challenge of developing synthetic routes of perhaps greater practicality than has been typical in the past.

As a medicinal chemist, I have had several opportunities to observe first-hand the benefits of an early and close collaboration between discovery and process chemists and I fully concur with Federsel's point of view. However, I believe that the message needs to be communicated more effectively to those outside of the community of organic chemists to cultivate a more sympathetic audience, a task of far greater difficulty.

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Too many targets, not enough target validation – Reply ▲

Initial letter: Szymkowski, D.E. (2001) *Drug Discov. Today* 6, 398–399

Response from Leodevico L. Ilag

In a recent letter, David E. Szymkowski rightfully argued for the dire need to link the causal role of a gene or gene product to a particular disease or functional context. It is essential to directly identify the function of each protein because proteins often represent the functional representative of a gene and over 90% of the targets for marketed drugs are proteins and not the DNA or RNA[1]. Furthermore, it is becoming clear that proteins can perform multiple functions depending on the cellular location of the protein, cell type where the protein is expressed, multimeric state of the protein, and substrate bound to the protein[2]. Moreover, human genes are composed of multiple protein domains providing additional functional versatility compared with the worm and fly[3].

Inactivating protein function

Since the protein is the more appropriate target for a drug, it is imperative that the methods that directly inactivate protein function are used. Szymkowski provided a table listing several approaches (i.e. knockout/transgenic mice and antisense oligonucleotides/ribozymes) that act at the DNA or RNA levels and not directly at the protein level. Although inactivation strategies based on these nucleotide-based knockout or knockdown strategies are popular, interpretation of the results from these methods are complicated by genetic compensation and polar mutation effects. Since the expression of several gene products or proteins that can be translated from the disrupted DNA or RNA is affected, it will be difficult to assign their respective function(s).

Among the other approaches listed, neutralizing antibodies and small-molecule agonists/antagonists represent methods that directly inactivate protein function. Although antibodies provide an efficient way of obtaining specific binders to proteins, the success rate of obtaining neutralizing antibodies is unsatisfactorily low[4]. However, small-molecule agonists/antagonists are available only for a few classes of molecules that are related to existing drug targets. It will be difficult to obtain small-molecule agonists/antagonists for totally novel proteins with undefined functions.

Alternative approach

An alternative approach to assess the causative role(s) of novel protein targets, not mentioned by Szymkowski, is a method called chromophore-assisted laser inactivation (CALI). CALI was developed as a method to determine the *in situ* function of proteins in cellular processes[5]. CALI converts a non-function-blocking antibody into a blocking reagent with a high probability.

To perform CALI, a ligand (e.g. antibody) is labeled with the dye malachite green (MG) and binds to the protein of interest, and the complex irradiated with 620 nm laser light[5,6]. At this wavelength, cellular components are not damaged, but the light is absorbed by the dye, which in turn generates hydroxyl radicals that selectively inactivate the protein bound by the MG-labeled ligand[7]. Unbound MG-labeled reagents do not cause significant damage because the effects of CALI are spatially restricted[8] to within 15 Å of the dye moiety due to the short lifetime of the hydroxyl radical[7]. Furthermore, CALI does not lead to obvious nonspecific damage to cells or tissues and CALI has been tested against a diverse array of both intracellular and extracellular proteins in primary cells, cell lines and model organisms[9]. Moreover, the high spatial and temporal resolution of the method offers several advantages over the other approaches.

Thus, CALI represents one of the tools that can provide causal relationships between correlative data and function leading to the selection of the few targets that are worthy of a full drug development effort.

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