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Biology

Microbiology

From ubiquity to specificity in drug target

18 million people in the world are suffering from the protozoan infection, Chagas' disease and, in the absence of vaccines and cures, there is an urgent need to identify protein targets for the development of therapeutic agents. Essential proteins that are ubiquitous are often neglected as targets for drug design. However, resolution of the fine structure can show some specificity that could be used to avoid cross-reactivity in drug targeting. Harkiolaki *et al.* now report the crystal structure of the dUTPase from *Trypanosoma cruzi* in its native state and complexed to dUDP [1]. By comparison of structures, the authors concluded that this ubiquitous and essential protein could represent a potential target in the treatment of Chagas' disease.

The major observation was that the dUTPase of *T. Cruzi*, which is a dimer, presents no structural similarities with the other known dUTPases (which are trimeric for the most part). Hence, it represents a novel protein fold. Additionally, despite a similar function at comparable kinetics, the dUTPase from *T. cruzi* could present a catalytic mechanism that is quite different from the other dUTPases.

Intriguingly, the substrate is bound to the active site in a different manner than for the other dUTPases and when the authors superimposed the nucleotide models derived from complexes of trimeric dUTPases and dimeric *T.cruzi* dUTPases, they observed a different nucleotide conformation. Specifically, on the uracil moiety, the deoxyribose of dUDP in the *T.cruzi* complex deviates significantly from all other deoxyriboses. Similarly, when the superimposition is performed on the deoxyribose and α-phosphate atoms, the plane of the uracil moieties of dUDP in the *T.cruzi* enzyme is rotated relative to the planes of the uracil moieties of nucleotides in complex with trimeric dUTPases.

Theses differences could be exploited in drug design for the production of a nucleotide mimic to bind and selectively inactivate the dimeric dUTPase. Moreover, as in *T.cruzi,* this enzyme also exists in *Trypanosoma brucei* and *Leishmania major* and no trimeric dUTPase homolog has been identified in their genomes. Consequently, this protein could be considered as a good potential target for drug design against protozoan infections.

1 Harkiolaki, M. *et al.* (2004) The crystal structure of *Trypanosoma cruzi* dUTPase reveals a novel dUTP/dUDP binding fold. *Structure* 12, 41–53

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Staphylococcal protein A induces inflammation in the airways

Immunoglobulin-binding surface proteins are widespread among Gram-positive pathogens and are considered important for pathogenesis. The prototypical protein A from *Staphylococcus aureus* interferes with opsonization by binding the Fc portion of immunoglobulins. *S. aureus* isolated from airway infections show increased expression of protein A, suggesting a role for this protein in interactions with airway epithelium in the absence of immunoglobulins. In staphylococcal pneumonia, neutrophils (PMNs) infiltrate to control the bacteria, but also cause epithelial damage and impairment of lung function.

Gómez *et al.* [2] studied the interactions between purified protein A, protein A-expressing and non-expressing *S. aureus* strains with airway epithelial cells. Protein A or protein A-overexpressing strains, but not non-expressing strains, induced interleukin 8 (IL-8) through mitogen-activated kinases (MAPKs). By using a protein A magnetic bead screening assay of cell lysates, TNFR1, the receptor for tumor necrosis factor-α (TNF-α), was identified as the protein A receptor. Soluble protein A or S. aureus stimulated surface expression and shedding of TNFR1 and induced inflammation by mimicking TNF-α activation through TNFR1. A model of pneumonia in mice showed that lack of either protein A in bacteria or TNFR1 in mice resulted in decreased virulence, and administration of soluble TNFR1 blocked protein A-induced inflammation, including PMN recruitment.

In summary, this study identifies an important new interaction between staphylococcal protein A and a receptor (TNFR1) in airway epithelium that directly stimulates inflammatory responses with neutrophil recruitment known to contribute to the pathology seen in pneumonia. This information provides opportunities for development of therapeutic strategies, and it is encouraging that the soluble form of the receptor functions as an inhibitor of the inflammation induced by staphylococci.

2 Gómez, M.I. *et al.* (2004) *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. *Nat. Med.* doi: 2010.1038/nm1079 (E-publication ahead of print; http://www.nature.com)

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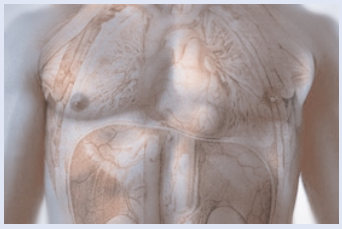
Cancer biology

Alternatives to Herceptin

The monoclonal antibody Herceptin (Trastuzumab) has become an important

Physiology

Cardiac hypertrophy: role of ligand-independent activation of AT_1 receptor.



Cardiac hypertrophy is one of the most influential clinical complications of cardiovascular disorders. Several pieces of evidence have suggested that mechanical stretch itself is an initial factor for cardiac hypertrophy in response to haemodynamic overload. However, it has been shown that the local renin–angiotensin system in cardiomyocytes is linked to the formation of pressure-overload hypertrophy. Intriguingly, pretreatment with angiotensin II (AII) type 1 receptor (AT_1-R) blockers significantly attenuates all mechanical stress-induced events, suggesting the participation of AII in these effects.

Although it has been reported that AII is stored in cardiomyocytes and that mechanical stress induces the secretion of stored AII, Zou *et al.* [3] recently examined the possibility that mechanical stress can directly activate the AT_1 receptor without the involvement of AII. They showed that, without the involvement of angiotensin II, mechanical stress not only activates extracellular-signal-regulated kinases and increases phosphoinositide production *in vitro*, but also induces cardiac hypertrophy *in vivo*. Mechanical stress also induced the association of the AT_1 receptor with Janus kinase 2, and translocation of G proteins into the cytosol.

All of these events were inhibited by the AT_1 receptor blocker candesartan, providing evidence that the AT_1 receptor is a 'mechanical sensor' that converts mechanical stress into biochemical signals inside the cell.

3 Zou, Y. *et al.* (2004) Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat. Cell Biol.* 6, 499–506

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drug in the treatment of metastatic breast cancer. Therapy is indicated in those 20–30% of breast cancer patients who overexpress the *Her2* gene. High concentration of the Her2 receptor causes spontaneous ligand-independent receptor homodimerization and activation of subsequent downstream signalling pathways.

In contrast to Herceptin, the novel monoclonal antibody Pertuzumab (2C4, Omnitarg) is supposed to be effective in patients without *Her2* overexpression. It is currently being evaluated in several phase II clinical trials. Pertuzumab binds to a region that is necessary for Her2 heterodimerization with EGFR/Her1 and Her3, thus preventing growth factor-induced signalling.

In an *in vitro* assay, Nahta *et al.* examined combination effects of Herceptin and Pertuzumab on breast cancer cells [4]. Drug combination inhibited survival of BT474 breast cancer cells, partially because of increased apoptosis. This was due to blockade of receptor signalling through AKT but not MAP kinase pathways. In combination with Herceptin, Pertuzumab synergistically increased downregulation of total and phosphorylated Her2 in a dose-dependent manner.

Klinger *et al.* embarked on another strategy by using antibodies directed against the Lewis-Y modification of ErbB receptors [5]. The Lewis-Y sugar moiety is expressed predominately during embryogenesis and in adult tissues restricted to granulocytes and epithelial surfaces. It can also be found in the majority of carcinomas including breast and ovary cancers. In the cancer cell lines SBKR-3 and A431, anti LeY monoclonal antibodies decreased EGF and heregulin induced signalling via the MAP kinase pathway and altered the recycling dynamics of EGF receptors.

Pertuzumab and eventually anti-LeY-antigen antibodies might provide a broader efficacy than currently used therapeutics like Herceptin for the majority of cancer patients without amplification of the *Her2* gene.

4 Nahta. R. *et al.* (2004) The HER2-targeting antibodies Trastuzumab and Pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res.* 64, 2343–2346
5 Klinger, M. *et al.* (2004) Antibodies directed against Lewis-Y antigen inhibit signaling of Lewis-Y modified ErbB receptors. *Cancer Res.* 64, 1087–1093

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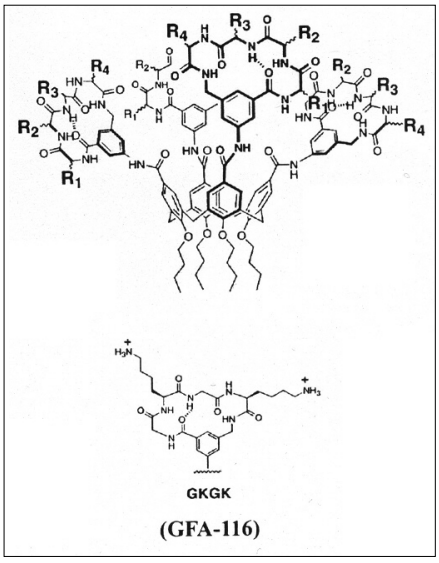
Blocking angiogenesis in tumors

As tumors increase in size, microenviromental conditions, for instance hypoxia, require the formation of new blood vessels. To survive, tumors secrete several proangiogenic factors. Among them, vascular endothelial growth factor (VEGF) is believed to play a crucial role in initiation of angiogenesis. Several new drugs are under investigation for their ability to disturb VEGF interaction with its receptor, Flk-1. Recently, the monoclonal anti-VEGF antibody Bevacizumab (Avastin) has received FDA approval for the treatment of metastatic colorectal cancer.

In a recent report, Sun *et al.* describe a new synthetic agent that can disrupt VEGF–Flk-1 interaction [6]. They designed a small library of calixarenes containing four peptide domain loops, the sequences of which can be varied to optimize binding abilities. The molecule growth factor antagonist-116 (GFA-116) containing the amino acid sequence GKGK linked to a calix[4]arene scaffold, exhibited remarkable specificity to block VEGF-driven receptor activation.

In several *in vitro* assays, GFA-116 efficiently inhibited capillary network formation and migration of human endothelial cells as well as microvessel outgrowth in an *ex vivo* rat aortic ring model. *In vivo* models revealed inhibition of rat cornea angiogenesis. In another assay, nude mice implanted with human lung cancer A-549 cells subcutaneously were treated with GFA-116. Tumor size and angiogenesis were significantly decreased, compared with untreated mice. In an immunocompetent mouse model, GFA-116 showed ability to inhibit tumor



growth and metastasis of highly metastatic B16-F10 melanoma cells.

Taken together, the results show that the synthetic molecule GFA-116 is specific for and effective in blocking VEGF-dependent signaling and could therefore be a potent new anticancer drug candidate.

6 Sun, J. *et al.* (2004) Blocking angiogenesis and tumorigenesis with GFA-116, a synthetic molecule that inhibits binding of vascular endothelial growth factor to its receptor. *Cancer Res.* 64, 3586–3592

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Metastasis, with a Twist

Despite being a major cause of cancer mortality, the mechanisms regulating tumor metastasis remain poorly understood. In an effort to address this deficiency, a team led by Robert Weinberg has identified Twist as a key player in this process [7].

The researchers exploited a mouse model comprising four distinct cell lines derived from the same spontaneous mammary tumor. When implanted into a host mammary gland, these lines form primary tumors differing in their metastatic potential. Using microarray technology, Twist was identified in three of the cell lines, suggesting a role in the early stages of metastasis. This was confirmed using siRNAs to inhibit Twist expression.

During *Drosophila* gastrulation, Twist is involved in the epithelial-mesenchymal transition (EMT). Intriguingly, Twist was also able to promote EMT in human mammary epithelial cells. This was associated with increased expression of mesenchymal markers at the expense of epithelial cell markers, including E-cadherin.

Because EMT occurs during epithelial carcinoma metastasis, the researchers speculated that Twist contributes to this process by inducing EMT. Twist was present in several metastatic tumor cell lines but undetectable in non-metastatic and normal cells. In addition, Twist was overexpressed in 70% of invasive lobular carcinomas, which display many features of EMT. Indeed, an inverse correlation between Twist and E-cadherin expression was established in these tumors.

In conclusion, during metastasis, tumor cells appear to subvert factors usually active in development. At this point, the involvement of additional components of the Twist pathway cannot be ruled out; however, identification of Twist provides a valuable tool for further dissection of the metastatic process.

7 Yang, J. *et al.* (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117, 927–939

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Business

Collaborations

Cellomics and GE collaborate for cellular screening solutions

With the aim of accelerating and enhancing drug-discovery programs, Cellomics (http://www.cellomics.com) and the General Electric Company (GE; http://www.ge.com) have announced details of a software development and commercialization agreement. The agreement outlines how the two companies will collaborate to develop high-content screening tools, using Cellomics' high-content informatics (HCi™) platform and GE Healthcare's sub-cellular imaging instruments.

The software developed by Cellomics will be developed to analyse information generated by GE Healthcare's IN Cell Analyzer 3000 and IN Cell Analyzer 1000 sub-cellular-imaging systems. Such software will permit the interrogation of this data using Cellomics® Store and vHCS™ Discovery ToolBox. As a result of these developments, it is thought that scientists will more easily be able to store, mine and interpret data. Such a system should permit more rapid and better-informed decisions to be made.

Michael Evans, Vice President of marketing and strategy, Discovery Systems, GE Healthcare, commented: 'This collaboration with Cellomics supports GE Healthcare's strategy of helping customers improve efficiencies in gathering, analyzing, and understanding complex drug development information,' and continued 'We are pleased that we will be able to combine our informatics solution expertise with GE Healthcare's cellular analysis products to drive towards the ultimate goal of cutting drug development time.'

Vernalis and Biogen Idec to collaborate on Parkinson's disease

Vernalis (http://www.vernalis.com) and Biogen Idec (http://www.biogen.com) have agreed to collaborate in a joint venture to develop and commercialize the Vernalis lead compound, V2006. The compound has recently completed Phase I clinical trials

Under the terms of the agreement, Vernalis will immediately receive US$10 million license fee and a series of other payments, dependant upon achieving specific milestones. Biogen Idec will have the right to develop one V2006 back up compound. They will invest to the tune of US$6 million through subscription of 6,218,487 new Vernalis ordinary shares, at a price of 53 pence per share.

Simon Sturge, chief executive officer, Vernalis commented 'This is an important program for Vernalis, and we are delighted to have attracted a partner of Biogen Idec's calibre to help us take it forward,' To which James Mullen, president and chief executive officer of Biogen Idec, added 'We look forward to applying the expertise we have developed with our neurology franchise to V2006, a promising product that will bolster our growing small molecule portfolio. In addition, this

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