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Enrichr-KG: bridging enrichment analysis across multiple libraries

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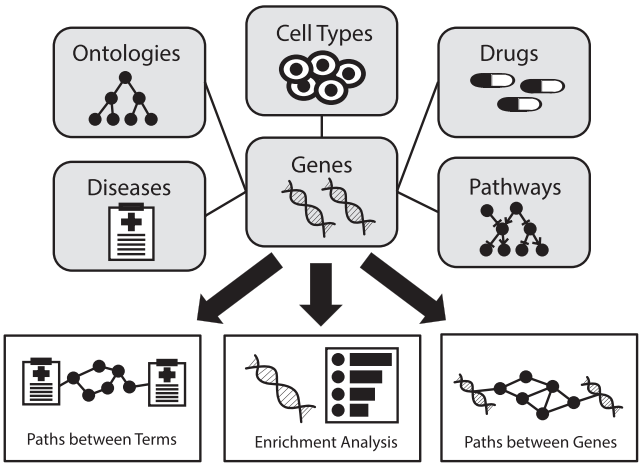
ABSTRACT

Gene and protein set enrichment analysis is a critical step in the analysis of data collected from omics experiments. Enrichr is a popular gene set enrichment analysis web-server search engine that contains hundreds of thousands of annotated gene sets. While Enrichr has been useful in providing enrichment analysis with many gene set libraries from different categories, integrating enrichment results across libraries and domains of knowledge can further hypothesis generation. To this end, Enrichr-KG is a knowledge graph database and a web-server application that combines selected gene set libraries from Enrichr for integrative enrichment analysis and visualization. The enrichment results are presented as subgraphs made of nodes and links that connect genes to their enriched terms. In addition, users of Enrichr-KG can add gene-gene links, as well as predicted genes to the subgraphs. This graphical representation of cross-library results with enriched and predicted genes can illuminate hidden associations between genes and annotated enriched terms from across datasets and resources. Enrichr-KG currently serves 26 gene set libraries from different categories that include transcription, pathways, ontologies, diseases/drugs, and cell types. To demonstrate the utility of Enrichr-KG we provide several case studies. Enrichr-KG is freely available at: https://maayanlab.cloud/enrichr-kg.

GRAPHICAL ABSTRACT



INTRODUCTION

Gene and protein set enrichment analysis provides context for genes and proteins identified in omics experiments using prior knowledge (1). Enrichment analysis involves querying a gene set against a catalog of annotated gene sets to find significant overlap between the input set and the annotated prior-knowledge gene sets. The results are ranked associated terms such as pathways, transcription factors, small molecules, diseases and other phenotypes, cell lines, cell types and tissues, and other biological and biomedical terms.

Enrichr (2–4) is a widely popular search engine for gene sets, performing enrichment analysis instantly against many annotated gene sets. In the past 10 years, over 59 million gene sets have been submitted as queries to Enrichr; and as of mid-2023, Enrichr has grown to host over ∼400 000 annotated gene sets from ∼200 gene set libraries. Such a resource provides a comprehensive collection of knowledge about genes, including their transcriptional and translational regulation, membership in pathways and biological processes, regulation and binding to drugs, association with

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diseases and other phenotypes, and expression across cell types, tissues, and cell lines. While Enrichr has been a valuable resource for hypothesis generation for many studies, there is still an opportunity to improve its functionality by, for example, integrating enrichment results across libraries and domains of knowledge. This can be achieved by viewing results of the enrichment analysis across libraries as an integrated network of genes and their annotations.

Network representation of biological molecular systems have been widely applied in biomedical research for abstracting connections between molecular entities (5–10). At the same time, many widely used web-based tools have been developed for network visualization and analysis. For example, STRING provides network visualizations of known and predicted associations between proteins, including physical protein-protein interactions (11). Genes2Networks (G2N) returns a protein interaction subnetwork that connects a set of input genes/proteins based on known protein-protein interactions (12). Another example is GeneMania (13) which visualizes associations between genes using evidence from across domains of knowledge such as co-expression, physical interaction, pathway membership, and shared structural domains. Other notable examples are HumanNet (14) and the DisGeNet Cytoscape app (15) which provide integrated network visualizations centered on disease genes and include predictions and prioritization of gene-disease associations.

Recently, knowledge graphs have gained popularity for integrating and generating hypotheses from connected data (16,17). Knowledge graphs have been used for studying disease mechanisms (18,19), mining small molecules for drug discovery (20,21), and analyzing connections between authors and biomedical entities using PubMed (22). Recently, there was an attempt to create a massive knowledge graph that integrates biomedical data for precision medicine (23). Within knowledge graphs data is stored as triples that describe how a subject entity is related to an object entity. For example, in the statement 'Drug A' targets 'Protein B', 'Drug A' is the subject, and 'Protein B' is the object, and the connection between them is described by the verb 'targets'. Generating a collection of these triples made of different types of entities forms a network of knowledge that can be navigated, becoming the subject for application of graph traversal algorithms, and graph completion prediction algorithms. However, one of the challenges with knowledge graphs is that their size grows rapidly and querying the graph for useful applications becomes challenging. At the same time, biomedical and biological knowledge about genes and proteins, as well as other molecular entities, can be stored as annotated gene sets. Such gene sets are useful for performing gene set enrichment analysis (1). Many tools and databases have been developed for performing gene set enrichment analysis, for example, DAVID (24), g:Profiler (25), WebGestalt (26), MSigDB-GSEA (27) and Enrichr (3). Currently, most enrichment analysis tools and databases store knowledge as gene set libraries. While such a storage schema has benefits, for example, performing fast overlap analysis across thousands of gene sets instantly, the comparison of enrichment results across multiple gene set libraries is not trivial. To solve this, tools such as EnrichmentMaps (28) visualize gene set enrichment analysis results as ball-and-stick subgraphs that connect genes to their enriched terms. Hence, several gene set enrichment analysis tools with network visualization already exist, each providing different features and advantages. A collection of such tools with a comparison of their features is provided (Table 1).

Here we describe a web-server application called Enrichr Knowledge Graph (Enrichr-KG) which combines enrichment analysis with a knowledge graph data representation to query a large collection of processed datasets made of associations between genes and many biological and biomedical terms. To create Enrichr-KG we converted selected gene set libraries from Enrichr into triples for ingestion into a knowledge graph database. Each triple represents the membership of a gene in an annotated gene set. Thus, performing enrichment analysis with Enrichr-KG returns an integrated network containing the top enriched terms from across multiple libraries connected to their overlapping genes. To preserve the breadth of knowledge offered by the Enrichr libraries, libraries were selected by their diversity, level of prior use, and uniqueness (Table 2).

MATERIALS AND METHODS

Collecting and processing the Enrichr libraries for knowledge graph database ingestion

We first selected 26 gene set libraries from Enrichr (2–4) for ingestion into the knowledge graph (Table 2). To preserve the variety of library types, we selected representative libraries from each Enrichr category as follows: transcription (ARCHS4 TFs (29), ChEA3 (30), FANTOM6 (31) and TRRUST (32)); pathways (KEGG (33,34), PFOCR (35), Reactome (36), the Kinase Library (37), and WikiPathways (38)); ontologies (Gene Ontology (39,40), Human Phenotype Ontology (41), Jensen DISEASES (42), and MGI Mammalian Phenotypes (43)); diseases/drugs (Project Achilles (44), LINCS L1000 perturbation signatures (45), and Drug Perturbation Proteome Atlas (46)), cell types (CCLE (47), Descartes (48), Human Gene Atlas (49), Tabula Muris (50), and Tabula Sapiens (51)); and other (Pfam (52)). Persistent IDs were then assigned to each term and gene. For genes, gene names were mapped to Entrez gene symbols (53). Genes that do not have a matching Entrez gene symbol, or a synonym, were discarded. The following method was employed for obtaining persistent identifiers for a term: (i) parsing the gene set term with a regular expression to extract the ID from the term; (ii) parsing data tables from the resource that was used to create the gene set library utilizing available APIs or downloadable mapping files; (iii) using ontologies and controlled vocabularies such as UBERON (54), Cell Ontology (55), Cellosaurus (55,56), PubChem (57) and Entrez Gene (53) and (iv) using the term as the persistent ID for those terms that failed the mapping methods described above. When possible, additional metadata elements were resolved from the gene set terms using regular expressions.

Building the graph database

Enrichr-KG visualizes the connections between enriched terms and genes across multiple selected gene set libraries.

Table 1. A comparison of features from resources providing enrichment analysis with network representations. If a resource had a broken URL, its features were taken from the relevant literature. Column values are: **A**: Interactive web server, **B**: Number of libraries, **C:** Statistical method, **D:** Cytoscape enabled, **E:** Gene set augmentation/predictions, **F:** URL to site works, **G**: PPIs, **H**: Co-expression correlations, **I**: Multiple edge types, **J**: Different node types in the same graph, **K**: Provides enrichment analysis

Resource	URL	PMID	A	B	C	D	E	F	G	H	I	J	K
Enrichr-KG	maayanlab.cloud/enrichr-kg		✓	24	Fisher exact test	✓	✓	✓	✓	✓	×	✓	✓
EnrichmentMap	baderlab.org/Software/EnrichmentMap	21085593	×	NA	NA	✓	×	✓	×	×	×	✓	×
BioGraph	biograph.pa.icar.cnr.it	30458802	✓	9	Fisher exact test	✓	×	×	×	×	×	✓	✓
MELODI	melodi.biocompute.org.uk	29342271	✓	5	Fisher exact test	×	×	✓	×	×	✓	✓	✓
Reactome graph database	reactome.org/dev/graph-database	29377902	×	1	NA	×	×	✓	×	×	×	×	×
GREG	www.moralab.science/GREG	32055858	✓	6	NA	×	×	×	✓	×	✓	✓	×
Bio4j	bio4j.github.io	NA	×	5	NA	✓	×	✓	✓	×	✓	✓	×
cyNeo4j	apps.cytoscape.org/apps/cyneo4j	26272981	×	NA	NA	✓	×	✓	×	×	✓	✓	×
DGLinker	dglinker.rosalind.kcl.ac.uk	34125897	✓	12	Fisher exact test	×	✓	✓	✓	✓	×	×	✓
AmiGO	amigo.geneontology.org/amigo	19033274	✓	2	Hypergeometric	✓	×	✓	×	×	×	×	✓
Genes2FANs	actin.pharm.mssm.edu/genes2FANs	22748121	✓	15	NA	✓	×	×	✓	✓	×	✓	×
STRING	string-db.org	36370105	✓	12	Kolmogorov–Smirnov	✓	×	✓	✓	✓	✓	×	✓
GeneMANIA	genemania.org	29912392	✓	20	Fisher	✓	✓	✓	✓	✓	✓	×	✓
DAVID	david.ncifcrf.gov	35325185	✓	16	EASE score	✓	×	✓	×	×	×	×	✓
ClueGO	apps.cytoscape.org/apps/cluego	19237447	×	3	Hypergeometric	✓	×	✓	×	×	×	✓	✓
Metascape	metascape.org	30944313	✓	24	Custom*	✓	×	✓	×	×	×	✓	✓
NetworkAnalyst	www.networkanalyst.ca	30931480	✓	11	GSEA	×	×	✓	✓	×	×	✓	✓
MSigDB-GSEA	www.gsea-msigdb.org	26771021	✓	15	GSEA	×	×	✓	✓	×	×	×	✓

*Explained in this blog post: https://metascape.org/blog/?p=122

Table 2. Gene set libraries stored in the Enrichr-KG database, their category, and term and gene coverage counts

Resource	Category	Terms	Gene coverage
Achilles (44)	Diseases/Drugs	216	4779
ARCHS4 (29)	Transcription	1724	22 226
ASCT+B (103)	Cell Types	777	12 531
CCLE (47)	Cell Types	378	11 710
ChEA3 (30)	Transcription	757	18 364
Descartes (48)	Cell Types	172	9515
DisGeNET (104)	Diseases/Drugs	9828	17 266
FANTOM6 (31)	Transcription	206	13 682
Gene Ontology (39,40)	Ontologies	6036	14 929
GWAS Catalog (105)	Diseases/Drugs	1737	15 296
Human Gene Atlas (49)	Cell Types	84	12 087
Human Phenotype Ontology (41)	Ontologies	1779	3077
DISEASES (42)	Ontologies	1811	15 141
KEGG (33,34)	Pathways	320	8073
LINCS (CRISPR KO) (45)	Diseases/Drugs	5212	9440
LINCS (Small Molecule) (45)	Diseases/Drugs	5425	9525
MGI Mammalian Phenotype (43)	Ontologies	4601	9756
Pfam (52)	Misc	608	8975
PFOCR (35)	Pathways	17 326	12 765
Reactome (36)	Pathways	1818	10 489
Tabula Muris (50)	Cell Types	106	3857
Tabula Sapiens (51)	Cell Types	469	1509
TRRUST (32)	Transcription	571	3126
WikiPathways (38)	Pathways	622	7151

Such visualization is achieved by storing the serialized processed gene set libraries in a Neo4j database (58). Specifically, the gene set libraries from Enrichr are converted into nodes and links where the nodes are either gene set terms or genes. If a gene is part of a gene set, then an edge is added to the network. Such information is stored in separate CSV files. The conversion of a gene set library into nodes and edges CSV files requires the construction of three files: (i) a term node CSV file containing the term identifiers, the term string, and any additional metadata; (ii) a unified gene node CSV file containing all the genes that appear in the gene set libraries and their respective identifiers and (iii) an edge CSV file that contains information on the connection between the genes and the terms, not including the weights which are associated with the enrichment results. These files are ingested into the Neo4j database using the py2neo bulk import function. Querying the database is achieved via the Cypher query language (59).

Creating the web-server application

While Neo4j comes with a console to query the database, it is not very customizable, and it is difficult to export as an open web-server application that can be shared publicly without a login requirement. To provide a public facing open customizable interface that enables users to interact with the data, we constructed a web application that uses Cytoscape.js (60) to visualize the results from the Cypher queries. Next.js and React were used to build the Enrichr-KG website. To provide enrichment analysis, Enrichr-KG communicates with the Enrichr API (2–4) to perform enrichment analysis. The results are then queried against the Neo4j database and visualized as a network.

Adding protein-protein interactions and gene-gene co-expression correlations

To include protein-protein interactions as an option for inclusion in subnetworks, human protein-protein interactions were downloaded from the STRING database (11). The top-scored 150 000 protein-protein interactions (PPIs) that

share a physical complex ranked by the combined score are included in the Enrichr-KG database. To include gene-gene co-expression correlations, the co-expression correlation matrix from ARCHS4 (29) was used. The top 10 co-expressed genes for each gene are extracted and included in the database. Correlations are computed and ranked by the Pearson correlation coefficient.

Augmenting subnetworks with predicted genes

To augment the genes in the subnetworks with additional genes based on co-expression, the genes in the subnetwork are used as the input. These genes are submitted to the Geneshot API (61) to obtain genes that on average are mostly co-expressed with the genes in the subnetwork using the co-expression matrix from ARCHS4 (29). The Cypher query is then updated to identify connections between the augmented co-expressed genes and the enriched terms.

Free text descriptions of subgraphs

To produce free text descriptions of the visualized subgraph, we developed templates that describe each type of association. The templates are then filled with the text describing genes and gene sets. For example, for gene-structural domain associations from Pfam (52), the template is: 'the gene products ${genes} have the structural domain ${term}'.

RESULTS

Interacting with the Enrichr-KG web-server application

Enrichr-KG is a gene set enrichment analysis tool that visualizes enrichment results as an interactive web-based network that connects genes to enriched terms, for example, pathways, biological processes, or phenotypes. To create Enrichr-KG, we serialized gene set libraries into CSV files that are ingested into a Neo4j database. The Enrichr-KG web interface is a customizable general-purpose UI that is built on top of the Neo4j database. As such, the UI component of Enrichr-KG can be reused for other related bioinformatics projects. The Enrichr-KG UI enables users to interact with the underlying data stored in the Neo4j database by performing gene set enrichment analyses on their input gene sets with various customization and interactive features (Figure 1). First, users can submit gene sets to perform enrichment analysis against a maximum of five selected gene set libraries. Input genes are validated against a dictionary of Entrez gene symbols and users are informed in real-time whether the gene is in the database (Supplementary Figure S1). Upon pressing the submit button, Enrichr-KG returns a subgraph that displays the top enriched terms per library as well as the genes that overlap across these terms (Supplementary Figure S2). Users can tweak the settings to control the subgraph content by adjusting various parameters such as the maximum node degree, maximum subgraph size, the gene set libraries to use, and the number of top terms to include from each library. The subnetwork layout can be changed to force-directed, circular, or hierarchical layouts. Additionally, the subnetwork can be downloaded as an image, as a story described in free-text, or as a serialized CSV file. Users can also view the enrichment results in a table or a bar chart that summarizes the results across libraries (Supplementary Figure S3). In addition, users can augment the subnetworks with additional predicted genes based on co-expression correlations; and add to the subnetworks known gene-gene links based on protein-protein interactions and/or co-expression correlations.

The term and gene search tab in Enrichr-KG enables users to query the database to identify specific genes or terms. The single term or single gene search queries display the immediate neighbors of that node. For example, known annotations for the gene APOE are displayed as a star subnetwork (Supplementary Figure S4). APOE is known to be associated with Alzheimer's disease and cholesterol metabolism (62–66). The two-term search feature of Enrichr-KG returns a subgraph that contains the shortest paths between two nodes. Shortest paths can be used to find connections between pairs of gene-gene, term-term, or gene-term nodes. This type of query returns the shared genes between two gene sets, or shared annotations between two genes. Such queries can illuminate connections between a gene and a gene set even if the gene is not a member of the set. For example, the subgraph that connects the two genes HNF1B and KCNJ11 shows their shared annotations such as decreased ß-cell function and decreased insulin sensitivity (67) (Supplementary Figure S5). Like the enrichment analysis subgraphs, the layout of these subgraphs can be changed, the subgraph can be augmented with additional genes, enriched with known protein-protein interactions, and made available for download as a CSV file, or as a story written in free text.

Case study 1: exploring knowledge about the APOE4 variant

APOE is a polymorphic gene associated with the risk of late-onset Alzheimer's disease (AD) (62–65). To understand the mechanisms of APOE4, the highest risk polymorphic form of APOE, Blanchard *et al.* (66) performed single cell RNA-seq profiling of post-mortem human brains of APOE4 carriers vs. non-carriers. Their findings show altered cell signaling of pathways involved in cholesterol homeostasis and transport. From this study, we submitted the top 100 up-regulated genes in the APOE4 carriers compared to the non-carriers for analysis with Enrichr-KG (Figure 2). Consistent with the reported findings, Enrichr-KG also identifies cholesterol-related enriched pathways from KEGG and WikiPathways. We also found enrichment for terms related to regulation of the cell cycle and immune activation. It is well accepted that inflammation and immune response activation is the key molecular mechanism of AD (68). The Enrichr-KG subnetwork can be used to narrow down mechanisms to the specific genes, pathways, transcription factors, and cell types that may be involved.

Case study 2: exploring molecular mechanisms of diabetic nephropathy

In the US, diabetic nephropathy (DN) is a common complication of diabetes that often leads to end-stage renal disease (ESRD) (69). Several studies examined the progression

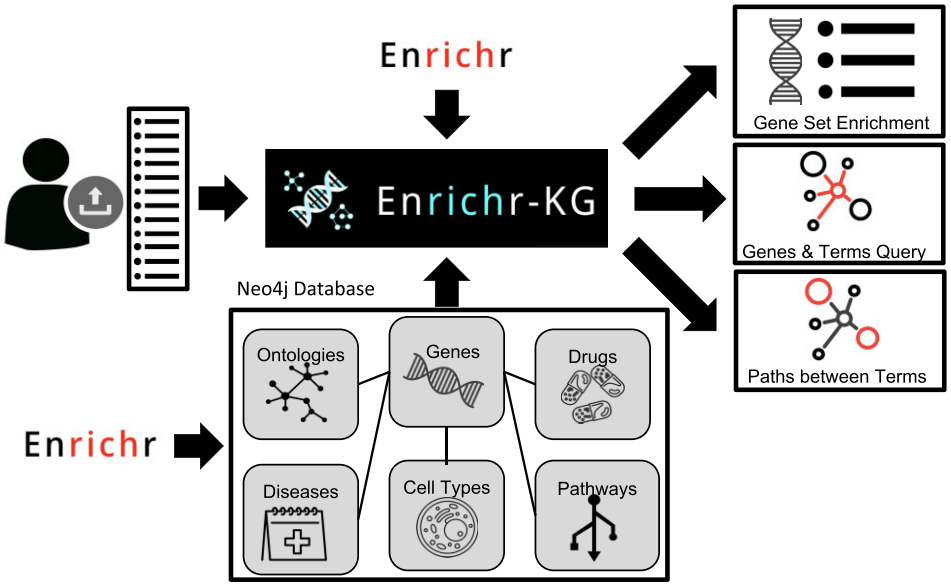

Figure 1. The Enrichr-KG workflow. Enrichr gene set libraries were serialized to nodes and edges where a gene is connected to a term if it is a member of the annotated gene set. The resulting network is made of links that connect genes to drugs, diseases, cell types, pathways, transcription factor regulators, and biological processes. A public facing web interface is provided to interact with the data. The interface enables users to input gene sets to perform integrated gene set enrichment analysis. The returned result is a subnetwork containing the top enriched terms from the libraries selected connected to the overlapping genes across libraries. Bar graphs and tables are also provided to visualize the top enriched terms. Gene and term search functionality can be used to query the immediate neighbors of a node. This is useful for finding shortest paths between two genes, two terms, or a gene and a term. All subnetworks can be downloaded as CSV files containing the nodes and edges of the subnetwork, as well as a free text description of the contents of the subnetwork.

of DN in hopes of finding targets and therapeutics to intervene with the progression of the disease to prevent or postpone ESRD (70–74). In a recent study, Fan et. al. (70) compared the transcriptomics profiles of early stage DN to advanced DN. 270 genes were identified to have lower expression level during early stage DN compared to the advanced stage, while 148 genes are up-regulated in early DN but are lowly expressed in advanced DN. The authors concluded that up-regulated genes in late DN are mainly related to inflammation and increased immune response. On the other hand, several genes that are downregulated in the late stage are reno-protective with RDH8, RDH12 and RBP4 being part of the retinoic acid pathway. The results from this study suggest that increasing the expression of these genes may help in preventing the progression of diabetic nephropathy. To further demonstrate the functionality of Enrichr-KG, we submitted the 148 genes that are down-regulated in advanced DN compared with early DN for analysis with Enrichr-KG. To perform the pathway enrichment analysis of these genes, we selected the KEGG and WikiPathways gene set libraries. Consistent with the published study, we find that the genes RDH8, RDH12 and RBP4, as well as CYP2E1 are enriched for the Vitamin A and carotenoid metabolism pathways; meanwhile RDH8, RDH12 and UGT1A7 overlapped with the retinol metabolism pathway in KEGG (Figure 3). Interestingly, we found that the 148 genes are also enriched for alanine, aspartate, and glutamate metabolism (GPT, NAT8L and AGXT), alanine and aspartate metabolism (GPT, and AGXT), tryptophan metabolism (CYP2E1, CYP4F12, and CYP2J2), and steroid hormone biosynthesis (CYP2E1, UGT1A7, and HSD3B1). It has been shown that a high ratio between aspartate aminotransferase to alanine aminotransferase can be a risk factor for DN (75). Furthermore, low levels of tryptophan were also identified as a prognostic marker for DN (76). Another study reported that impairment of renal steroidogenesis has a probable role in diabetes related kidney damage in rats (77). Lastly, selecting the SigCom LINCS (45) L1000 chemical perturbation consensus signatures resource, Enrichr-KG reports small molecules that may up-regulate the reno-protective genes. These genes include RDH8, RDH12, RBP4 and GLP1R from the original paper, as well as CYP2E1 and UGT1A7 that also overlap with the vitamin A and retinol related pathways. We found three small molecules that up-regulate some of these genes (Figure 3). One of them is Pinitol, a known anti-diabetic agent extracted from the plant Bougainvillea spectabilis. It has been shown to improve glycaemic control in mice (78). Recently, it has been shown to also have a reno-protective effect on diabetic rats (79). Another compound that up-regulates the reno-protective genes is the ChEBI classified SA-1938862 (CHEBI:126863, BRD-K21368140-001-01-0) which is a harmala alkaloid (CHEBI:61379) (80). Harmala alkaloids are alkaloids extracted from the Peganum harmala plant (81). It has been shown that seed extracts from Peganum harmala mitigate kidney damage in diabetic rats (82). In addition, the anti-inflammatory drug bethametasone is also enriched for up regulating the reno-protective genes. It is known that bethametasone causes spikes in blood sugar (83). However, in general, glucocorticoids have been used alone or in combination with other drugs to treat glomerular diseases (84).

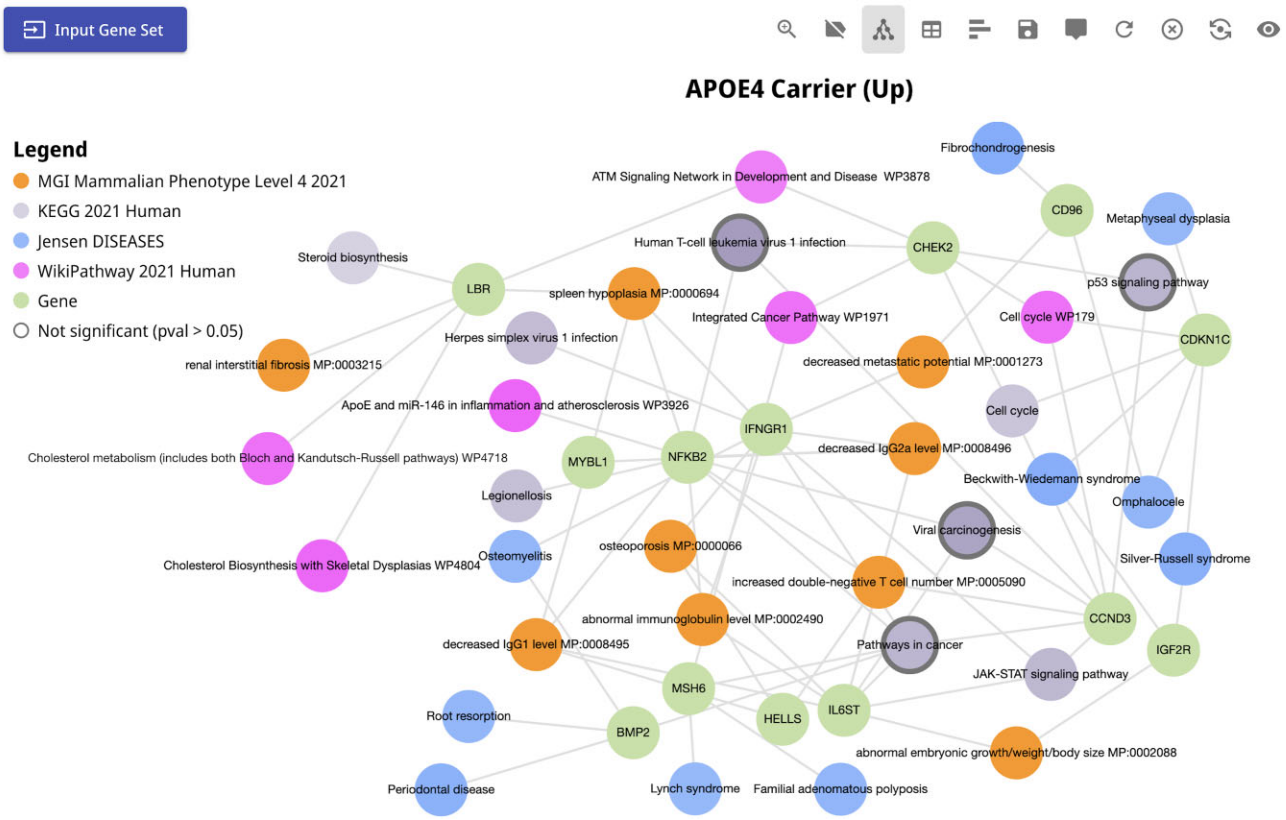

Figure 2. Subnetwork from the APOE4 case study. Enrichment analysis was performed on the top 100 up-regulated genes of postmortem brains from APOE4 carriers compared with the non-carriers extracted from the Blanchard et al. study. The KEGG Pathways, WikiPathways, DISEASES, DisGeNET and MGI Mammalian Phenotype gene set libraries were selected for the analysis. A subnetwork that connects the top enriched terms and the up-regulated genes that appear in at least two libraries is shown. The query can be accessed via the following URL: https://maayanlab.cloud/turl/199918c6.

Case study 3: exploring phenotypes, kinases, and drugs related to type 2 diabetes mellitus utilizing the gene set augmentation feature of Enrichr-KG

Type 2 diabetes mellitus is a common metabolic disease characterized by an inability to secrete insulin by pancreatic beta cells, and/or a loss in the ability of cells and tissues to respond to insulin (85). We can examine genes associated with type 2 diabetes mellitus with Enrichr-KG by using the gene set search feature in the input form of the application. Searching for 'type 2 diabetes' in the term search feature of Enrichr-KG, we identified a gene set sourced from ClinVar (86) which provides a list of genes with mutations and other various are known to be associated with the disease. Next, we selected the MGI Mammalian Phenotype Library (43) to view mouse phenotypes associated with this gene set, the Kinase Library (37) to view related kinases that phosphorylate gene products from the gene set, and the Proteomics Drug Atlas (46) to prioritize drugs that may induce or suppress the expression of the genes in the set (Figure 4). Examining the resultant subgraph, we observe phenotypes such as decreased pancreatic beta cell number, hyperglycemia, and impaired glucose tolerance. These terms are connected to the IRS2 gene, which encodes the insulin receptor substrate. IRS2 is also the substrate of multiple kinases including MEKK6, MAP3K15, PDK1 and CK1A. Impaired IRS2 function is crucial in the development of type 2 diabetes (87,88). Additionally, the drug AZD8055 is identified as an up-regulator of IRS2, IRS1 and TCF7L2 at the protein level. AZD8055 is an mTOR inhibitor that was shown to induce insulin resistance *in vivo* (89). It is unclear how this seemingly conflicting evidence is resolved. Selecting the 'augment gene set' feature of Enrichr-KG, we can add co-expressed genes into the displayed subnetwork. One of the genes that are added to the network is USH1C. This gene was identified to play a role in hearing and vision (90), but the observation that it is highly co-expressed with the genes in the subnetwork, and the observation that mice with this gene knocked out display a decrease in circulating insulin levels, suggest that it is likely also playing an important role in diabetes. Vision impairments are a known phenotype of type 2 diabetes, and USH1C function is likely involved.

Case study 4: exploring phenotypes, drugs, genes and kinases related to cellular senescence

Cellular senescence is a state of permanent cell cycle arrest of somatic cells (91) and is implicated in aging-related pathologies and cancer (92,93). A gene set containing 301 genes called SenoRanger was established by identifying genes upregulated in RNA-seq profiles of senescent cells from a variety of studies compared to expression from multiple atlases containing normal expression, retaining genes

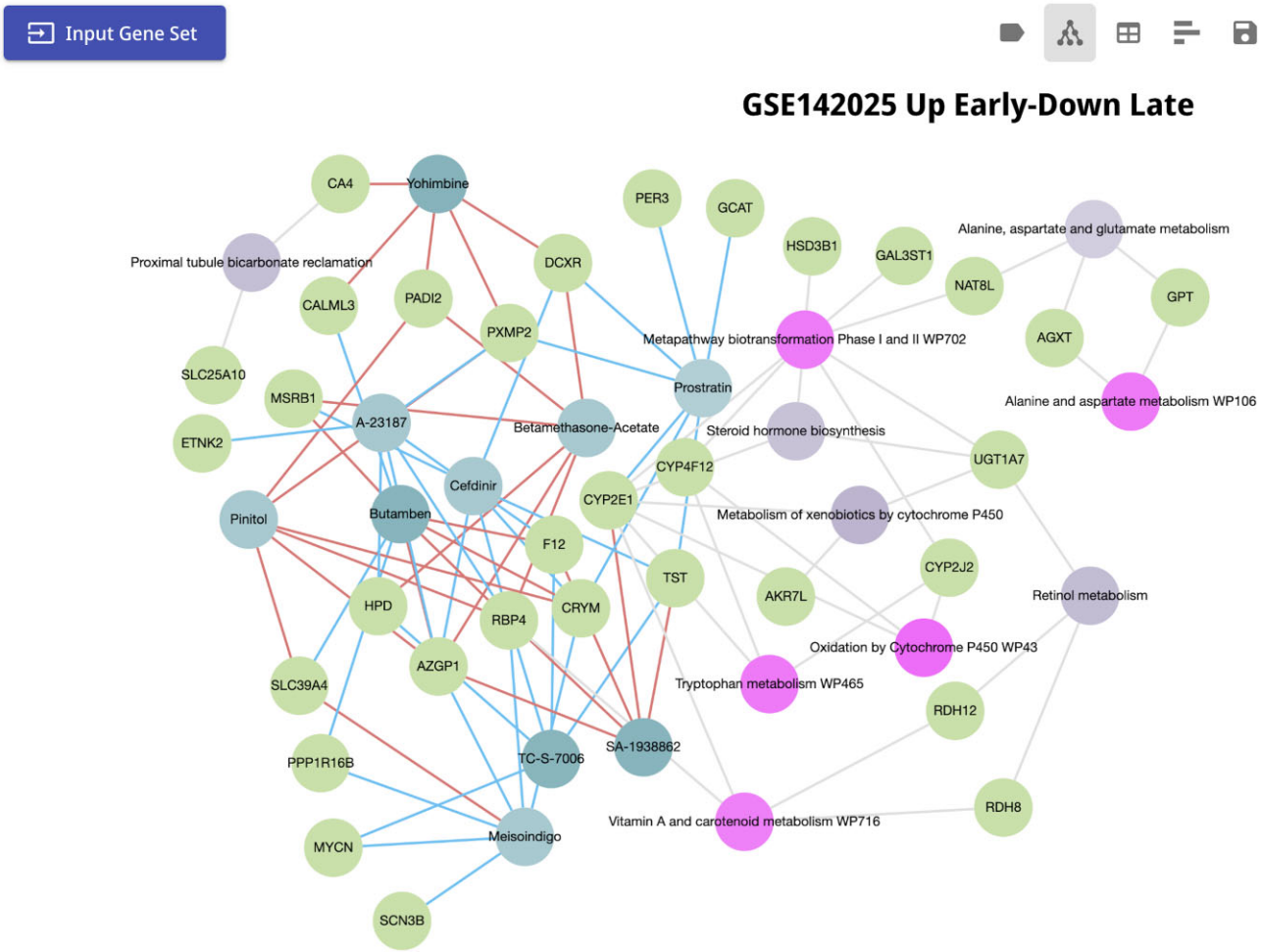

Figure 3. Enriched terms for the diabetic nephropathy case study. 148 genes that are down-regulated in advanced stage DN compared with early stage were submitted to Enrichr-KG with the selected gene set libraries: KEGG pathways, WikiPathways and LINCS L1000 consensus chemical perturbations. Genes appear as green nodes, WikiPathways pathways are pink nodes, KEGG pathways are in light purple, and LINCS L1000 compounds are in turquoise and light blue. Small molecules that up regulate a gene are linked to the gene via a red link, and those that down-regulate the genes are in blue. The query can be accessed via the following URL: https://maayanlab.cloud/turl/d9e0a6f3.

identified in multiple of these comparisons (94). To identify mouse phenotypes enriched in the SenoRanger gene set, we submitted it to Enrichr-KG (Figure 5) and selected the MGI mouse phenotypes (43) gene set library. Several phenotypes related to skin appear in the subnetwork, namely, 'abnormal cutaneous collagen fibril morphology MP:0008438', 'decreased skin tensile strength MP:0003089', and 'abnormal dermal layer morphology MP:0001243'. At the same time, using the SigCom LINCS resource (45), maxacalcitol, a derivative of vitamin D used to treat skin disorders (95) is identified as the only drug that up-regulates many of the genes in the subnetwork. This observation is in concordance with prior literature where vitamin D analogs have been shown to cause DNA damage and cellular senescence in epithelial type II cells (96).

The SenoRanger genes were also enriched for genes downregulated in several CRISPR KO signatures including those of GPR25, RGS1, CLCNKB, and L1CAM. RGS1 is a regulator of T-cell migration and exhaustion and has been investigated as a target for treating multiple cancers (97). Its knockdown in cervical cancer cell lines led to increased apoptosis and inhibition of cell proliferation and migration (98). L1CAM, a cell adhesion molecule, has been previously identified as an overrepresented cell surface maker in senescence cells. Additionally, its expression is associated with metabolic changes and enhanced migration and adhesion (99). Finally, selecting the Kinase Library (37) we observed multiple significantly enriched kinases including TGFBR2, ANKRD3, GRK1, GRK2 and ALK4. GRK2 is involved in cell cycle regulation and progression and its increased expression may induce cellular senescence through cell cycle arrest mediated by increased p53 phosphorylation (100,101). Additionally, TGFRBR2, the TGF-β receptor, was identified and included in the subnetwork. TGF-β signaling plays an important role in cellular senescence as well as age-related pathologies such as obesity and Alzheimer's disease (102). Overall, this and the other use cases demonstrate how Enrichr-KG can be used to confirm existing knowledge and to form new hypotheses via integrative analysis and visualization.

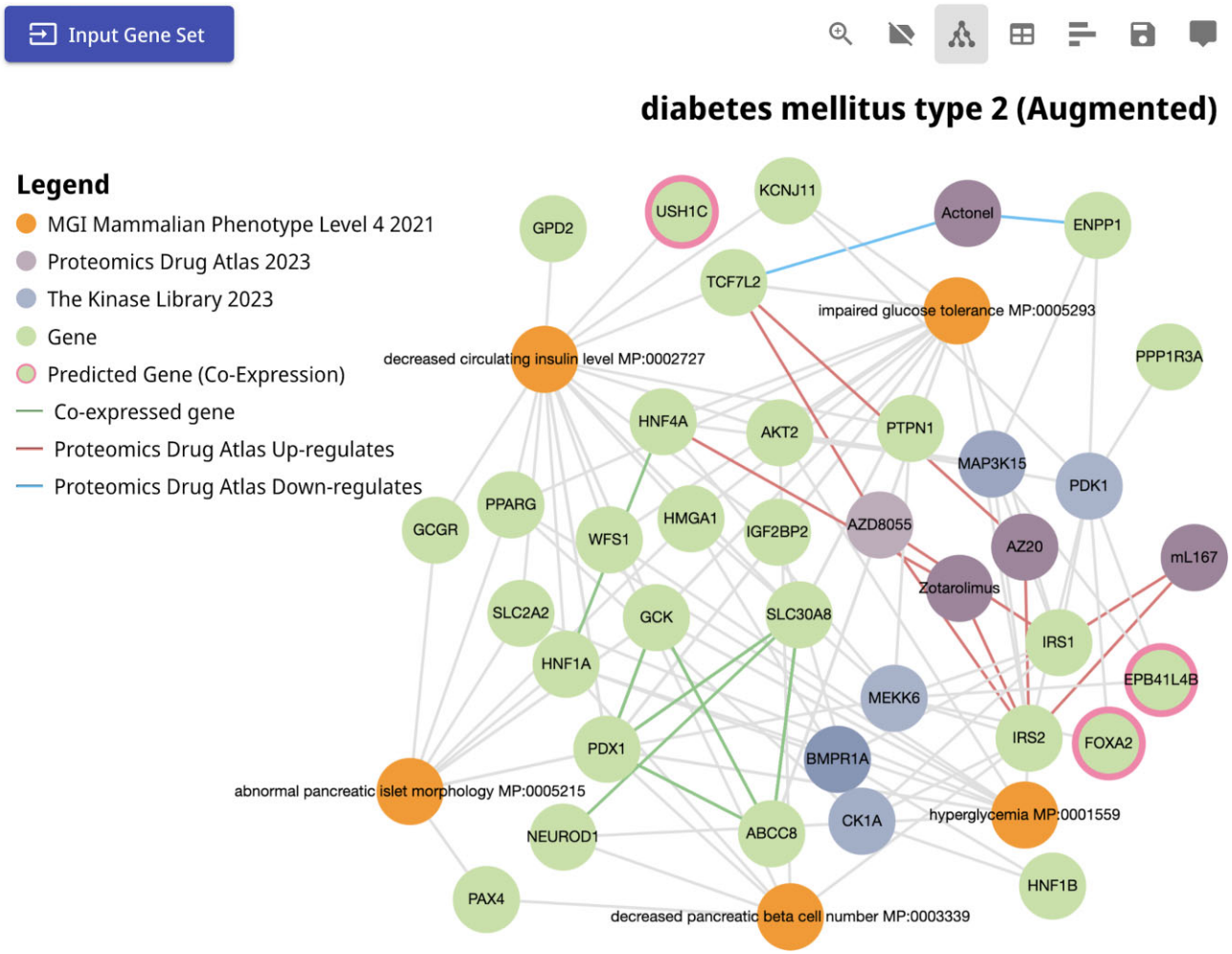

Figure 4. Subnetwork for the type 2 diabetes mellitus case study. A gene set made of 28 genes was fetched from the ClinVar 2019 gene set library with the annotation term 'diabetes mellitus type 2' and was submitted to Enrichr-KG. The MGI Mammalian Phenotype, the Kinase Library, and the Proteomics Drug Atlas gene set libraries were selected for the analysis. The resultant subnetwork that connects the top enriched terms and the up-regulated genes that appear in at least two libraries is visualized. Gene set augmentation was selected to add highly co-expressed genes. The query can be accessed via the following URL: https://maayanlab.cloud/turl/8b005a41.

DISCUSSION

Here, we present Enrichr-KG, a web-server application that extends Enrichr's gene set enrichment analysis by bridging results from across multiple gene set libraries. To achieve this, we converted gene set libraries into a bipartite graph where genes are connected to their annotation terms. Such a representation can be ingested into a knowledge graph database for fast querying. Importantly, to distill the most useful information from this knowledge graph, the queries are coupled with gene set enrichment analysis results. The networked approach facilitates querying for paths between genes and annotation terms that might otherwise be difficult to extract. Some of the features available from Enrichr-KG that are not part of Enrichr are infusion of known gene-gene associations from protein interactions and co-expression resources, augmentation with additional relevant genes based on co-expression, and textual summaries of the contents of the subnetworks produced by Enrichr-KG.

Because Enrichr-KG relies on Enrichr for the gene set enrichment analysis component, it also shares some of the limitations of Enrichr. First, human and mouse genes are merged to simplify the gene search space, which could be a disadvantage for some analysis contexts. While the Enrichr API supports the upload of a gene set background, this feature is not currently implemented for Enrichr-KG. In addition, Enrichr-KG currently contains a small subset of all the gene set libraries available from Enrichr. Apart from these limitations, which will be mitigated in future releases, we also plan on extending the knowledge graph's functionality by better predicting additional links using more sophisticated graph completion machine learning algorithms. Such functionality will further assist with hypothesis generation. In addition, we also plan on utilizing existing large language models (LLM) to produce improved textual descriptions with references to better describe the resultant subnetworks extracted from the enrichment analysis results.

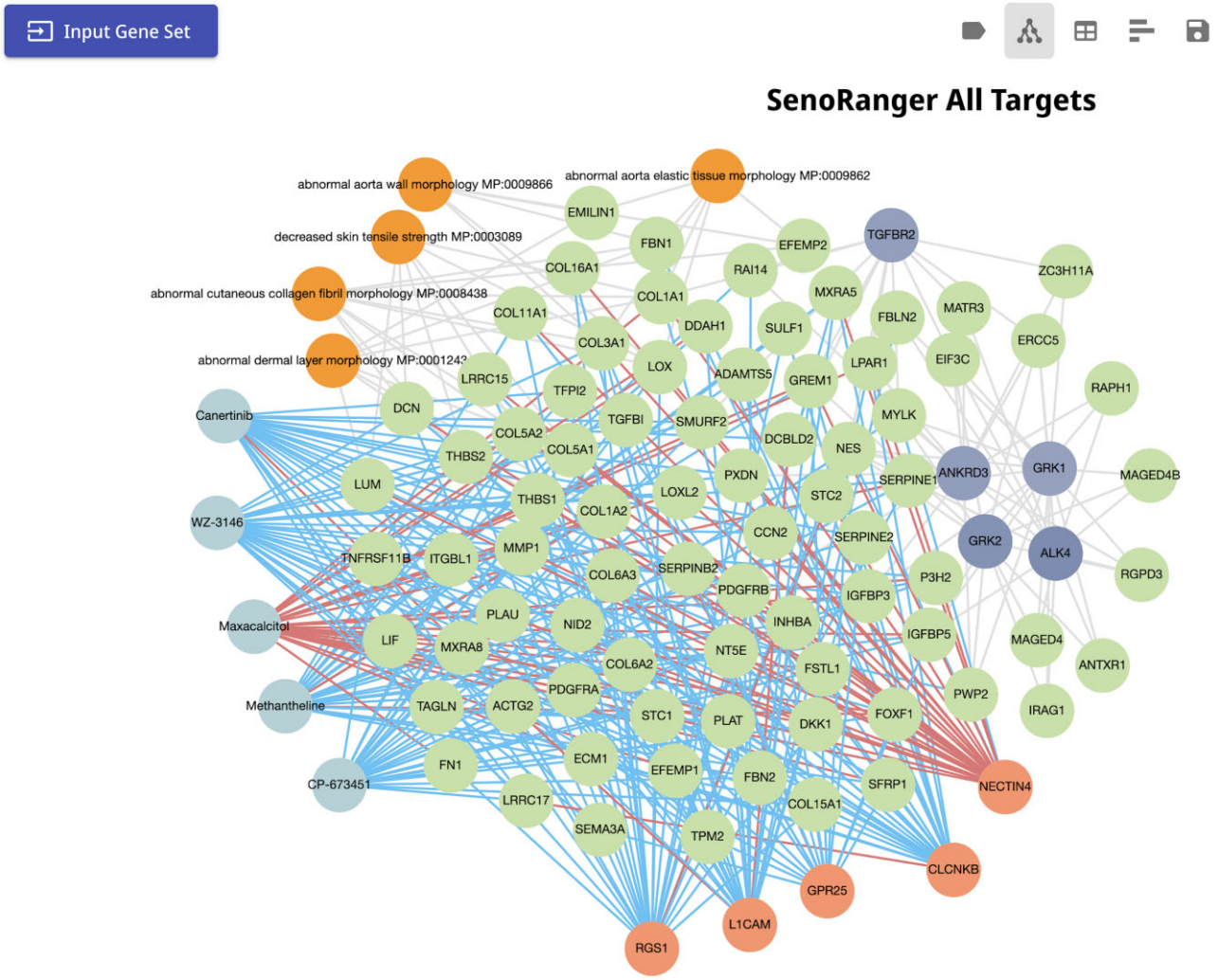

Figure 5. Subnetwork for the cellular senescence case study. A gene set containing 301 genes called SenoRanger [Deng *et al.* in press] was established by identifying genes upregulated in RNA-seq profiles of senescent cells from several in-vitro studies. The set of 301 genes was submitted to Enrichr-KG for analysis. The MGI Mammalian Phenotype, the Kinase Library, and SigCom LINCS gene set libraries were selected for the analysis. A subnetwork that connects the top enriched terms and the up-regulated genes that appear in at least two libraries is automatically generated. The query can be accessed via the following URL: https://maayanlab.cloud/turl/43025ee5.

DATA AVAILABILITY

The Enrichr-KG web-server application is available at: https://maayanlab.cloud/enrichr-kg. The processed datasets for ingestions into a knowledge graph database are available as CSV files for download from: https://maayanlab.cloud/enrichr-kg/downloads.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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