Published in final edited form as:
*Curr Opin Chem Biol*. 2009 December ; 13(5-6): 549–555. doi:10.1016/j.cbpa.2009.08.003.

# Identifying Druggable Disease-Modifying Gene Products

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## Summary

Many disease genes encode proteins that are difficult to target directly using small molecule drugs. Improvements in libraries based on synthetic compounds, natural product and other types of molecules may ultimately allow some challenging proteins to be successfully targeted; however, these developments alone are unlikely to be sufficient. A complementary strategy exploits the functional interconnectivity of intracellular networks to find druggable targets, lying upstream, downstream or in parallel to a disease-causing gene, where modulation can influence the disease process indirectly. These targets can be selected using prior knowledge of disease-associated pathways or identified using phenotypic chemical and genetic screens in model organisms and cells. These approaches should facilitate the development of effective drugs for many genetic disorders.

### Keywords

druggable genome; chemical genetics; small molecule; RNAi; synthetic lethal

## A. Disease and the Druggable Genome

The development of drugs to combat human genetic disorders, including cancer and neurodegenerative disease, is a high priority. In recent years, new DNA sequencing and genotyping technologies have enabled the discovery of a slew of novel disease-causing mutations and disease-associated DNA sequence variants [1–5]. Transforming this knowledge into a set of validated drug targets poses a significant challenge. It is sobering to consider that, to date, only ~2% of all predicted human gene products (260–400) have been successfully targeted with small molecule drugs [6,7] (Figure 1a). In part, this may reflect the fact that only 10–15% of all human genes (2,200–3,000) are thought to be in principle ‘druggable’ (e.g. encode proteins similar in sequence to those that have already been targeted with small molecules) [8,9], and that the overlap between druggable genes and known disease genes is only on the order of 25% [10,11] (Figure 1a). Adding to the challenge, mutated human disease genes can give rise to protein targets that differ only subtly in structure or abundance compared to their wild-type counterparts, or eliminate the production of a specific gene product altogether (e.g. due to mRNA destabilization or gene deletion) (Figure 1b). These considerations suggest

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Conflict of Interest Statement

The authors are not aware of any conflicts of interest that would materially impact the content of this work.

that few gene products, disease-associated or not, are likely to represent direct small molecule drug targets. How then can we find targets that are disease-specific, druggable, and that can be modulated with small-molecule-based drugs or other reagents to bring about a desired therapeutic effect. Here we review several strategies used to discover useful molecular targets for human genetic disorders.

## B. Direct Targeting of Disease Gene Products

Conceptually, the simplest approach to treating a genetic disorder is to modulate the function of a disease-causing gene product directly (Figure 1b), as illustrated by the use of the small molecule imatinib (Gleevec) to inhibit the constitutively active kinase produced by the *BCR-ABL1* fusion gene found in patients with chronic myeloid leukemia [12]. The number of disease-associated gene products considered druggable is small (see above) but continues to slowly expand. For example, in early surveys, the disease-associated E3 ubiquitin ligase Mdm2, which is amplified in many cancers, was thought to be undruggable [8]. It has since been shown that the crucial Mdm2-p53 binding interface can be disrupted by the nutlin family of small molecule inhibitors, leading to stabilization of p53 and cancer cell death [13] (Figure 2a). These results suggest that extensive searching of existing chemotypes may yield direct modulators of additional disease gene products.

Both re-screening of existing chemotypes, and *de novo* computationally-assisted drug design [14], will be facilitated by new models of protein structures and protein-interaction interfaces. For example, starting with an existing model of the Jak3 tyrosine kinase domain, Sayyah *et al* first used orthology modeling to develop a model of the Jak2 kinase domain, which is mutated in several cancers [15]. This model was then used to screen 20,000 known compounds *in silico* for those likely to bind adjacent to the ATP binding site and inhibit kinase activity [15]. This screen resulted in the identification of six candidate compounds, one of which, Z3, was subsequently shown to specifically inhibit Jak2 function in several cell culture and disease models [15] (Figure 2b). The application of similar approaches to other targets may greatly decrease the amount of actual screening that needs to be performed in the future, and help characterize novel direct modulators of disease gene product function.

## C. Exploiting the Functional Interconnectivity of Biological Systems to Find Alternate Druggable Targets

It is not always possible to target a disease gene product itself directly. However, normal and disease genes do not function in isolation: genes, gene products and metabolites interact with one another to form functionally interconnected genetic, protein and metabolic interaction networks of exquisite complexity [16–19]. Genetic diseases perturbing one or more genes alter the connectivity of these networks, as reflected in disease-specific patterns of gene expression, protein-protein interactions and metabolite production [20–22]. Changes in network connectivity induced by disease gene activity (or lack thereof) may expose unique genetic or chemical sensitivities due to a loss of biological redundancy, feedback regulation and/or the up- or down-regulation of alternate, druggable target genes [23–26]. If suitable drugs are available to modulate these indirect targets, it becomes possible to exploit acquired chemical sensitivities to achieve a desired phenotypic outcome, such as cancer-cell selective cell death [27–29]. Indirect targets can be identified using a number of approaches that embrace the functional connectivity of cellular networks (see below).

### Target selection guided by existing knowledge of disease-associated pathways

Tractable indirect targets can be selected using knowledge of disease-associated biological processes. A recent example involves the link between cancer, protein degradation and the cell

cycle. Cullin-RING type E3 ubiquitin ligases regulate the degradation of numerous cell cycle proteins. To function properly, these enzymes require post-translational modifications by the ubiquitin-like protein NEDD8. It was hypothesized that inhibiting the NEDD8 modification of Cullin-RING E3 ubiquitin ligases would specifically disrupt the proteolytic turnover of cell cycle proteins, thereby inhibiting the growth of tumor cells [30]. High-throughput small molecule screening was used to identify compounds specifically inhibiting the NEDD8-activating enzyme (NAE), which is required to conjugate NEDD8 to target proteins [30]. One small molecule, MLN4924, was identified as a potent inhibitor of NAE and shown to be effective in preventing the growth of tumor cell lines and human tumor xenografts in mice [30] (Figure 2c). This study illustrates the power of using existing knowledge to guide the selection of novel druggable targets.

## Target selection guided by systematic genetic and biochemical screens

The selection of candidate indirect targets need not be guided solely by educated guesswork; systematic high-throughput genetic and biochemical methods can be used to pinpoint targets functionally connected with disease genes. Follow-up studies can then assess the druggability of candidate ‘hits’.

For example, genome-wide RNAi libraries, based on endoribonuclease-prepared or chemically synthesized small interfering RNAs (esiRNAs or siRNAs), or virally-encoded short hairpin RNAs (shRNAs) [31–34], can be used in screens to identify synthetic lethal genetic interactions with cancer-specific mutations. This involves searching for gene products that, when depleted, cause lethality only in the presence of a second genetic alteration; this demonstrates a degree of functional connectivity between the depleted mRNA and the genetic change. One screen examined multiple shRNAs targeting 1,006 human genes, including 571 kinases, to identify genes that were synthetic lethal with oncogenic Ras alleles in two different cell lines [35]. This screen identified a single gene, *CSNK1E*, that when disrupted resulted in apoptosis in cancer cells, but not in matched non-tumorigenic cells [35]. Subsequently, it was demonstrated that inhibition of the protein product of this gene, casein kinase 1 epsilon, by the small molecule inhibitor IC261, recapitulated the effects of shRNA-mediated knockdown, suggesting that inhibition of this kinase may be an effective method to kill some tumor cells [35]. More recently, larger screens examining more cell lines [36] or a larger number of shRNAs [37] have been undertaken to find *KRAS*-specific synthetic lethal interactions. This work identified *STK33*, encoding a serine/threonine kinase, and *PLK1*, encoding a Polo-like kinase, as oncogenic *KRAS* synthetic lethal interactors in several different cancers. Notably, both genes are good candidate drug targets. One interesting application of this general approach is to tailor drug treatment to individual cancer patients based on cellular profiles of RNAi sensitivity [38].

In addition to genetic approaches, target selection can be guided by high-throughput biochemical profiling. Recently, the activation status of 46 tyrosine kinases was profiled in a large panel of cancer cell lines using a high-throughput, bead-based immunosandwich assay [39]. This screen determined that the non-receptor tyrosine kinase Src is activated in a large proportion of human tumor cell lines [39]. This was unexpected, because there is little evidence for mutation or amplification of the *SRC* gene in human cancers [39]. It was subsequently shown that the previously developed small molecule tyrosine kinase inhibitor Dasatanib could be used to inhibit Src activity and that this treatment killed glioblastoma tumors, where Src was active [39]. These results demonstrate the power of systematic functional studies to identify druggable, indirect targets. Crucially, functional screens allow for the identification of targets that would not be predicted from DNA sequencing alone.

### Target Identification Using Unbiased Phenotypic Screens

Complementary to the above approaches are those involving phenotype-based chemical screening. In this approach, a screen is conducted to identify a small molecule or other chemical perturbagen that yields a desired phenotypic outcome, such as cell death or an alternative cell fate. Importantly, small molecules can induce both loss- and gain-of-function phenotypes in target proteins, potentially broadening the number of observable phenotypes compared to perturbagens such as RNAi, which can only induce loss-of-function effects in targets. Follow-up studies can then determine the relevant protein target and mechanism of action. Overall, the most compelling advantage of this approach is that it can potentially identify useful drug leads directly.

**Cell death—**It is possible to identify small molecules that are synthetically lethal in cancer cells in the context of specific gene mutations. For example, screening of ~ 70,000 compounds using lung, bone and engineered cancer cells harboring oncogenic gain-of- function *KRAS*, *NRAS* and *HRAS* alleles, respectively, identified three compounds exhibiting tumor-cell selective synthetic lethality: erastin, RSL3 and RSL5 [40–42] (Figure 2d,e). The mode of cell death induced by these compounds is a novel form of oxidative death distinct from necrosis or apoptosis [40,42]. The cellular targets of two of these compounds (erastin and RSL5) were identified as mitochondrial Voltage Dependent Anion Channels 2 and 3 (VDAC2/3), which are upregulated by oncogenic Ras [40]. How erastin modulation of VDAC2 and VDAC3 brings about tumor-cell-specific cell death is under investigation, but appears to involve iron and mitochondrial respiratory activity, as well as VDACs [42]. VDAC2 and 3 represent examples of targets that would not have been selected as relevant to cancer *a priori*, because they themselves are not mutated in disease. Similar phenotype-driven approaches have recently been used to identify compounds synthetically lethal in *VHL* null and *KRAS* mutant cells [43, 44], suggesting that this approach will be useful for a broad range of mutations.

**Chemical Suppression—**Cell death is only one potentially useful phenotype. In other cases, it is sufficient or preferable to channel the cell to an alternate cell fate. One recent example involves synthetic interactions in a model of acute myelogenous leukemia (AML). In one form of AML, caused by the AML1-ETO oncogene, the differentiation of granulocytic blast cells is slowed, resulting in their overproduction, which in turn may serve as a source of stem cells contributing to cancer [45]. Recently, a screen of 2,000 bioactive small molecules conducted using a transgenic zebrafish model of AML1-ETO-induced AML determined that nimesulide, an inhibitor of the prostaglandin-endoperoxide synthase (PTGS) family of enzymes, suppressed AML1-ETO-oncogene-mediated cell fate transformation by preventing prostaglandin E2 synthesis [46] (Figure 2f). It was subsequently shown that a related compound could inhibit cell fate transformation in human cells expressing this oncogene [46]. In both zebrafish and human cells, PTGS enzymes are upregulated at the transcriptional level, possibly accounting for why they become useful targets [46]. This screen is also notable for the use of a live animal model, which allows for the effects of drugs on whole-animal development and physiology to be assessed. Chemical suppressor screens have also been used with cultured cells to identify small molecules that suppress cell death in models of neurodegenerative disorders, such as Huntington's disease and Parkinson's Disease [47–50], suggesting that phenotypic suppression may be a useful general approach to discovering small molecules with therapeutic potential.

## D. Conclusions & Future Directions

Finding new drugs to treat genetic disorders presents a grand challenge for 21st century medicine. No one existing approach is likely to be suitable in all cases and each has distinct disadvantages. It is conceivable that many disease genes will continue to elude efforts at direct

targeting with existing chemical libraries. Functional-connectivity-based approaches to target identification, even though guided by high-throughput genetic and biochemical screening technologies, may simply suggest undruggable proteins; thus improved chemical libraries will always be useful. The effectiveness of small molecules discovered through phenotype-based approaches will only be as good as the cellular models used to discover them, and drug-target identification remains a technical hurdle.

These concerns are being addressed in a number of ways. For example, new compound collections can provide access to previously uncharted regions of chemical space, potentially opening up the possibility of finding new direct or indirect modifiers for a given gene product or disease model. These libraries may be based on proven ligand scaffolds, or structures found in natural products or generated through novel small molecule synthetic routes [12,51–54] (Figure 3). Fragment-based screening [55] and *de novo* computational design [56] may also be helpful in broadening the number of targets that can be modulated (Figure 3). Non-small molecule-based reagents, such as stabilized, stapled peptide helices [57,58], cyclic peptides, peptide-like molecules, DNA and RNA aptamers [59] or even siRNA molecules, may also prove useful in some circumstances to inactivate the function of specific gene products (Figure 3). From a screening perspective, the development of new functional RNAi screening approaches, such as genome-wide pooled screens [36,60] and *in vivo* screens [61], is opening the door to comprehensive annotation of the role of most gene products and broadening the potential for finding useful candidate druggable genes. New proteomic methods to identify the targets of small molecule ligands should speed the process of characterizing the function of small molecules discovered in unbiased phenotypic screens [62]. Together, these improvements in small molecule library design, high-throughput target identification and functional connectivity screening should improve our ability to take full advantage of the druggable genome to treat disease.

## Acknowledgments

The authors thank Andras Bauer and David Clarke for comments on the manuscript. S.J.D. is supported by a postdoctoral fellowship from the Canadian Institutes of Health Research and by a grant to B.R.S. (R01CA097061). B.R.S. is funded by NIH (R01GM085081, R01CA097061), NYSTAR and the Arnold and Mabel Beckman Foundation.

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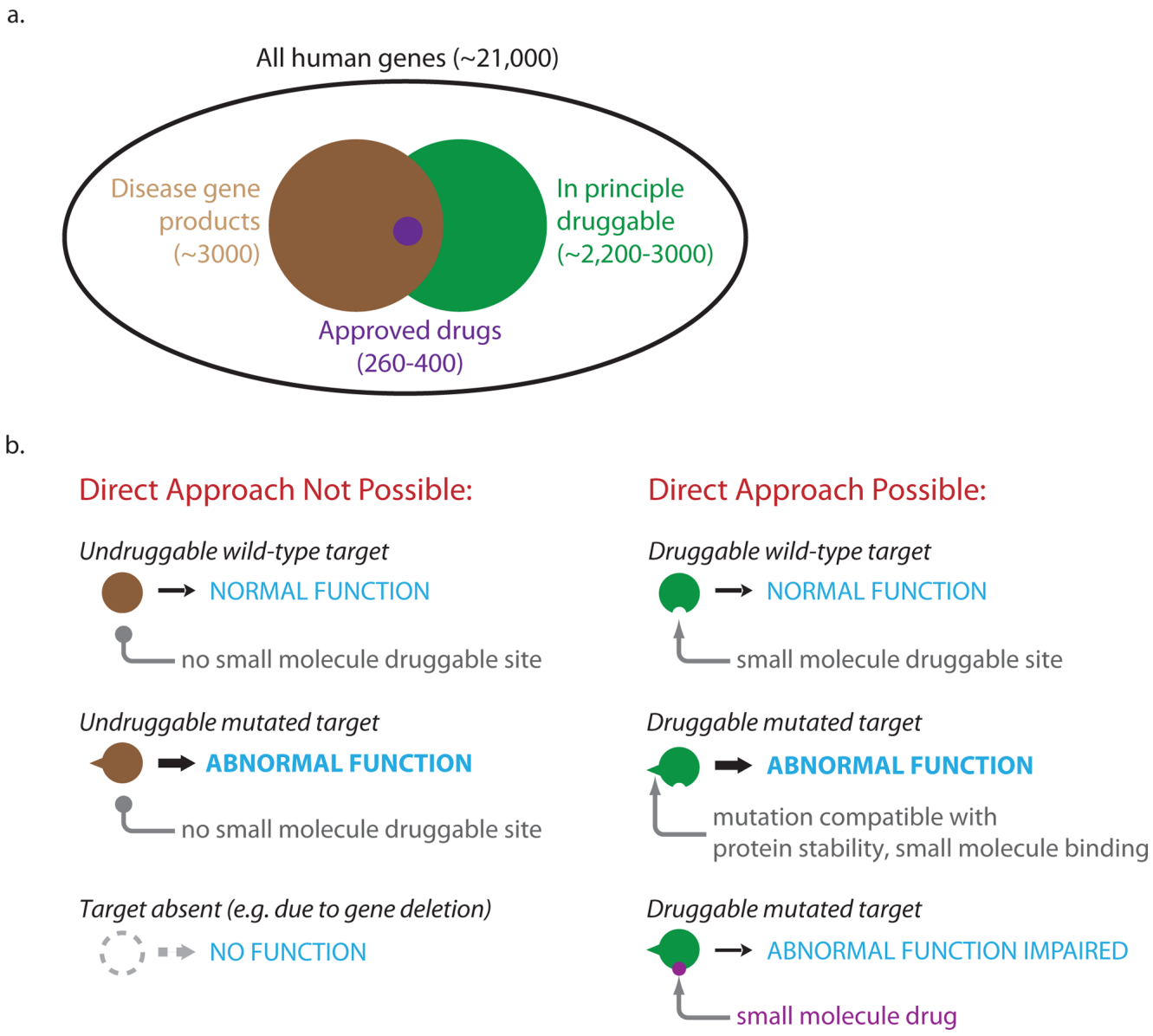


**Figure 1.**

The druggable genome in relation to disease. **a**. Venn diagram illustrating the relationship between all potential human proteins, those proteins that are in principle druggable (green), those proteins encoded by disease genes (brown), and those proteins targeted by approved therapeutics (purple). The size of the ovals approximates the number of gene products in each category. While not considered here, it should be noted that one gene may give rise to multiple gene products through alternate splicing. **b**. Cartoon depicting disease gene products that are in principle undruggable, either because a suitable drug-binding fold is not present or because the disease-causing mutation eliminates protein production, and gene products that are druggable (e.g. accessible to a small molecule modulator). Small molecule modulators of druggable targets could in principle act to either impair the abnormal function of a target resulting from a gain of function mutation, or restore the impaired function of a target resulting

from a partial loss of function mutation (not shown). Part (a) is in part adapted from Reference 11.

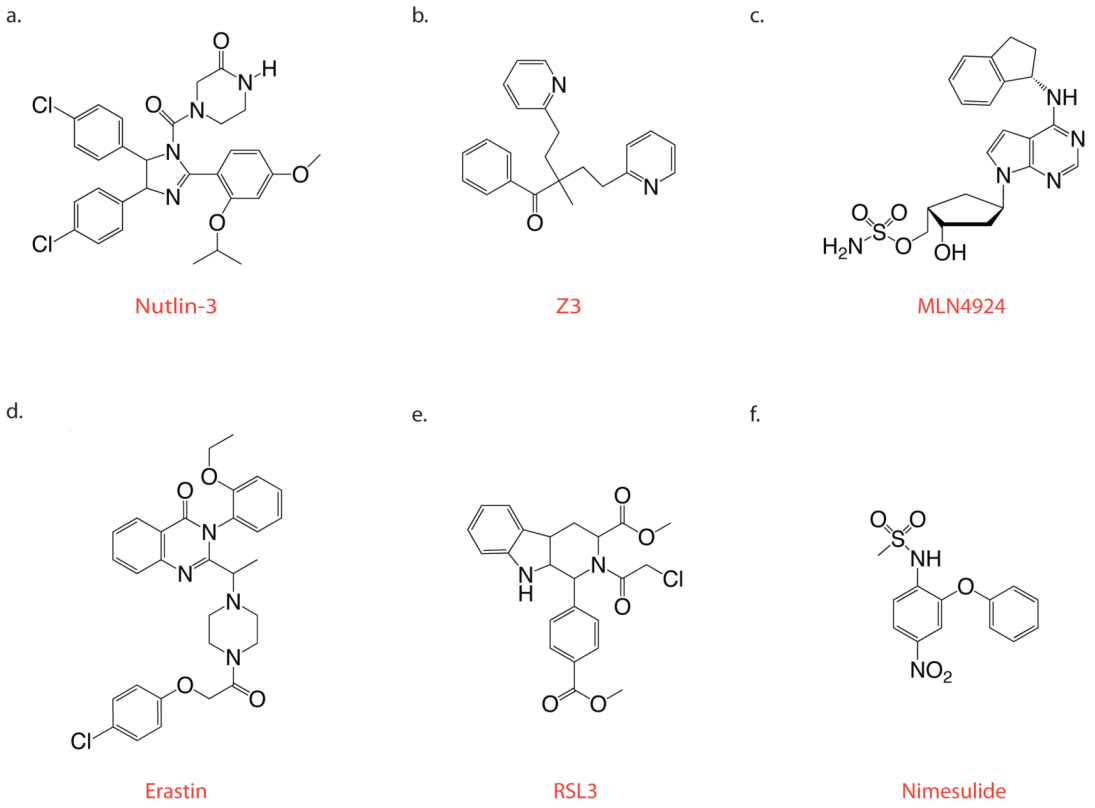


**Figure 2.**

Chemical structures of small molecule compounds discussed in the text. **a**. Nutlin-3 directly inhibits the binding of p53 to Mdm-2. **b**. Z3 is a Janus kinase (Jak) inhibitor discovered through *in silico* screening. **c**. MLN4924 is a novel anti-cancer agent that selectively inhibits the NEDD8 activating enzyme (NAE). **d**. Erastin is an oncogenic Ras-selective lethal compound discovered through unbiased phenotypic screening. Erastin kills tumor cells through binding to the mitochondrial voltage-dependent anion channels 2 and 3. **e**. RSL3 is functionally similar to erastin but structurally distinct. The RSL3 target is unknown. **f**. Nimesulide is active in a zebrafish model of AML, counteracting the effects of the AML1-ETO oncogene on granulocytic blast cell differentiation.

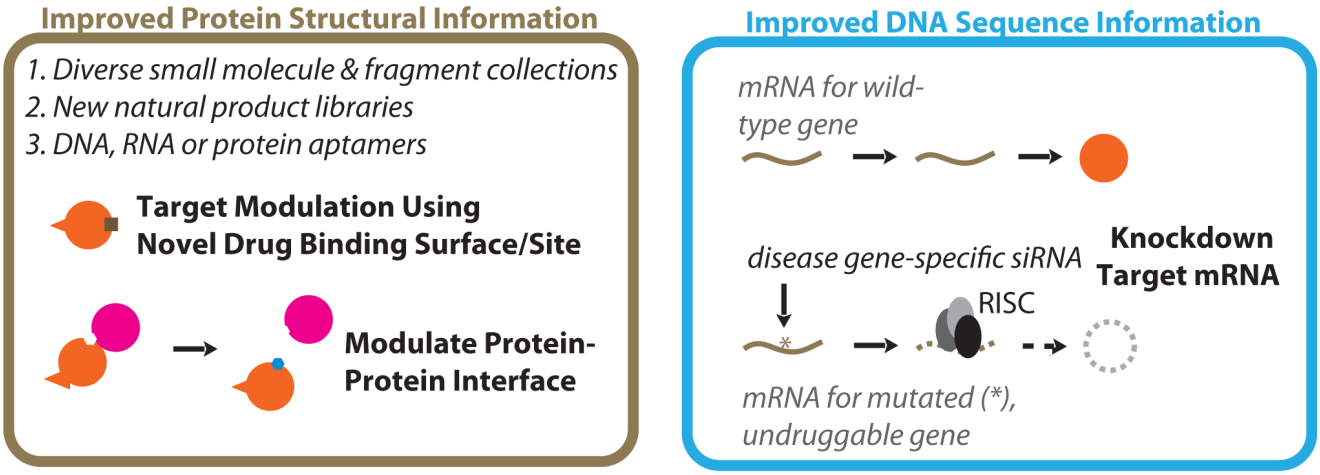


**Figure 3.**

Approaches to increase the size of the druggable genome, thereby facilitating both direct and indirect disease gene targeting. New chemical libraries, based on small molecule, fragment, or aptamer approaches may allow new protein folds or interaction interfaces to be targeted. In cases where these approaches fail, purely genetic approaches, involving the delivery of siRNAs directly to cells to silence mRNA expression may prove availing. RISC = RNAi-induced silencing complex, which binds and cleaves siRNA-mRNA double-stranded RNA duplexes.