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Validation of genomics-derived drug targets using yeast

The steady flow of novel genes from genomics provides a great opportunity for increasing the number of therapeutic targets, a limited resource for those engaged in drug discovery. The paucity of valid drug targets limits the impact the pharmaceutical and biotechnological industries can have on disease states and places constraints on the future improvement of health care worldwide. Realization of the potential of a decoded human genome will require a concerted effort by researchers to elucidate the physiological roles of newly identified proteins in normal and diseased processes. As the investigation of fundamental disease mechanisms is time-intensive, strategies that accelerate the evaluation of candidate molecules as disease-relevant targets must be devised.

The broad impact of genomics on drug development will be expeditiously handled only by establishing a clear focus, whether that is within a molecular class, a therapeutic area, an organ system, a type of tissue or a disease process. In addition, it will be important to take advantage of any strategy that is able to side-step obstacles to target validation. The yeast-based system described here is an example of a focused, alternative approach designed to expedite the process of moving from the DNA sequence to its biological relevance to the production of drugs.

Saccharomyces cerevisiae

The yeast *Saccharomyces cerevisiae* is being exploited to obtain the tools required for discerning whether a given orphan G protein-coupled receptor (GPCR) is a suitable drug target. The prediction that the growing collection of orphan GPCRs will be a rich source of targets for therapeutic intervention is based on the recognition that a significant portion of the drugs currently on the market target this receptor family[1]. The potential functional areas of GPCRs for which natural ligands have recently been identified also support the prediction (Table 1). For example, with the identification of the motilin receptor[2] comes the possibility of new treatments for gastrointestinal disorders.

Surrogate ligands

To embark on an investigation of receptor biology, it might be ideal to have the natural ligand, but this is not essential. Surrogate ligands have facilitated receptor research in the past (e.g. nicotine, muscarine, NMDA, kainate) and are especially useful in enabling target validation in this era of genomics, where ligand identification is lagging, at times by many years, behind the cloning of new receptors. A potent, selective surrogate agonist enables the elucidation of signalling pathways, an assessment of biological function and the discovery of receptor antagonists. These tools, agonist and antagonist, facilitate the study of receptor function in the absence of the natural ligand.

Functional expression of mammalian homologues

Yeasts are amenable to the functional expression of mammalian homologues of yeast proteins. This fact, together with the rich history of yeast genetics and the completion of the sequencing of the yeast genome, has made *Saccharomyces cerevisiae* an ideal organism to use in augmenting other approaches to target validation. Yeast screens can be engineered that permit rapid identification of surrogate agonists and receptor antagonists of orphan GPCRs while offering distinct advantages. By deleting endogenous pheromone receptors, yeasts can be made to contain a null background for the expression of human receptors. This

Table 1. 'De-orphanized' G protein-coupled receptors

Receptor	Ligand	Predominant receptor localization	Potential functional area
GPR38/MTL-R1A	Motilin	Duodenum, colon	Gastrointestinal motility
GPR9–GPR6	TECK	Thymus	Thymocyte development
Edg-1	Sphingosine-1-phosphate	Endothelial cells	Angiogenesis
HGFAN72/OX$_1$R	Orexin	Brain	Feeding
HGR3	Prolactin-releasing peptide	Pituitary	Pregnancy, lactation
APJ	Apelin	CNS	Unknown

Abbreviations: GPR, G-protein receptor; HGR, human glucocorticoid receptor; MTLB, mannitol regulator; TECK, thymus-expressed chemokine.

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is an advantage when compared with mammalian cells that express multiple GPCRs whose identities are often incompletely characterized. The null background has proven useful in the dissection of the ligand specificity of lipid receptors[3]. The existence of multiple, co-expressed lipid receptors with overlapping ligand specificity made this dissection difficult in mammalian cells.

High-throughput screening

Yeast assays can be adapted for the high-throughput screening of small-molecule libraries to identify receptor agonists and antagonists[4]. Fifty receptor–G protein combinations can be screened against 30,000 compounds in a single agonist search. In yeast assays formatted for antagonist discovery, 25,000 compounds can be screened per week. By assembling compound banks that contain molecules that are or could be natural ligands for GPCRs, it has also been possible to identify natural ligands using yeasts (Klein, C. *et al.*, unpublished observations). Multiple isogenic yeast strains, differing only with respect to the expressed GPCR, can easily be screened in parallel, providing internal controls for receptor specificity.

Yeasts provide a universal readout of signal transduction from an activated receptor, regardless of the G protein that acts as the transducer[5,6]. This is achieved by coupling mammalian receptors to the yeast pheromone response pathway, a mitogen-activated protein (MAP) kinase cascade homologous to that found in mammalian cells. The pheromone signalling pathway has been exploited not only for the functional expression of receptors but also for ligand expression. The genetic malleability of yeasts provided an opportunity to design a unique, genetic selection of peptide agonists for GPCRs (Ref. 7). Highly engineered yeast strains expressing random peptide libraries have been used to discover surrogate peptide agonists for co-expressed orphan receptors[8]. This peptide selection strategy extends the use of yeasts beyond the identification of small-molecule surrogate or natural ligands for these receptors.

The future

Exploitation of yeasts represents one strategy for facilitating the study of the biology of novel targets. Obtaining an understanding of function is time-intensive but essential to envisioning novel therapies, as only with a thorough knowledge of disease mechanisms can selection of those warranting drug development be made from the multitude of targets. The challenge of transmuting DNA sequences into disease-relevant targets has provoked the creation of new technologies and will continue to be a driving force in biomedical research for the foreseeable future.

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