their clustering into chromocentres. To test whether increased MeCP2 was responsible for clustering or simply coincidental, the group transfected undifferentiated myoblasts with fluorescently labelled MeCP2 protein and then scored the number of chromocentres. A clear negative correlation was observed, that is, brighter cells had fewer chromocentres confirming that increasing MeCP2 did indeed increase clustering.

Interestingly the group found that mice that lack functional MeCP2 have normal muscle development and centromeric clustering comparable with wild-type mice. This suggested that other factors might compensate the loss of MeCP2 and the group found that MBD2, another methyl-DNA binding protein, could also induce clustering when transfected into myoblasts. The finding that clustering could be induced by alternative mechanisms and/or factors indicates that this process of large-scale centromere reorganization is functionally significant, perhaps reflecting the need for cells to establish repressive domains for gene silencing during differentiation.

1 Brero, A. *et al.* (2005) Methyl CpG-binding proteins induce large-scale chromatin reorganization during terminal differentiation. *J. Cell Biol.* 169, 733–743

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one strain, but not to all tested strains. Combined immunization with all four antigens conferred protection against 12/12 strains of six different serotypes. Generated antiserum had a significantly higher opsonophagocytic activity against a strain expressing all four antigens than when immunized with the antigens separately.

In a spin-off study of this vaccine screen Lauer *et al.* [3] used antiserum against GBS80 and GBS104 to detect the proteins on the bacteria and in surface extracts. In addition to the monomeric forms of the proteins, both antisera recognized ladders of high molecular weight bands indicating protein polymers. Electron microscopy using gold-labeled antibodies confirmed the presence of polymers and identified thin extended pilus-like structures containing both antigens on the bacterial surface. Even though pilus-like structures have been described in Gram-positive bacteria, this is the first example in pathogenic streptococci.

This study elegantly demonstrates the power of multiple genome scans in identifying vaccine candidates, especially in multiserotype bacterial pathogens. It also demonstrates that functional testing and imaging of proteins found in genome scans can reveal novel bacterial structures and organelles.

2 Maione, D. *et al.* (2005) Identification of a universal group B *Streptococcus* vaccine by multiple genome screen. *Science* 309, 148–150
3 Lauer, P. *et al.* (2005) Genome analysis reveals Pili in group B *Streptococcus*. *Science* 309, 105

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Swimming under the radar – motile bacteria that evade Toll-like Receptor 5

Motility is an essential feature of many pathogenic bacteria. Propulsion is imparted by the bacterial flagellum which is composed of several thousand flagellin subunits, each organized into three regions, D0D1, D2D3 and D0D1. The highly conserved D0D1 domains are stacked upon each other in the core of the flagellar filament, whereas the variable D2D3 region is located on the outer surface of the filament [4]. Bacterial flagellins are recognized by Toll-like receptor 5 (TLR-5), a component of the innate immune system. Binding of flagellin monomers to TLR-5 on epithelial cells lining mucosal surfaces triggers a proinflammatory response.

TLR-5 specifically recognizes a portion of the flagellin D0D1 domain that is also involved in flagellum assembly. As a result, single mutations in *Salmonella typhimurium* flagellin that abolish recognition by TLR-5 also render the organism nonmotile. In contrast, several important gastrointestinal pathogens, including the phylogenetically distant *Campylobacter jejuni* and *Helicobacter pylori*, are highly motile, even though their flagellins are not recognized by TLR-5. To better understand the evolution of these stealthy flagellins, Andersen-Nissen *et al.* correlated sequence differences between *Salmonella* and *Helicobacter* flagellins with the 3D structure of *Salmonella* flagellin (fliC) and used this information to construct a *Salmonella* mutant that retains motility but is not recognized by TLR-5 [5]. Substitution of Ala for Ile at position 411 in the C-terminal conserved region of fliC abolishes motility and causes a 30-fold decrease in stimulation of TLR-5. Substitution of the *H. pylori* sequence for the *Salmonella* sequence at positions 58 and 59 (K58S, G59S) in the N-terminal conserved domain did not affect motility or stimulation of the TLR-5 pathway. When all three mutations were introduced into fliC (I411A, K58S, G59S) motility was restored; however, the mutant still exhibited markedly reduced stimulation of the TLR-5 pathway. These experiments demonstrate that compensatory mutations can produce functional flagellins that are not recognized by TLR-5. Natural flagellins with this property are only found in specific bacterial lineages, which suggests that TLR-5 plays an important host defense role that is not easily circumvented.

4 Ramos, H.C. *et al.* (2004) Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa. *Trends Microbiol.* 11, 509–517
5 Andersen-Nissen, E. *et al.* (2005) Evasion of Toll-like receptor 5 by flagellated bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 102, 9247–9252

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Docking of artemisinins to the serca-like Ca^{2+}ATPase PfATP6 predicts their antimalarial activities

The artemisinins are arguably the most important group of antimalarials today owing to their activity against chloroquine-resistant parasites and their relatively low cost. The parent compound, artemisinin is a sesquiterpene found in the sweet wormwood plant, *Artemisia annua*. A number of semi-synthetic antimalarial drugs, including artemether, arteether and artesunate are derived from it. The plant, also known as qinghaosu, has been used as an antipyretic for

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CANCER BIOLOGY

Cancer drug delivery: patch and deliver

In normal tissues and organs, interstitial fluid, filtered from blood vessels, is drained by lymphatic vessels to maintain the interstitial fluid pressure (IFP) close to zero (mm Hg). By contrast, in tumours, IFP is normally altered due to impaired lymphatic function and abnormalities in vascular structure and function. Elevated interstitial fluid pressure, a hallmark of solid tumors, can lead to compromize the delivery of therapeutics to tumours because the oncotic and hydrostatic pressures in the microvascular and interstitial spaces are at equilibrium. Tong *et al*. [8] have now shown that, by blocking vascular endothelial growth factor (VEGF) signaling with a VEGF-receptor-2 antibody (DC101), it is possible to decrease IFP, not by restoring lymphatic function, but by producing a morphologically and functionally 'normalized' vascular network around human xenograft tumours.

The normalization process was demonstrated to prune immature vessels and improve the integrity and function of the remaining vasculature by promoting perivascular cell and basement membrane coverage. In essence, the weak and loose 'bits' were shed off and the remaining vessels were given a make-over. DC101 administration (40mg/kg intraperitoneally) also induced a hydrostatic pressure gradient across the vascular wall, which led to a deeper penetration of administered molecules into the tumour tissue. It is important to point out that the permeability of such normalized vessels is still higher than that of normal vessels, thus allowing selective drug extravasation into the neoplastic area. There is now the need to test this mode of better drug delivery in clinically-relevant animal models.

8 Tong, R.T. *et al*. (2004). Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res*. 64, 3731–3736

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2000 years in China, but extraction and modification of artemisinin dates only to the 1970s when these compounds were extensively studied by Chinese scientists. International clinical use of artemisinins started only in the 1990s. The mechanism of action of artemisinins has been a matter of considerable controversy and remains contentious. However, in 2003 Eckstein-Ludwig *et al*. [6] provided compelling evidence that a sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) orthologue in the parasite called PfATP6 is the target.

Now a computational study by Jung *et al*. [7] has demonstrated a correlation between the docking indices of 74 artemisinin derivatives and their antimalarial biological activities. The authors have performed a BLAST search revealing that SERCA is the only similar protein in the Protein Data Bank database of crystal structures and has 43.5 % sequence homology. This was used to produce a three-dimensional homology-modelled structure. The model was found to preserve the thapsigargin-binding site of the SERCA crystal structure. Molecular docking studies were then performed with the artemisinin derivatives, which were found to bind to this site mainly by hydrophobic interaction. The LUDI scores for the docking of these derivatives were found to correlate with biological activity. This strongly supports the proposal that PfATP6 is indeed the target of these drugs and points the way to rational design of new derivatives. Intriguingly, the endoperoxide group that is essential for the activity of these compounds is exposed to the exterior of the binding pocket. It is believed that this group is activated by Fe^{2+}. The result of this activation and the reason that it is essential for antimalarial activity remains to be explained.

6 Eckstein-Ludwig, U. *et al*. (2003) Artemisinins target the SERCA of *Plasmodium falciparum*. *Nature* 424, 957–961

7 Jung, M. *et al*. (2005) Three-dimensional structure of *Plasmodium falciparum* Ca2+-ATPase (PfATP6) and docking of artemisinin derivatives to PfATP6. *Bioorg. Med. Chem. Lett*. 15, 2994–2997

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