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Review

Single-Cell Techniques and Deep Learning in Predicting Drug Response

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Rapidly developing single-cell sequencing analyses produce more comprehensive profiles of the genomic, transcriptomic, and epigenomic heterogeneity of tumor subpopulations than do traditional bulk sequencing analyses. Moreover, single-cell techniques allow the response of a tumor to drug exposure to be more thoroughly investigated. Deep learning (DL) models have successfully extracted features from complex bulk sequence data to predict drug responses. We review recent innovations in single-cell technologies and DL-based approaches related to drug sensitivity predictions. We believe that, by using insights from bulk sequence data, deep transfer learning (DTL) can facilitate the use of single-cell data for training superior DL-based drug prediction models.

Highlights

A comprehensive understanding of heterogeneous tumor subpopulations will benefit drug sensitivity prediction and combination drug treatment design.

Deep learning models are powerful and extensively used in drug sensitivity prediction and in inferring drug–target interactions.

Single-cell sequencing techniques offer precise and accurate profiling of tumor subpopulations and reveal subtle differences in their response to drug treatments.

Applying deep transfer learning to predict drug sensitivity allows us to not only take advantage of prior knowledge obtained from massive bulk sequencing data but also utilize the heterogeneous landscapes generated by single-cell sequencing techniques.

The integration of single-cell multi-omic data for drug sensitivity prediction using transfer learning methods poses a special challenge.

Overview of Current Research Methodologies for Investigating Drug Resistance

Treatments for cancers have undergone major advances with the development of molecularly targeted therapy, immunotherapy, chemotherapy, and radiotherapy [1]. Targeted drugs – monoclonal antibodies and small molecules that are used by molecularly targeted therapies – achieve the highest level of cytotoxicity in tumors because they are able to precisely target cancer cells [2]. The availability of these treatments has dramatically improved patient prognoses. Moreover, if the feature targeted by drug treatment is shared by all the tumor subpopulations, the targeted drugs can produce complete remission of the disease. However, cancerous tumors are rarely homogenous. They are composed of diverse cell subpopulations demarcated by distinct genomes and transcriptomes, each of which can yield a unique response and sensitivity to a given drug [3]. As a result, heterogeneous cancer subpopulations with extraordinarily dynamic characteristics often exhibit resistance to single-drug treatments, preventing complete eradication of the disease [4]. Once the vast majority of the tumor has been eliminated, a small number of remaining cancerous cells – **minimal residual disease** (**MRD**, see Glossary) – survive and continue proliferating [4]. The inevitable relapse features a disease now largely resistant to the initial treatment. In addition to some tumor subpopulations that possess inherent resistance to select treatments, cancer cells can also acquire resistance via multiple mechanisms such as **drug inactivation**, **target alternation**, and **drug efflux** [1,5,6]. Insensitivity to treatment is now responsible for up to 90% of cancer-related deaths [7]. Thus, it is imperative to increase our understanding of the mechanisms by which resistance is propagated, and accurately predict which **combinational drug treatment** will be the most effective against specific cancers.

High-throughput sequencing techniques such as DNA-sequencing, RNA-sequencing, **assay for transposase-accessible chromatin with high-throughput sequencing** (**ATAC-seq**), and **ChIP-seq** can characterize the genomic, transcriptomic, and epigenetic landscapes of tumors. These profiles are indispensable for gleaning insight into resistant tumors. For example, whole-genome characterization of resistant ovarian cancer by DNA-seq directly led to the identification of tumor repressors that, when inactivated, result in **drug resistance** [8]. Advances in single-cell techniques, including **single-cell DNA sequencing (scDNA-seq)** and **single-cell RNA sequencing (scRNA-seq)**, have enabled scientists to analyze the genomic and transcriptomic

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profiles of individual cells. Such innovation allows researchers to better investigate cancer heterogeneity and deduce the culprits of drug resistance. Moreover, the findings produced by studying these concepts facilitate **drug sensitivity** predictions for independent cancer subpopulations. For example, the key regulators (*KDM5A/B*) of therapeutic resistance in breast cancer subsets have been identified via scRNA-seq and bulk ChIP-seq [9].

Deep learning (DL) models have also successfully predicted **drug responses** (Figure 1, Key Figure). However, vast amounts of genomic and transcriptomic data are necessary to produce meaningful and generalizable DL prediction tools. Many DL models have benefited from the vast libraries of drug-, protein-, and gene-related data from many disease (sub)types that are available in the public domain. Specifically, databases such as The Cancer Genome Atlas, Genomics of Drug Sensitivity in Cancer [10], The Cancer Cell Line Encyclopedia [11], Cancer Target Discovery and Development [12], and the University of California, Santa Cruz TumorMap [13] are all commonly utilized to train DL models.

Even though single-cell sequencing can enhance the resolution by which heterogeneity is studied, the data generated by these techniques are of large volume and high complexity. The copious number of cells increases the dimensionality and scale of the data. Both make it challenging to use single-cell sequence data to connect the distinct modalities they encode and construct heterogeneous biological networks. However, recent work in classifying cell types has highlighted DL architectures which can process the large-scale single-cell data and effectively extract high-dimensional features. Nevertheless, the small amount of data available at single-cell resolution currently precludes the active development of DL models for drug-related predictions.

DL Models Can Accurately Predict Drug–Target Interactions and Drug Sensitivity

Using publicly available data, DL models have boasted modest success in predicting the sensitivity of a novel cell type to drug treatment options. DL models use artificial networks to emulate

Key Figure

Combining Single-Cell and Deep Learning (DL) Models in Drug Sensitivity Prediction

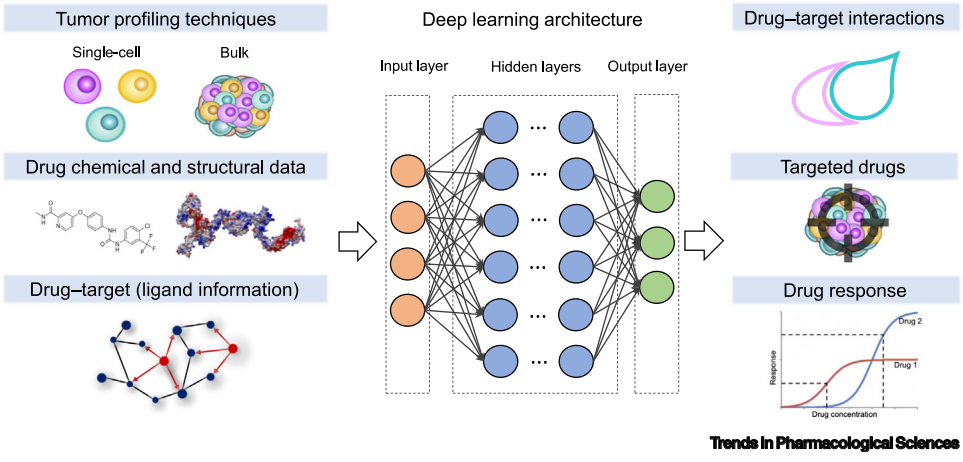

Figure 1. DL models are typically trained on tumor profiles, chemical and structural information of drugs, and drug–target data. By extracting high-dimensional features through multilayer perceptrons, DL models can infer drug–target interactions, purpose new drugs, and predict drug resistance.

Glossary

ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing): a technique to determine chromatin accessibility across the genome.
Batch effect: external factors associated with experiments can influence the data produced and result in inaccurate conclusions. This effect represents the systematic technical differences when samples are processed and measured in different batches.
Bulk sequencing: examines the sequence information of bulk samples, usually containing multiple cells.
Cell type-specific regulon: the full complement of transcriptional targets that are regulated by a protein. These can include either direct physical targets, transcription factors and cofactors, or indirect targets for signal transduction.
ChIP-seq: chromatin immunoprecipitation with high-throughput sequencing, a technique to identify genome-wide binding sites in DNA for transcription factors and other proteins.
Combinational drug treatment: the use of more than one drug to treat a disease; this usually reduces the development of drug resistance.
Deep belief network (DBN): a network constructed on several layers of restricted Boltzmann machines.
Deep learning (DL): an artificial intelligence function that mimics the workings of the human brain in processing unstructured data through many layers of neural networks.
Drug efflux: cells express efflux pumps that are able to move drugs out of the cell.
Drug inactivation: cancer cells may express enzymes to break down or modify drugs, leading to their loss of function.
Drug resistance: a reduction in the effectiveness of a medication, such as an antimicrobial or an antineoplastic, in treating disease.
Drug response: the pharmacodynamic (PD) response to a drug; this includes all the effects of the drug on any physiological and/or pathological processes.
Drug sensitivity: the concentration of a drug that inhibits cell growth.
Fluorescent *in* situ hybridization (FISH): a molecular technique that uses fluorescent probes that can specifically bind to DNA/RNA/proteins to visualize the location of those targets.

biological neural networks and learn patterns from the source data. DL has gained popularity owing to its ability to analyze and extract insights from exceedingly large amounts of unstructured or unlabeled data [14]. The insights derived from these networks can be built into models that are used for a variety of tasks, including data denoising, cell clustering, phenotype prediction, and image processing. The unstructured and multidimensional datasets produced by high-throughput sequencing have been utilized by DL models to predict drug sensitivities in cancer and other diseases [15]. Numerous DL models have been developed to make these predictions. The most common classes of DL models tasked with evaluating anticancer treatment options have been deep neural networks (DNN), convolutional neural networks (CNNs), recurrent neural networks (RNNs), and graph convolutional networks (GCNs) (Box 1). Specifically, Table 1 presents a nonexhaustive overview of DL approaches that have been used to address current limitations in predicting drug efficacy.

As the table highlights, DNN is one of the most popular DL architectures for predicting drug–target interactions (DTIs) using both existing and novel molecules. For example, DeepDTIs employed a **deep belief network (DBN)** model to extract features from drug–target data in the DrugBank database [16] and predicted the interaction likelihood of drug–target pairs [17]. However, DeepDTIs creates inferences that introduce unwanted noise into the model, reducing its performance. Three additional DL models have since been built, claiming to outperform DeepDTIs: (i) DeepCPI, another DNN-based model, showed superior predictive performance and scalability for large-scale compound affinity data [18]; (ii) DeepAffinity predicts compound–protein interaction but utilizes RNN and GCN models to capture long-term, nonlinear dependencies among residues/atoms in proteins/compounds [19]. DeepAffinity also employs a DTL framework to facilitate the enhancement in predicting DTIs from limited labeled protein–compound interaction data compared with DeepDTIs; and DeepConv-DTI captures local residue patterns of proteins participating in DTIs and detects the binding sites of DTIs [20]. DeepConv-DTI uses a CNN model which is also relatively successful at extracting meaningful features from sparse interactions, making it an ideal model for predicting DTIs. One reason for the enhanced performance of DeepConv-DTI over

Immune checkpoint blockade (ICB): immune checkpoints are accessory molecules that regulate the activation and silencing of T cells. ICB treatment can release inherent limits on the activation and maintenance of T cell effector function by inhibiting the immune checkpoints.
Minimal residual disease (MRD): the small number of cancer cells that survive drug treatment and usually result in relapse.
Single-cell B/T cell receptor sequencing (scBCR/TCR-seq): a genomic method for analyzing B/T cell receptors that are uniquely expressed on the B/T cell surface. The diverse range of BCRs/TCRs expressed by the total B/T cell population of an individual is termed the B/T cell receptor repertoire.
Single-cell RNA/DNA sequencing (scRNA-seq/scDNA-seq): examines sequence information from individual cells with optimized next-generation sequencing technologies, providing higher resolution of cellular diversity.
Target alternation: cancer cells may modify or downregulate the expression of proteins that are targeted by drugs.
Visualized neural network (VNN): an analytical method that can simulate not only system function but also system structure.

Box 1. DL Models and Frameworks

Four DL models that have been applied in drug prediction are DNN, CNN, RNN, and GCN (Figure IA). These are discussed here.

DNN

This is a feed-forward neural network consisting of input, hidden, and output layers that are densely connected. For drug sensitivity prediction, it is necessary to construct a function from tumor drug profiles to sensitivity. During the model training, the hidden layers are able to perform data abstractions and transformations that identify parameters in the function.

CNN

Operates in a manner inspired by the human visual cortex; it consists of convolutional (feature extraction) and pooling (dimension reduction) layers. The convolution and pooling layers help to extract all information in the datasets without consuming too much time or computational sources.

RNN

A type of artificial neural network in which connections between nodes form a directed graph along a temporal sequence.

GCN

A type of neural network architectures that can leverage the graph structure and aggregate node information from neighbors in a convolutional fashion. It has natural advantages when dealing with graph-based data.

Two further frameworks, DTL and VAE, can be combined with DL models (Figure IB). DTL transfers store knowledge gained while solving one problem and applying it to a different but related problem. VAE consists of an encoder, a decoder, and a loss function. The encoding distribution is regularized by a standard deviation vector and a mean vector during the training to ensure that its latent space has adequate properties for generating new data.

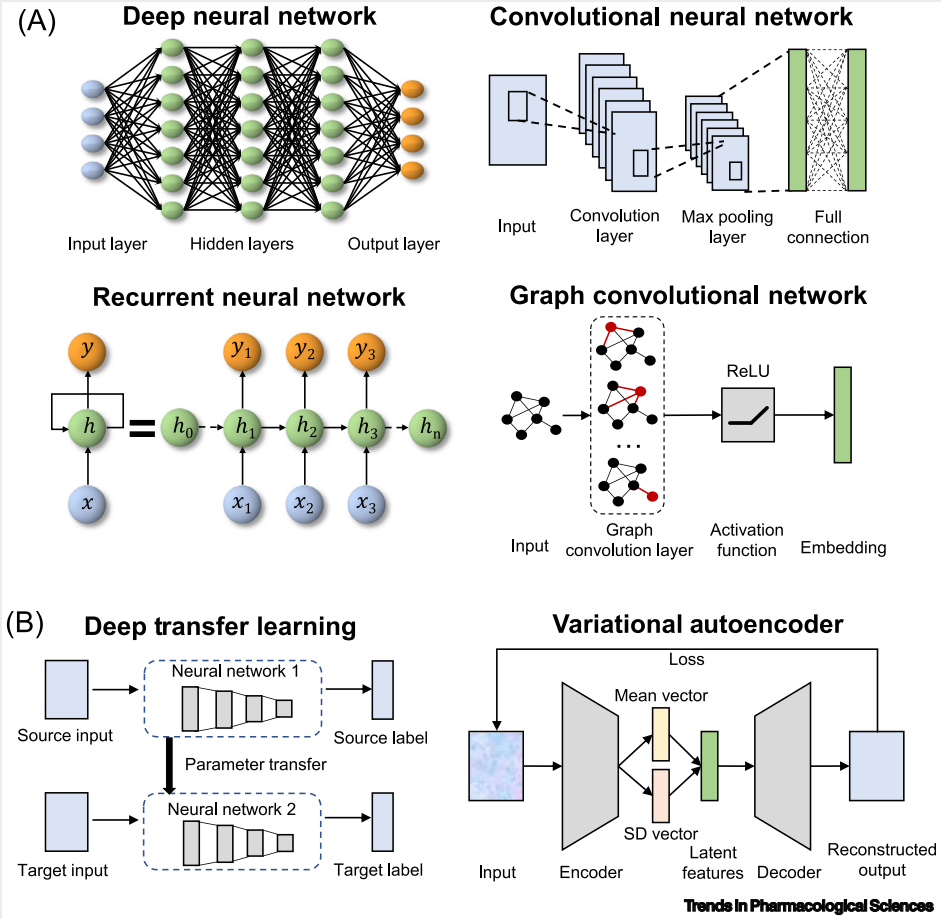

Figure I. Scheme of Deep Learning (DL) Models and Frameworks. (A) Four DL models that have been applied in drug prediction, including deep neural network (DNN), convolutional neural network (CNN), recurrent neural network (RNN) (x is the input layer, h is the hidden layer, y is the output layer), and graph convolutional network (GCN). (B) Two frameworks that can be combined with DL models. Abbreviations: ReLU, rectified linear units; SD vector, vector of the standard deviations.

DeepDTIs is that the DeepConv-DTI CNN architecture does not require entire structure or sequence data to learn DTIs [17,20]. In other words, the model in DeepDTI learns from sequences not involved in DTIs; these irrelevant features detract from its prediction accuracy. AtomNet [21] also implements a CNN model to analyze feature locality and hierarchical composition to predict the bioactivity of small-molecule DTIs. DEEPScreen takes advantage of CNN's excellence in image analysis to predict DTIs from simple 2D images of compounds [22]. DeepChem does this also, but is built on a GCN model instead [23]. DeepAMPEP30 is another published CNN-based model. Instead of identifying DTIs from known drugs and proteins, this model suggests novel drugs that have potential to target affected cells [24]. Specifically, DeepAMPEP30 proposes short peptide sequences that exhibit optimized antimicrobial properties and can be used as a targeted treatment for a wide breadth of diseases, including bacterial infections and cancers. In a proof-of-concept experiment, some of the proposed peptides were as effective as ampicillin at treating multiple bacterial infections [24].

In addition to predicting DTIs, the aforementioned models demonstrate a capacity for inferring novel drug indications or novel uses for existing drugs. This practice is referred to as drug

Table 1. DL Approaches and Examples in Predicting Drug Treatment Efficacy

Model	Tools	Input data	Purpose	Refs
Drug–target interactions				
DBN	DeepDTIs[i]	Drug–target pair information, drug structure, and protein sequence	The probability of interaction for any given drug–target pair was inferred by DeepDTIs based on external, experimental drug–target pairs. Among the top 10 predicted drug–target interactions, four had been previously reported, and one was found to have a low binding affinity for the glucocorticoid receptor.	[17]
DNN	DeepCPI[ii]	Drug structure and protein sequence for drug–target pairs	Drug–target interactions were predicted by DeepCPI. The inferred interactions between small molecules and glucagon-like peptide 1 receptor, glucagon receptor, and vasoactive intestinal peptide receptor were experimentally validated.	[18]
DNN	DeepDTnet[iii]	Drug–target pair information, drug similarity, and target similarity	DeepDTnet can identify targets of known drugs using a heterogeneous drug–gene–disease network embedding 15 types of chemical, genomic, phenotypic, and cellular network profiles. A new, direct inhibitor of human retinoic acid receptor related orphan receptor γt, topotecan, was predicted by deepDTnet, and then experimentally validated by the authors.	[26]
CNN	DeepConv-DTI[iv]	Raw protein sequences and drug–target pair data	Local residue patterns from proteins in drug–target interactions were captured by DeepConv-DTI via the convolution on various lengths of amino acid subsequences. This model achieved higher accuracy than DBN-based DeepDTI and CNN-based DeepDTA.	[20]
CNN	DEEPScreen[v]	2D structure of compounds and protein structure	Drug–target interactions were predicted based on 704 target proteins and the 2D structure of compounds. Janus kinase (JAK) proteins were predicted by the model as new targets of drug cladribine and experimentally validated *in vitro*.	[22]
CNN	AtomNet	3D structure of target proteins and small molecules	Applying local convolutional filters to extract target structural information, AtomNet successfully predicted new active molecules for targets such as wee1 and 1qzy that previously had no known modulators.	[21]
GCN	DeepChem[vi]	Compound structure	GCN and long short-term memory were employed to optimize small molecule-based drug discovery by predicting the toxicity and bioactivity of candidate drugs using their structural data.	[23]
RNN CNN GCN DTL	DeepAffinity[vii]	Raw protein sequences and compound sequences (2D information)	Bidirectional RNN was utilized to capture nonlinear joint dependencies among either protein residues or compound atoms that are sequentially distant. DeepAffinity unified RNN-CNN/RNN-GCN to predict drug–target interactions. The model outperformed conventional models in achieving relative error in the half-maximal inhibitory concentration within fivefold for test cases and 20-fold for protein classes not included in training.	[19]
DNN	Deep-AmPEP30[viii]	Genomic sequence data and known antimicrobial peptide (AMP) sequences	AMPs to treat a variety of diseases such as cancer and infections were identified based on known AMP sequences. One peptide (FWELWKFLKSLWSIFPRRRP) the model produced proved to have the same antibacterial efficacy as ampicillin.	[24]
Drug repurposing				
DNN		Transcriptional response to drug exposure	The therapeutic categories of drugs were exclusively identified from transcriptional profiles; 26 420 drug perturbation samples were analyzed for three cell lines and then assigned to one of 12 therapeutic categories.	[29]
VAE	deepDR[ix]	Drug-disease pairs and drug chemical information	Fourteen candidates of the top 20 drug candidates to treat Parkinson's disease predicted by deepDR were validated by previous studies.	[28]
Drug response				
DNN	RefDNN[x]	Drug structure and gene expression data before drug exposure	RefDNN learned representations for a high-dimensional gene expression vectors and molecular structure vectors of drugs to predict drug response; then labeled and identified biomarkers contributing to drug resistance. Among the top 10 genes identified by RefDNN, six (high expression patterns of *MYOF*, *UBC*, *NQO1*, and *LGALS3*, and low expression patterns of *RACK1* and *RPS23*) were experimentally proven to be associated with nilotinib resistance.	[30]
DNN VAE	DeepDR	Genomic and transcriptomic profiles before and after drug treatments	The drug response of tumors was predicted from integrated genomic profiles. Specifically, DeepDR improves the prediction of drug response and the identification of novel therapeutic options. The model was applied to predict drug response in 9059 tumors from 33 cancer types. The resulting predictions include known therapies, such as epidermal growth factor receptor (EGFR) inhibitors in non-small cell lung cancer and tamoxifen in breast cancer, as well as novel drug targets such as vinorelbine for TTN-mutated tumors.	[33]

Table 1. (continued)

Model	Tools	Input data	Purpose	Refs
DNN RNN CNN		HIV genome sequence and drug sensitivity data	HIV-related drug resistance was predicted by three DL models. Of the 20 most important features predicted by the models, 18, 9, and 16 known drug resistance mutation positions were identified by using CNN, DNN, and RNN models, respectively.	[32]
VNN	Dcell[xi]	Genotype data	The phenotypic resulting from individual gene perturbations in eukaryotic cells was transparently simulated. During the simulation, 80% of growth prediction was captured by 484 subsystems.	[31]
Drug–drug interactions				
DNN	DeepSynergy[xii]	Drug chemical data and transcriptional data	DeepSynergy distinguished between different cancer cell lines and found specific drug combinations with maximal efficacy against a given cancer cell line through the incorporation of genomic information with compound information.	[34]
GCN	Decagon[xiii]	Drug–drug interaction, protein–drug interaction, protein–protein interaction, and side effects	Decagon constructed a large two-layer multimodal graph of protein–protein interactions, drug–target interactions, and drug–drug interactions to predict the potential side effects of drug pairs. Decagon accurately predicted polypharmacy side effects, outperforming baselines by up to 69%. It had the best performance in modeling side effects with strong apparent molecular underpinnings; for example, in mumps and carbuncle.	[84]
Other				
CNN	DeepMACT[xiv]	3D image data	DeepMACT performed image recognition to track the biodistribution of antibody-based agents. Trained on an MDA-MB-231 cancer cell-based tumor model, DeepMACT has 80% accuracy to detect metastasis.	[85]

repurposing. DeepCPI, for example, highlighted several drugs as candidates to be repurposed for neural pharmacology [18]. One such drug, oxazepam, is traditionally used to treat alcohol withdrawal. DeepCPI found that it might also impact on intramitochondrial cholesterol transfer, implicating its potential application in the treatment of Alzheimer's disease [18,25]. In another attempt to enhance drug repurposing efforts by predicting DTIs, Zeng *et al.* proposed deepDTnet: a DNN for graphical representation [26]. The model created a drug–gene–disease network that successfully predicted topotecan – traditionally used to treat ovarian and lung cancers – as a drug repurposing candidate to treat multiple sclerosis. Specifically, deepDTnet found that human retinoic acid receptor orphan receptor γt, whose overexpression can lead to the development of multiple sclerosis, can be inhibited by topotecan [26]. Similarly to deepDTnet, arbitrary-order proximity embedded deep forest (AOPEDF) is also proposed for drug–gene–disease network prediction using a deep forest model [27]. Although this model is not expressly a DL model, we have included it in our discussion because it offered better performance than deepDTnet. In addition, AOPEDF requires fewer manual adjustments of the parameters controlling the model's learning process than DNNs, such as deepDTnet, to achieve high performance [27].

Although DTI models can be applied to drug repurposing research, other approaches have been devised for this exclusive purpose. For example, a DNN-based model, deepDR [28], employs a variational autoencoder (VAE) framework to extract high-dimensional features from low-dimensional representations of drugs, and reported drug–disease pairs and inferred candidates for repurposing (Box 1). Aliper *et al.* also used a DNN model to classify known drugs into therapeutic categories based on the effect that drug exposure has on gene expression [29]. The team found that 'misclassified' drugs might not be misclassified, but instead represent novel drug indications. Two such 'misclassified' drugs, otenzepad and pinacidil, support their hypothesis [29]. These drugs were classified as central nervous system drugs; however, to date, both are exclusively used to treat cardiovascular conditions. That said, previous studies provide evidence confirming that both drugs might also impact on brain function. For instance, although pinacidil targets K channels in the treatment of cardiovascular diseases, K channels

are also extremely prevalent in the brain and are required for signal transduction [29]. The prevalence of K channels in the brain would make pinacidil an excellent candidate to treat some neural conduction disorders.

Once novel drug treatment options have been identified, it becomes necessary to evaluate and predict disease response and sensitivity to each treatment. Multiple DL models, including RefDNN, have been produced to make these predictions. RefDNN identified drugs capable of improving the sensitivity of hepatocellular carcinoma (HCC) to sorafenib, the only approved treatment for HCC [30]. Sorafenib typically elicits only a modest response in HCC; however, the proposed drugs were experimentally validated to either synergize with sorafenib or target other key HCC regulatory pathways. Dcell, another DL model, predicts drug responses via a **visualized neural network (VNN)**. Although it is a unique approach, the VNN allowed Ma *et al.* to glean novel biological perspectives [31]. Dcell predicts the impact that modulating gene function has on cell phenotypes, and depicts the underlying mechanisms and pathways through an easily interpreted hierarchal visualization [31]. Dcell is a notable improvement in terms of the transparency and interpretability of DL models.

Although more traditional DL models have enabled researchers to glean biological insights and predict drug responses, these models are not transparent. The underlying mechanisms producing these insights often remain obscured by the black boxes through which DL models make their predictions until they are ascertained by either *in vitro* or *in vivo* experimentation. DL models have also been limited by the suboptimal accuracy of drug sensitivity predictions in diseases with high mutation rates, including HIV and cancers [32]. This is due in part to the lack of relevant data that are necessary for DL models to create generalizable inferences [30,32,33]. For these hypermutable disorders, heterogeneous disease states can also influence the accuracy of DL models. Currently, many DL models are trained using **bulk sequencing** data, but these have insufficient cellular resolution for effectively analyzing complex heterogeneity. Models, such as DeepSynergy, which attempt to identify combinations of drugs that maximize treatment sensitivity in all tumor subpopulations while minimizing systemic side effects, could indubitably benefit from enhanced resolution [34].

Single-Cell Technologies Discern the Effect of Heterogeneity on Drug Resistance

Cellular heterogeneity inferred from single-cell data can greatly facilitate the accurate prediction of drug sensitivity and help the design of combinational drugs. Although still a relatively new technology, researchers are using single-cell technologies with an increasing frequency to study heterogeneous disorders. This increased popularity reflects the ability of single-cell sequencing to capture subtle differences in genomic and transcriptional states of heterogeneous subpopulations, whereas bulk sequencing merely produces an aggregate estimate of gross cellular features [35]. Moreover, the high-resolution data provided by single-cell technologies allow researchers to harness single-cell sequencing [36] to individually profile the genomic and transcriptomic heterogeneity of cells within tumor subpopulations. Gene subnetworks identified from scRNA-seq profiles can be highly correlated with a patient's survival and drug response to cancer [37]. A nonexhaustive list of the recent applications of available single-cell technologies is given in Table 2 to provide examples of what methodologies scientists have at their disposal to investigate the intricacies of single-cell environments.

Traditionally, single-cell technologies have primarily been utilized to characterize distinct cell types [9,38]. scRNA-seq has been the obvious candidate for such tasks because distinct cell types possess unique transcriptional profiles [38]. Both scRNA-seq and scDNA-seq have been employed to characterize the distinct cell subpopulations within tumors [38–42]. ScDNA-seq is

Table 2. Overview of Available Single-Cell Technologies to Study Drug Resistance in Heterogeneous Disorders

Technology	Purpose	Refs
Targeted drugs		
scDNA-seq	scDNA-seq was performed on 510 circulating tumor cells and 189 leukocytes. Microheterogeneity analysis of individual CTCs discerned cells present before drug exposure that were resistant to *ERBB2*-targeted therapies.	[40]
scRNA-seq Bulk ATAC-seq	scRNA-seq of paired drug naïve and resistant acute myeloid leukemia patient samples highlighted regulators of enhancer function as important modulators of the resistant cell state. Inhibition of Lsd1 facilitated the binding of pioneer factor and cofactor to nucleate new enhancers, overcoming stable epigenetic-derived resistance.	[45]
CROP-seq	Pooled CRISPR screening was combined with scRNA-seq to facilitate high-throughput functional dissection of complex regulatory mechanisms and heterogeneous cell populations. *ETS1*, *RUNX1*, and *GATA3* were found to be essential for Jurkat T cell function.	[66]
Single-cell FISH	Single-cell FISH visualized transcriptional variability at the single-cell level; this was used to predict drug resistance development. It was found that the addition of drugs induces epigenetic reprogramming in some cells, converting a transient transcriptional state to a stable state. Reprogramming began with a loss of *SOX10*-mediated differentiation followed by activation of new signaling pathways, partially mediated by Jun/AP-1 and TEAD transcription factors.	[43]
sci-Plex scRNA-seq	'Nuclear hashing' was used to quantify global transcriptional responses in thousands of independent perturbations at a single-cell resolution. sci-Plex was employed to screen three cancer cell lines exposed to 188 compounds. Approximately 650 000 single-cell transcriptomes across ~5000 independent samples were profiled in one experiment. The similarity in the single-cell transcriptomes following treatment with distinct compounds highlighted drugs that target convergent molecular pathways.	[35]
scRNA-seq	A candidate tumor cell subgroup associated with anticancer drug resistance was identified using scRNA-seq on viable patient-derived xenograft (PDX) cells. Fifty tumor-specific single-nucleotide variations were observed to be heterogeneous in individual PDX cells after performing scRNA-seq on 34 PDX tumor cells from a lung adenocarcinoma patient. PDX cells that survived *in vitro* anticancer drug treatment displayed transcriptome signatures consistent with the group characterized by $KRAS^{G12D}$.	[39]
scRNA-seq	A total of 4645 cells isolated from 19 patients, including malignant, immune, stromal, and endothelial cells, were profiled using scRNA-seq. Malignant cells within the same tumor displayed transcriptional heterogeneity associated with drug resistance. Analysis of tumor-infiltrating T cells revealed exhaustion mechanisms that were connected to T cell activation and clonal expansion, and their variability across patients.	[41]
RT-qPCR	Single-cell RT-qPCR of the luminal-type breast cancer cell line MCF7 and its derivatives, including docetaxel-resistant cells, showed that epithelial-to-mesenchymal transition and stemness-related genes were upregulated in drug-resistant cells and cell cycle-related genes were downregulated. Both were primarily regulated by *LEF1*.	[46]
Microfluidic platform	An integrated microfluidic platform was built to construct single-cell arrays that could analyze drug resistance. A proof-of-concept experiment was implemented by determining the vincristine resistance of single glioblastoma cells with different biomechanical properties. The results indicated that the biomechanics of tumor cells has significant implications for cell drug resistance.	[86]
MULTI-seq	MULTI-seq (multiplexing using lipid-tagged indices for single-cell and single-nucleus RNA sequencing) reagents were shown to be able to barcode any cell type or nucleus from any species provided that there was an accessible plasma membrane.	[69]
Single-cell barcoding	Transient transfection with short barcode oligonucleotides simultaneously analyzed multiple samples with scRNA-seq. The accuracy of the method was validated and its ability to identify multiplets and negatives was confirmed by analyzing samples from a 48-plex drug treatment experiment.	[74]

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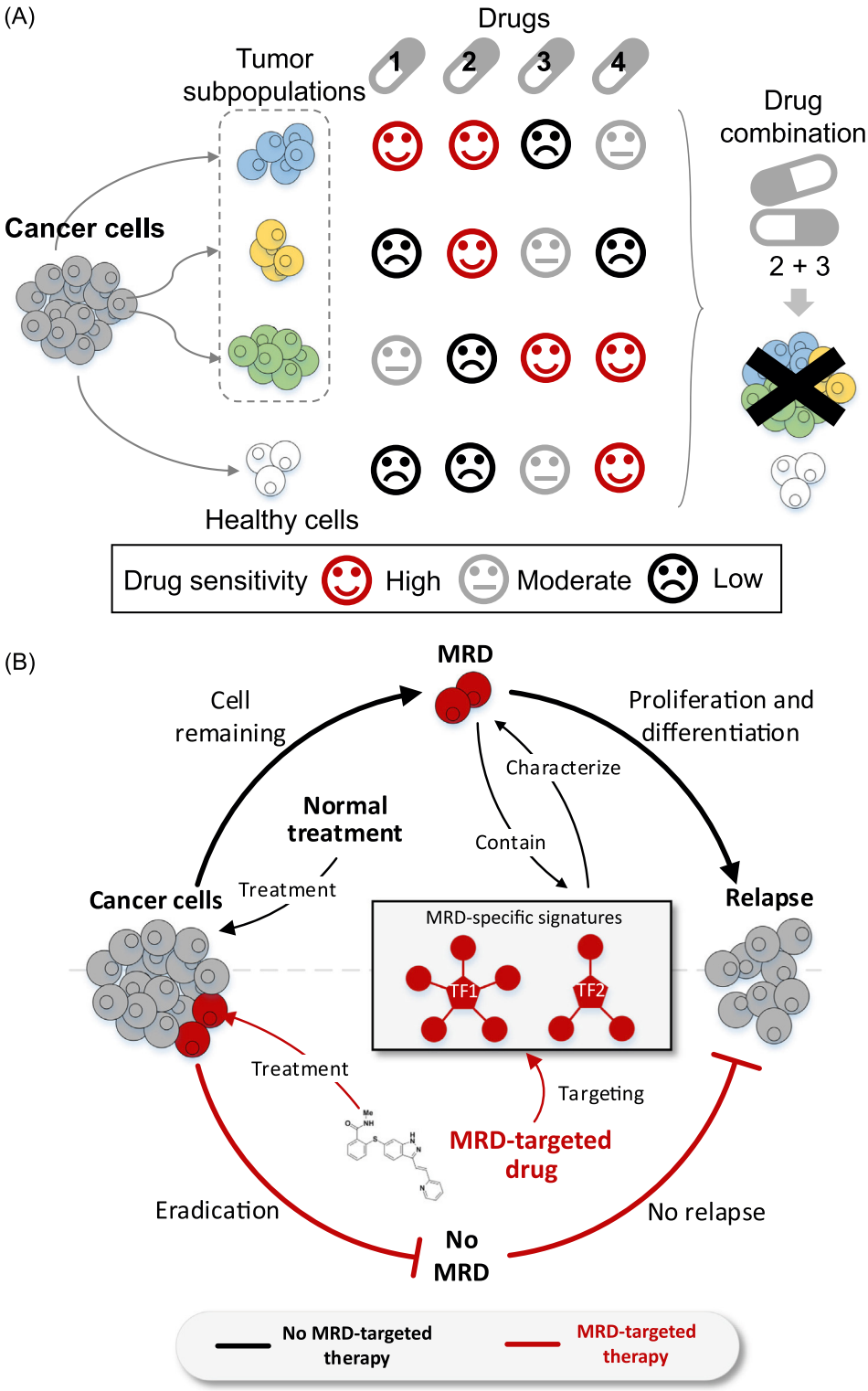
Table 2. (continued)

Technology	Purpose	Refs
Immunotherapy		
TCR-seq scRNA-seq	By performing single-cell RNA sequencing on 5063 single T cells and coupled TCR-seq, 11 T cell subsets were distinguished based on their molecular and functional properties which also delineated their developmental trajectory. The gene layilin was found to be upregulated in both activated $CD8^+$ T cells and regulatory T cells (Tregs), (represses $CD8^+$ T cell functions *in vitro*).	[58]
LIBRA-seq (scBCR-seq)	The antigen specificity of thousands of B cells from two HIV-infected subjects was mapped. The predicted antigen specificities were confirmed for several HIV- and influenza-specific antibodies.	[60]
RAGE-seq (scTCR-seq plus scBCR-seq)	A total of 7138 cells sampled from the primary tumor and draining lymph node of breast cancer were used to infer B cell clonal evolution and identify alternatively spliced BCR transcripts.	[61]
Perturb-seq	Perturb-seq was performed on 200 000 immune cells, identifying transcription factors that regulate the response of dendritic cells to lipopolysaccharide. Perturb-seq was shown to accurately identify individual gene targets, gene signatures, and cell states affected by both individual perturbations and their genetic interactions.	[67]
scRNA-seq	By combing single-cell RNA-seq and TCR analysis, TCR signal intensity was found not to affect resting/activated Treg proportions, but activated Treg programs.	[57]

beneficial when attempting to discern genomic heterogeneity among cancers. For example, Yang and colleagues used scDNA-seq analysis to highlight variants in genes, including *MLL2,* as key drivers of the growth and survival of bladder cancer stem cells [42]. Likewise, the transcriptional variation identified through scRNA-seq is vital for understanding the mechanism underlying drug resistance. Kim *et al.* found that scRNA-seq could characterize and predict the most aggressive tumor subpopulations in lung adenocarcinomas [39]. Of note, when they compared the results of their single-cell approach with conventional bulk tumor analysis, they discovered that the transcriptional profiles of the resistant subpopulations are often masked by more prominent subpopulations, thus obscuring meaningful insights. This highlights the necessity to transition from bulk methods to single-cell analyses when investigating heterogeneous cells. Consideration of circulating tumor cells (CTCs) strengthens this notion. It is suggested that clinicians may benefit from using scDNA-seq to detect and profile CTCs in peripheral blood for staging cancers and monitoring their progression [40,43]. Even in patients with advanced metastatic cancers, CTCs are only present at a magnitude of fewer than 100 cells among the 5 million total cells in 1 ml of whole blood [44]. Single-cell technologies now have the sensitivity to accurately detect CTCs at this extremely low concentration, which is something that bulk analyses will never be able to replicate.

Because they can successfully distinguish unique cells, the focus of single-cell studies has shifted from merely characterizing cell types (Figure 2A) to elucidating the biological mechanisms responsible for the development of drug resistance in previously characterized, resistant subpopulations. Drug resistance can be caused by genetic and non-genetic factors. Genetic resistance arises when a heritable mutation takes place in the DNA of a cell, making it and its progeny resistant to the current drug treatment [40,42]. Using scDNA-seq, genetic alterations can be quickly identified, and the treatment can be adjusted to target the novel subpopulation [40,42]. In drug-resistant cell lineages without a novel mutation, discerning how the resistance developed is a more challenging endeavor.

Previous research using single-cell sequencing has identified two general mechanisms by which non-genetic resistance may develop. The first involves the development of transient resistance



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originating from cells within a tumor in a 'persister' state [39]. Simply put, persister cells exhibit atypical growth and metabolism that enable higher tolerance to drug treatment. Interestingly, following drug withdrawal, persister cells lose their resistance and can be eradicated by the original drug treatment [39]. The other mechanism generates cells that enter a transcriptionally stable end-state. Such cells exhibit sustained resistance in lieu of spontaneous resensitization following drug withdrawal [39,45].

Unfortunately, the mechanism driving this sustained resistance has remained far less clear, precluding informed drug selection. However, two hypotheses have been posited to explain the development of sustained resistance. The first involves a rare subpopulation of resistant cells that are already present in the drug-naïve tumor [46]. Drug treatment selects for these resistant cells while eradicating sensitive cell populations [43]. The continued proliferation of the MRD post-treatment leads to inevitable relapse (Figure 2B). Indeed, single-cell RT-qPCR has confirmed that, in some breast cancers, drug-resistant subpopulations existed before drug exposure [46]. Detecting the biomarkers of these refractory subpopulations during initial treatment can improve outcomes by informing initial drug selections to target all subpopulations [40]. The other hypothesis involving acquired resistance is seemingly the more common phenomenon. Tumor subpopulations that acquire sustained resistance display transcriptional plasticity during drug exposure [43,45]. Mathematical modeling, using single-cell data, has suggested that spatiotemporal heterogeneity might drive transcriptional plasticity towards a sustained resistance state during drug treatment [47]. In turn, changes in gene expression, identified by scRNA-seq, scATAC-seq, and **fluorescence *in situ* hybridization (FISH)**, modulate pathways controlling functions such as epigenetic remodeling and immune response so as to initiate and propagate resistance [35,43,45,48–51]. scRNA-seq, combined with scATAC-seq, has implicated chromatin modulation in the continuation of sustained resistance [45]. Altered chromatin structure can enable proliferation by driving cellular metabolism towards retention of acetate, a significant source of nutrition for tumor cells [35,52]. In selected acute myeloid lymphomas, resistance is initiated through the recruitment of novel enhancers to enable continued expression of genes vital to the survival of disease cells [45]. When cancer cells circumvent gene inhibition through the use of alternative enhancers, different transcription factors are then required for continued expression of these genes. This suggests that cancer cells can switch pathways leading to the expression of key regulators of tumor survival. Single-cell sequencing also provides an assessment of the immunological contribution to drug resistance in tumors. $CD8^+$ T cells are recruited to the tumor by macrophages [50]. **Single-cell T cell receptor sequencing (scTCR-seq)** assays found that the expression of genes such as *PD1* by tumor cells results in the exhaustion of tumor-infiltrating T cells [48,50]. This hampers many of the typical responses of effector T cells, including cytotoxicity [41,49].

Novel insights obtained from mechanistic analyses have informed current research trends and have led to the development of additional technologies to enhance the evaluation of drug response at the single-cell level. Regarding the contribution of the immune system, a novel therapeutic approach called **immune checkpoint blockade (ICB)** has been employed [48,53–55]. ICB drugs both stimulate T cell infiltration and block the molecules inducing T cell exhaustion [41]. ICB therapies have demonstrated surprising efficacy in resensitizing the exhausted T cell tumor infiltrate and, in doing so, represent a remarkable step for immuno-oncology [56–58]. Similar

Figure 2. Prediction of Drug Sensitivity at the Single-Cell Level. (A) Tumor subpopulations maintain diverse sensitivity to different drugs (1–4). Treatments with single drugs may obtain less treatment efficacy. Knowing drug sensitivity at the single-cell level can guide the development of a combination treatment that maximizes the efficiency of killing tumor cells while minimizing damage to healthy cells. (B) Minimal residual disease (MRD) cells will proliferate and differentiate into a new tumor population that induces cancer relapse. The understanding of specific signatures characterized in MRD cells can help to discover novel drugs that specifically target MRD. MRD-targeted drug(s) administered in combination with conventional treatments can cure cancer and prevent relapse.

pursuits, including the use of **single-cell B cell receptor sequencing (scBCR-seq)** to study antigen specificity relevant to antibody therapies, have proven that immuno-oncology is a viable treatment option that deserves extensive study [59–65]. Drug discovery methods have also been adapted to utilize single-cell technologies. For example, the single-cell barcoding technique together with CRISPR/Cas9 is employed to introduce gene perturbations into individual cells and evaluate the transcriptional implications of these perturbations in a high-throughput manner [45,66–68]. As a result, it is possible to associate phenotypes with genetic and transcriptional perturbations at the single-cell level. Such insights will be harnessed in the future to make predictions regarding drug responses.

Challenges and Future Perspectives in Single-Cell Based Drug Prediction

Given that single-cell sequencing remains a relatively nascent field, there is immense potential for further growth and discovery. For scRNA-seq, signal dropouts remain a major issue – where a gene is observed at a low or moderate expression level in one cell but detected as zero in another [69]. Imputation and normalization have been utilized to correct this issue as well as **batch effects** with modest success [70]. Batch effects are differences in measurements that arise from variations in non-biological factors, such as laboratory conditions, reagents, or instruments. Both the dropouts and batch effects introduce noise into the real values, leading to distorted drug response readouts or misclassified cell types. DL models have proved to be an outstanding way to extract accurate high-dimensional features from sequencing data and infer intrinsic gene relations, especially in large datasets at the single-cell level [71–73]. Recovering true values and removing batch effects enable more accurate cell type annotations and more efficient integration of large datasets from different samples and sequencing runs, which improves drug response predictions.

In addition, barcoding methodologies have also been developed to further overcome the challenges introduced by the technical zero and batch effect [35,69,74]. The use of sample-specific barcodes in scRNA-seq enables multiplexing that reduces batch effects. One such barcoding technique, MULTI-seq, claims to even address dropout issues by distinguishing between low-RNA expression and low-quality cells [69]. Another limitation of scRNA-seq is that the assumption that mRNA levels correlate with protein translation is not always true. FISH and western blot assays have confirmed multiple instances in which overexpression of genes was accompanied by little to no subsequent translation [45,46]. Unfortunately, although fields such as proteomics and metabolomics have begun to implement single-cell technologies, they have not progressed rapidly enough [75]. As such, they are not currently scalable, and it would not be feasible to employ them in conjunction with high-throughput techniques such as scRNA-seq to explore this phenomenon.

Facilitating Single-Cell-Level Drug Predictions Using DTL

DL models have previously been utilized in single-cell clustering analysis, batch effect correction, and denoising [76–79], but have not been used for DTI prediction or drug responses. Because single-cell sequencing is still in its infancy relative to bulk sequencing, no large public repositories are so far available for drug-related single-cell data, limiting the training power of DL models. To overcome this limitation, we could borrow the information contained in bulk sequencing data and transfer to the single-cell level.

DTL preserves previously learned features and trained parameters that can be applied to the testing dataset with a similar task. This has been shown to improve prediction performance, especially when only limited data are available for the new task and the model was originally trained on large amounts of data. We could first train out similar features from both bulk and single-cell

data by passing a discriminator covered in a generative adversarial network. A DTL framework can then be applied to transfer known drug–feature relationships at the bulk level to individual cells, resulting in predicted drug sensitivities for each cell type (Figure 3A). Such pursuits promise to yield significant advances in the treatment of heterogeneous disorders and in turn will improve the prognosis of patients afflicted by these disorders. In a more advanced way, bulk data can be used to regularize inferences transferred between two single-cell datasets to improve the accuracy of drug sensitivity prediction (Figure 3B).

Integration of Single-Cell Multi-Omic Data for Drug prediction

Single-cell multi-omic technologies (scMulti-omics) simultaneously measure multiple modalities within an individual cell, including features from genomics, epigenomics, transcriptomes, and proteomics. Such approaches profile cell behavior and identity more comprehensively than was possible with previous individual methodologies. When multiple omic profiles are used in conjunction, one profile can recover the cellular characteristics – such as DNA methylation, gene expression, chromatin accessibility, or protein abundance – that might be lost by another sequencing technique. Furthermore, scMulti-omics can also validate conclusions drawn from other omic profiles. The DL-based integrative analysis could accurately answer biological questions, including tumor

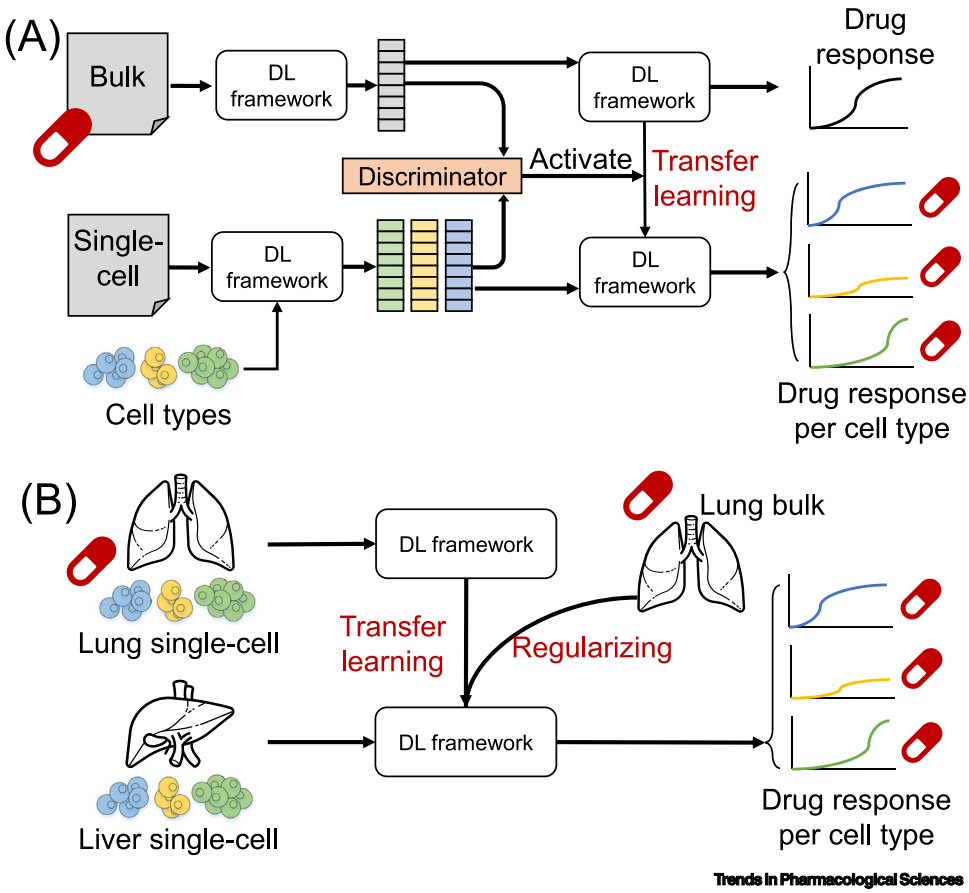

Figure 3. Potential Applications of a Deep Transfer Learning (DTL) Framework to Single-Cell Data for Drug Sensitivity Prediction. (A) The combination of a generative adversarial network and the DTL framework transfers the drug sensitivity established at the bulk level to the single-cell level. (B) A more advanced application of DTL transferred drug sensitivity between two single-cell datasets and uses bulk-level information as a regularizer to constrain the deep learning (DL) parameters.

type classification and prognosis prediction [80,81]. Thus, a unified multimodal learning framework can be expected to incorporate the integrative analysis of scMulti-omic data, protein structure, drug structure, and side-effect information. Such a framework can build intrinsic links between genomic variations and phenotypic phenomena induced by drugs, and thus enhance the accuracy and efficiency of drug sensitivity prediction. However, there is an immense computational burden that must be addressed and overcome. Although several methods are available to help integrate and analyze scMulti-omic data, including factorization, Bayesian modeling, and network-based modeling, integrating the data from two or more technologies only exacerbates computational issues encountered during analysis [82,83]. Another challenge is related to the analytical abilities of integrative tools. Existing computational methods cannot simultaneously perform functions such as identifying *cis*-regulatory motifs, finding **cell type-specific regulons**, and inferring gene regulatory networks. A robust benchmarking pipeline of integrative scMulti-omic analytic methods will be necessary to rectify this. In addition, there is also room for improvement in DL models. The attention mechanisms can be embedded into DL models to make more accurate inferences.

Outstanding Questions

How best can we choose an appropriate DL model when dealing with several data categories?

How best can we incorporate the analysis of drug–target interactions, structural information, and sequencing data using a DTL model?

What algorithm would help to solve the 'black box' problem in artificial intelligence and DL?

Other than drug sensitivity prediction and drug–target interaction, what field will benefit from DTL?

How can single-cell multi-omics provide insights into heterogeneous responses across tumor cell subpopulations?

What methods could we use to minimize the loss encountered during transfer of bulk data knowledge to single-cell analysis?

Concluding Remarks

The variability in the genomic and transcriptomic profiles of heterogeneous tumor subpopulations prevents the development of effective drug regimens for cancer patients. Most targeted cancer therapies and drugs exhibit diverse responses in patients, leading to low cure rates and high relapse rates. It is impossible to experimentally test and validate drug responses *in vivo*. As such, determining how to assess drug responses accurately and effectively *in silico* is crucial for continued drug development. By training and applying advanced DL models, scientists can rapidly predict potential drug targets for novel treatments, simulate drug response under millions of conditions, and discover new purposes for existing drugs. We have reviewed six DL model types and summarized the application of DL-based tools to drug discovery and prediction of drug responses. Using these DL models, researchers have successfully improved the accuracy of the predictions they make.

However, accuracy is inherently limited by the resolution of the data generated by conventional bulk sequencing methods. One trend in alleviating this limitation is the transition to advanced single-cell techniques for drug response predictions. Single-cell technologies comprehensively profile the heterogeneity of cancer cells to identify targeted treatment options and assess the risk of developing drug resistance. Owing to the high dimensionality and large sample sizes of single-cell data, DL models are naturally well suited for single-cell analyses. However, currently, the quantity of available benchmarked, drug-related, single-cell data limits the application power of DL models. To fully address the limitations of both single-cell sequencing and DL models, and to maximize their functionality in predicting drug responses, several key points must be considered (see Outstanding Questions). We further propose that the substantial application of DL, especially the DTL framework, presents an immediate solution to enable single-cell-informed drug response predictions by first learning drug–target information from bulk data. We believe that although admittedly there is a long way to go, the combination of DL and single-cell technologies will eventually reshape how drug development and target therapies are conducted.

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Resources

[i]https://github.com/Bjoux2/DeepDTIs_DBN

[ii]https://github.com/FangpingWan/DeepCPI

[iii]https://github.com/ChengF-Lab/deepDTnet

[iv]https://github.com/GIST-CSBL/DeepConv-DTI

[v]https://github.com/cansyl/DEEPScreen

[vi]https://github.com/deepchem/deepchem

[vii]https://github.com/Shen-Lab/DeepAffinity

[viii]https://cbbio.cis.um.edu.mo/AxPEP/

[ix]https://github.com/ChengF-Lab/deepDR

[x]https://github.com/mathcom/RefDNN

[xi]https://github.com/idekerlab/DCell

[xii]https://github.com/KristinaPreuer/DeepSynergy

[xiii]https://github.com/mims-harvard/decagon

[xiv]http://discotechnologies.org/DeepMACT/

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