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An Insight Into the Molecular Mechanism of Berberine Towards Multiple Cancer Types Through Systems Pharmacology

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Over the past several decades, natural products with poly-pharmacological profiles have demonstrated promise as novel therapeutics for various complex diseases, including cancer. Berberine (PubChem CID: 2353), a soliloquies quaternary alkaloid, has been validated to exert powerful effects in many cancers. However, the underlying molecular mechanism is not yet fully elucidated. In this study, we summarized the molecular effects of berberine against multiple cancers based on current available literatures. Furthermore, a systems pharmacology infrastructure was developed to discover new cancer indications of berberine and explore their molecular mechanisms. Specifically, we incorporated 289 high-quality protein targets of berberine by integrating experimental drug–target interactions (DTIs) extracted from literatures and computationally predicted DTIs inferred by network-based inference approach. Statistical network models were developed for identification of new cancer indications of berberine through integration of DTIs and curated cancer significantly mutated genes (SMGs). High accuracy was yielded for our statistical models. We further discussed three typical cancer indications (hepatocarcinoma, lung adenocarcinoma, and bladder carcinoma) of berberine with new mechanisms of actions (MOAs) based on our systems pharmacology framework. In summary, this study systematically provides a powerful strategy to identify potential anti-cancer effects of berberine with novel mechanisms from a systems pharmacology perspective.

Keywords: berberine, cancer, systems pharmacology, drug–target interactions, significantly mutated genes

INTRODUCTION

Natural products with diverse chemical scaffolds have been recognized as an invaluable source of candidates in drug discovery and development for multiple complex diseases, including cancer. Berberine, a plant-derived compound isolated from medicinal plants such as Coptis chinensis and Hydrastis canadensis, had a long history of medicinal application in traditional Chinese medicine

(Ayati et al., 2017). As one of the main alkaloids, berberine has been reported to exert potentially beneficial effects on many cancer types, including breast cancer (Kim et al., 2008), bladder cancer (Yan et al., 2011), and hepatocarcinoma (Liu et al., 2011; Zhu et al., 2016). For example, berberine had shown significant inhibitory effect on hepatocellular carcinoma cells and could reduce the volume and weight of tumors in an H22 transplanted tumor model in mice (Li et al., 2015).

Based on collection of hundreds of berberine-related pharmacological literatures, we systematically summarized eight key mechanisms of anti-cancer effects of berberine, including cell death, cell invasion and metastasis, cell cycle arrest, cell growth, transcription factors, inflammatory factors, angiogenic, chemo-sensitivity, and radio-sensitivity (**Figure 1** and **Supplementary Table S1**). Specifically, apoptosis (programmed cell death) plays a vital role in tumor cell development, differentiation, and proliferation (Ola et al., 2011). Recent study has revealed that berberine could induce apoptosis of human osteosarcoma U2OS cells through inhibiting the PI3K/Akt signaling pathway activation (Chen, 2016). In addition, anti-angiogenesis is a promising strategy for prevention and treatment of multicancer in preclinical or clinical studies in terms of many natural products (Khalid et al., 2016; Kotoku et al., 2016). Previous *in vitro* and *in vivo* experiments have validated that berberine exerted anti-angiogenic effect through inhibiting various proinflammatory and pro-angiogenic factors, including vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-2 (IL-2), and metalloproteinase inhibitor (TIMP) (Hamsa and Kuttan, 2012).

Collectively, berberine with polypharmacology has demonstrated its broad anti-cancer properties through targeting various oncogenic pathways and targets. Therefore, systematic exploration of the drug targets of berberine is of great significance for understanding its anti-cancer mechanisms of action (MOAs) and for further excavating its novel cancer indications.

Systems pharmacology-based approaches, as an emerging interdiscipline that combines experimental assays and computational tools, have provided an alternative to understand the therapeutic mechanisms of complex diseases (Fang et al., 2018). Recent studies have demonstrated advanced discovery of new indications for natural products based on systems pharmacology approaches (Fang et al., 2017b; Fang et al., 2019). For example, novel molecular mechanisms of several effective natural products (e.g., resveratrol, quercetin, caffeic acid, and wogonoside) for multiple complex diseases including multi-cancer types and age-related disorders have been identified and validated by various literatures and *in vitro* and *in vivo* experiments (Fang et al., 2017a; Huang et al., 2019). Collectively, systems pharmacology-based approaches have been proved as an effective tool for exploring the poly-pharmacological actions of natural products towards various complex diseases.

In this study, we proposed a systems pharmacology infrastructure to identify new cancer indications of berberine and explore their molecular mechanisms (**Figure 2**). Specifically, we constructed a global DTI network of berberine by integrating both experimentally reported DTIs obtained from literatures and DTIs computationally predicted by our previous predictive network models (Fang et al., 2017c). Besides, a high-quality

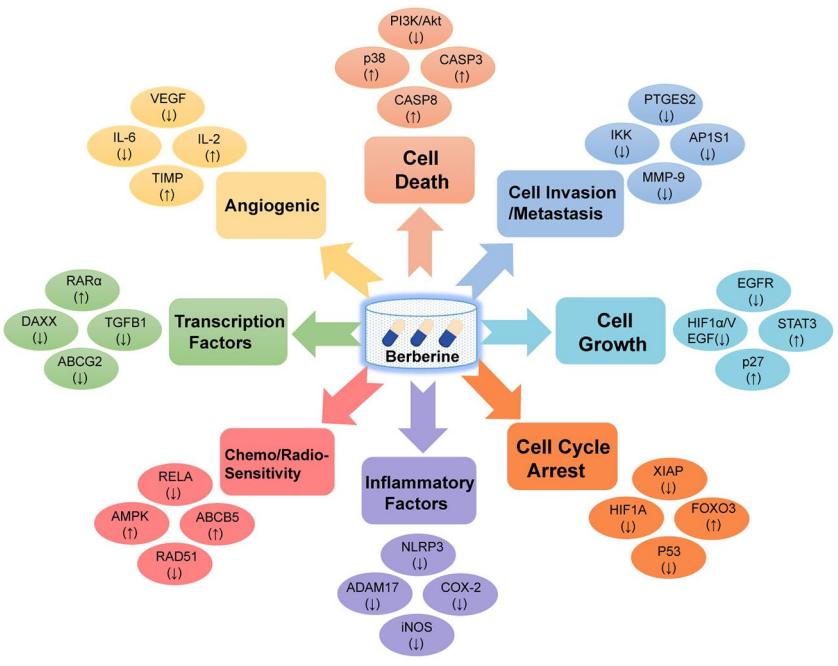

FIGURE 1 | Diagram illustrating the eight potential anti-cancer effects of berberine. Berberine exerts anti-cancer activities *via* targeting various cancer key protein targets, related to cell death, cell invasion and metastasis, cell cycle arrest, cell growth, transcription factors, inflammatory factors, angiogenic, chemo-sensitivity, and radio-sensitivity.

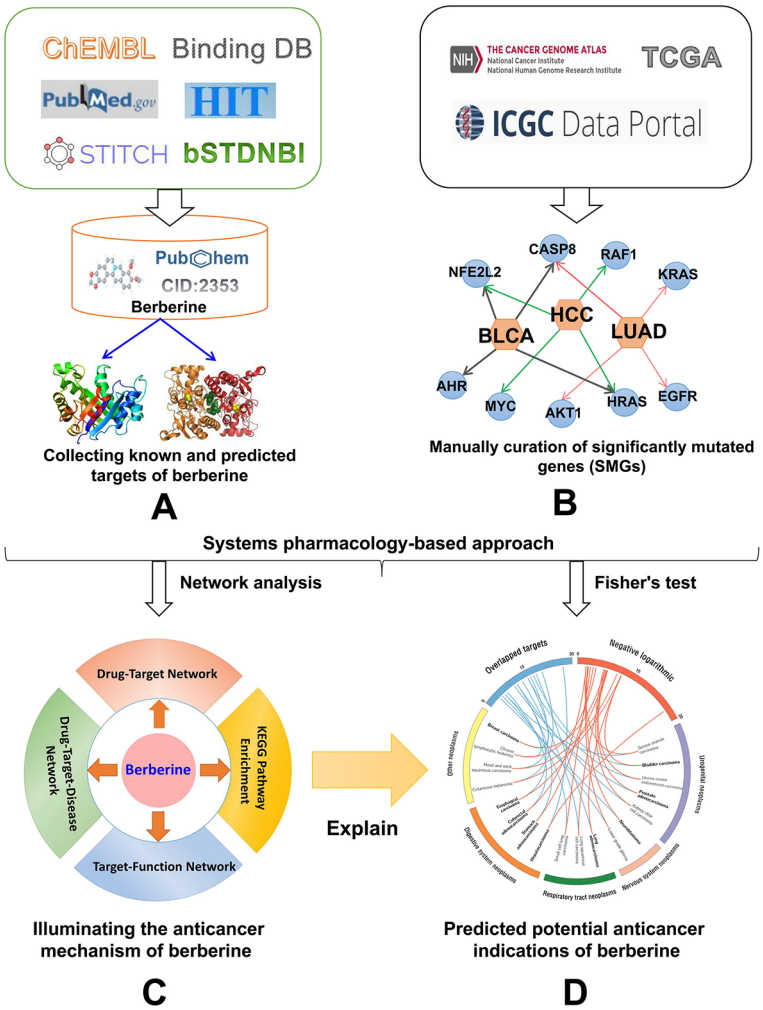

FIGURE 2 | Workflow of a systems pharmacology infrastructure for the identification of cancer indications and exploration of molecular mechanisms of berberine. **(A)** Construction of drug–target network for berberine, **(B)** manual curation of cancer significantly mutated genes (SMGs) for multiple cancer types, **(C)** performing network analyses to explore the anti-cancer mechanism of berberine, and **(D)** statistical network models for prioritizing novel anti-cancer indication of berberine through integrating computationally predicted and known drug targets into the curated cancer SMGs.

collection of significantly mutated genes (SMGs) for multiple cancer types was manually collected. On the basis of curated cancer SMGs and DTIs, we built statistical network models with high accuracy to prioritize new cancer indications of berberine and showcased its potential mechanisms. Overall, this study provides a useful systems pharmacology framework to interpret the multi-scale MOAs of berberine in multiple cancer type management, which may give some enlightenment for further treatment of cancer-associated diseases.

MATERIALS AND METHODS

Collection of Known Targets for Berberine

Known targets of berberine were collected by extracting data from four data sources, including HIT (Ye et al., 2011), STITCH (Kuhn et al., 2014), BindingDB (accessed June 2016) (Gilson et al., 2016), and ChEMBL (Bento et al., 2014). For STITCH, we only kept the targets with experimental evidence score higher than 0.7. We totally obtained 66 known targets *via* integrating the four available databases. Besides, we further gathered 238 extra targets of berberine by manually retrieving large-scale pharmacological literatures from PubMed (https://www.ncbi.nlm.nih.gov) with "berberine [title] and cancer" as search terms (**Supplementary Table S2**). After duplicated targets and DTIs were eliminated from non-*Homo sapiens*, 275 high-quality known DTIs were selected for further study (**Supplementary Table S3**).

Network-Based Target Prediction for Berberine

In a previous study, we have developed statistical network models to predict targets of natural products through a balanced

substructure–drug–target network-based inference (bSDTNBI) approach (Fang et al., 2018). The bSDTNBI method utilizes resource-diffusion processes to prioritize potential targets for natural products through integrating known DTI network, drug–substructure linkages, and new input drug–substructure linkages (Wu et al., 2017). For a new input chemical, each of its substructures equally spreads resources to its neighbor nodes layer by layer, and targets obtaining final resources could be regarded as the potential targets of the new chemical. Four parameters ($\alpha = \beta = 0.1$, $\gamma = -0.5$, and $k = 2$) of bSDTNBI were adopted based on a previous study (Wu et al., 2016). Among them, parameter α was introduced to balance the initial resource allocation of different node types, while β was used to adjust weighted values of different edge types. The third parameter γ was imported to balance the influence of hub nodes in resource-diffusion processes, and the fourth parameter κ denotes the number of resource-diffusion processes. We calculated four substructure items for each compound based on four types of molecular fingerprints from PaDEL-Descriptor (version 2.18) (Yap, 2011), including Substructure (FP4), Klekota-Roth (KR), MACCS, and PubChem. Among the four network models generated with different types of fingerprints, bSDTNBI_KR performed best with the highest values of precision ($P = 0.049$), recall ($R = 0.752$), precision enhancement (Ep = 27.02), recall enhancement (eR = 27.24), and the area under the receiver operating characteristic curve (AUC = 0.959). Finally, the best model built based on KR molecular fingerprint was selected to predict the new targets of berberine. The top 20 predicted candidates were used for further study (**Supplementary Table S3**).

Significantly Mutated Genes (SMG) for Multiple Cancer Types

We collected 804 SMGs for 28 cancer types/subtypes from a previous study (Cheng et al., 2016), including glioblastoma multiforme (GBM), serous ovarian adenocarcinoma (SOC), stomach adenocarcinoma (STAD), colorectal adenocarcinoma (CRAC), breast carcinoma (BRCA), uterine corpus endometrioid (UCEC), medulloblastoma (MBL), acute myeloid leukemia (AML), cutaneous melanoma (CM), lung squamous cell (SQCC), thyroid carcinoma (THCA), lung adenocarcinoma (LUAD), kidney clear cell (CCSK), head and neck squamous (HNSCC), small cell lung (SCLC), lower grade glioma (LGG), bladder carcinoma (BLCA), esophageal carcinoma (EC), prostate adenocarcinoma (PRAD), hepatocarcinoma (HCC), neuroblastoma (NBL), chronic lymphocytic leukemia (CLL), pancreas adenocarcinoma (PAC), multiple myeloma (MM), acute lymphocytic leukemia (ALL), non-small cell lung (NSCLC), diffuse large B-cell lymphoma (DLBCL), and pilocytic astrocytoma (PA). Considering a lack of statistical power if the number of SMG for specific cancer types is lower than 20, we further excluded ALL, NSCLC, DLBCL, and PA. All SMGs are annotated using gene Entrez ID, chromosome location, and the official gene symbols from the National Center for Biotechnology Information (NCBI) database (Zhe and Huang, 2002). Finally, 24 cancer types/subtypes covering 804 SMGs were selected for further study (**Supplementary Table S4**).

Prioritizing Cancer Indications of Berberine

In this study, an integrated statistical network model was generated to prioritize cancer indication of berberine based on drug–target network and cancer SMGs (Cheng et al., 2016; Jiang et al., 2018). We assumed that berberine would exert high potential for the treatment of a specific cancer type if its targets tend to be SMGs of this cancer. For each cancer type/subtype, Fisher's exact test was utilized to calculate the statistical significance of the enrichment of SMGs for each cancer type in target profiles of berberine. The *P*-values were corrected by Benjamini–Hochberg method (Benjamini and Yekutieli, 2001). We set a cutoff adjusted *P*-value threshold (q) < 0.05 to define significantly predicted drug–cancer pairs.

Network Construction

To further explore the multi-scale MOAs of berberine in treating multiple cancer types, three types of networks were constructed by Cytoscape 3.2.1 software (Shannon et al., 2003): 1) drug–target (D-T) network, which presents the relationship between berberine and its targets; 2) target–function (T-F) network, which illustrates the relationship between cancer-related biological processes and SMGs; and 3) drug–target–disease (D-T-D) network, which reflects a global view of the molecular mechanism of berberine against multiple cancer types. After network analysis, the SMGs were further mapped to DAVID database (https://david.ncifcrf.gov/summary.jsp) for extracting the canonical pathways that were highly associated with these targets (Dennis et al., 2003). Finally, circos plot was used to visualize the predicted cancer indications.

RESULTS AND DISCUSSION

Construction of the Drug–Target (D-T) Network for Berberine

The constructed drug–target interaction network (**Figure 3**) of berberine contains 289 interactions, including 275 known targets and 20 predicted targets (**Supplementary Table S3**). *In vitro* and *in vivo* assays in previous studies have validated that five out of the 20 predicted targets could be mediated by berberine, indicating high accuracy of our target prediction approach. These five predicted targets are caspase-3 (CASP3) (Okubo et al., 2017), cellular tumor antigen p53 (TP53) (Qing et al., 2014), caspase-9 (CASP9) (Zhao et al., 2017), nuclear factor NF-kappa-B p105 subunit (NFKB1) (Yu et al., 2014), and mitogen-activated protein kinase 1 (MAPK1) (Song et al., 2015).

We further mapped the 289 protein targets of berberine into the curated cancer SMGs, resulting in 51 cancer-related targets encoded by SMGs (**Supplementary Table S3**). Accumulating evidences indicate that berberine may exert anti-cancer effects through regulating these targets. For instance, signal transducer and activator of transcription 3 (STAT3) are important in

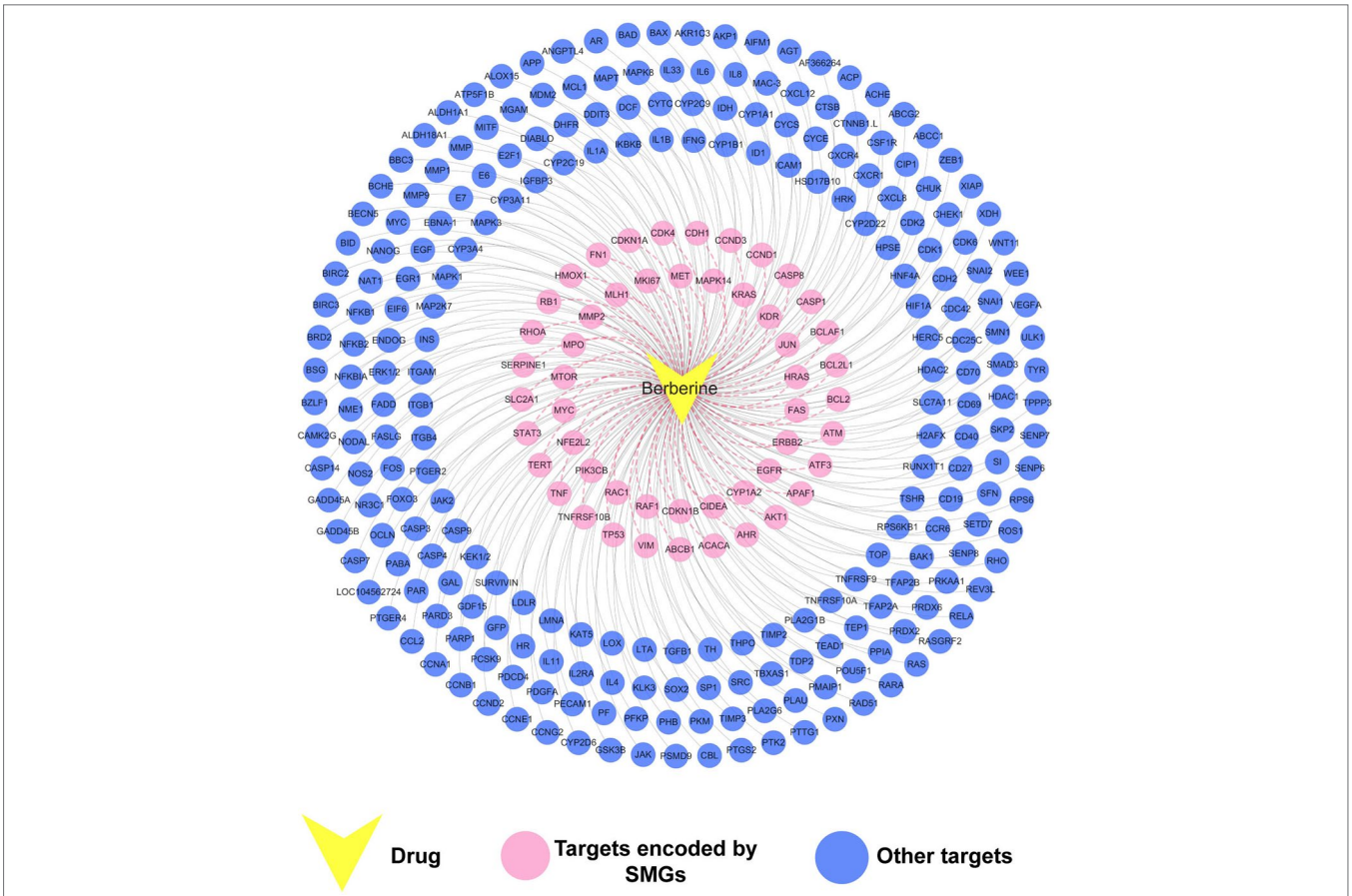

FIGURE 3 | Drug–target (D-T) network of berberine composed of known and predicted targets. The predicted targets were obtained by a balanced substructure–drug–target network-based inference (bSDTNBI) approach. This network includes 289 drug–target interactions connecting berberine and 51 protein targets encoded by significantly mutated genes (SMGs).

various phases of the tumor development, including tumor cell proliferation, survival, invasion, immunosuppression, and inducing and maintaining a pro-carcinogenic inflammatory microenvironment (Fan et al., 2013). A previous study has showed that berberine suppressed tumorigenicity and growth of nasopharyngeal carcinoma (NPC) cells by inhibiting STAT3 activation (Tsang et al., 2013). Recently, a strategy targeting tumor suppressors and apoptosis-related genes provides a rationale for developing more effective approaches and agents for cancer prevention (Sun et al., 2017; López-Cortés et al., 2018; Yamaguchi et al., 2019). Berberine has been observed to activate expression of many tumor apoptosis-related proteins, including caspase-8 (CASP8), tumor necrosis factor-a (TNF-a), and p38 MAPK, and thus induced apoptosis of HeLa cells (Lu et al., 2010). Besides, it has been reported that berberine can decrease expression of mitochondrial-dependent anti-apoptotic factors such as B-cell lymphoma-2 (Bcl-2) and Bcl-2-like protein 1 (BCL2L1) in KB human oral cancer cells (Kim et al., 2015).

Taken together, the observed polypharmacological profiles of berberine motivated us to elucidate its anti-cancer mechanism through systems pharmacology analysis on the interaction between berberine and 51 SMGs.

Elucidating Molecular Mechanisms of Berberine in Cancer Prevention and Treatment

Target–Function Network

As depicted in **Figure 4**, the target–function (T-F) network is composed of 230 T-F pairs connecting 51 SMG targets and 8 cancer-related functional modules based on the DAVID analysis (**Supplementary Table S5**). The eight functional modules include anti-cancer action associated with sustaining proliferative signaling (Huang et al., 2015), resisting cell death (Chidambara Murthy et al., 2012), deregulating cellular energetics (Tan et al., 2015), enabling replicative immortality (Xiong et al., 2015), avoiding immune destruction (Jiang et al., 2017), genome instability and mutation (Li et al., 2014), angiogenesis (Jie et al., 2011), and activating invasion and metastasis (Tang et al., 2009). On average, each SMG target is involved in six cancer-related functional modules. We found that 25 out of 51 SMG targets are associated with more than five functional modules, indicating the higher potential role of these SMG targets related to cancers. Previous studies of berberine in cancer validated the functional analysis of our T-F network. For instance, berberine could induce cell cycle arrest involved in sustaining proliferative signaling in

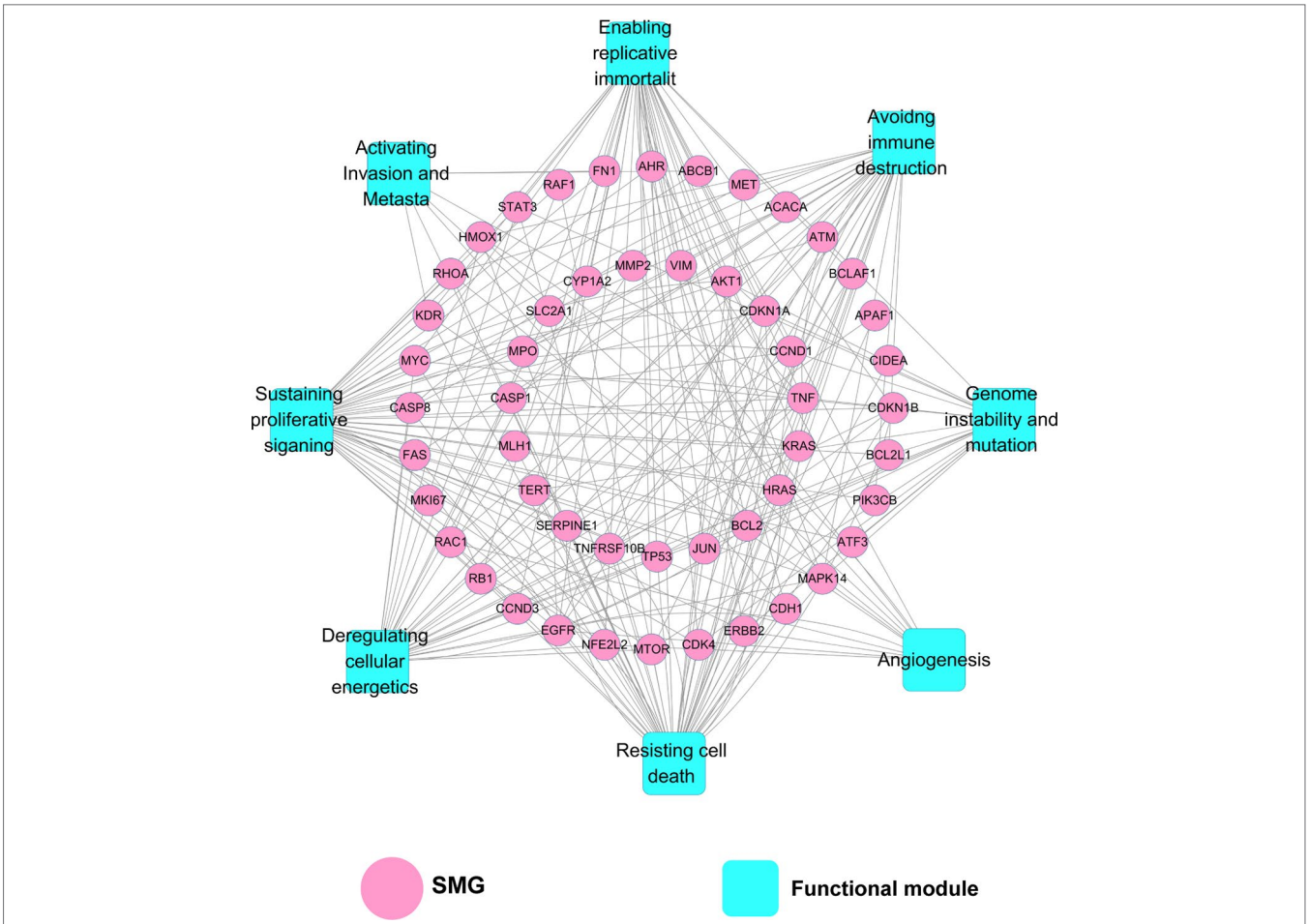

FIGURE 4 | Target–function (T-F) network demonstrating the relationship between cancer-related biological processes and SMGs. A functional module is linked to a target if the target is involved in mechanism of anti-cancer action.

cholangiocarcinoma KKU-213 and KKU-214 cell lines (Puthdee et al., 2017). Berberine was reported to inhibit metastasis and tumor-induced angiogenesis in human cervical cancer cells as well (Chu et al., 2014).

KEGG Enrichment Analysis

In order to further elucidate molecular mechanisms of berberine in cancer prevention and treatment, we performed KEGG pathway enrichment analysis based on the 51 SMGs. After pathways with adjusted P (q) value higher than 0.05 were excluded, 56 enriched pathways related to cancer pathogenesis were obtained (**Supplementary Table S6**).

Among 56 pathways, PI3K-Akt (hsa04151; $q = 2.0 \times 10^{-12}$), p53 (hsa04115; $q = 2.7 \times 10^{-9}$), HIF-1 (hsa04066; $q = 3.9 \times 10^{-9}$), FoxO (hsa04068; $q = 4.9 \times 10^{-9}$), VEGF (hsa04370; $q = 5.7 \times 10^{-7}$), MAPK (hsa04010; $q = 2.5 \times 10^{-6}$), Ras (hsa04014; $q = 6.4 \times 10^{-6}$), Jak-STAT (hsa04630; $q = 9.9 \times 10^{-4}$), mTOR (hsa04150; $q = 1.5 \times 10^{-2}$), AMPK (hsa04152; $q = 1.9 \times 10^{-2}$), and NF-kappa B (hsa04064; $q = 4.0 \times 10^{-2}$) signaling pathways have been confirmed to be associated with berberine in previous literatures (**Table 1**). For example, berberine was reported to inhibit cellular growth and promotes apoptosis by down-regulating PI3K/Akt signaling pathway in breast cancer SKBR-3 cells and hepatoma HepG2 cells (Liu et al., 2011; Kuo et al., 2011). *In vitro* and *in vivo* assays revealed that berberine sensitized drug-resistant breast cancer to doxorubicin (DOX) chemotherapy and directly induced apoptosis through the dose-orchestrated AMPK signaling pathway (Pan et al., 2017). Berberine also induces autophagic cell death through inhibition of mTOR-signaling pathway by suppressing Akt activity and up-regulating P38 MAPK signaling in HepG2 and MHCC97-L cells (Wang et al., 2010). The rest of the 45 enriched pathways prompt the potential anti-cancer acting mechanisms that may be mediated by berberine, which deserve to be validated by experimental assays in the future.

Drug–Target–Diseases Network

We further built a drug–target–diseases (D-T-D) network *via* mapping 51 SMGs targeted by berberine into multiple cancers. As shown in **Figure 5**, the 51 SMGs are related to 24 types of cancer. On average, each cancer links to nine SMGs, while each SMG is connected to 4.6 cancer types. Network analysis showed that the top 6 SMGs connected to the largest number of cancer types are

TABLE 1 | Summary of the 11 enriched pathways validated to be mediated by berberine in previous literatures.

Pathway ID	Pathway name	Genes	*P*-value	PMID
hsa04151	PI3K-Akt signaling pathway	EGFR, HRAS, PIK3CB, MET, TP53, RAF1, BCL2L1, CDK4, KDR, AKT1, CDKN1A, CCND1, KRAS, CDKN1B, CCND3, BCL2, RAC1, MTOR, MYC, FN1	2.03E−12	27081456|25212656
hsa04115	p53 signaling pathway	CDKN1A, CCND1, CCND3, CASP8, SERPINE1, TP53, APAF1, FAS, CDK4, ATM	2.66E−09	20455200
hsa04066	HIF-1 signaling pathway	AKT1, EGFR, HRAS, CCND1, KRAS, PIK3CB, ERBB2, TP53, RAF1, RB1, CDK4	3.89E−09	28775788
hsa04068	FoxO signaling pathway	AKT1, EGFR, HRAS, CCND1, KRAS, PIK3CB, ERBB2, TP53, RAF1, MLH1, CDH1, MYC	4.88E−09	24766860|29360760
hsa04370	VEGF signaling pathway	TNF, MAPK14, BCL2, RAC1, TP53, APAF1, BCL2L1, CASP1	5.72E−07	23869238
hsa04010	MAPK signaling pathway	AKT1, EGFR, HRAS, CCND1, KRAS, PIK3CB, ERBB2, TP53, RAF1, MLH1, CDH1, MYC	2.45E−06	19492307|25212656
hsa04014	Ras signaling pathway	AKT1, EGFR, HRAS, CCND1, KRAS, PIK3CB, ERBB2, TP53, RAF1, RB1, CDK4	6.42E−06	25212656|23159854
hsa04630	Jak-STAT signaling pathway	AKT1, HRAS, KRAS, PIK3CB, JUN, RAC1, RAF1	9.90E−04	26463023
hsa04150	mTOR signaling pathway	TNF, CASP8, APAF1, CASP1	1.50E−02	23159854|20830746
hsa04152	AMPK signaling pathway	EGFR, MAPK14, JUN, RAC1, MET	1.88E−02	28775788
hsa04064	NF-kappa B signaling pathway	TNF, CASP8, APAF1, CASP1	3.97E−02	19107816

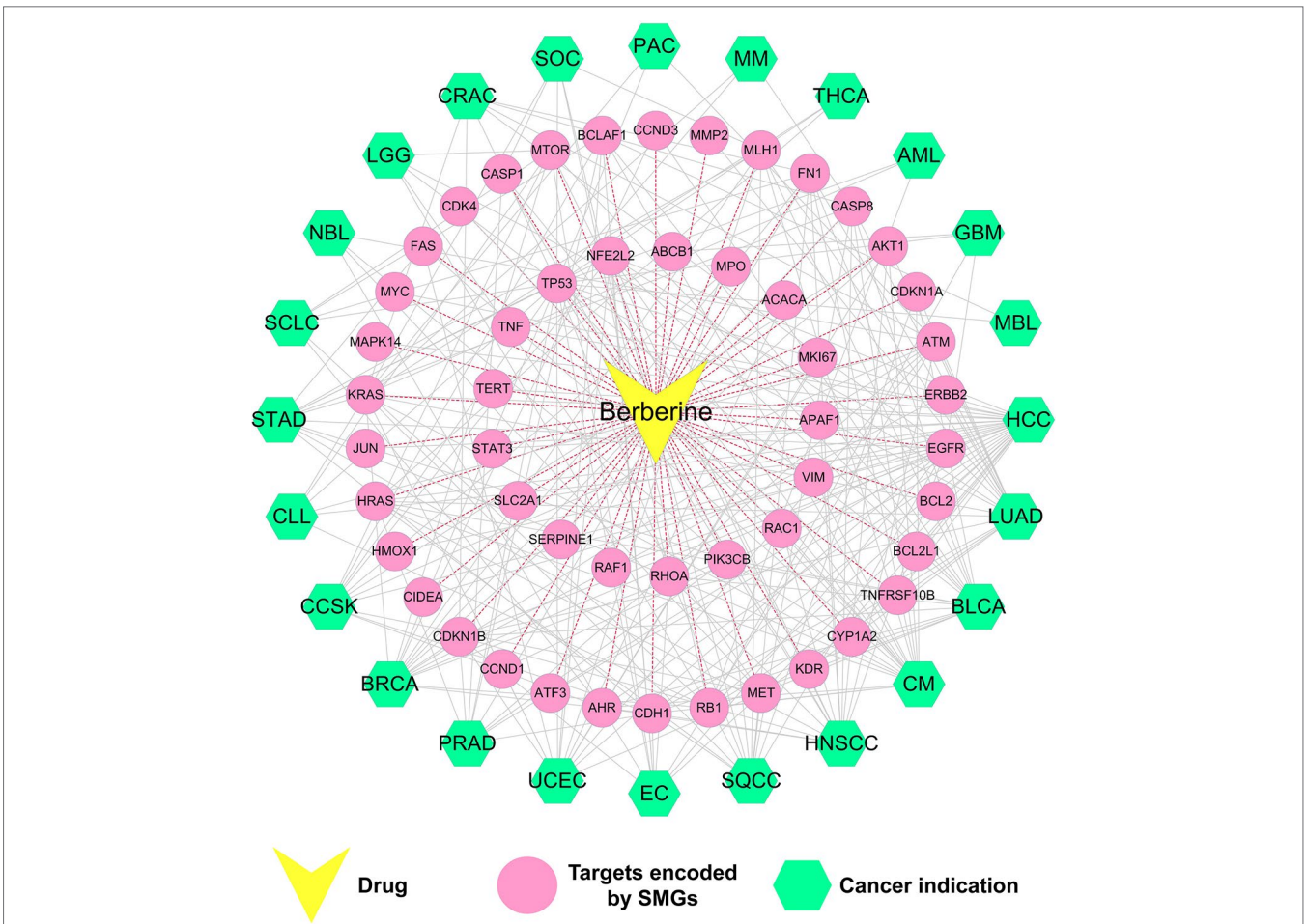

FIGURE 5 | Drug–target–disease (D-T-D) network of berberine. This network shows 51 proteins of berberine encoded by SMGs of 24 types of cancer.

cellular tumor antigen p53 (TP53), gTPase KRas (KRAS), epidermal growth factor receptor (EGFR), retinoblastoma-associated protein (RB1), serine-protein kinase ATM (ATM), and cadherin-1 (CDH1). Among them, EGFR, a key significantly mutated gene of cancer, is involved in the pathological mechanism of 13 cancer types, including LUAD, HNSCC, SQCC, EC, UCEC, PRAD, BRCA, CCSK, CLL,

STAD, LGG, CRAC, and GBM. Previous studies confirmed that berberine can inhibit EGFR signal pathway in several cancer types, including STAD (Wang et al., 2016), PRAD (Huang et al., 2015), and CRAC (Wang et al., 2013). Besides, berberine acts in specific tumor by regulating multiple SMGs. For instance, cellular tumor antigen p53 (TP53) (Wilson et al., 2010), RAC-alpha serine/threonine-protein kinase (AKT1) (López-Cortés et al., 2018), and cyclin-dependent kinase inhibitor 1B (CDKN1B) (Cusan et al., 2018) are highly correlated with breast cancer. Accumulating evidences demonstrated that berberine can inhibit breast cancer by acting on SMGs such as TP53 (Kim et al., 2012; Tan et al., 2015), AKT1 (Kuo et al., 2011), and CDKN1B (Patil et al., 2010).

Briefly, the D-T-D network demonstrated that SMGs targeted by berberine were closely related to multi-cancer types. In the following part, statistical systems pharmacology approach was employed to identify novel cancer indications of berberine and explore the molecular mechanisms.

Systems Pharmacology-Based Prediction of Cancer Indications for Berberine

As shown in **Figure 6**, a statistical systems pharmacology framework is proposed to prioritize novel cancer indications of berberine based on Fisher's exact test analysis. We calculated the therapeutic potential of berberine in 24 cancer indications and obtained 18 cancer indications of which adjusted P (q) values are lower than 0.05 ($q < 0.05$), including HCC ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 19.25$), LUAD ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 9.35$), BLCA ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 9.31$), CM ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 9.29$), HNSCC ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 8.52$), SQCC ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.74$), EC ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.66$), UCEC ($q < 1.0 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.52$), PRAD ($q = 1.15 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.32$), BRCA ($q = 1.33 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.26$), CCSK ($q = 2.30 \times 10^{-5}$; $-\text{Log10}\ (q) = 6.02$), CLL ($q = 0.55 \times 10^{-3}$; $-\text{Log10}\ (q) = 4.64$), STAD ($q = 1.76 \times 10^{-3}$; $-\text{Log10}\ (q) = 4.14$), SCLC ($q = 5.33 \times 10^{-3}$; $-\text{Log10}\ (q) = 3.65$), NBL ($q = 1.29 \times 10^{-2}$; $-\text{Log10}\ (q) = 3.27$), LGG ($q = 1.67 \times 10^{-2}$; $-\text{Log10}\ (q) = 3.16$), CRAC ($q = 3.21 \times 10^{-2}$; $-\text{Log10}\ (q) = 2.87$), and SOC ($q = 3.36 \times 10^{-2}$; $-\text{Log10}\ (q) = 2.85$) (**Supplementary Table S7**). As listed in **Table 2**, 10 out of the 18 predicted cancer indications of berberine were validated by reported experimental evidences, including HCC, LUAD, BLCA, EC, PRAD, BRCA, STAD, CRAC, and SOC, indicating the high accuracy of our systems pharmacology-based predictive method (success rate = 55.6%).

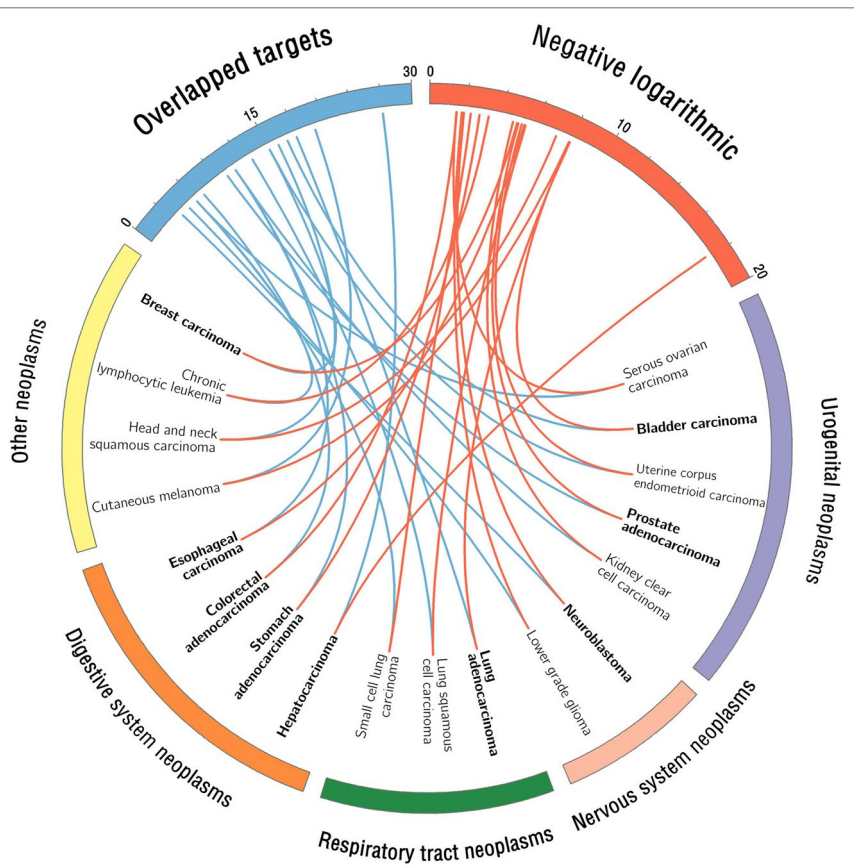

FIGURE 6 | Circos plot visualizes the predicted cancer indications of berberine. The red connected lines represent the calculated $-\text{Log}_{10}(q)$ value of each berberine-cancer type pair based on Fisher's exact test, while the blue ones represent the corresponding number of overlapped targets. The predicted cancer indications with literature validation were highlighted in bold font. We classified the 18 predicted cancer indications into four neoplasm categories according to Medical Subject Headings (MeSH) system (https://www.ncbi.nlm.nih.gov/mesh/68009371).

Among the 18 cancer indications, CM, HNSCC, SQCC, UCEC, CCSK, CLL, SCLC, NBL, and LGG are the unreported cancer indications of berberine, which deserve further preclinical validation. For example, cutaneous melanoma (CM), one of the most aggressive types of cancer, represents a major problem worldwide due to its high incidence and elevated degree of heterogeneity (Jemal et al., 2010; Coricovac et al., 2018). Based on our predictive model, berberine exerted a high potential for anti-CM, with a significant q value [$q < 1.23 \times 10^{-8}$; $-\text{Log10}\ (q) = 9.29$]. Therefore, the potential of berberine in the prevention and treatment of CM deserves to be further validated.

Case Study: Exploring the MOAs of Berberine on Hepatocarcinoma (HCC), Lung Adenocarcinoma (LUAD), and Bladder Carcinoma (BLCA)

To further validate the accuracy of statistical network models and predicted anti-cancer targets of berberine, we selected three typical cancer types [HCC ($q = 5.63 \times 10^{-20}$), LUAD ($q = 4.52 \times 10^{-10}$), and BLCA ($q = 4.92 \times 10^{-10}$)] as case studies to illustrate their anti-cancer MOAs **(Figure 7)**.

Hepatocellular Carcinoma

HCC, the third leading cause of cancer death worldwide, has become one of the most common and prevalent human malignancies in the world (Okubo et al., 2017). *In vitro* assays revealed that berberine can inhibit autophagy in hepatoma cell lines (e.g., HepG2 cells and MHCC97-L cells) by regulating multiple proteins [e.g., mitogen-activated protein kinase 14 (MAPK14), TP53, and phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform (PIK3CB)] and pathways (e.g., P38 MAPK signaling), stimulating further development of derivatives for drug-base cancer prevention and treatment (Wang et al., 2010; Liu et al., 2011; Wang et al., 2014). In this study, Fisher's test showed that berberine played a significant role in treatment of liver cancer ($q = 5.63 \times 10^{-20}$). In addition, network analysis revealed that berberine bound with 27 HCC-related SMG targets, suggesting its underlying anti-cancer mechanisms of berberine (**Figure 7**). *In vivo* or *in vitro* data have demonstrated that these SMGs are closely relevant to the treatment of cancer by berberine. For example, berberine can inhibit cell proliferation of HepG2, Hep3B, and SNU-182 through up-regulating protein expression of tumor suppressor genes, such as activating transcription factor 3 (ATF3) (Chuang et al., 2017). Furthermore, study revealed that berberine inhibited expression of BCL2, thus reducing autophagic cell death and mitochondrial apoptosis in liver cancer cells, such as HepG2 and MHCC97-L cells (Hur et al., 2010).

Lung Adenocarcinoma

LUAD is one of the leading causes of cancer-related death both men and women in the United States. Approximately two million people are diagnosed with lung cancer each year (Torre et al., 2016). Berberine was predicted to have anti-LUAD potential ($q = 4.52 \times 10^{-10}$). Some previous *in vivo* and *in vitro* studies confirmed our prediction (Mitani et al., 2001; Zheng et al., 2014). Furthermore, berberine is currently being assessed as an anti-LUAD drug in clinical trials (NCT03486496). As shown in **Figure 7**, berberine interacts with 13 LUAD-related SMGs (e.g., matrix metalloproteinase-2), indicating the underlying MOAs of anti-LUAD of berberine. Matrix metalloproteinases (MMPs), one target displayed in our network, is the major protease of LUAD and is associated with tumor invasion and metastasis (Herbst et al., 2000). Study on human lung cancer cell line A549 confirmed that berberine inhibited invasion and growth of tumor cells through decreasing productions of matrix metalloproteinase-2 (MMP2) (Peng et al., 2006).

Bladder Carcinoma

BLCA is the most common cancer of the urinary system in the United States (Kaufman et al., 2009). In our network model, berberine is predicted to have a significant relationship with

TABLE 2 | Relevant literature evidences of the 18 predicted cancer indications of berberine.

Cancer type	*P*-value (Fisher test)	Adj-*P*	Negative logarithmic	PMID
HCC	5.63E−20	1.35E−18	17.87	26081696|25496992|24942805
LUAD	4.52E−10	1.08E−08	7.96	24766860|26672764|26503561
BLCA	4.92E−10	1.18E−08	7.93	21545798|23065570|10418949
CM	5.12E−10	1.23E−08	7.91	N/A
HNSCC	3.03E−09	7.27E−08	7.14	26503508
SQCC	1.82E−07	4.37E−06	5.36	N/A
EC	2.18E−07	5.23E−06	5.28	28465635|26667771|21858113
UCEC	3.03E−07	7.27E−06	5.14	N/A
PRAD	4.77E−07	1.15E−05	4.94	16505103|26698234|25572870
BRCA	5.53E−07	1.33E−05	4.88	29143794|29414799|28926092
CCSK	9.58E−07	2.30E−05	4.64	N/A
CLL	2.28E−05	5.47E−04	3.26	N/A
STAD	7.32E−05	1.76E−03	2.76	27142767|25837881|18468407
SCLC	2.22E−04	5.33E−03	2.27	N/A
NBL	5.36E−04	1.29E−02	1.89	27235712|19189664|19096576
LGG	6.95E−04	1.67E−02	1.78	N/A
CRAC	1.34E−03	3.21E−02	1.49	23604974|26463023|25954974
SOV	1.40E−03	3.36E−02	1.47	N/A

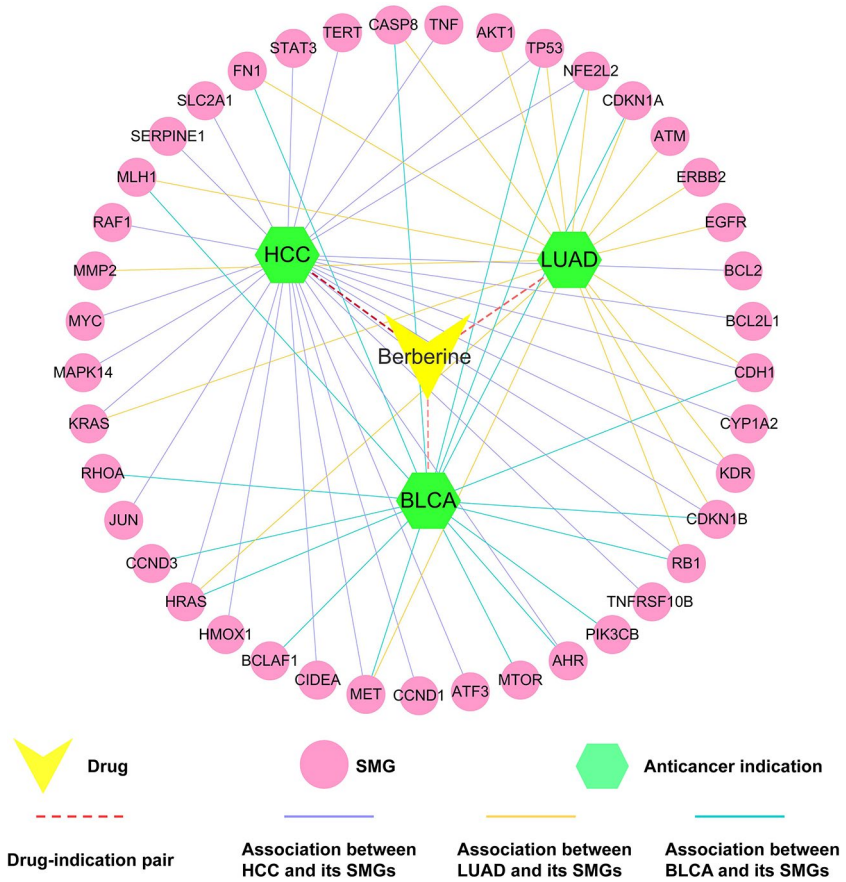

FIGURE 7 | Drug–target–disease (D-T-D) network of berberine on hepatocarcinoma (HCC), lung adenocarcinoma (LUAD), and bladder carcinoma (BLCA). The thickness of the red dotted line represents the predicted association between berberine and three types of tumors.

BLCA ($q = 4.92 \times 10^{-10}$). Meanwhile, our network indicated that berberine interacts with 17 BLCA-related SMGs (e.g., HRAS). According to previous study, the oncogenic ras genes GTPase HRas (HRAS) mutations, endogenously expressed in T24 bladder cancer cell line, were associated with grades and stages of BLCA detected in more than 35% of patients (Buyru et al., 2003). Berberine inhibited cell proliferation and induced cell cycle arrest and apoptosis in BLCA by inhibiting oncogenic H-Ras pathway in BIU-87 and T24 cell lines (Yan et al., 2011).

Taken together, these three case studies against different cancer types (HCC, LUAD, and BLCA) indicate that systems pharmacology approach applied in this study is an effective method for exploring molecular mechanisms of anti-cancer effect of berberine. Meanwhile, the newly predicted tumor types might be promising to further investigate MOAs of berberine.

CONCLUSION

Berberine had been observed to exert multiple biological and pharmacological activities with potential benefits to a variety of complex diseases, including cancer. In this study, we proposed an integrated systems pharmacology infrastructure to identify cancer indications of berberine and explore the underlying molecular mechanisms. This work explores the following new anti-cancer characteristics of berberine: i) Through literature mining, we summarize eight mechanisms of anti-cancer effect of berberine; ii) global drug–target network of berberine is constructed by integrating large-scale experimentally reported targets and computationally predicted targets. Mechanisms of action (MOAs) of various anti-cancer effects of berberine are discussed through current drug–target network; iii) a statistical model is developed to prioritize novel cancer indications of berberine through integrating target profiles of berberine and significantly mutated genes in cancer.

Yet several limitations of our approach should be acknowledged. First, although we have integrated a wide range of DTIs from published literatures and publicly available databases, the incompleteness of current drug–target networks may still exist. Recent studies proved that integration of large-scale gene expression profiles of natural products may help to improve the performance of drug–target network model (Yamanishi et al., 2010; Cheng et al., 2012). Second, as it is extremely difficult to obtain information on the active sites of berberine and mutated domain of proteins from

public sources, the current study could not explain the MOAs from a microcosmic point of view. Third, experimental assays should be performed to further validate the predicted targets and MOAs of anti-cancer effects of berberine in the future.

In summary, the systems pharmacology framework in this study has provided potential strategies to discover the polypharmacology effects of berberine for the prevention and treatment towards multiple cancers.

AUTHOR CONTRIBUTIONS

JF and YZ provided the concept and designed the study. PG and CC conducted the experiments and wrote the manuscript. XW, JZ, XF, QWu, YH, WZ, WH, and FZ participated in the experiments. JF and QWa contributed to revision and proofreading of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00857/full#supplementary-material

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