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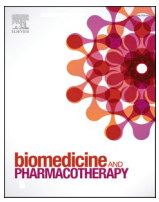

Short communication

Identification of difluorinated curcumin molecular targets linked to traumatic brain injury pathophysiology

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ABSTRACT

Traumatic brain injury (TBI) affects approximately 50% of the world population at some point in their lifetime. To date, there are no effective treatments as most of the damage occurs due to secondary effects through a variety of pathophysiological pathways. The phytoceutical curcumin has been traditionally used as a natural remedy for numerous conditions including diabetes, inflammatory diseases, and neurological and neurodegenerative disorders. We have carried out a system pharmacology study to identify potential targets of a difluorinated curcumin analogue (CDF) that overlap with those involved in the pathophysiological mechanisms of TBI. This resulted in identification of 312 targets which are mostly involved in G protein-coupled receptor activity and cellular signalling. These include adrenergic, serotonergic, opioid and cannabinoid receptor families, which have been implicated in regulation of pain, inflammation, mood, learning and cognition pathways. We conclude that further studies should be performed to validate curcumin as a potential novel treatment to ameliorate the effects of TBI.

1. Introduction

Worldwide, approximately 70 million people suffer a traumatic brain injury (TBI) each year and about half the global population will have one or more traumatic brain injuries of varying severities over their lifetimes [1,2]. TBI is one of the leading causes of disability across all age groups throughout the world [2]. The estimated cost of TBI is approximately 400 billion US dollars each year and the most usual causes are automobile accidents and falls [1,2]. Following the primary brain injury, clinical management of TBI has focussed mainly on preventing or minimising the effects of secondary injuries [3]. The main approach to treating TBI involves damage control and includes surgery and post-operative care [4]. Currently, there are no effective pharmaceutical treatments available for TBI [5,6]. Thus, there is an urgent need to increase our understanding of the pathophysiology underlying TBI to identify new and effective treatment approaches.

In TBI, inflammation associated with the primary lesion can aggravate the injury and lead to a poorer prognosis [7]. Secondary injuries can evolve from this early damage and advance to altered calcium signalling, excessive free radical generation, widespread cell damage due to necrosis or apoptosis, and consequential neurodegeneration [8]. In turn, these effects could lead to cognitive impairment [9,10]. However, diseases such as TBI are complex and heterogeneous due to the involvement of a range of secondary injury mechanisms [11]. Therefore, strategies aimed at targeting multiple pathways are likely to be needed for more effective treatment of this condition. Several natural products and herbal medicines have been tested for the ability to ameliorate disorders such as neuroinflammatory conditions, which are marked by perturbed oxidative-reductive balance and hyper-inflammation [12–22].

Curcuma longa L. is a member of the Zingiberaceae (ginger) family, which has shown benefits in a number of metabolic conditions as well as neurological and neurodegenerative disorders [23–28]. Its anti-inflammatory effects are known to occur through decreasing the

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activity of NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3) inflammasomes, which are responsible for mounting inflammatory responses [29]. Therefore, given the pivotal role of inflammation in TBI, treatment with curcumin may offer a therapeutic alternative for improved patient outcomes.

The standard use of curcumin in medicine has been limited due to its low bioavailability [30]. Thus several strategies have been employed to improve its biological activity and pharmacokinetic profile, including generation of a difluorinated curcumin analogue (curcumin-difluorinated, CDF) [31–33]. Substitution with fluorine has been shown to improve characteristics of small molecules such as intrinsic potency, membrane permeability and blood-brain barrier penetration [34,35]. These attributes are of particular importance in the case of compounds aimed at central nervous system (CNS) targets.

Recent advances in computational biology have led to drastic improvements in our ability to predict drug-target interactions [36,37] and to decipher the molecular signature of a given disease [38,39]. Here, we have used a system pharmacology approach to identify potential molecular targets of CDF that overlap with those involved in the pathophysiological mechanisms of TBI. The main objectives were to identify potential CNS targets of CDF for ameliorating the detrimental outcomes of TBI and to assess the potential use of curcumin-related analogues as a new treatment approach.

2. Materials and methods

2.1. Identification of curcumin-difluorinated targets

For this study, Simplified Molecular Input Line Entry System (SMILES) of curcumin-difluorinated in Pubchem (CID: 54597187) for prediction of molecular targets using the Similarity Ensemble Approach (SEA; https://sea.bkslab.org/), Targetnet (http://targetnet.scbdd.com) and SwissPrediction (http://swisstargetprediction.ch) databases were used. The SEA database relates proteins based on similarities of their ligands and Targetnet can predict the binding of targets with a given molecule. We limited the searches to "*Homo sapiens*" with a probability score > 0.05. After the data were combined and duplicates eliminated, we used Uniprot (https://www.uniprot.org/) to unify the gene identification codes and remove any further duplicates.

2.2. Targets identification

For identification of TBI-related targets, we used: 1) Comparative Toxicogenomics Dataset (CTD; http://ctdbase.org/); 2) the Online Mendelian Inheritance in Man (OMIM; https://omim.org/); and 3) Genecard/Malacards (https://www.malacards.org/). The CTD and OMIM databases link diseases with specific genes and the Malacards database is a human disease compendium with diverse clinical and genetic annotations. We used the search terms "human" or "*Homo sapiens*" or "traumatic brain injury" to assemble a list of targets.

2.3. Gene function and GO pathways and KEGG enrichment

Gene/protein enrichment and biological annotations were performed using the freely available ClueGO [40] database in cytoscape. The common genes between the TBI and CDF searches were imported into the database, using both gene ontology (GO) analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations. The top 10 common genes in each category were selected for further analysis. For inclusion, we used a p-value cut-off of 0.05.

2.4. Protein-Protein Interaction (PPI) network

We identified potential clusters in the common genes between the TBI and CDF groups using MCODE in Cytoscape and carried out PPI network and topological analyses from the top-ranked cluster using Genemania to explore predictable protein-protein interactions with a confidence ratio > 0.5. We also used the Network Analyzer plugin to determine the level of confidence and importance of protein interaction pathways in relation to the number of degrees (interactions).

3. Results

3.1. Potential targets of CDF in TBI

The use of the combined prediction tools described in methods led to identification of a total of 665 potential targets for the CDF compound. *In silico* analysis of the pathophysiological pathways altered in TBI resulted in the identification of 2700 targets in total. Of the targets predicted for TBI, 312 (10.2%) were common to those predicted for CDF (Fig. 1 and Suppl Table 1**)**.

3.2. PPI analysis and cluster subnetwork

To identify and document the characteristics of the 312 targets, we performed PPI analysis using Cytoscape. This resulted in a total of 332 nodes, and 15,489 edges, with an average number of 72,736 neighbours, a diameter of 4, and a clustering coefficient of 0.347. To classify and identify possible clusters within this network that could represent CDF targets for treatment of TBI, we used the MCODE plugin, which assesses clusters according to significance and degree of interaction and classifies these using scores. Cluster 1 contained 60 nodes (Table 1) with 2234 edges, with a score of 59.729. The seed for this cluster was the hypocretin receptor 2 (HCRTR2), a G protein-coupled receptor involved in binding of orexins as regulators of feeding behaviour (Fig. 2**A**). Cluster 2 contained 23 nodes, 316 edges with a score of 16.636 (Fig. 2**B**). For this cluster, the seed was the dual-specificity tyrosine phosphorylation regulated Kinase 1A (DYRK1A), thought to have a role in cell proliferation and brain development. Cluster 3 contained 29 nodes and 418 edges, and had a score of 15.429 (Fig. 2**C**). The seed for cluster 3 seed was the estrogen receptor alpha (ESR1), which regulates multiple pathways such as cell differentiation, proliferation and migration in neurological and neurodegenerative conditions. Cluster 4 contained 27 nodes, 358 edges and had a score of 14.462 (Fig. 2**D**). The seed was the glutamate ionotropic receptor NMDA type subunit 2B (GRIN2B), an ionotropic receptor involved in glutamate signalling in brain development, neuronal plasticity and differentiation.

Since cluster 1 retrieved the highest score, we explored this subnetwork further with PPI analysis using Genemania in Cytoscape. This showed that the cluster had an average of 58,733 neighbours, 73.11%

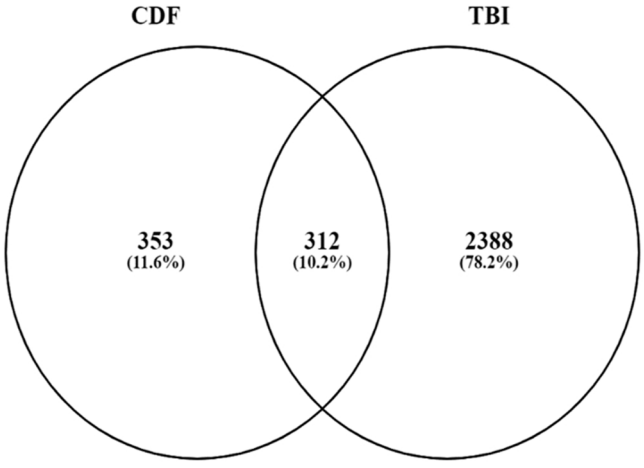

Fig. 1. Shared genes in CDF and TBI lists. A total of 312 genes (10.2%) are common to both lists.

Table 1
60 genes comprising cluster 1.

Gene ID	Full name
ADRA1A	Alpha-1A adrenergic receptor
ADRA1B	Alpha-1B adrenergic receptor
ADRA1D	Alpha-1D adrenergic receptor
ADRA2A	Alpha-2A adrenergic receptor
ADRA2B	Alpha-2B adrenergic receptor
ADRA2C	Alpha-2C adrenergic receptor
ADORA1	Adenosine receptor A1
ADORA2A	Adenosine receptor A2a
ADORA3	Adenosine receptor A3
AGTR1	Type-1 angiotensin II receptor
AGTR2	Type-2 angiotensin II receptor
ADRB1	Beta-1 adrenergic receptor
ADRB3	Beta-3 adrenergic receptor
AVPR1A	Vasopressin V1a receptor
AVPR1B	Vasopressin V1b receptor
AVPR2	Vasopressin V2 receptor
BDKRB2	B2 bradykinin receptor
CCR2	C-C chemokine receptor type 2
CCR4	C-C chemokine receptor type 4
CCR5	C-C chemokine receptor type 5
CCKBR	Gastrin/cholecystokinin type B receptor
CHRM1	Muscarinic acetylcholine receptor M1
CHRM2	Muscarinic acetylcholine receptor M2
CNR1	Cannabinoid receptor 1
CNR2	Cannabinoid receptor 2
CXCR1	C-X-C chemokine receptor type 1
CXCR2	C-X-C chemokine receptor type 2
CXCR3	C-X-C chemokine receptor type 3
CXCR4	C-X-C chemokine receptor type 4
CYSLTR1	Cysteinyl leukotriene receptor 1
DRD1	D(1A) dopamine receptor
DRD2	D(2) dopamine receptor
DRD3	D(3) dopamine receptor
DRD4	D(4) dopamine receptor
EDNRA	Endothelin-1 receptor
EDNRB	Endothelin receptor type B
F2R	Proteinase-activated receptor 1
FPR1	fMet-Leu-Phe receptor
GHSR	Growth hormone secretagogue receptor type 1
HCRTR1	Orexin receptor type 1
HCRTR2	Orexin receptor type 2
HTR1A	5-hydroxytryptamine receptor 1A
HTR1B	5-hydroxytryptamine receptor 1B
HTR1D	5-hydroxytryptamine receptor 1D
HTR2A	5-hydroxytryptamine receptor 2A
HTR2C	5-hydroxytryptamine receptor 2C
HTR6	5-hydroxytryptamine receptor 6
OPRD1	Delta-type opioid receptor
OPRK1	Kappa-type opioid receptor
OPRL1	Opioid Related Nociceptin Receptor 1
OPRM1	Mu-type opioid receptor
OXTR	Oxytocin receptor
P2RY12	P2Y purinoceptor 12 (P2Y12)
PTGER2	Prostaglandin E2 receptor EP2 subtype
PTGER3	Prostaglandin E2 receptor EP3 subtype
S1PR1	Sphingosine 1-phosphate receptor 1
S1PR3	Sphingosine 1-phosphate receptor 3
TACR1	Substance-P receptor
TSHR	Thyrotropin receptor
TACR1	Substance-P receptor

shared protein domains with 10.06% co-expression, and 5.31% shared physical interactions and 0.35% common pathways. In terms of number of interactions, the top 10 targets in this cluster were the 5-hydroxytryptamine (serotonin) receptor 1D (HTR1D; degree = 93), 5-hydroxytryptamine receptor 1 A (HTR1A; degree = 90), kappa opioid receptor (OPRK1; degree = 88), alpha-1D adrenergic receptor (ADRA1D; degree = 84), delta opioid receptor (OPRD1; degree = 84), opioid receptor-like 1 (OPRL1; degree = 83), cannabinoid receptor 2 (CNR2; degree = 80), dopamine receptor D3 (DRD3; degree = 80), 5-hydroxytryptamine receptor 6 (HTR6; degree = 79) and endothelin receptor type B (EDNRB; score = 79) (Table 2).

3.3. GO enrichment analysis

3.3.1. Top molecular functions

The 60 genes in cluster 1 were further investigated for functional annotations using ClueGO in Cytoscape (Fig. 3**A**). The top enriched GO molecular function terms were G protein-coupled receptor activity [-log (p value) = 78,927], transmembrane signalling receptor activity [-log(p value) = 66,531), molecular transducer activity [(-log(p value) = 61,544], signalling receptor activity [(-log(p value) = 61,544], G protein-coupled peptide receptor activity [(-log(p value) = 44,354], peptide receptor activity [(-log(p value) = 43,642], G protein-coupled neurotransmitter receptor activity [(-log(p value) = 17,535], neurotransmitter receptor activity [(-log(p value) = 17,232], regulation of catalytic activity [(-log(p value) = 12,764], and G protein-coupled receptor binding [(-log(p value) = 11,701] (Fig. 3**B**).

3.3.2. Top biological processes

For the GO biological process terms (Fig. 4**A**), the top 10 were G protein-coupled receptor activity [-log(p value) = 79,153], G protein-coupled receptor signalling pathway [-log(p value) = 66,309], G protein-coupled receptor signalling pathway, coupled to cyclic nucleotide second messenger [-log(p value) = 55,767], phospholipase C-activating G protein-coupled receptor signalling pathway [-log(p value) = 40,948], cellular ion homoeostasis [(-log(p value) = 39,161], second messenger-mediated signalling [-log(p value) = 38,462], cellular chemical homoeostasis [-log(p value) = 37,325], blood circulation [-log (p value) = 36,241], regulation of tube size [-log(p value) = 35,804] and cellular homoeostasis [-log(p value) = 34,285] (Fig. 4**B**). The terms with the highest number of targets were G protein-coupled receptor activity and G protein-coupled receptor signalling pathway with 60 targets each (Table 3).

3.3.3. Top cellular compartments

The top 10 GO cellular compartments were intrinsic component of the plasma membrane [-log(p value) = 50,250], integral component of the plasma membrane [-log(p value) = 49,442], integral component of membrane [-log(p value) = 30,660], plasma membrane [-log(p value) = 30,208], intrinsic component of membrane [-log(p value) = 29,963], cell periphery [-log(p value) = 29,669], intrinsic component of synaptic membrane [-log(p value) = 18,474], intrinsic component of the presynaptic membrane [-log(p value) = 17,957], membrane [-log(p value) = 16,166] and intrinsic component of postsynaptic membrane [-log(p value) = 15,577] (Fig. 5**A, B**). According to the number of genes in each term, the integral component of the membrane was identified as the top-ranked cellular compartment (Table 3).

3.4. Pathway analysis using KEGG

Analysis of the molecular interactions with the targets present in cluster 1 using KEGG in ClueGO showed that the terms with the highest percentage of genes were neuroactive ligand-receptor interaction (KEGG:04080), followed by calcium signalling (KEGG:04020), cAMP signalling (KEGG:04024) and cGMP-PKG signalling (KEGG:04022) (Fig. 6**A**). Assessment of the percentage of terms per group showed that the cGMP-PKG signalling pathway was the most enriched (26.67%) and the second-highest was renin secretion (13.36%) (Fig. 6**B**).

4. Discussion

This is the first *in silico* study to attempt to identify commonalities between the pathophysiological pathways affected in TBI and the molecular targets of the curcumin analogue CDF. This revealed that the top common molecular functions and biological processes were related to G protein-coupled receptor activity and cellular signalling. Consistent with this, the top cellular compartment identified was the plasma membrane, including pre-and post-synaptic membranes involved in neuronal

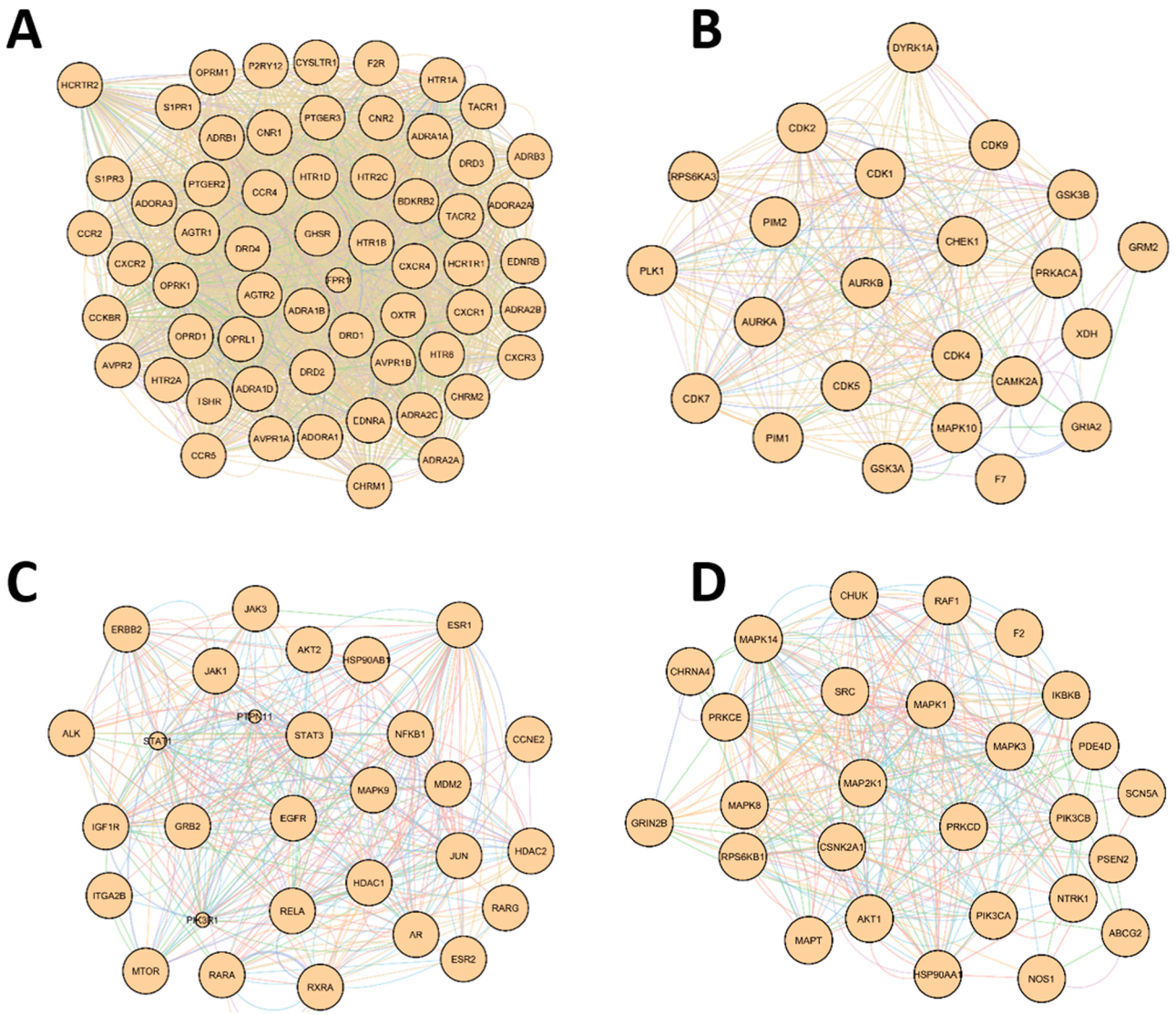

Fig. 2. Most significant clusters within the protein-protein network with shared genes in TBI and CDF lists (total of 312). Cluster 1 has 60 nodes (A), while cluster 2 (23 nodes, B), cluster 3 (C, 29 nodes) and cluster 4 with 27 nodes (D).

Table 2
Top 10 genes according to the number of interactions within the cluster 1 subnetwork.

Gene name	Degree
HTR1D (5-Hydroxytryptamine Receptor 1D)	93
HTR1A (5-Hydroxytryptamine Receptor 1A)	90
OPRK1 (Kappa opioid receptor)	88
ADRA1D (Alpha-1D adrenergic receptor)	84
OPRD1 (Delta opioid receptor)	84
OPRL1 (Opioid receptor-like 1)	83
CNR2 (Cannabinoid receptor 2)	80
DRD3 (Dopamine receptor D3)	80
HTR6 (5-Hydroxytryptamine Receptor 6)	79
EDNRB (Endothelin Receptor Type B)	79

transmission and synaptic connectivity. The finding that G protein-coupled receptors were the main target identified is of interest as most of the currently approved drugs target this family of receptors [41].

In the most significant cluster of overlapping targets, the 5-hydroxytryptamine 1D (5-HT1D) receptor was identified as the top candidate. Several studies suggested that perturbations in the function of this receptor are involved in the pathophysiology of migraine [42], a common consequence of TBI. However, it is still controversial as to whether migraine results from a vascular or neurological dysfunction since 5-HT can act as both a vasoconstrictor and neurotransmitter and its levels are known to be decreased in migraine [43]. Furthermore, 5-HT agonists, such as the tryptan family of molecules, are primarily used to treat migraine [44]. The activity of these compounds includes vasoconstriction of dilated cerebral blood vessels, inhibition of vasoactive neuropeptide release and blockade of neurotransmission involved in nociception. This is of relevance in this study as TBI can produce delayed secondary ischaemic infarcts in affected individuals and blocking the spread with a 5HT1D agonist may be a means of limiting injury expansion and cell death [45]. It is also of interest in this regard that curcumin is known to have vasoactive effects that are concentration and time-dependent [46]. Curcumin has also been used to treat migraine via reduction of neuroinflammatory effects [47] and as a hydrogel for alleviation of the reactive oxygen species generated in TBI [48]. Another study showed that curcumin treatment ameliorated spatial memory deficits in a rat TBI model via decreased chronic neuroinflammation and increased signalling in pathways involved in hippocampal neurogenesis [49]. In a similar way, curcumin may alleviate inflammation in TBI by acting on p38, leading to a significant decrease in release of the pro-inflammatory cytokines IL-6, IL-1β and TNF-α [50]. In clinical

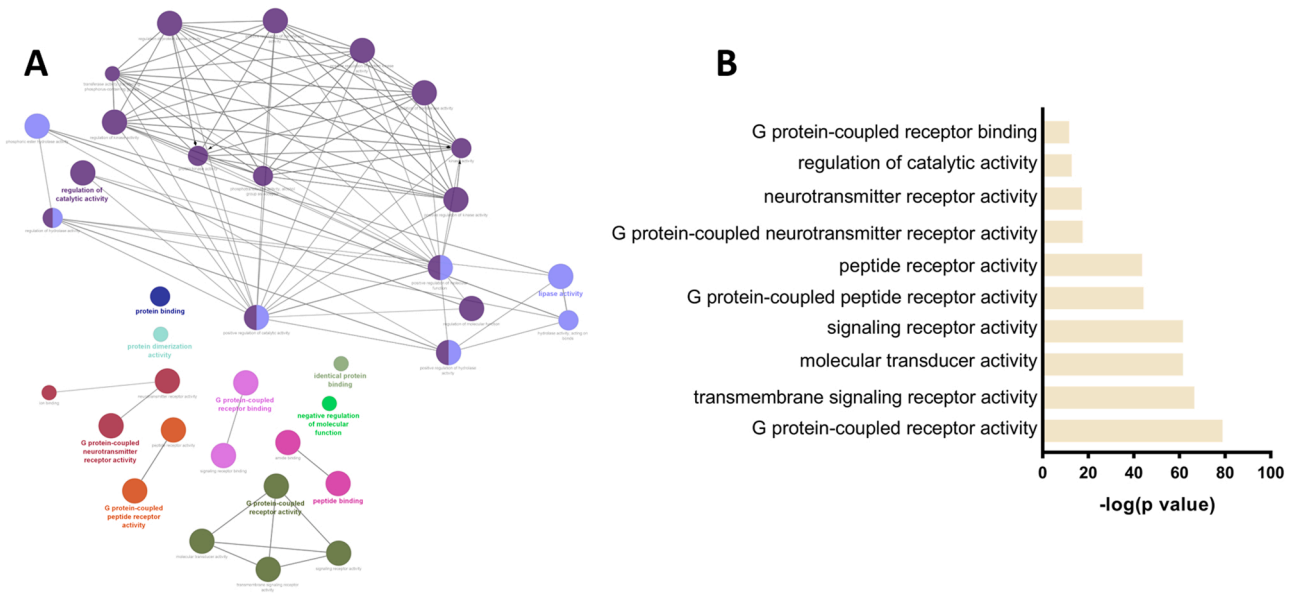

Fig. 3. Protein-protein interaction (PPI) and molecular function enrichment of cluster 1 genes. 60 genes belonging to cluster 1 were networked (A) and submitted to GO enrichment pathways analysis (B).

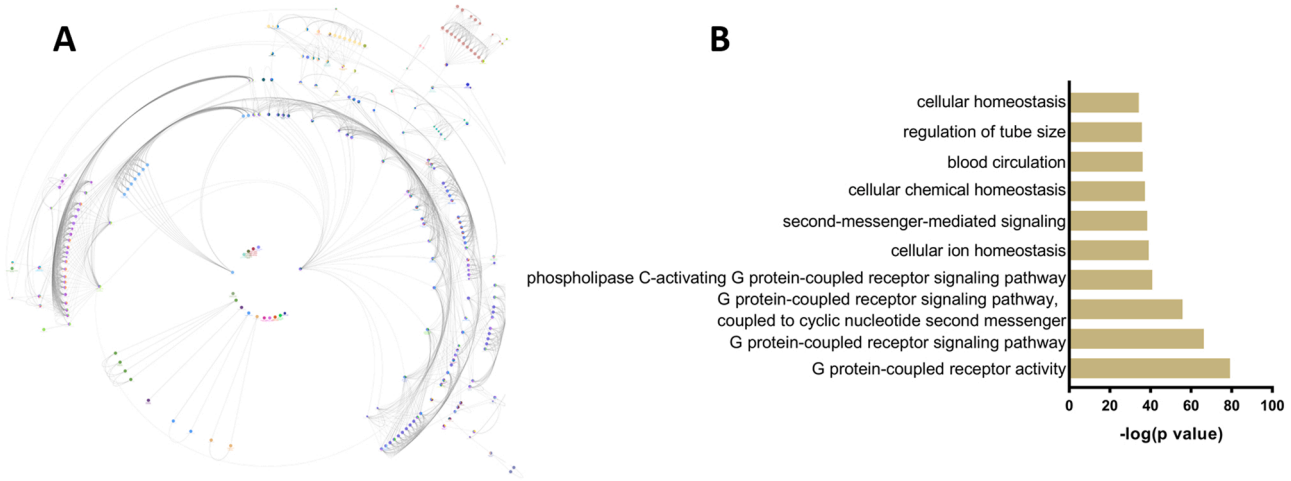

Fig. 4. GO enrichment of cluster 1 genes. Network (A) and top regulated biological processes in cluster 1 (B).

settings, TBI patients given a single dose of 500 mg curcuminoids present lower serum levels of IL-6 and TNF- α, MCP-1 and c reactive protein (CRP), improving APACHEII and NUTRIC scores, two indices that measure overall physiology and nutritional health in severe affected patients [51].

The next most significant overlapping targets of TBI and CDF in the top cluster were HT1A, OPRK1 and ADRA1D G protein-coupled receptors. The 5-HT1A receptor is widely distributed on postsynaptic neurons in brain regions such as the hippocampus, cerebral cortex and basal forebrain, which are sensitive to neuronal damage following ischaemic or traumatic insults to the brain [52]. As 5-HT1A receptors inhibit the firing activity of neurons in these brain regions, agonists of this receptor may offer neuroprotection in neurodegenerative conditions such as stroke and TBI [52]. The OPRK1 receptor is expressed in the brain and periphery, where it modulates the perception of pain, stress and reward states [53]. For these reasons, OPRK1 receptor agonists have been widely explored as a treatment for pain and psychiatric conditions such as depression and drug addiction. The ADRA1D receptor is discretely expressed in brain regions such as the olfactory bulb, cerebral cortex, hypothalamus and hippocampus, as well as in the periphery [54]. ADRA1D receptor expression in the paraventricular nucleus of the hypothalamus suggests that it may play a role in regulation of the stress response [55].

Other G protein-coupled receptors localised to the brain and identified in the top cluster included the OPRD1, OPRL1, CNR2, DRD3, HTR6 and EDNRB receptors. The OPRD1 and ORPL1 receptors both appear to be involved in reward pathways and regulation of mood and contextual learning [56,57]. Dysregulation of the CNR2 receptor is involved in various neurodegenerative disorders and up-regulation appears to be neuroprotective [58]. The DRD3 receptor is expressed in limbic regions of the brain and may be involved in the modulation of memory, cognition, emotions and motivation [59]. The HTR6 receptor has been found to regulate neurogenesis, mood, cognition and memory performance [60]. Finally, the EDNRB receptor appears to be involved in the regulation of astrocytic functions in the maintenance of blood-brain barrier integrity [61,62].

In line with these findings, the top biological processes associated with the TBI-CDF overlapping targets included G protein-receptor activity and signalling pathways, and the top cellular compartments were intrinsic components of plasma membranes (in particular pre-and post-

Table 3
GO enrichment analysis.

	GO ID	GO term	Number of genes
Molecular function	GO:0004930	G protein-coupled receptor activity	60
	GO:0004888	transmembrane signalling receptor activity	60
	GO:0060089	molecular transducer activity	60
	GO:0038023	signalling receptor activity	60
	GO:0005515	protein binding	54
	GO:0065009	regulation of molecular function	36
	GO:0050790	regulation of catalytic activity	33
	GO:0008528	G protein-coupled peptide receptor activity	29
	GO:0001653	peptide receptor activity	29
	GO:0044093	positive regulation of molecular function	22
Biological process	GO:0004930	G protein-coupled receptor activity	60
	GO:0007186	G protein-coupled receptor signalling pathway	60
	GO:0007165	signal transduction	60
	GO:0023052	signalling	60
	GO:0007154	cell communication	60
	GO:0051716	cellular response to stimulus	60
	GO:0050896	response to stimulus	60
	GO:0050794	regulation of cellular process	60
	GO:0050789	regulation of biological process	60
	GO:0065007	biological regulation	60
Cellular compartment	GO:0016021	integral component of membrane	60
	GO:0005886	plasma membrane	60
	GO:0031224	intrinsic component of membrane	60
	GO:0071944	cell periphery	60
	GO:0016020	membrane	60
	GO:0031226	intrinsic component of plasma membrane	55
	GO:0005887	integral component of plasma membrane	54
	GO:0030054	cell junction	27
	GO:0042995	cell projection	27
	GO:0045202	synapse	26

synaptic membranes). Furthermore, the KEGG analysis confirmed that the molecular interactions of the targets in cluster 1 showed the highest scores for neuroactive ligand-receptor interaction and calcium, cAMP signalling and cGMP-PKG signalling. All of these findings are consistent with the main targets being identified as G protein-coupled receptors and signalling pathways. The effects of curcumin on these receptors may be mediated through indirect means or via direct binding and activation of the relevant second messenger systems. A recent study which carried out competitive binding assays, docking analysis, confocal fluorescence microscopy and cAMP receptor activation assays found that curcumin analogues bound with μM affinity to the ADRA2A and ADRA2B receptors [63]. Another study found that curcumin binds to CB1 receptors with nM affinity and with μM affinity to CB2 receptors [64]. Also, a curcumin analogue was found to bind to the HT1A receptor with high affinity [65]. All of these receptors were identified as components of cluster 1 in the present study.

Lastly, we found that the most enriched terms in the KEGG analysis

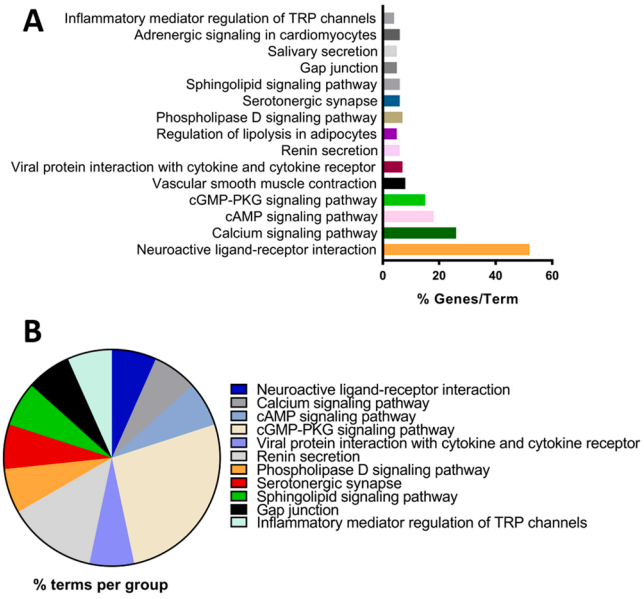

Fig. 6. Pathway analysis of cluster 1 using KEGG. Percentage of genes in each term (A) and percentage of terms per group (B).

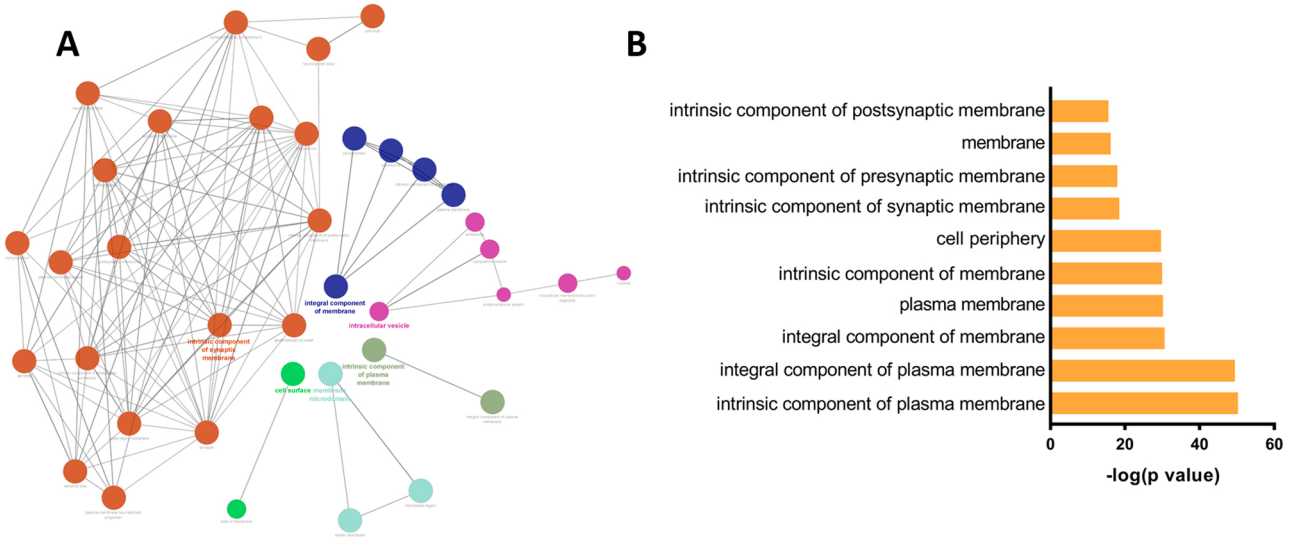

Fig. 5. GO enrichment with ClueGO for cellular compartments. Cluster 1 genes were submitted for enrichment analysis showing an intricate network (A) and top regulated cellular compartments (B).

of cluster 1 were cGMP-protein kinase G (PKG) signalling and renin secretion. This is interesting since the cGMP-PKG pathway appears to be involved in preventing activation of proapoptotic pathways in promotion of neuronal survival [66,67]. Likewise, multiple components of the renin-angiotensin system have been considered as potential targets for alleviating the damaging effects of stroke, cerebral aneurysm and TBI [68,69]. Taken together, these findings suggest that the broad spectrum of activities of the phytochemical curcumin make it a prime candidate to be considered in the treatment of TBI.

4.1. Conclusions and future perspectives

The last two decades have shown a shift in the increasing use of *in silico* target prediction and such studies are expected to become even more useful as the relevant databases become more populated [70]. It is critical that all targets identified should be tested in vitro to ensure that they are not false positives. Furthermore, in vivo testing must be performed to determine if there are any off-target toxicities. Nevertheless, the present findings warrant further preclinical and clinical investigations of curcumin and its analogues as a potential novel treatment to ameliorate the often-devastating effects of TBI.

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CRediT authorship contribution statement

Design and data collection: **George E. Barreto** and **Amirhossein Sahebkar**. Drafting: **George E. Barreto, Amirhossein Sahebkar** and **Thozhukat Sathyapalan**. Revision of drafted version: **George E. Barreto, Amirhossein Sahebkar, Paul C. Guest** and **Thozhukat Sathyapalan**. All authors agree with submission and publication of manuscript.

Consent to participate

Not Applicable.

Consent for publication

Not applicable.

Competing Interests

The authors claim no competing interests.

Availability of data and material

Available within the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.112770.

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