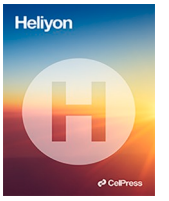
Heliyon 10 (2024) e24196

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

A label free chemoproteomic-based platform to disclose cannabidiol molecular mechanism of action on chronic myelogenous leukemia cancer cells

Sara Ceccacci [a,b], Lorenzo Corsi [c,d], Lucio Spinelli [a,e], Clarissa Caroli [c,d], Matilde Marani [c], Lisa Anceschi [c,d], Matteo Mozzicafreddo [f], Federica Pellati [c], Maria Chiara Monti [a,*]

[a] Department of Pharmacy, Università di Salerno, Via Giovanni Paolo II, 84084, Fisciano, Italy
[b] PhD Program in Drug Discovery and Development, Department of Pharmacy, Università di Salerno, Via Giovanni Paolo II, 84084, Fisciano, Italy
[c] Department of Life Sciences, University of Modena and Reggio Emilia, Via G. Campi 103/287, 41125, Modena, Italy
[d] Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Via Campi 287, 41125, Modena, Italy
[e] Department of Pharmacy, Università degli Studi di Napoli 'Federico II', Via Domenico Montesano, 49, 80131, Napoli, Italy
[f] Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, 60126, Ancona, Italy

ARTICLE INFO

Keywords:
Cannabidiol
Chronic myelogenous leukemia
Drug Affinity Responsive Target Stability
Limited proteolysis
Chemo-proteomics
Mass spectrometry
Multi-target activity

ABSTRACT

The discovery of the interactome of cannabidiol (CBD), a non-psychoactive cannabinoid from *Cannabis sativa* L., has been here performed on chronic myelogenous leukemia cancer cells, using an optimized chemo-proteomic stage, which links Drug Affinity Responsive Target Stability with Limited Proteolysis Multiple Reaction Monitoring approaches. The obtained results showed the ability of CBD to target simultaneously some potential protein partners, corroborating its well-known poly-pharmacology activity. In human chronic myelogenous leukemia K562 cancer cells, the most fascinating protein partner was identified as the 116 kDa U5 small nuclear ribonucleoprotein element called EFTUD2, which fits with the spliceosome complex. The binding mode of this oncogenic protein with CBD was clarified using mass spectrometry-based and *in silico* analysis.

1. Introduction

The identification of protein receptors of bioactive natural products (NPs) is one of the key point in the field of target discovery, since it represents a pivotal step to elucidate the mechanism of action of these molecules [1]. This process is mainly important when a NP shows an interesting bioactive profile but its main target is still unknown. Besides, the discovery of other targets may be useful to identify the so-called "off-targets"; their identification is important to decrease the undesired biological effects. However, the aptitude of NPs to link a few proteins simultaneously (poly-pharmacology) may also produce either additive or synergistic effects, leading to a higher efficacy and reduced toxicity.

At present, among the existing strategies for target identification, chemical proteomics looks to be the most hired one [2]. In particular, the recent chemo-proteomic-based approaches, such as Drug Affinity Responsive Target Stability (DARTS), may surpass the

* Corresponding author.
E-mail address: mariachiara.monti@unina.it (M.C. Monti).

https://doi.org/10.1016/j.heliyon.2024.e24196
Received 27 October 2023; Received in revised form 4 January 2024; Accepted 4 January 2024
Available online 5 January 2024

drawbacks of the conventional affinity purification (AP), since they use the bioactive small molecule in its unmodified form. Mostly, the DARTS principle relies on the protection against limited proteolysis events of a certain protein upon the ligand interaction: usually, a protein is in balance among numerous conformations with the same energy states but, upon binding with the ligand, the balance moves to the more stable state and the resistance to proteolysis is enlarged [3,4]. Indeed, in the DARTS workflow, the "native" small molecule is mixed with an opportune cell lysate for a certain time, before the limited proteolysis step. The ligand-stabilized proteins, which represent in principle the whole ligand interactome, are simply visualized by gel electrophoresis (SDS-PAGE) and, after tryptic digestion *in situ*, identified by proteomics [5,6]. Furthermore, a more exhaustive picture of the binding region(s) of the putative targets to the bioactive compound can be achieved by a newly-established strategy named targeted-Limited Proteolysis-Mass Spectrometry (t-LiP-MS), a gel-free DARTS-like approach grounded on a double-protease digestion and the filtering power of Multiple Reaction Monitoring (MRM)-MS analysis [7–9]. t-LiP-MS lets to know which areas of the protein targets showed conformational fluctuations due to the ligand, which alters the proteases accessibility.

In this set-up, we have performed the *targetomics* analysis of cannabidiol (CBD), a non-psychoactive cannabinoid from *Cannabis sativa* L., endowed with a wide spectrum of biological activities [10,11]. Contrarily to Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD is not able to bind CB1 receptors with high affinity [12], since it adopts a bent and not planar conformation, which does not allow the recognition of CB1 receptors [13].

Besides this aspect, CBD has a multi-targets action on different proteins or receptors, mainly G protein-coupled receptor 55 (GPR55), transient receptors potential vanilloid (TRPVs), peroxisome proliferator-activated receptor gamma (PPARγ) and serotonin 5-HT_{1A} receptors. As a value of its several interactions, CBD shows many interesting beneficial properties, counting high anti-oxidant and anti-inflammatory potential, neuroprotective, anxiolytic and anti-convulsant properties [11].

Recent studies exposed that CBD decreases the proliferation of human chronic myelogenous leukemia K562 cancer cells by prompting apoptosis, occurring by targeting the cytochrome *c* in the cytosol together with the reduction of the mitochondrial membrane potential and the activation of caspase 3 and 7 [14]. To shed light on CBD apoptotic action mechanism, this study is focalized on the identification of its interactome in K562 cancer cells by using a functional proteomic platform initially based on the DARTS. Next, DARTS results were completed by t-LiP-MS analysis, validated by immunoblotting assays and further explained by *in silico* experiments. *In cell* experiments were also performed.

2. Materials and methods

2.1. Identification of CBD interactome by Drug Affinity Responsive Target Stability (DARTS)

K562 cell pellets were mechanically lysate in PBS (Phosphate Buffered Saline) at pH 7.4 containing 0.1 % IGEPAL and protease inhibitors (Sigma-Aldrich); the debris were wasted after the centrifugation at 1000×*g* and 4 °C for 10 min. The protein concentration was determined by Bradford spectrophotometric assays up to 3 μg/μL. 90 μg of protein mixtures were raised for 1 h at room temperature with DMSO or with CBD (1, 10 and 100 μM in DMSO). The DMSO in each sample is 1 % (v/v). Next, limited proteolysis has been performed as reported in Refs. [5,6]. More in details, two different appropriate controls were also prepared: the first is the lysate treated with 1 % DMSO without CBD and digested by subtilisin (negative control), the second is the lysate treated with 1 % DMSO without CBD and undigested by subtilisin (positive control). Two independent experiments were repeated on two independent cell lysates.

MS data, obtained on tryptic digest of the DARTS samples loaded on SDS-PAGE as reported by Ref. [15], were then processed using the parameters reported in Ref. [5]. The mass spectrometry proteomics data have been placed in the ProteomeXchange Consortium via the PRIDE [16] partner repository with the dataset identifier PXD036650.

2.2. Corroboration of DARTS results by immunoblotting

Samples (10 μg) derived from the above described DARTS experiments were investigated by immunoblotting analysis, using primary antibodies specific for DYNC1H1 (1:1000 v/v in 5 % milk; Proteintech), SNRNP200 (1:1000 v/v; Raybiotech), TOP2A (1:1000 v/v; Biorad) and EFTUD2 (1:1000 v/v; Proteintech), GAPDH (1:2000 v/v, Invitrogen) or ACTB (1:500 v/v; Santa Cruz Biotechnology). A rabbit/mouse peroxidase-conjugated secondary antibody (1:2500 v/v, Thermo Fisher Scientific) was added to detect the signal using LAS 4000 (GE Healthcare) digital imaging system.

2.3. T-LiP-MRM analysis

EFTUD2 tryptic peptides were selected to get the full methods listing EFTUD2 peptides and their best fragmentations as reported in Ref. [6]. Then, these methods were tested on K562 cell lysate upon an *in solution* tryptic digestion as reported in Ref. [6]. K562 cell lysate aliquots (200 μg) were incubated (1 h, room temperature, 300 RPM) either with DMSO or CBD (1, 10 and 100 μM in DMSO). The DMSO concentration in each sample was 1 % (v/v). Samples were then doubly digested: first with subtilisin (enzyme to proteins ratio of 1:500 w/w, 30 min, room temperature, 300 RPM) and after with the specific protease trypsin, extensively (enzyme to proteins ratio of 1:100 w/w, overnight, room temperature, 300 RPM). Peptides were then run on a UPLC-ESI-MS system using MRM methods. Each area was measured using the Analyst Software from AB Sciex. Two independent experiments were repeated on two independent cell lysates.

2.4. In silico prediction of the CBD/EFTUD2 complex

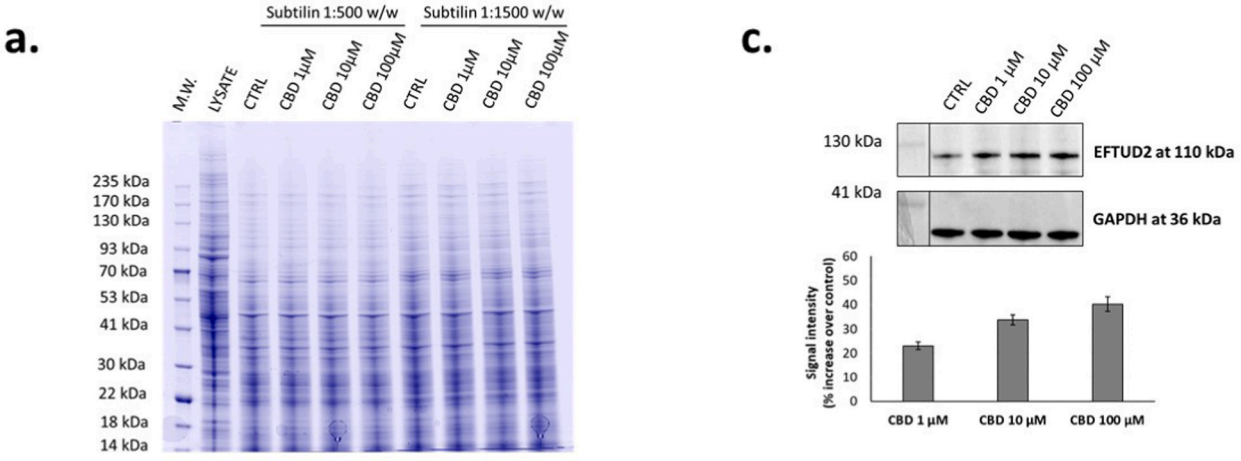
The binding between CBD and EFTUD2 was predicted *in silico* using molecular docking analysis. The crystal structure of EFTUD2 was obtained by extracting chain C from the PDB file 6ID1 [17]. We chose the crystallographic structure 6ID1 because it is the most complete and it has the highest resolution (2.86 Å). The 3D structure of CBD was designed and energy minimized as reported in Refs. [18,19].

This procedure followed the method previously reported by Del Gaudio et al. [20] employing the server SwissDock [21] as the docking algorithm. All parameters were set to their default values, including the docking zone covering the entire protein. The optimal final complex geometry was rendered using PyMol software and the 3D representation of the interaction was obtained through the Protein-Ligand Interaction Profiler (PLIP) web server [22]. The predicted equilibrium dissociation constant ($K_{D,pred}$) of this complex was obtained from the standard equation $K_{D,pred} = e^{\Delta G^{1000}_{RT}}$.

To check the stability of the predicted complex, a molecular dynamics (MD) analysis of the complex between CBD and EFTUD2 was conducted using GROMACS version 2023.1.

2.5. Evaluation of EFTUD2 expression levels in K562 cells upon CBD treatment by immunoblotting

K562 cells were grown as request. For the following Western blot experiments performed on protein extracts obtained from K562 cell line, cells were treated for 24 h with CBD 20 μM, corresponding to the IC_{25} as optimized by Ref. [14]. Proteins from both the control and the treated K562 cancer cell line were obtained by lysing and homogenized (in ice) the samples in RIPA buffer with 2 mM phenylmethylsulfonyl fluoride (Merck Life Science S.r.l., Italy). The samples were, then, quantified using BCA protein assay (ThermoFisher, Milan, Italy). SDS-PAGE were carried out and transferred to nitrocellulose membrane incubated with primary antibody anti-EFTUD2 (1:1000) and then with HRP-conjugated anti-rabbit antibody (Invitrogen, Milan, Italy) and visualized using chemiluminescence method. Three replicates were performed on four independent experiments.



b.

Accession	Mass	Description	% Protection		
			CBD 1μM	CBD 10μM	CBD 100μM
Q14204	534809	Cytoplasmic dynein 1 heavy chain 1	38.0	78.6	102.0
Q15029	110336	116 kDa U5 small nuclear ribonucleoprotein comp.	46.8	90.7	92.4
P11388	175017	DNA topoisomerase 2-alpha	41.2	50.2	55.1
O75643	246006	U5 small nuclear ribonucleoprotein 200 kDa helicase	8.4	18.3	31.1
O60306	172270	RNA helicase aquarius	14.5	19.2	29.9
P19367	103561	Hexokinase-1	14.8	26.0	28.8
Q92878	154823	DNA repair protein RAD50	1.1	19.2	27.3
P35579	227646	Myosin-9	0.6	3.9	23.8

Fig. 1. (**a**) Coomassie stained gel of DRATS experiment using subtilisin at 1:500 and 1:1500 w/w (enzyme to proteins ratio). (**b**) List of putative targets protected by CBD in a concentration dependent mode in two independent DARTS experiments together with the percentage of protection. (**c**) Immunoblotting and densitometric analysis of DARTS experiments revealing EFTUD2 as protected by increasing amount of CBD The bars represent the standard deviations.

3. Results

3.1. CBD cellular target(s)

For the identification of CBD targets, DARTS experiments were performed. As first step, proteome was obtained under native conditions from K562 cancer cells and it was divided into aliquots incubated with increasing concentrations of either CBD (1, 10, 100 μM diluted in DMSO) or the vehicle (negative control). The DMSO concentration was 1 % v/v. Next, limited proteolysis was carried out by treatment with the nonspecific protease subtilisin, except for one sample which was untreated (positive control). After the quenching of the enzyme, an SDS-PAGE was performed and revealed by Coomassie blue staining (Fig. 1a). Then, the gel was cut in pieces, the bands were digested *in situ* using trypsin and nano-LC-MS/MS analysis were performed (15). The succeeding data analysis was finalized using Mascot search engine software able to afford a putative list of CBD protein interactors. To evaluate the protection degree to proteolysis events due to CBD, Mascot matches outputs were considered, linking the results of the treated samples with those of the negative and positive controls (Table S1). All putative targets protected by CBD in a concentration-dependent way, in two DARTS independent experiments, are shown in Fig. 1b. Definitely, they are the following ones: cytoplasmic dynein 1 heavy chain 1 (DYNC1H1), 116 kDa U5 small nuclear ribonucleoprotein component (EFTUD2), DNA topoisomerase 2-alpha (TOP2A), U5 small nuclear ribonucleoprotein 200 kDa helicase (SNRNP200), RNA helicase aquarius (AQR), Hexokinase-1 (HK1), DNA repair protein RAD50 (RAD50) and Myosin-9 (MYH9).

Four of these proteins were the most protected by CBD at its highest concentration (protection by CBD at 100 μM > 30 %) and our attention was placed on these putative receptors. Indeed, the direct interaction between CBD and DYNC1H1, EFTUD2, TOP2A and SNRNP200 was validated thought immunoblotting, carried out on the above described DARTS samples, to measure the CBD protection using a method independent by mass spectrometry. A truthful densitometric analysis was carried out by means of GAPDH (glyceraldehyde 3-phosphate dehydrogenase) as loading normalizer. As shown in Fig. 1c, Figs. S1a–c and Fig. S2, DYNC1H1, SNRNP200, TOP2A and EFTUD2 signals rise their intensity consequently to CBD amounts, thus settling proteomics data.

These data are in accordance with the poly-pharmacological activity of CBD: indeed, it is a common fact that this natural compound has multi-targets interactions [11] and, here, several possible binders were revealed. As an additional step, we focused on EFTUD2 binding depiction, since this protein has been described as an oncogenic one, stimulating the survival of several cancer cells by STAT3 activation [23] and p53 signaling pathway inhibition [24]. A CBD-based variation of this pathway could be of relevant scientific interest. Regarding the other targets, future insights will be arranged to detail different interaction modes.

3.2. t-LiP MRM and molecular docking of CBD-EFTUD2 complex

To go deep into the interaction features of CBD-EFTUD2 linkage, a t-LiP-MRM strategy was useful. It lets the prompt appreciation of the target/ligand interaction regions (also called LIP peptides) looking at the protein conformational changes in a whole cell lysate [7, 8].

Proteome cell extracts were mixed with or without CBD and a double digestion step has been finalized: first, they were treated in native conditions using subtilisin to produce structure-specific protein peptides and, next, using trypsin extensively to obtain peptides amenable to the subsequent MRM-MS analysis.

t-LiP-MRM is fully complementary to DARTS approach, since it investigates changes in the conformational features on the target proteins 3D structure, due to CBD binding and, therefore, looking for alteration of the LiP peptides patterns. Indeed, the CBD-treated

a.

Q1_mz	Peptide	Rt (min)	Fold change		
			CBD 1 μM	CBD 10 μM	CBD 100 μM
628.86	S[183-194]K	6.10	1.26	1.57	2.13
947.95	I[326-341]K	9.13	2.28	1.73	1.96
696.89	I[590-602]K	5.73	1.27	1.47	1.92

b.

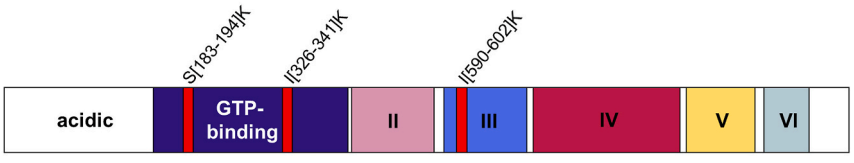


Fig. 2. (**a**) Selected EFTUD2 peptides shown with their Q1 *m/z* value, length, retention time and fold changes at three CBD concentrations; (**b**) EFTUD2 schematic cartoon covering different domains with LiP-protected peptides highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and the control samples changed in the area of the peaks related to the fully tryptic peptides (embedding LiP sites), mapping for target regions structurally affected by metabolite binding.

EFTUD2 plays a critical part in the catalytic splicing by facilitating the GTP-dependent dissociation of U4 and U6 snRNPs [25,26]. Like other EF-related proteins, EFTUD2 contains a GTP-binding domain and extra conserved domains II–VI. Furthermore, it has an N-terminal acidic domain whose function is still not known and which has not been found in other group members.

Initially, K562 native lysate was incubated either with CBD (at 1, 10, 100 μM concentration in DMSO) or with the vehicle and with subtilisin at 1:500 w/w (enzyme to proteins ratio). After the quenching of this first step, samples were fully digested by trypsin, giving peptide mixtures separated by UPLC-MRM-MS analysis. An evaluation of the fully tryptic peptides intensities in different samples shed light on which EFTUD2 regions were affected by CBD binding: those peptides whose peak area raised up upon CBD treatment were symptomatic of CBD protection. t-LiP-MRM results showed that those regions protected by CBD belong to the GTP binding domain (S[183–194]K and I[326–341]K) and to the domain III (I[590–602]K), as shown in Fig. 2a and b.

A molecular docking analysis of CBD on EFTUD2 was done in parallel, using the 3D structure of the human protein. Based on the predicted affinity, CBD exhibited its most favorable pose interacting with the amino acids LYS98, PHE102, LYS646, and GLU680, with a $K_{D,pred}$ value of 5.21 μM (Fig. 3a and b). Furthermore, a molecular dynamics (MD) analysis of the complex between CBD and EFTUD2 was performed indicating that the complex achieves sufficient stability (RMSD = 1.5 Å) within 1 ns, a relatively fast timeframe, and maintains this stability over the subsequent nanoseconds (Fig. S3). Therefore, associating t-LIP-MRM data with molecular docking, we can speculate that CBD-protected peptide in the domain III maps very close to EFTUD2 interaction site as disclosed by docking analysis. On the contrary, the GTP binding domain seems to be affected by CBD thought a long-range conformational change.

3.3. Modulation of EFTUD2 protein expression by CBD in K562 cell line

To investigate a conceivable modulation of EFTUD2 by CBD in the human chronic myelogenous leukemia K562 cancer cell line, the expression of this spliceosome protein was assessed by incubating cells with 20 μM CBD for 24 h. As shown in Fig. 4, the protein expression of EFTUD2 was slightly reduced by the compound, even if the densitometric analysis did not show any significant variation in comparison to the untreated control. This result is fully in agreement with DARTS and LIP data since CBD can directly target EFTUD2, changing its conformation and/or activity without any variation in its expression.

4. Discussion and conclusions

Bioactive NPs protein receptors identification is a decisive stage to fully elucidate NPs mechanism of action, above all when they show a poly-pharmacology profile in order to clarify their additive and/or synergistic actions. In such a set-up, DARTS and LIP-MRM strategies were optimized and applied to the case of CBD, a non-psychoactive cannabinoid from *C. sativa*, with a wide biological and

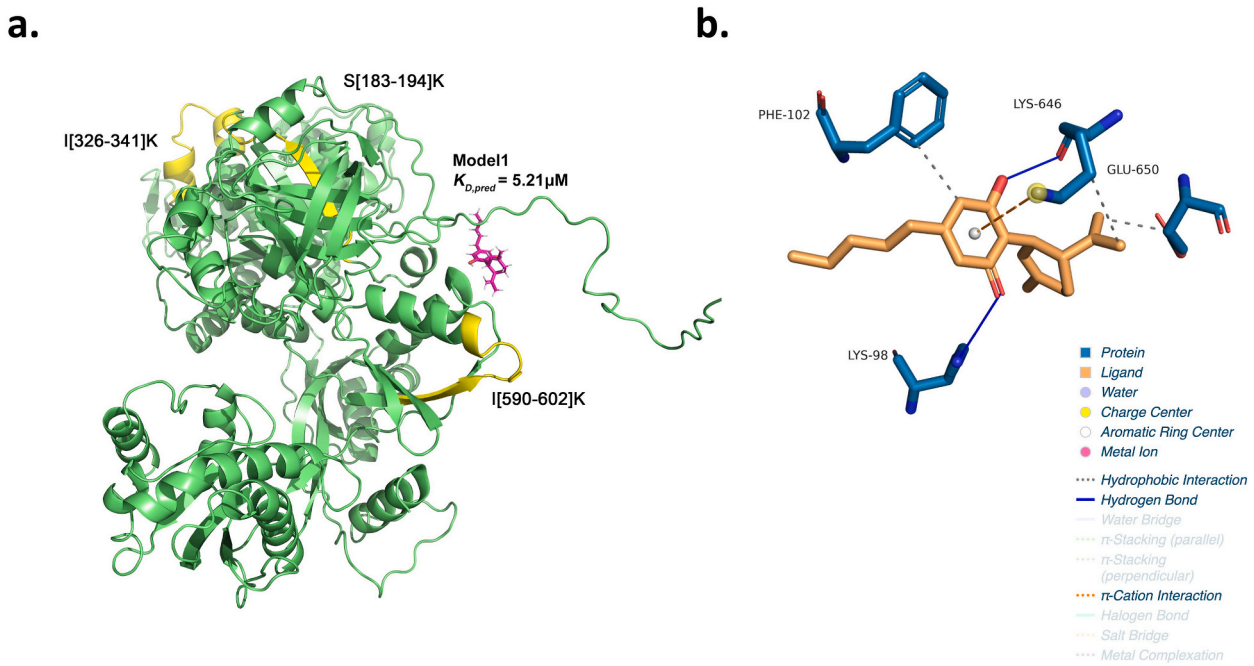


Fig. 3. (**a**) Best predicted complex between CBD and EFTUD2, obtained from molecular docking. EFTUD2 is showed in green. t-LiP-MRM CBD-protected peptides are in yellow. CBD is in purple. (**b**) The residues involved in the complex formation are labeled and shown in stick representation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

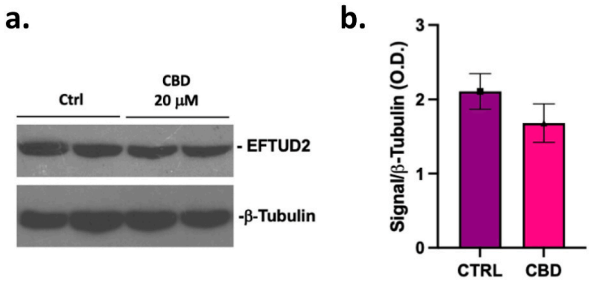


Fig. 4. Representative Western blot (**a**) and respective densitometric analysis (**b**) of EFTUD2 protein level K562 cancer cell line treated or not (CTRL) with 20 μM CBD for 24 h.

pharmacological spectrum of activities.

Even though several researches defined the CBD-mediated anti-proliferative activity in cancer cell lines through the action on its putative endogenous receptors CB1 and CB2 and on GPR55 or TRPV1 [27], the exact molecular mechanism of action of this compound is still unknown. Recent interesting studies have demonstrated that CBD can inhibit the proliferation of human chronic myelogenous leukemia K562 cancer cells by inducing apoptosis, together with a drop of the mitochondrial membrane potential and the stimulation of caspase 3 and 7 [14]. Remarkably, the protein expression levels of the above-mentioned receptors were low or absent in the K562 cell line as far as regard the CB2 and thus they cannot be accountable of the antiproliferative activity of CBD (https://www.proteinatlas.org).

Therefore, for the first time, high- and low-resolution proteomic approaches were applied here for the identification of CBD targets (untargeted proteomics) and for the investigations of the target regions involved in the protein/ligand interaction (targeted proteomics) on K562 chronic myelogenous leukemia cells.

Our DARTS results pointed towards the aptitude of CBD to bind concurrently multiple targets, thus corroborating its known polypharmacology activity. In particular, the 116 kDa U5 small nuclear ribonucleoprotein component called EFTUD2 was chosen for further investigations and, in this paper, we have also labeled how CBD modifies EFTDU2 structure upon its interaction, both by changing the conformation of the GTP binding domain and of the domain III, as also confirmed by *in silico* analysis. Besides, cell assays have shown that CBD does not influence the expression levels of EFTUD2, thus corroborating the idea that CBD is able to directly network with this protein and not with its transcriptomic regulators. EFTUD2 controls the spliceosome dissociation and an anomalous alternative splicing is a hallmark of cancer [28,29].

The application of a strategy which combines untargeted DARTS and targeted LIP-MRM strategies allowed us to discover in an unbiased way which are the most affine interactors of CBD in a K562 cell lysates obtained in mild pseudo-physiological conditions, keeping the proteins in their native form and the macromolecular complexes unbroken. This is the main strength of this work, since both the natural compound and the putative targets do not have any constrain during the interaction events in solution. Moreover, a detailed mechanism of protein-ligand interaction can be also provided. Nevertheless, it has also been considered that this approach is able to detect almost exclusively the soluble cytoplasmatic protein receptors giving no information on the membrane ones, thus underestimating the involvement of such putative targets.

In conclusion, we propose that the anti-cancer activity of CBD could, in turn, be also related to an alteration of the spliceosome functionality mediated by the interaction of the NPs with the EFTUD2 factor. This is an element of high novelty since the role of EFTUD2 has been underestimated in the field of cancer research in comparison to others small nuclear ribonucleoprotein such as U2AF1 and SF3B1. Indeed, up to now, only four papers have been published describing the involvement of EFTUD2 in the colitis-associated tumorigenesis [30], in the endometrial cancer [31] and in the HCC [32,33].

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for- profit sectors. Part of this research was supported by FAR2022 Life Sciences Department UNIMORE Project entitled: “Study of the effect of the PyK2 protein on the activity of TRPC receptors in cellular models of glioblastoma multiforme (GBM): new pharmacological targets"(PI Prof. Lorenzo Corsi).

Data availability

The mass spectrometry proteomics data were deposited on the public repository ProteomeXchange Consortium via the PRIDE partner repository [16] with the dataset identifier PXD036650.

CRediT authorship contribution statement

Sara Ceccacci: Writing - original draft, Investigation. **Lorenzo Corsi:** Methodology, Formal analysis, Data curation, Conceptualization. **Lucio Spinelli:** Investigation, Data curation. **Clarissa Caroli:** Investigation. **Matilde Marani:** Investigation. **Lisa Anceschi:**

Visualization, Investigation. **Matteo Mozzicafreddo:** Writing - original draft, Investigation, Data curation, Conceptualization. **Federica Pellati:** Writing - review & editing, Writing - original draft, Supervision, Conceptualization. **Maria Chiara Monti:** Writing - review & editing, Writing - original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24196.

References

[1] S. Ziegler, V. Pries, C. Hedberg, H. Waldmann, Target identification for small bioactive molecules: finding the needle in the haystack, Angew Chem. Int. Ed. Engl. 52 (2013) 2744–2792, https://doi.org/10.1002/anie.201208749.

[2] U. Rix, G. Superti-Furga, Target profiling of small molecules by chemical proteomics, Nat. Chem. Biol. 5 (2009) 616–624, https://doi.org/10.1038/nchembio.216.

[3] B. Lomenick, R.W. Olsen, J. Huang, Identification of direct protein targets of small molecules, ACS Chem. Biol. 6 (2011) 34–46, https://doi.org/10.1021/cb100294v.

[4] M.Y. Pai, B. Lomenick, H. Hwang, R. Schiestl, W. McBride, J.A. Loo, J. Huang, Drug Affinity Responsive Target Stability (DARTS) for small-molecule target identification, Methods Mol. Biol. 1263 (2015) 287–298, https://doi.org/10.1007/978-1-4939-2269-7_22.

[5] S. Ceccacci, J. Deitersen, M. Mozzicafreddo, E. Morretta, P. Proksch, S. Wesselborg, B. Stork, M.C. Monti, Carbamoyl-phosphate synthase 1 as a novel target of phomoxanthone A, a bioactive fungal metabolite, Biomolecules 10 (2020) E846, https://doi.org/10.3390/biom10060846.

[6] E. Morretta, R. Belvedere, A. Petrella, A. Spallarossa, F. Rapetti, O. Bruno, C. Brullo, M.C. Monti, M.C. Novel insights on the molecular mechanism of action of the anti-angiogenic pyrazolyl-urea GeGe-3 by functional proteomics, Bioorg. Chem. 115 (2021) 105168, https://doi.org/10.1016/j.bioorg.2021.105168.

[7] Y. Feng, G. De Franceschi, A. Kahraman, M. Soste, A. Melnik, P.J. Boersema, P.P. de Laureto, Y. Nikolaev, A.P. Oliveira, P. Picotti, Global analysis of protein structural changes in complex proteomes, Nat. Biotechnol. 32 (2014) 1036–1044, https://doi.org/10.1038/nbt.2999.

[8] S. Schopper, A. Kahraman, P. Leuenberger, Y. Feng, I. Piazza, O. Müller, P.J. Boersema, P. Picotti, Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry, Nat. Protoc. 12 (2017) 2391–2410, https://doi.org/10.1038/nprot.2017.100.

[9] P. Picotti, R. Aebersold, Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions, Nat. Methods 9 (2012) 555–566, https://doi.org/10.1038/nmeth.2015.

[10] S. Burstein, Cannabidiol (CBD) and its analogs: a review of their effects on inflammation, Bioorg. Med. Chem. 23 (2015) 1377–1385, https://doi.org/10.1016/j.bmc.2015.01.059.

[11] J. Peng, M. Fan, C. An, F. Ni, W. Huang, J. Luo, A narrative review of molecular mechanism and therapeutic effect of cannabidiol (CBD), Basic Clin. Pharmacol. Toxicol. 130 (2022) 439–456, https://doi.org/10.1111/bcpt.13710.

[12] R.G. Pertwee, The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin, Br. J. Pharmacol. 153 (2008) 199–215, https://doi.org/10.1038/sj.bjp.0707442.

[13] H. Chung, A. Fierro, C.D. Pessoa-Mahana, Cannabidiol binding and negative allosteric modulation at the cannabinoid type 1 receptor in the presence of delta-9-tetrahydrocannabinol: an in Silico study, PLoS One 14 (2019) e0220025, https://doi.org/10.1371/journal.pone.0220025.

[14] L. Anceschi, A. Codeluppi, V. Brighenti, R. Tassinari, V. Taglioli, L. Marchetti, L. Roncati, A. Alessandrini, L. Corsi, F. Pellati, Chemical characterization of non-psychoactive Cannabis sativa L. extracts, in vitro antiproliferative activity and induction of apoptosis in chronic myelogenous leukaemia cancer cells, Phytother Res. 36 (2022) 914–927, https://doi.org/10.1002/ptr.7357.

[15] A. Shevchenko, H. Tomas, J. Havli, J.V. Olsen, M. Mann, In-gel digestion for mass spectrometric characterization of proteins and proteomes, Nat. Protoc. 1 (2006) 2856–2860, https://doi.org/10.1038/nprot.2006.468.

[16] Y. Perez-Riverol, J. Bai, C. Bandla, D. García-Seisdedos, S. Hewapathirana, S. Kamatchinathan, D.J. Kundu, A. Prakash, A. Frericks-Zipper, M. Eisenacher, M. Walzer, S. Wang, A. Brazma, J.A. Vizcaíno, The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences, Nucleic Acids Res. 50 (2021), https://doi.org/10.1093/nar/gkab1038. D543–D552.

[17] X. Zhang, X. Zhan, C. Yan, W. Zhang, D. Liu, J. Lei, Y. Shi, Structures of the human spliceosomes before and after release of the ligated exon, Cell Res. 29 (2019) 274–285, https://doi.org/10.1038/s41422-019-0143-x.

[18] M.D. Hanwell, D.E. Curtis, D.C. Lonie, T. Vandermeersch, E. Zurek, G.R. Hutchison, Avogadro: an advanced semantic chemical editor, visualization, and analysis platform, J. Cheminf. 4 (2012) 17, https://doi.org/10.1186/1758-2946-4-17.

[19] M. Mozzicafreddo, M. Cuccioloni, L. Bonfili, V. Cecarini, F.A. Palermo, P. Cocci, G. Mosconi, A. Capone, I. Ricci, A.M. Eleuteri, M. Angeletti, Environmental pollutants directly affect the liver X receptor alpha activity: kinetic and thermodynamic characterization of binding, J. Steroid Biochem. Mol. Biol. 152 (2015) 1–7, https://doi.org/10.1016/j.jsbmb.2015.04.011.

[20] F. Del Gaudio, F. Pollastro, M. Mozzicafreddo, R. Riccio, A. Minassi, M.C. Monti, Chemoproteomic fishing identifies arzanol as a positive modulator of brain glycogen phosphorylase, Chem. Commun. 54 (2018) 12863–12866, https://doi.org/10.1039/c8cc07692h.

[21] A. Grosdidier, V. Zoete, O. Michielin, SwissDock, a protein-small molecule docking web service based on EADock DSS, Nucleic Acids Res. 39 (2011) W270–W277, https://doi.org/10.1093/nar/gkr366.

[22] M.F. Adasme, K.L. Linnemann, S.N. Bolz, F. Kaiser, S. Salentin, V.J. Haupt, M. Schroeder, Plip 2021: expanding the scope of the protein-ligand interaction profiler to DNA and RNA, Nucleic Acids Res. 49 (2021), https://doi.org/10.1093/nar/gkab294. W530–W534.

[23] M. Tu, L. He, Y. You, J. Li, N. Yao, C. Qu, W. Huang, L. Xu, R. Luo, J. Hong, EFTUD2 maintains the survival of tumor cells and promotes hepatocellular carcinoma progression via the activation of STAT3, Cell Death Dis. 11 (2020) 1–12, https://doi.org/10.1038/s41419-020-03040-5.

[24] G. Niu, K.L. Wright, Y. Ma, G.M. Wright, M. Huang, R. Irby, J. Briggs, J. Karras, W.D. Cress, D. Pardoll, R. Jove, J. Chen, H. Yu, Role of Stat3 in regulating p53 expression and function, Mol. Cell Biol. 25 (2005) 7432–7440, https://doi.org/10.1128/MCB.25.17.7432-7440.2005.

[25] M.A. Lines, L. Huang, J. Schwartzentruber, S.L. Douglas, D.C. Lynch, C. Beaulieu, M.L. Guion-Almeida, R.M. Zechi-Ceide, B. Gener, G. Gillessen-Kaesbach, C. Nava, G. Baujat, D. Horn, U. Kini, A. Caliebe, Y. Alanay, G.E. Utine, D. Lev, J. Kohlhase, A.W. Grix, D.R. Lohmann, U. Hehr, D. Böhm, , FORGE Canada Consortium, J. Majewski, D.E. Bulman, D. Wieczorek, K.M. Boycott, Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly, Am. J. Hum. Genet. 90 (2012) 369–377, https://doi.org/10.1016/j.ajhg.2011.12.023.

[26] A. Jacob, J. Pasquier, R. Carapito, F. Auradé, A. Molitor, P. Froguel, K. Fakhro, N. Halabi, G. Viot, S. Bahram, A. Rafii, A de novo synonymous variant in EFTUD2 disrupts normal splicing and causes mandibulofacial dysostosis with microcephaly: case report, BMC Med. Genet. 21 (2020) 182, https://doi.org/10.1186/s12881-020-01121-y.
[27] L. Milian, M. Mata, J. Alcacer, M. Oliver, M. Sancho-Tello, J.J.M. de Llano, C. Camps, J. Galbis, J. Carretero, C. Carda, Cannabinoid receptor expression in non-small cell lung cancer. Effectiveness of tetrahydrocannabinol and cannabidiol inhibiting cell proliferation and epithelial-mesenchymal transition in vitro, PLoS One 15 (2020) e0228909, https://doi.org/10.1371/journal.pone.0228909.
[28] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70, https://doi.org/10.1016/s0092-8674(00)81683-9.
[29] S. Oltean, D.O. Bates, Hallmarks of alternative splicing in cancer, Oncogene 33 (2014) 5311–5318, https://doi.org/10.1038/onc.2013.533.
[30] Z. Lv, Z. Wang, L. Luo, Y. Chen, G. Han, R. Wang, H. Xiao, X. Li, C. Hou, J. Feng, B. Shen, Y. Wang, H. Peng, R. Guo, Y. Li, G. Chen, Spliceosome protein Eftud2 promotes colitis-associated tumorigenesis by modulating inflammatory response of macrophage, Mucosal Immunol. 12 (2019) 1164–1173. https://doi:10.1038/s41385-019-0184-y.
[31] S. Beyer, L. Müller, S. Mitter, L. Keilmann, S. Meister, C. Buschmann, F. Kraus, N.E. Topalov, B. Czogalla, F. Trillsch, A. Burges, S. Mahner, E. Schmoeckel, S. Löb, S. Corradini, M. Kessler, U. Jeschke, T. Kolben J, High RIG-I and EFTUD2 expression predicts poor survival in endometrial cancer Cancer, Res Clin Oncol 149 (2023) 4293–4303. https://doi:10.1007/s00432-022-04271-z.
[32] M. Tu, L. He, Y. You, J. Li, N. Yao, C. Qu, W. Huang, L. Xu, R. Luo, J. Hong EFTUD2 maintains the survival of tumor cells and promotes hepatocellular carcinoma progression via the activation of STAT3, Cell Death Dis. 11 (2020) 830. https://doi:10.1038/s41419-020-03040-5.
[33] C. Lv, X.J. Li, L.X. Hao, S. Zhang, Z. Song, X.D. Ji, B. Gong, Over-activation of EFTUD2 correlates with tumor propagation and poor survival outcomes in hepatocellular carcinoma, Clin. Transl. Oncol. 24 (2022) 93–103. https://doi:10.1007/s12094-021-02673-y.