Hepatoblastoma (HB) is the main paediatric liver cancer, but it is a very rare disease. Despite significant improvements in the treatment of children diagnosed with HB, limited treatment options exist for patients with advanced tumours. Besides, survivors generally have long-term adverse effects derived from treatment such as ototoxicity, cardiotoxicity, delayed growth, and secondary tumours. Accordingly, there is an urgent need to define new and efficient therapeutic strategies for patients with HB. Computational methods to predict drug sensitivity from a tumour's transcriptome have been successfully applied for some common adult malignancies, but specific efforts in paediatric cancers are lacking because of paucity of data. In this study, we computationally screened the efficacy of drugs in HB patients with the aggressive C2 subtype and poor clinical outcome starting from their transcriptome. Our method utilized publicly available collections of pan-cancer transcriptional profiles and drug responses across 36 tumour types and 495 compounds. The drugs predicted to be most effective were experimentally validated using patient-derived xenograft (PDX) models of HB grown in vitro and in vivo. We thus identified two CDK9 inhibitors, alvocidib and dinaciclib as potent HB growth inhibitors for the high-risk C2 molecular subtype. We also found that in a cohort of 46 patients with HB, high CDK9 tumour expression was significantly associated with poor prognosis. Our work proves the usefulness of computational methods trained on pan-cancer datasets to reposition drugs in rare paediatric cancers such as HB, and to help clinicians in choosing the best treatment options for their patients.

Myeloid phagocytes of the respiratory immune system, such as neutrophils, monocytes, and alveolar macrophages, are essential for immunity to Aspergillus fumigatus, the most common etiologic agent of mold pneumonia worldwide. Following the engulfment of A. fumigatus conidia, fusion of the phagosome with the lysosome is a critical process for killing conidia. TFEB and TFE3 are transcription factors that regulate lysosomal biogenesis under stress and are activated by inflammatory stimuli in macrophages, but it is unknown whether TFEB and TFE3 contribute to anti-Aspergillus immunity during infection. We found that lung neutrophils express TFEB and TFE3, and their target genes were upregulated during A. fumigatus lung infection. In addition, A. fumigatus infection induced nuclear accumulation of TFEB and TFE3 in macrophages in a process regulated by Dectin-1 and CARD9. Genetic deletion of Tfeb and Tfe3 impaired macrophage killing of A. fumigatus conidia. However, in a murine immune-competent Aspergillus infection model with genetic deficiency of Tfeb and Tfe3 in hematopoietic cells, we surprisingly found that lung myeloid phagocytes had no defects in conidial phagocytosis or killing. Loss of TFEB and TFE3 did not impact murine survival or clearance of A. fumigatus from the lungs. Our findings indicate that myeloid phagocytes activate TFEB and TFE3 in response to A. fumigatus, and while this pathway promotes macrophage fungicidal activity in vitro, genetic loss can be functionally compensated in the lung, resulting in no measurable defect in fungal control and host survival.

To fulfill their function, pancreatic beta cells require precise nutrient-sensing mechanisms that control insulin production Transcription factor EB (TFEB) and its homolog TFE3 have emerged as crucial regulators of the adaptive response of cell metabolism to environmental cues. Here, we show that TFEB and TFE3 regulate beta-cell function and insulin gene expression in response to variations in nutrient availability. We found that nutrient deprivation in beta cells promoted TFEB/TFE3 activation, which resulted in suppression of insulin gene expression. TFEB overexpression was sufficient to inhibit insulin transcription, whereas beta cells depleted of both TFEB and TFE3 failed to suppress insulin gene expression in response to amino acid deprivation. Interestingly, ChIP-seq analysis showed binding of TFEB to super-enhancer regions that regulate insulin transcription. Conditional, beta-cell-specific, Tfeb-overexpressing, and Tfeb/Tfe3 double-KO mice showed severe alteration of insulin transcription, secretion, and glucose tolerance, indicating that TFEB and TFE3 are important physiological mediators of pancreatic function. Our findings reveal a nutrient-controlled transcriptional mechanism that regulates insulin production, thus playing a key role in glucose homeostasis at both cellular and organismal levels.

Birt-Hogg-Dubé (BHD) syndrome is an inherited familial cancer syndrome characterized by the development of cutaneous lesions, pulmonary cysts, renal tumors and cysts and caused by loss-of-function pathogenic variants in the gene encoding the tumor-suppressor protein folliculin (FLCN). FLCN acts as a negative regulator of TFEB and TFE3 transcription factors, master controllers of lysosomal biogenesis and autophagy, by enabling their phosphorylation by the mechanistic Target Of Rapamycin Complex 1 (mTORC1). We have previously shown that deletion of Tfeb rescued the renal cystic phenotype of kidney-specific Flcn KO mice. Using Flcn/Tfeb/Tfe3 double and triple KO mice, we now show that both Tfeb and Tfe3 contribute, in a differential and cooperative manner, to kidney cystogenesis. Remarkably, the analysis of BHD patient-derived tumor samples revealed increased activation of TFEB/TFE3-mediated transcriptional program and silencing either of the two genes rescued tumorigenesis in human BHD renal tumor cell line-derived xenografts (CDXs). Our findings demonstrate in disease-relevant models that both TFEB and TFE3 are key drivers of renal tumorigenesis and suggest novel therapeutic strategies based on the inhibition of these transcription factors.

The stress-responsive transcription factor EB (TFEB) is a master controller of lysosomal biogenesis and autophagy and plays a major role in several cancer-associated diseases. TFEB is regulated at the posttranslational level by the nutrient-sensitive kinase complex mTORC1. However, little is known about the regulation of TFEB transcription. Here, through integrative genomic approaches, we identify the immediate-early gene EGR1 as a positive transcriptional regulator of TFEB expression in human cells and demonstrate that, in the absence of EGR1, TFEB-mediated transcriptional response to starvation is impaired. Remarkably, both genetic and pharmacological inhibition of EGR1, using the MEK1/2 inhibitor Trametinib, significantly reduced the proliferation of 2D and 3D cultures of cells displaying constitutive activation of TFEB, including those from a patient with Birt-Hogg-Dubé(BHD) syndrome, a TFEB-driven inherited cancer condition. Overall, we uncover an additional layer of TFEB regulation consisting in modulating its transcription via EGR1 and propose that interfering with the EGR1-TFEB axis may represent a therapeutic strategy to counteract constitutive TFEB activation in cancer-associated conditions.

Obesity is a major risk factor for end-stage kidney disease. We previously found that lysosomal dysfunction and impaired autophagic flux contribute to lipotoxicity in obesity-related kidney disease, in both humans and experimental animal models. However, the regulatory factors involved in countering renal lipotoxicity are largely unknown. Here, we found that palmitic acid strongly promoted dephosphorylation and nuclear translocation of transcription factor EB (TFEB) by inhibiting the mechanistic target of rapamycin kinase complex 1 pathway in a Rag GTPase–dependent manner, though these effects gradually diminished after extended treatment. We then investigated the role of TFEB in the pathogenesis of obesity-related kidney disease. Proximal tubular epithelial cell–specific (PTEC-specific) Tfeb-deficient mice fed a high-fat diet (HFD) exhibited greater phospholipid accumulation in enlarged lysosomes, which manifested as multilamellar bodies (MLBs). Activated TFEB mediated lysosomal exocytosis of phospholipids, which helped reduce MLB accumulation in PTECs. Furthermore, HFD-fed, PTEC-specific Tfeb-deficient mice showed autophagic stagnation and exacerbated injury upon renal ischemia/reperfusion. Finally, higher body mass index was associated with increased vacuolation and decreased nuclear TFEB in the proximal tubules of patients with chronic kidney disease. These results indicate a critical role of TFEB-mediated lysosomal exocytosis in counteracting renal lipotoxicity.

Epithelial-mesenchymal transition (EMT) is a complex and pivotal process involved in organogenesis and is related to several pathological processes, including cancer and fibrosis. During heart development, EMT mediates the conversion of epicardial cells into vascular smooth muscle cells and cardiac interstitial fibroblasts. Here, we show that the oncogenic transcription factor EB (TFEB) is a key regulator of EMT in epicardial cells and that its genetic overexpression in mouse epicardium is lethal due to heart defects linked to impaired EMT. TFEB specifically orchestrates the EMT-promoting function of transforming growth factor (TGF) β, and this effect results from activated transcription of thymine-guanine-interacting factor (TGIF)1, a TGFβ/Smad pathway repressor. The Tgif1 promoter is activated by TFEB, and in vitro and in vivo findings demonstrate its increased expression when Tfeb is overexpressed. Furthermore, Tfeb overexpression in vitro prevents TGFβ-induced EMT, and this effect is abolished by Tgif1 silencing. Tfeb loss of function, similar to that of Tgif1, sensitizes cells to TGFβ, inducing an EMT response to low doses of TGFβ. Together, our findings reveal an unexpected function of TFEB in regulating EMT, which might provide insights into injured heart repair and control of cancer progression.

Successful elimination of bacteria in phagocytes occurs in the phago-lysosomal system, but also depends on mitochondrial pathways. Yet, how these two organelle systems communicate is largely unknown. Here we identify the lysosomal biogenesis factor transcription factor EB (TFEB) as regulator for phago-lysosome-mitochondria crosstalk in macrophages. By combining cellular imaging and metabolic profiling, we find that TFEB activation, in response to bacterial stimuli, promotes the transcription of aconitate decarboxylase (Acod1, Irg1) and synthesis of its product itaconate, a mitochondrial metabolite with antimicrobial activity. Activation of the TFEB–Irg1–itaconate signalling axis reduces the survival of the intravacuolar pathogen Salmonella enterica serovar Typhimurium. TFEB-driven itaconate is subsequently transferred via the Irg1-Rab32–BLOC3 system into the Salmonella-containing vacuole, thereby exposing the pathogen to elevated itaconate levels. By activating itaconate production, TFEB selectively restricts proliferating Salmonella, a bacterial subpopulation that normally escapes macrophage control, which contrasts TFEB's role in autophagy-mediated pathogen degradation. Together, our data define a TFEB-driven metabolic pathway between phago-lysosomes and mitochondria that restrains Salmonella Typhimurium burden in macrophages in vitro and in vivo.

Objective: Brown adipose tissue (BAT) thermogenesis offers the potential to improve metabolic health in mice and humans. However, humans predominantly live under thermoneutral conditions, leading to BAT whitening, a reduction in BAT mitochondrial content and metabolic activity. Recent studies have established mitophagy as a major driver of mitochondrial degradation in the whitening of thermogenic brite/beige adipocytes, yet the pathways mediating mitochondrial breakdown in whitening of classical BAT remain largely elusive. The transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy belonging to the MiT family of transcription factors, is the only member of this family that is upregulated during whitening, pointing toward a role of TFEB in whitening-associated mitochondrial breakdown. Methods: We generated brown adipocyte-specific TFEB knockout mice, and induced BAT whitening by thermoneutral housing. We characterized gene and protein expression patterns, BAT metabolic activity, systemic metabolism, and mitochondrial localization using in vivo and in vitro approaches. Results: Under low thermogenic activation conditions, deletion of TFEB preserves mitochondrial mass independently of mitochondriogenesis in BAT and primary brown adipocytes. However, this does not translate into elevated thermogenic capacity or protection from diet-induced obesity. Autophagosomal/lysosomal marker levels are altered in TFEB-deficient BAT and primary adipocytes, and lysosomal markers co-localize and copurify with mitochondria in TFEB-deficient BAT, indicating trapping of mitochondria in late stages of mitophagy. Conclusion: We identify TFEB as a driver of BAT whitening, mediating mitochondrial degradation via the autophagosomal and lysosomal machinery. This study provides proof of concept that interfering with the mitochondrial degradation machinery can increase mitochondrial mass in classical BAT under human-relevant conditions. However, it must be considered that interfering with autophagy may result in accumulation of non-functional mitochondria. Future studies targeting earlier steps of mitophagy or target recognition are therefore warranted.

Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent potentially lethal monogenic disorder. Mutations in the *PKD1* gene, which encodes polycystin-1 (PC1), account for approximately 78% of cases. PC1 is a large 462-kDa protein that undergoes cleavage in its N and C-terminal domains. C-terminal cleavage produces fragments that translocate to mitochondria. We show that transgenic expression of a protein corresponding to the final 200 amino acid (aa) residues of PC1 in two *Pkd1*-KO orthologous murine models of ADPKD suppresses cystic phenotype and preserves renal function. This suppression depends upon an interaction between the C-terminal tail of PC1 and the mitochondrial enzyme Nicotinamide Nucleotide Transhydrogenase (NNT). This interaction modulates tubular/cyst cell proliferation, the metabolic profile, mitochondrial function, and the redox state. Together, these results suggest that a short fragment of PC1 is sufficient to suppress cystic phenotype and open the door to the exploration of gene therapy strategies for ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD) is a life-threatening monogenic disease caused by mutations in PKD1 and PKD2 that encode polycystin 1 (PC1) and polycystin 2 (PC2). PC1/2 localize to cilia of renal epithelial cells, and their function is believed to embody an inhibitory activity that suppresses the cilia-dependent cyst activation (CDCA) signal. Consequently, PC deficiency results in activation of CDCA and stimulates cyst growth. Recently, re-expression of PCs in established cysts has been shown to reverse PKD. Thus, the mode of action of PCs resembles a 'counterbalance in cruise control' to maintain lumen diameter within a designated range. Herein we review recent studies that point to novel arenas for future PC research with therapeutic potential for ADPKD.

Drug screening data from massive bulk gene expression databases can be analyzed to determine the optimal clinical application of cancer drugs. The growing amount of single-cell RNA sequencing (scRNA-seq) data also provides insights into improving therapeutic effectiveness by helping to study the heterogeneity of drug responses for cancer cell subpopulations. Developing computational approaches to predict and interpret cancer drug response in single-cell data collected from clinical samples can be very useful. We propose scDEAL, a deep transfer learning framework for cancer drug response prediction at the single-cell level by integrating large-scale bulk cell-line data. The highlight in scDEAL involves harmonizing drug-related bulk RNA-seq data with scRNA-seq data and transferring the model trained on bulk RNA-seq data to predict drug responses in scRNA-seq. Another feature of scDEAL is the integrated gradient feature interpretation to infer the signature genes of drug resistance mechanisms. We benchmark scDEAL on six scRNA-seq datasets and demonstrate its model interpretability via three case studies focusing on drug response label prediction, gene signature identification, and pseudotime analysis. We believe that scDEAL could help study cell reprogramming, drug selection, and repurposing for improving therapeutic efficacy.

Inhibition of the overactivated alternative complement pathway in autosomal dominant polycystic kidney disease (ADPKD) retards disease progression in animal models; however, it remains unknown how complement factor B (CFB) is upregulated in ADPKD. Here, we showed that the overexpression of CFB in cystic kidneys is associated with increased JAK2/STAT1 activity and enhanced expression of the polycystin-1 C-terminal tail (PC1-CTT). Overexpression or blockage of STAT1 increased or decreased CFB expression and CFB promoter activity. Moreover, overexpression of PC1-CTT induced JAK2/STAT1 activation and CFB upregulation in renal tubular epithelial cells. Furthermore, PC1-CTT overexpression increased human CFB promoter activity, whereas dominant negative STAT1 plasmids or mutation of putative STAT1 responsive elements decreased PC1-CTT-induced CFB promoter activity. The effect of CFB on macrophage differentiation was tested on a mouse macrophage cell line. Bioactive CFB dose dependently promoted macrophage M2 phenotype conversion. In addition, conditioned media from renal epithelial cells promoted macrophage M2 phenotype conversion which was blocked by STAT1 inhibition in a dose-dependent manner. Conditioned media from PC1-CTT-transfected renal epithelial cells further promoted macrophage M2 phenotype conversion, which was suppressed by fludarabine or a CFB antibody. In addition, we show that NF-kB acts downstream of PC1-CTT and may partly mediate PC1-CTT-induced CFB expression. In conclusion, our study reveals possible mechanisms of CFB upregulation in ADPKD and a novel role of PC1-CTT in ADPKD-associated inflammation. Furthermore, our study suggests that targeting STAT1 may be a new strategy to prevent inflammation in the kidney of patients with ADPKD.

Polycystic kidney disease (PKD), comprising autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD), is characterized by incessant cyst formation in the kidney and liver. ADPKD and ARPKD represent the leading genetic causes of renal disease in adults and children, respectively. ADPKD is caused by mutations in PKD1 encoding polycystin1 (PC1) and PKD2 encoding polycystin 2 (PC2). PC1/2 are multi-pass transmembrane proteins that form a complex localized in the primary cilium. Predominant ARPKD cases are caused by mutations in polycystic kidney and hepatic disease 1 (PKHD1) gene that encodes the Fibrocystin/Polyductin (FPC) protein, whereas a small subset of cases are caused by mutations in DAZ interacting zinc finger protein 1 like (DZIP1L) gene. FPC is a type I transmembrane protein, localizing to the cilium and basal body, in addition to other compartments, and DZIP1L encodes a transition zone/basal body protein. Apparently, PC1/2 and FPC are signaling molecules, while the mechanism that cilia employ to govern renal tubule morphology and prevent cyst formation is unclear. Nonetheless, recent genetic and biochemical studies offer a glimpse of putative physiological malfunctions and the pathomechanisms underlying both disease entities. In this review, I summarize the results of genetic studies that deduced the function of PC1/2 on cilia and of cilia themselves in cyst formation in ADPKD, and I discuss studies regarding regulation of polycystin biogenesis and cilia trafficking. I also summarize the synergistic genetic interactions between Pkd1 and Pkhd1, and the unique tissue patterning event controlled by FPC, but not PC1. Interestingly, while DZIP1L mutations generate compromised PC1/2 cilia expression, FPC deficiency does not affect PC1/2 biogenesis and ciliary localization, indicating that divergent mechanisms could lead to cyst formation in ARPKD. I conclude by outlining promising areas for future PKD research and highlight rationales for potential therapeutic interventions for PKD treatment.

Mutations in polycystin-1 (PC1) lead to autosomal-dominant polycystic kidney disease (ADPKD), a leading cause of renal failure for which no treatment is available. PC1 is an integral membrane protein, which has been implicated in the regulation of multiple signaling pathways including the JAK/STAT pathway. Here we show that membrane-anchored PC1 activates STAT3 in a JAK2-dependent manner, leading to tyrosine phosphorylation and transcriptional activity. The C-terminal cytoplasmic tail of PC1 can undergo proteolytic cleavage and nuclear translocation. Tail-cleavage abolishes the ability of PC1 to directly activate STAT3 but the cleaved PC1 tail now coactivates STAT3 in a mechanism requiring STAT phosphorylation by cytokines or growth factors. This leads to an exaggerated cytokine response. Hence, PC1 can regulate STAT activity by a dual mechanism. In ADPKD kidneys PC1 tail fragments are overexpressed, including a unique ~15-kDa fragment (P15). STAT3 is strongly activated in cyst-lining epithelial cells in human ADPKD, and orthologous and nonorthologous polycystic mouse models. STAT3 is also activated in developing, postnatal kidneys but inactivated in adult kidneys. These results indicate that STAT3 signaling is regulated by PC1 and is a driving factor for renal epithelial proliferation during normal renal development and during cyst growth.

To date the coronavirus family is composed of seven different viruses which were commonly known as cold viruses until the appearance of the severe acute respiratory coronavirus (SARS-CoV) in 2002, the middle east respiratory syndrome coronavirus (MERS) in 2012 and the severe acute respiratory coronavirus 2 (SARS-CoV-2) which caused the COVID-19 global pandemic in 2019. Using bioinformatic approaches we tested the potential interactions of human miRNAs, expressed in pulmonary epithelial cells, with the available coronavirus genomes. Putative miRNA binding sites were then compared between pathogenic and non pathogenic virus groups. The pathogenic group shares 6 miRNA binding sites that can be potentially involved in the sequestration of miRNAs already known to be associated with deep vein thrombosis. We then analysed ∼100k SARS-CoV-2 variant genomes for their potential interaction with human miRNAs and this study highlighted a group of 97 miRNA binding sites which is present in all the analysed genomes. Among these, we identified 6 miRNA binding sites specific for SARS-CoV-2 and the other two pathogenic viruses whose down-regulation has been seen associated with deep vein thrombosis and cardiovascular diseases. Interestingly, one of these miRNAs, namely miR-20a-5p, whose expression decreases with advancing age, is involved in cytokine signaling, cell differentiation and/or proliferation. We hypothesize that depletion of poorly expressed miRNA could be related with disease severity.

The interaction between RNA and RNA-binding proteins (RBPs) has a key role in the regulation of gene expression, in RNA stability, and in many other biological processes. RBPs accomplish these functions by binding target RNA molecules through specific sequence and structure motifs. The identification of these binding motifs is therefore fundamental to improve our knowledge of the cellular processes and how they are regulated. Here, we present BRIO (BEAM RNA Interaction mOtifs), a new web server designed for the identification of sequence and structure RNA-binding motifs in one or more RNA molecules of interest. BRIO enables the user to scan over 2508 sequence motifs and 2296 secondary structure motifs identified in Homo sapiens and Mus musculus, in three different types of experiments (PAR-CLIP, eCLIP, HITS). The motifs are associated with the binding of 186 RBPs and 69 protein domains. The web server is freely available at http://brio.bio.uniroma2.it.

Background Intra-tumour heterogeneity (ITH) presents a significant obstacle in formulating effective treatment strategies in clinical practice. Single-cell RNA sequencing (scRNA-seq) has evolved as a powerful instrument for probing ITH at the transcriptional level, offering an unparalleled opportunity for therapeutic intervention. Results Drug response prediction at the single-cell level is an emerging field of research that aims to improve the efficacy and precision of cancer treatments. Here, we introduce DREEP (Drug Response Estimation from single-cell Expression Profiles), a computational method that leverages publicly available pharmacogenomic screens from GDSC2, CTRP2, and PRISM and functional enrichment analysis to predict single-cell drug sensitivity from transcriptomic data. We validated DREEP extensively in vitro using several independent single-cell datasets with over 200 cancer cell lines and showed its accuracy and robustness. Additionally, we also applied DREEP to molecularly barcoded breast cancer cells and identified drugs that can selectively target specific cell populations. Conclusions DREEP provides an in silico framework to prioritize drugs from single-cell transcriptional profiles of tumours and thus helps in designing personalized treatment strategies and accelerating drug repurposing studies. DREEP is available at https://github.com/gambalab/DREEP. Keywords Drug prediction, Single-cell transcriptomics, Precision oncology, Cancer

Cancer cells within a tumour have heterogeneous phenotypes and exhibit dynamic plasticity. How to evaluate such heterogeneity and its impact on outcome and drug response is still unclear. Here, we transcriptionally profile 35,276 individual cells from 32 breast cancer cell lines to yield a single cell atlas. We find high degree of heterogeneity in the expression of biomarkers. We then train a deconvolution algorithm on the atlas to determine cell line composition from bulk gene expression profiles of tumour biopsies, thus enabling cell line-based patient stratification. Finally, we link results from large-scale in vitro drug screening in cell lines to the single cell data to computationally predict drug responses starting from single-cell profiles. We find that transcriptional heterogeneity enables cells with differential drug sensitivity to co-exist in the same population. Our work provides a framework to determine tumour heterogeneity in terms of cell line composition and drug response.

Single-cell RNA sequencing (scRNA-seq) technology allows massively parallel characterization of thousands of cells at the transcriptome level. scRNA-seq is emerging as an important tool to investigate the cellular components and their interactions in the tumor microenvironment. scRNA-seq is also used to reveal the association between tumor microenvironmental patterns and clinical outcomes and to dissect cell-specific effects of drug treatment in complex tissues. Recent advances in scRNA-seq have driven the discovery of biomarkers in diseases and therapeutic targets. Although methods for prediction of drug response using gene expression of scRNA-seq data have been proposed, an integrated tool from scRNA-seq analysis to drug discovery is required. We present scDrug as a bioinformatics workflow that includes a one-step pipeline to generate cell clustering for scRNA-seq data and two methods to predict drug treatments. The scDrug pipeline consists of three main modules: scRNA-seq analysis for identification of tumor cell subpopulations, functional annotation of cellular subclusters, and prediction of drug responses. scDrug enables the exploration of scRNA-seq data readily and facilitates the drug repurposing process. scDrug is freely available on GitHub at https://github.com/ailabstw/scDrug.

To enable personalized cancer treatment, machine learning models have been developed to predict drug response as a function of tumor and drug features. However, most algorithm development efforts have relied on cross-validation within a single study to assess model accuracy. While an essential first step, cross-validation within a biological data set typically provides an overly optimistic estimate of the prediction performance on independent test sets. To provide a more rigorous assessment of model generalizability between different studies, we use machine learning to analyze five publicly available cell line-based data sets: National Cancer Institute 60, ancer Therapeutics Response Portal (CTRP), Genomics of Drug Sensitivity in Cancer, Cancer Cell Line Encyclopedia and Genentech Cell Line Screening Initiative (gCSI). Based on observed experimental variability across studies, we explore estimates of prediction upper bounds. We report performance results of a variety of machine learning models, with a multitasking deep neural network achieving the best cross-study generalizability. By multiple measures, models trained on CTRP yield the most accurate predictions on the remaining testing data, and gCSI is the most predictable among the cell line data sets included in this study. With these experiments and further simulations on partial data, two lessons emerge: (1) differences in viability assays can limit model generalizability across studies and (2) drug diversity, more than tumor diversity, is crucial for raising model generalizability in preclinical screening.

Background: Cancer cell lines are frequently used in research as in-vitro tumor models. Genomic data and large-scale drug screening have accelerated the right drug selection for cancer patients. Accuracy in drug response prediction is crucial for success. Due to data-type diversity and big data volume, few methods can integrative and efficiently find the principal low-dimensional manifold of the high-dimensional cancer multi-omics data to predict drug response in precision medicine. Method: A novelty k-means Ensemble Support Vector Regression (kESVR) is developed to predict each drug response values for single patient based on cell-line gene expression data. The kESVR is a blend of supervised and unsupervised learning methods and is entirely data driven. It utilizes embedded clustering (Principal Component Analysis and k-means clustering) and local regression (Support Vector Regression) to predict drug response and obtain the global pattern while overcoming missing data and outliers' noise. Results: We compared the efficiency and accuracy of kESVR to 4 standard machine learning regression models: (1) simple linear regression, (2) support vector regression (3) random forest (quantile regression forest) and (4) back propagation neural network. Our results, which based on drug response across 610 cancer cells from Cancer Cell Line Encyclopedia (CCLE) and Cancer Therapeutics Response Portal (CTRP v2), proved to have the highest accuracy (smallest mean squared error (MSE) measure). We next compared kESVR with existing 17 drug response prediction models based a varied range of methods such as regression, Bayesian inference, matrix factorization and deep learning. After ranking the 18 models based on their accuracy of prediction, kESVR ranks first (best performing) in majority (74%) of the time. As for the remaining (26%) cases, kESVR still ranked in the top five performing models. Conclusion: In this paper we introduce a novel model (kESVR) for drug response prediction using high dimensional cell-line gene expression data. This model outperforms current existing prediction models in terms of prediction accuracy and speed and overcomes overfitting. This can be used in future to develop a robust drug response prediction system for cancer patients using the cancer cell-lines guidance and multi-omics data.

Although an essential step, cell functional annotation often proves particularly challenging from single-cell transcriptional data. Several methods have been developed to accomplish this task. However, in most cases, these rely on techniques initially developed for bulk RNA sequencing or simply make use of marker genes identified from cell clustering followed by supervised annotation. To overcome these limitations and automatize the process, we have developed two novel methods, the single-cell gene set enrichment analysis (scGSEA) and the single-cell mapper (scMAP). scGSEA combines latent data representations and gene set enrichment scores to detect coordinated gene activity at single-cell resolution. scMAP uses transfer learning techniques to re-purpose and contextualize new cells into a reference cell atlas. Using both simulated and real datasets, we show that scGSEA effectively recapitulates recurrent patterns of pathways' activity shared by cells from different experimental conditions. At the same time, we show that scMAP can reliably map and contextualize new single-cell profiles on a breast cancer atlas we recently released. Both tools are provided in an effective and straightforward workflow providing a framework to determine cell function and significantly improve annotation and interpretation of scRNA-seq data.

Inducible gene expression systems can be used to control the expression of a gene of interest by means of a small-molecule. One of the most common designs involves engineering a small-molecule responsive transcription factor (TF) and its cognate promoter, which often results in a compromise between minimal uninduced background expression (leakiness) and maximal induced expression. Here, we focussed on an alternative strategy using quantitative synthetic biology to mitigate leakiness while maintaining high expression, without modifying neither the TF nor the promoter. Through mathematical modelling and experimental validations, we designed the CASwitch, a mammalian synthetic gene circuit based on combining two well-known network motifs: the Coherent Feed-Forward Loop (CFFL) and the Mutual Inhibition (MI). The CASwitch combines the CRISPR-Cas endoribonuclease CasRx with the state-of-the-art Tet-On3G inducible gene system to achieve high performances. To demonstrate the potentialities of the CASwitch, we applied it to three different scenarios: enhancing a whole-cell biosensor, controlling expression of a toxic gene and inducible production of Adeno-Associated Virus (AAV) vectors.

The provided text discusses the challenges and limited treatment options for Hepatoblastoma (HB), the main pediatric liver cancer. Despite improvements in treatment, survivors often face long-term adverse effects. The text emphasizes the need for new therapeutic strategies, particularly for patients with advanced tumors. It introduces computational methods to predict drug sensitivity from a tumor's transcriptome, highlighting the success of these methods in common adult malignancies but noting the lack of specific efforts in pediatric cancers due to data scarcity. In this study, the researchers computationally screened the efficacy of drugs for HB patients with the aggressive C2 subtype and poor clinical outcomes based on their transcriptome. They utilized publicly available collections of pan-cancer transcriptional profiles and drug responses across various tumor types and compounds. The most effective drugs predicted by the computational screening were experimentally validated using patient-derived xenograft (PDX) models of HB, both in vitro and in vivo. Two CDK9 inhibitors, alvocidib and dinaciclib, were identified as potent inhibitors for the high-risk C2 molecular subtype. Additionally, the study found that high CDK9 tumor expression in a cohort of 46 HB patients was significantly associated with poor prognosis. The research demonstrates the utility of computational methods trained on pan-cancer datasets to reposition drugs in rare pediatric cancers like HB. The goal is to assist clinicians in choosing optimal treatment options for their patients.

The immune response to Aspergillus fumigatus, a common cause of mold pneumonia, involves myeloid phagocytes in the respiratory system. Neutrophils, monocytes, and alveolar macrophages play a crucial role in the defense against A. fumigatus. When these phagocytes engulf A. fumigatus conidia, the fusion of the phagosome with the lysosome is a key process for eliminating the conidia. Transcription factors TFEB and TFE3, known regulators of lysosomal biogenesis under stress, are activated by inflammatory stimuli in macrophages. The study investigated whether TFEB and TFE3 contribute to anti-Aspergillus immunity. Lung neutrophils were found to express TFEB and TFE3, and their target genes were upregulated during A. fumigatus lung infection. Furthermore, A. fumigatus infection induced the nuclear accumulation of TFEB and TFE3 in macrophages, a process regulated by Dectin-1 and CARD9. The genetic deletion of Tfeb and Tfe3 impaired macrophage killing of A. fumigatus conidia. However, in a murine model with immune-competent Aspergillus infection and genetic deficiency of Tfeb and Tfe3 in hematopoietic cells, lung myeloid phagocytes surprisingly showed no defects in conidial phagocytosis or killing. Loss of TFEB and TFE3 did not impact murine survival or the clearance of A. fumigatus from the lungs. The findings suggest that myeloid phagocytes activate TFEB and TFE3 in response to A. fumigatus, and although this pathway promotes macrophage fungicidal activity in vitro, genetic loss can be functionally compensated in the lung. This compensation results in no measurable defect in fungal control and host survival.

Pancreatic beta cells play a critical role in maintaining glucose homeostasis by precisely sensing nutrients and regulating insulin production. Among the key regulators of the adaptive response to environmental cues, Transcription Factor EB (TFEB) and its homolog TFE3 have emerged as crucial players in controlling cell metabolism. This study explores the role of TFEB and TFE3 in regulating beta-cell function and insulin gene expression in response to variations in nutrient availability. The research reveals that nutrient deprivation in beta cells activates TFEB/TFE3, leading to the suppression of insulin gene expression. Notably, TFEB overexpression alone is sufficient to inhibit insulin transcription. Depletion of both TFEB and TFE3 in beta cells prevents the suppression of insulin gene expression in response to amino acid deprivation. ChIP-seq analysis uncovers the binding of TFEB to super-enhancer regions that regulate insulin transcription. In vivo experiments involving conditional, beta-cell-specific Tfeb-overexpressing mice and Tfeb/Tfe3 double-knockout mice demonstrate severe alterations in insulin transcription, secretion, and glucose tolerance. These findings underscore the physiological importance of TFEB and TFE3 as key mediators of pancreatic function. In summary, this study unravels a nutrient-controlled transcriptional mechanism orchestrated by TFEB and TFE3, shedding light on their pivotal role in regulating insulin production. The identified mechanism operates at both cellular and organismal levels, significantly contributing to our understanding of glucose homeostasis.

Birt-Hogg-Dubé (BHD) syndrome is an inherited familial cancer syndrome characterized by the development of cutaneous lesions, pulmonary cysts, and renal tumors and cysts. The syndrome is caused by loss-of-function pathogenic variants in the gene encoding the tumor-suppressor protein folliculin (FLCN). FLCN, acting as a negative regulator of the TFEB and TFE3 transcription factors, serves as a master controller of lysosomal biogenesis and autophagy. Its function involves enabling the phosphorylation of TFEB and TFE3 by the mechanistic Target Of Rapamycin Complex 1 (mTORC1). Previous studies demonstrated that the deletion of Tfeb rescued the renal cystic phenotype in kidney-specific Flcn knockout (KO) mice. Building upon this, the current research, utilizing Flcn/Tfeb/Tfe3 double and triple KO mice, reveals that both Tfeb and Tfe3 contribute differentially and cooperatively to kidney cystogenesis. Analysis of tumor samples derived from BHD patients further indicates increased activation of the TFEB/TFE3-mediated transcriptional program. Notably, silencing either of the two genes rescued tumorigenesis in human BHD renal tumor cell line-derived xenografts (CDXs). These findings, established in disease-relevant models, underscore the key roles of both TFEB and TFE3 as drivers of renal tumorigenesis. The study suggests novel therapeutic strategies that involve the inhibition of these transcription factors, providing potential avenues for the development of targeted treatments for BHD syndrome and related conditions.

The stress-responsive transcription factor EB (TFEB) serves as a master controller of lysosomal biogenesis and autophagy, playing a significant role in various cancer-associated diseases. While TFEB is well-known for its posttranslational regulation by the nutrient-sensitive kinase complex mTORC1, understanding its transcriptional regulation has been limited. In this study, integrative genomic approaches are employed to identify the immediate-early gene EGR1 as a positive transcriptional regulator of TFEB expression in human cells. The research demonstrates that in the absence of EGR1, the TFEB-mediated transcriptional response to starvation is impaired. Notably, both genetic and pharmacological inhibition of EGR1, achieved through the MEK1/2 inhibitor Trametinib, leads to a significant reduction in the proliferation of cells with constitutive activation of TFEB. This includes cells from a patient with Birt-Hogg-Dubé (BHD) syndrome, a TFEB-driven inherited cancer condition, cultured in both 2D and 3D environments. The findings unveil an additional layer of TFEB regulation involving the modulation of its transcription via EGR1. The study suggests that interfering with the EGR1-TFEB axis may represent a therapeutic strategy to counteract constitutive TFEB activation in cancer-associated conditions. This insight offers potential avenues for developing targeted therapeutic interventions for conditions associated with dysregulated TFEB activity.

Obesity stands out as a significant risk factor for end-stage kidney disease. Previous investigations from our group revealed that lysosomal dysfunction and impaired autophagic flux contribute to lipotoxicity in obesity-related kidney disease, observed in both human subjects and experimental animal models. However, the regulatory factors involved in mitigating renal lipotoxicity remain largely unknown. In this study, we discovered that palmitic acid strongly induced dephosphorylation and nuclear translocation of transcription factor EB (TFEB) by inhibiting the mechanistic target of rapamycin kinase complex 1 pathway. This effect occurred in a Rag GTPase–dependent manner, although it gradually diminished with extended treatment. The research then delved into the role of TFEB in the pathogenesis of obesity-related kidney disease. Mice with proximal tubular epithelial cell–specific (PTEC-specific) deficiency in Tfeb, fed a high-fat diet (HFD), exhibited increased phospholipid accumulation in enlarged lysosomes, presenting as multilamellar bodies (MLBs). Activated TFEB facilitated lysosomal exocytosis of phospholipids, aiding in the reduction of MLB accumulation in PTECs. Additionally, HFD-fed mice with PTEC-specific Tfeb deficiency displayed autophagic stagnation and worsened injury during renal ischemia/reperfusion. Finally, an association was observed between higher body mass index and increased vacuolation along with decreased nuclear TFEB in the proximal tubules of patients with chronic kidney disease. In summary, these findings highlight the critical role of TFEB-mediated lysosomal exocytosis in counteracting renal lipotoxicity, shedding light on potential therapeutic targets for obesity-related kidney disease.

The complex and pivotal process of epithelial-mesenchymal transition (EMT) plays a crucial role in organogenesis and is associated with various pathological processes, including cancer and fibrosis. During heart development, EMT facilitates the transformation of epicardial cells into vascular smooth muscle cells and cardiac interstitial fibroblasts. In this study, we highlight the oncogenic transcription factor EB (TFEB) as a key regulator of EMT in epicardial cells, and its genetic overexpression in mouse epicardium proves lethal due to heart defects linked to impaired EMT. TFEB takes center stage in orchestrating the EMT-promoting function of transforming growth factor (TGF) β, specifically through the activation of thymine-guanine-interacting factor (TGIF)1, a repressor in the TGFβ/Smad pathway. The activation of the Tgif1 promoter by TFEB is demonstrated, with in vitro and in vivo findings confirming increased Tgif1 expression upon Tfeb overexpression. Furthermore, Tfeb overexpression in vitro hinders TGFβ-induced EMT, and this effect is nullified by silencing Tgif1. Conversely, Tfeb loss of function, akin to Tgif1 deficiency, heightens cell sensitivity to TGFβ, inducing an EMT response even at low doses of TGFβ. In summary, these findings unveil an unexpected role of TFEB in regulating EMT, offering potential insights into the repair of injured hearts and the control of cancer progression. This study sheds light on the intricate mechanisms governing EMT, which could have implications for therapeutic interventions in conditions involving EMT dysregulation.

In the successful elimination of bacteria within phagocytes, the phago-lysosomal system plays a crucial role, but this process also relies on mitochondrial pathways. However, the communication between these two organelle systems is not well understood. This study identifies the lysosomal biogenesis factor transcription factor EB (TFEB) as a regulator for the crosstalk between phago-lysosomes and mitochondria in macrophages. Through a combination of cellular imaging and metabolic profiling, the research reveals that TFEB activation, triggered by bacterial stimuli, induces the transcription of aconitate decarboxylase (Acod1, Irg1) and the synthesis of its product itaconate. Itaconate is a mitochondrial metabolite with antimicrobial activity. The activation of the TFEB–Irg1–itaconate signaling axis proves effective in reducing the survival of the intravacuolar pathogen Salmonella enterica serovar Typhimurium. Subsequently, TFEB-driven itaconate is transferred via the Irg1-Rab32–BLOC3 system into the Salmonella-containing vacuole, exposing the pathogen to elevated itaconate levels. By activating itaconate production, TFEB selectively restricts the proliferation of Salmonella, a bacterial subpopulation that typically evades macrophage control. This contrasts TFEB's role in autophagy-mediated pathogen degradation. The data from this study define a TFEB-driven metabolic pathway between phago-lysosomes and mitochondria that effectively restrains the burden of Salmonella Typhimurium in macrophages both in vitro and in vivo.

Objective: The thermogenic activity of brown adipose tissue (BAT) holds the potential to enhance metabolic health in both mice and humans. However, the predominant exposure of humans to thermoneutral conditions often leads to BAT whitening, characterized by a reduction in mitochondrial content and metabolic activity. While mitophagy has been identified as a major contributor to mitochondrial degradation in the whitening process of thermogenic brite/beige adipocytes, the pathways involved in mitochondrial breakdown during the whitening of classical BAT remain largely unknown. The transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy belonging to the MiT family of transcription factors, stands out as the only member upregulated during whitening, suggesting a potential role for TFEB in whitening-associated mitochondrial breakdown. Methods: Brown adipocyte-specific TFEB knockout mice were generated, and BAT whitening was induced by thermoneutral housing. The study encompassed the characterization of gene and protein expression patterns, BAT metabolic activity, systemic metabolism, and mitochondrial localization using in vivo and in vitro approaches. Results: Under conditions of low thermogenic activation, the deletion of TFEB was found to preserve mitochondrial mass independently of mitochondriogenesis in BAT and primary brown adipocytes. However, this preservation did not translate into enhanced thermogenic capacity or protection from diet-induced obesity. TFEB-deficient BAT and primary adipocytes exhibited altered levels of autophagosomal/lysosomal markers. Lysosomal markers were observed to co-localize and copurify with mitochondria in TFEB-deficient BAT, indicating the trapping of mitochondria in late stages of mitophagy. Conclusion: The study identifies TFEB as a driver of BAT whitening, mediating mitochondrial degradation through the autophagosomal and lysosomal machinery. This research provides proof of concept that interfering with the mitochondrial degradation machinery can increase mitochondrial mass in classical BAT under human-relevant conditions. However, potential implications must be considered, as interfering with autophagy may lead to the accumulation of non-functional mitochondria. Future studies targeting earlier steps of mitophagy or target recognition are therefore warranted.

Autosomal dominant polycystic kidney disease (ADPKD) stands out as the most prevalent potentially lethal monogenic disorder. The majority of cases, approximately 78%, result from mutations in the PKD1 gene, responsible for encoding polycystin-1 (PC1). PC1, a sizable 462-kDa protein, undergoes cleavage in both its N and C-terminal domains. Notably, C-terminal cleavage yields fragments that translocate to mitochondria. In this study, transgenic expression of a protein corresponding to the final 200 amino acid (aa) residues of PC1 is demonstrated to suppress the cystic phenotype and preserve renal function in two Pkd1-KO orthologous murine models of ADPKD. This suppression is contingent upon an interaction between the C-terminal tail of PC1 and the mitochondrial enzyme Nicotinamide Nucleotide Transhydrogenase (NNT). The interaction plays a crucial role in modulating tubular/cyst cell proliferation, the metabolic profile, mitochondrial function, and the redox state. Collectively, these findings suggest that a short fragment of PC1 holds the potential to effectively suppress the cystic phenotype, opening avenues for exploring gene therapy strategies for ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD) represents a life-threatening monogenic condition stemming from mutations in PKD1 and PKD2, the genes encoding polycystin 1 (PC1) and polycystin 2 (PC2). PC1 and PC2 are localized to the cilia of renal epithelial cells, and their function is understood to involve an inhibitory activity that suppresses the cilia-dependent cyst activation (CDCA) signal. The deficiency of PCs leads to the activation of CDCA, thereby stimulating cyst growth. Recent studies have demonstrated that re-expression of PCs in established cysts can reverse PKD, suggesting a mode of action akin to a 'counterbalance in cruise control' to maintain lumen diameter within a designated range. This review explores recent findings pointing to novel avenues for future research on PCs with therapeutic potential for treating ADPKD.

The text discusses the utilization of drug screening data from extensive bulk gene expression databases for analyzing and determining the optimal clinical application of cancer drugs. With the increasing availability of single-cell RNA sequencing (scRNA-seq) data, there is an opportunity to gain insights into improving therapeutic effectiveness by studying the heterogeneity of drug responses among cancer cell subpopulations. The authors propose a computational approach called scDEAL, a deep transfer learning framework designed for predicting cancer drug responses at the single-cell level. This is achieved by integrating large-scale bulk cell-line data. The key feature of scDEAL is its ability to harmonize drug-related bulk RNA-seq data with scRNA-seq data. It achieves this by transferring a model trained on bulk RNA-seq data to predict drug responses in scRNA-seq. Another notable aspect of scDEAL is its use of integrated gradient feature interpretation to infer the signature genes associated with drug resistance mechanisms. The authors benchmarked scDEAL on six scRNA-seq datasets and demonstrated its model interpretability through three case studies. These case studies focused on drug response label prediction, gene signature identification, and pseudotime analysis. The text concludes by expressing the belief that scDEAL could significantly contribute to the study of cell reprogramming, drug selection, and repurposing, ultimately leading to improvements in therapeutic efficacy.

In autosomal dominant polycystic kidney disease (ADPKD), the inhibition of the overactivated alternative complement pathway has been shown to retard disease progression in animal models. However, the mechanisms underlying the upregulation of complement factor B (CFB) in ADPKD remained unknown. This study unraveled the association between CFB overexpression in cystic kidneys and increased JAK2/STAT1 activity, coupled with enhanced expression of the polycystin-1 C-terminal tail (PC1-CTT). The research demonstrated that manipulating STAT1 levels could reciprocally modulate CFB expression and CFB promoter activity. Additionally, overexpression of PC1-CTT induced JAK2/STAT1 activation and upregulated CFB in renal tubular epithelial cells. Further experiments revealed that PC1-CTT overexpression increased human CFB promoter activity, and this effect was mediated through putative STAT1 responsive elements. The impact of CFB on macrophage differentiation was explored, showing that bioactive CFB promoted macrophage M2 phenotype conversion. Conditioned media from renal epithelial cells, particularly those overexpressing PC1-CTT, further facilitated macrophage M2 phenotype conversion, and this effect was suppressed by fludarabine or a CFB antibody. Moreover, the study identified NF-kB as acting downstream of PC1-CTT, partly mediating PC1-CTT-induced CFB expression. In conclusion, this study sheds light on the mechanisms of CFB upregulation in ADPKD, highlighting the novel role of PC1-CTT in ADPKD-associated inflammation. The findings also suggest that targeting STAT1 could be a promising strategy to mitigate inflammation in the kidneys of ADPKD patients.

Polycystic kidney disease (PKD), encompassing both autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD), is characterized by the continuous formation of cysts in the kidneys and liver. ADPKD, prevalent in adults, results from mutations in PKD1 (polycystin1) and PKD2 (polycystin 2). On the other hand, ARPKD, affecting children, is primarily caused by mutations in the PKHD1 gene, encoding the Fibrocystin/Polyductin (FPC) protein, and a smaller subset by mutations in the DAZ interacting zinc finger protein 1 like (DZIP1L) gene. PC1/2 and FPC are transmembrane proteins located in the primary cilium, suggesting their role as signaling molecules. The precise mechanisms through which cilia regulate renal tubule morphology and prevent cyst formation remain unclear. Nevertheless, recent genetic and biochemical studies have provided insights into potential malfunctions and pathomechanisms underlying these diseases. This review summarizes genetic studies elucidating the functions of PC1/2 and cilia in cyst formation in ADPKD. It also discusses studies on the regulation of polycystin biogenesis and cilia trafficking. Additionally, it highlights synergistic genetic interactions between Pkd1 and Pkhd1, along with unique tissue patterning events controlled by FPC, not PC1. Notably, DZIP1L mutations compromise PC1/2 cilia expression, while FPC deficiency does not impact PC1/2 biogenesis and ciliary localization, indicating divergent mechanisms leading to cyst formation in ARPKD. The review concludes by outlining promising areas for future PKD research and suggesting potential therapeutic interventions for PKD treatment.

Mutations in polycystin-1 (PC1) give rise to autosomal-dominant polycystic kidney disease (ADPKD), a leading cause of renal failure without an available treatment. PC1, an integral membrane protein, is implicated in regulating various signaling pathways, including the JAK/STAT pathway. This study reveals that membrane-anchored PC1 activates STAT3 in a JAK2-dependent manner, resulting in tyrosine phosphorylation and transcriptional activity. The C-terminal cytoplasmic tail of PC1 can undergo proteolytic cleavage and nuclear translocation. Tail-cleavage eliminates PC1's direct activation of STAT3, but the cleaved PC1 tail now coactivates STAT3 in a mechanism requiring STAT phosphorylation by cytokines or growth factors. This leads to an exaggerated cytokine response. Thus, PC1 can regulate STAT activity through a dual mechanism. In ADPKD kidneys, PC1 tail fragments, including a unique ~15-kDa fragment (P15), are overexpressed. STAT3 is robustly activated in cyst-lining epithelial cells in human ADPKD, as well as in orthologous and nonorthologous polycystic mouse models. While STAT3 is activated in developing and postnatal kidneys, it is inactivated in adult kidneys. These findings suggest that STAT3 signaling is regulated by PC1 and serves as a driving factor for renal epithelial proliferation during normal renal development and cyst growth.

In the coronavirus family, there are currently seven different viruses, initially known as common cold viruses. This classification changed with the emergence of severe acute respiratory coronavirus (SARS-CoV) in 2002, Middle East respiratory syndrome coronavirus (MERS) in 2012, and severe acute respiratory coronavirus 2 (SARS-CoV-2), which caused the global COVID-19 pandemic in 2019. Utilizing bioinformatic methods, we investigated potential interactions between human miRNAs expressed in pulmonary epithelial cells and available coronavirus genomes. We compared putative miRNA binding sites between pathogenic and non-pathogenic virus groups. The pathogenic group shares six miRNA binding sites that may be involved in sequestering miRNAs associated with deep vein thrombosis. Additionally, we analyzed approximately 100,000 SARS-CoV-2 variant genomes for potential interactions with human miRNAs. This analysis revealed a group of 97 miRNA binding sites present in all analyzed genomes. Among these, six miRNA binding sites were specific to SARS-CoV-2 and other pathogenic viruses, with down-regulation linked to deep vein thrombosis and cardiovascular diseases. Notably, one of these miRNAs, miR-20a-5p, whose expression decreases with age, is involved in cytokine signaling, cell differentiation, and/or proliferation. We hypothesize that the depletion of poorly expressed miRNAs could be related to disease severity."

The interaction between RNA and RNA-binding proteins (RBPs) plays a crucial role in gene expression regulation, RNA stability, and various biological processes. RBPs execute these functions by binding to target RNA molecules through specific sequence and structure motifs. Identifying these binding motifs is essential for enhancing our understanding of cellular processes and their regulation. Introducing BRIO (BEAM RNA Interaction mOtifs), a new web server designed to identify sequence and structure RNA-binding motifs in one or more RNA molecules of interest. BRIO allows users to scan over 2508 sequence motifs and 2296 secondary structure motifs identified in Homo sapiens and Mus musculus, obtained from three different types of experiments (PAR-CLIP, eCLIP, HITS). These motifs are associated with the binding of 186 RBPs and 69 protein domains. The web server is freely accessible at http://brio.bio.uniroma2.it.

The provided text discusses the challenges posed by intra-tumour heterogeneity (ITH) in devising effective treatment strategies in clinical practice. Single-cell RNA sequencing (scRNA-seq) is acknowledged as a powerful tool for exploring ITH at the transcriptional level, offering a unique opportunity for therapeutic intervention. The results section introduces the emerging field of research focused on drug response prediction at the single-cell level, aiming to enhance the efficacy and precision of cancer treatments. The text then introduces a computational method named DREEP (Drug Response Estimation from single-cell Expression Profiles). DREEP utilizes publicly available pharmacogenomic screens from GDSC2, CTRP2, and PRISM, along with functional enrichment analysis, to predict single-cell drug sensitivity from transcriptomic data. Extensive in vitro validation of DREEP is conducted using multiple independent single-cell datasets comprising over 200 cancer cell lines, demonstrating the method's accuracy and robustness. Furthermore, DREEP is applied to molecularly barcoded breast cancer cells, leading to the identification of drugs capable of selectively targeting specific cell populations. In the conclusion, DREEP is highlighted as an in silico framework providing a means to prioritize drugs based on single-cell transcriptional profiles of tumors. The tool is positioned as valuable for designing personalized treatment strategies and expediting drug repurposing studies. The availability of DREEP is mentioned, with a link to its GitHub repository. Keywords such as "Drug prediction," "Single-cell transcriptomics," "Precision oncology," and "Cancer" summarize the key aspects covered in the text.

The text discusses the challenge of evaluating heterogeneity and its impact on outcomes and drug responses in cancer cells within a tumor, given their diverse phenotypes and dynamic plasticity. To address this, the authors perform transcriptional profiling on 35,276 individual cells from 32 breast cancer cell lines, resulting in a comprehensive single-cell atlas. The findings reveal a high degree of heterogeneity in the expression of biomarkers among the individual cells. Subsequently, the authors employ a deconvolution algorithm trained on the atlas to identify the cell line composition from bulk gene expression profiles of tumor biopsies. This approach enables patient stratification based on the cell line composition. Furthermore, the study links results from large-scale in vitro drug screening in cell lines to the single-cell data, allowing for the computational prediction of drug responses starting from single-cell profiles. A key observation is that transcriptional heterogeneity allows cells with varying drug sensitivity to coexist within the same population. In summary, the work establishes a framework for determining tumor heterogeneity by assessing cell line composition and drug response. This approach offers valuable insights into the coexistence of cells with differential drug sensitivity within a population, contributing to our understanding of cancer cell dynamics.

The text introduces the significance of Single-cell RNA sequencing (scRNA-seq) technology, which allows for the parallel characterization of thousands of cells at the transcriptome level. This technology is becoming a crucial tool for investigating cellular components and their interactions within the tumor microenvironment. Additionally, scRNA-seq is employed to unveil associations between tumor microenvironmental patterns and clinical outcomes, as well as to dissect cell-specific effects of drug treatments in complex tissues. Despite the proposal of methods for predicting drug response using gene expression data from scRNA-seq, there is a need for an integrated tool that spans from scRNA-seq analysis to drug discovery. In response to this, the authors present scDrug, a bioinformatics workflow encompassing a one-step pipeline for cell clustering in scRNA-seq data and two methods for predicting drug treatments. The scDrug pipeline consists of three primary modules: scRNA-seq analysis for identifying tumor cell subpopulations, functional annotation of cellular subclusters, and prediction of drug responses. This integrated tool, scDrug, allows for the exploration of scRNA-seq data and streamlines the drug repurposing process. Importantly, scDrug is made freely available on GitHub at https://github.com/ailabstw/scDrug, providing accessibility for researchers and practitioners in the field.

In the quest for personalized cancer treatment, machine learning models have been developed to predict drug response based on tumor and drug features. However, the majority of algorithm development efforts have relied on cross-validation within a single study for assessing model accuracy. While cross-validation within a biological dataset is a crucial initial step, it often provides an overly optimistic estimate of prediction performance when applied to independent test sets. To address this limitation and offer a more rigorous assessment of model generalizability across different studies, machine learning is employed to analyze five publicly available cell line-based datasets: National Cancer Institute 60, Cancer Therapeutics Response Portal (CTRP), Genomics of Drug Sensitivity in Cancer, Cancer Cell Line Encyclopedia, and Genentech Cell Line Screening Initiative (gCSI). The analysis considers observed experimental variability across studies and explores estimates of prediction upper bounds. The study reports the performance results of various machine learning models, with a multitasking deep neural network demonstrating the best cross-study generalizability. Notably, models trained on CTRP yield the most accurate predictions on the remaining testing data, and gCSI stands out as the most predictable among the cell line datasets included in the study. Through experiments and simulations on partial data, two key lessons emerge: (1) differences in viability assays can limit model generalizability across studies, and (2) drug diversity, rather than tumor diversity, plays a crucial role in enhancing model generalizability in preclinical screening.

In the realm of cancer research, cell lines serve as common in-vitro tumor models. The utilization of genomic data and large-scale drug screening has expedited the selection of the right drugs for cancer patients. The accuracy of drug response prediction is paramount for the success of precision medicine. Given the diversity of data types and the vast volume of big data, few methods efficiently integrate and identify the principal low-dimensional manifold in high-dimensional cancer multi-omics data to predict drug response. To address this challenge, a novel k-means Ensemble Support Vector Regression (kESVR) method is developed. This method predicts drug response values for individual patients based on cell-line gene expression data. kESVR combines supervised and unsupervised learning, relying on embedded clustering (Principal Component Analysis and k-means clustering) and local regression (Support Vector Regression). It is entirely data-driven and is designed to handle missing data and noise from outliers. Comparative analyses are conducted, pitting kESVR against four standard machine learning regression models: simple linear regression, support vector regression, random forest (quantile regression forest), and backpropagation neural network. The results, based on drug response across 610 cancer cells from Cancer Cell Line Encyclopedia (CCLE) and Cancer Therapeutics Response Portal (CTRP v2), showcase kESVR's superior accuracy, as indicated by the smallest mean squared error (MSE) measure. Furthermore, kESVR is compared with 17 existing drug response prediction models, encompassing a range of methods such as regression, Bayesian inference, matrix factorization, and deep learning. Upon ranking these models based on prediction accuracy, kESVR emerges as the top performer in the majority (74%) of cases. Even in the remaining cases (26%), kESVR consistently ranks within the top five models. In conclusion, the introduced model, kESVR, proves to be a powerful tool for drug response prediction using high-dimensional cell-line gene expression data. It outperforms current existing prediction models in terms of accuracy and speed, overcoming overfitting challenges. The potential application of kESVR in developing a robust drug response prediction system for cancer patients, guided by cancer cell-lines and multi-omics data, is highlighted for future exploration.

While cell functional annotation is a crucial step, it often presents challenges when dealing with single-cell transcriptional data. Several existing methods attempt to address this task, but many rely on techniques initially designed for bulk RNA sequencing or simply utilize marker genes identified through cell clustering followed by supervised annotation. To address these limitations and streamline the process, we introduce two novel methods: single-cell gene set enrichment analysis (scGSEA) and single-cell mapper (scMAP). The scGSEA method leverages latent data representations and gene set enrichment scores to identify coordinated gene activity at the resolution of individual cells. On the other hand, scMAP employs transfer learning techniques to repurpose and contextualize new cells within a reference cell atlas. Through the use of both simulated and real datasets, we demonstrate that scGSEA effectively captures recurrent patterns of pathway activity shared by cells from different experimental conditions. Additionally, scMAP reliably maps and contextualizes new single-cell profiles using a breast cancer atlas recently released by our team. Both tools are integrated into an efficient and straightforward workflow, providing a comprehensive framework for determining cell function. The introduction of scGSEA and scMAP significantly enhances the annotation and interpretation of single-cell RNA sequencing (scRNA-seq) data, offering valuable insights into cell functionality.

Gene expression systems that are inducible offer control over the expression of a target gene using a small molecule. One common approach involves engineering a transcription factor (TF) responsive to a small molecule, along with its corresponding promoter. However, this design often faces challenges in balancing minimal uninduced background expression (leakiness) with maximal induced expression. In this study, an alternative strategy is explored using quantitative synthetic biology to mitigate leakiness while maintaining high expression without modifying either the TF or the promoter. The focus of the research is the development of the CASwitch, a mammalian synthetic gene circuit designed through a combination of two well-known network motifs: the Coherent Feed-Forward Loop (CFFL) and the Mutual Inhibition (MI). This innovative approach involves combining the CRISPR-Cas endoribonuclease CasRx with the Tet-On3G inducible gene system to achieve optimal performance. The CASwitch is designed through mathematical modeling and validated experimentally. To showcase the potential of the CASwitch, it is applied to three distinct scenarios: enhancing a whole-cell biosensor, controlling the expression of a toxic gene, and inducing the production of Adeno-Associated Virus (AAV) vectors. The results demonstrate the versatility and efficacy of the CASwitch in diverse applications, emphasizing its value in synthetic biology and controlled gene expression systems.