Pharmacological Research 176 (2022) 106059

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Brusatol has therapeutic efficacy in non-small cell lung cancer by targeting Skp1 to inhibit cancer growth and metastasis

Shangping Xing [a,b], Feifei Nong [c], Yaqin Wang [a,b], Da Huang [a,b], Jialiang Qin [a,b], Yu-Fei Chen [a,b], Dan-Hua He [a,b], Pei-En Wu [a,b], Huicai Huang [a,b], Ruoting Zhan [a,b], Hui Xu [a,b], Yong-Qiang Liu [a,b,*]

[a] Research Center of Chinese Herbal Resources Science and Engineering, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[b] Key Laboratory of Chinese Medicinal Resource from Lingnan, Ministry of Education, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[c] Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, Guangzhou 510006, China

ARTICLE INFO

Keywords:
Brusatol
NSCLC
Skp1
Skp2
SCF complex
Metastasis

ABSTRACT

Skp1-Cul1-F-box protein (SCF) ubiquitin E3 ligases play important roles in cancer development and serve as a promising therapeutic target in cancer therapy. Brusatol (Bru), a known Nrf2 inhibitor, holds promise for treating a wide range of tumors; however, the direct targets of Bru and its anticancer mode of action remain unclear. In our study, 793 Bru-binding candidate proteins were identified by using a biotin-brusatol conjugate (Bio-Bru) followed by streptavidin-affinity pull down-based mass spectrometry. We found that Bru can directly bind to Skp1 and disrupt the interactions of Skp1 with the F-box protein Skp2, leading to the inhibition of the Skp2-SCF E3 ligase. Bru inhibited both proliferation and migration via promoting the accumulation of the substrates p27 and E-cadherin; Skp1 overexpression attenuated while Skp1 knockdown enhanced these effects of Bru in non-small cell lung cancer (NSCLC) cells. Moreover, Bru binding to Skp1 also inhibited the β-TRCP-SCF E3 ligase. In both subcutaneous and orthotopic NSCLC xenografts, Bru significantly inhibited the growth and metastasis of NSCLC through targeting SCF complex and upregulating p27 and E-cadherin protein levels. These data demonstrate that Bru is a Skp1-targeting agent that may have therapeutic potentials in lung cancer.

1. Introduction

The ubiquitin proteasome system (UPS) regulates cellular protein homeostasis through the ubiquitination and degradation of various substrate proteins [1]. Proteasome inhibitors have shown great success in the treatment of multiple myeloma and other tumors [2–4]. Ubiquitin E3 ligases (more than 600 members) play key roles in specifically determining the substrates for ubiquitination and further degradation, and SCF complex is one of the major classes of E3 ligase [5]. SCF E3 ligase contains four components, including the variable F-box proteins (e.g., Skp2, β-TRCP and Fbxw7) that recognize specific substrates of the SCF complex, the adaptor protein Skp1 that links F-box proteins to Cullin1 (Cul1), the scaffold protein Cul1 that recruits Rbx1 and E2 conjugating enzyme, and the RING-finger protein Rbx1/ROC1 [6]. Accumulative evidence demonstrates that SCF complex plays pivotal roles in tumorigenesis, cancer development, and tumor metastasis [7–9]. F-box proteins such as Skp2 and β-TRCP, promote tumor growth and metastasis via degrading their substrate proteins, including p27, E-cadherin, and IκB-α [10,11]. Cul1 is also highly expressed in tumor tissue and serves as a therapeutic target [12]. These evidence demonstrates that components of SCF complex can be exploited as promising therapeutic targets in cancer treatments.

SCF E3 ligase-targeted therapy have shown promising therapeutic efficacy in multiple cancers. Skp2-targeting drugs such as compound A, compound #25 and betulinic acid, can dissociate Skp2 from SCF complex and inhibit Skp2-SCF E3 ligase [13–15]; Cul1 neddylation inhibitor

Abbreviations: Bru, Brusatol; Bio-Bru, Biotin-Brusatol; MS, mass spectrometry; NSCLC, non-small cell lung cancer; Skp1, S phase kinase-associated protein 1; Skp2, S phase kinase-associated protein 2; SCF, Skp1-Cul1-F-box protein; EMT, epithelial-mesenchymal transition; SA, Streptavidin; CHX, Cycloheximide; EdU, 5-ethynyl-2'-deoxyuridine; CDDP, cisplatin; CETSA, cellular thermal shift assay; H&E, hematoxylin and eosin; IHC, immunohistochemistry; Ub, ubiquitin; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; CR, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

* Correspondence to: School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou 510006, China.
E-mail address: liuyq@gzucm.edu.cn (Y.-Q. Liu).

https://doi.org/10.1016/j.phrs.2022.106059
Received 10 December 2021; Received in revised form 30 December 2021; Accepted 3 January 2022
Available online 5 January 2022