Alternative treatment method for gastric cancer by using Cymbopogon citratus through structural molecular biology and computer assisted drug design.

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Abstract

Background: The Chinese medicine Cymbopogon citratus is formulated to treat stomach cancer caused by H. pylori. Along with smoking, drinking alcohol and partial gastric surgery for ulcers this is the leading cause of stomach cancer. However, the underlying molecular processes active components of this prescription and possible targets remain unclear. The identification of molecular processes of Cymbopogon citratus is important.

Methods: TCMSP database and Analysis software is used to find all the active ingredients present in Cymbopogon citratus. TCMSP and STITCH databases are used to know about the possible active sites in the structure of Cymbopogon citratus. A PPI network was built with both putative C. citratus targets and known gastric cancer therapeutic targets. Molecular docking is used to validate the ability of Cymbopogon citratus compounds to bind with the possible target molecules for treatment of gastric cancer by screening them.

Findings: The methods to study the mechanisms of Cymbopogon citratus in the treatment of stomach cancer is done by researchers by using network pharmacology methods which includes construction of network, prediction of target, functional enrichment analysis and molecular docking. Molecular docking simulation revealed that the seven putative targets had a high affinity for the corresponding compounds.

Conclusion: Cymbopogon citratus' fundamental pharmacological actions and underlying mechanisms in the treatment of stomach cancer were comprehensively clarified by this innovative and scientific network pharmacology-based investigation.

Keywords: Gastric Cancer, Cymbopogon citratus, Discovery Studio, CB Dock, Network pharmacology, Molecular Docking, Mechanisms.

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Introduction

Gastric cancer contributes significantly to the global incidence of cancer and cancer-related deaths. Gastric cancer is also more common in Latin America, Northern Europe, and the Far East. However, it continues to be the second-leading global cause of cancer mortality (Yunbo Chen et al.,2018). The preferred course of therapy for stomach cancer is surgery. Currently, treatment options for gastric cancer peritoneal metastasis include systemic chemotherapy, cytoreductive surgery, biotherapy, and intraperitoneal chemotherapy, although the curative outcomes are often dismal (Lu Zhao et al.,2014). The attention of academic departments is brought to research and use of traditional Chinese medicine to find effective and secure alternative methods of treatment for stomach cancer. (Zao Hui Li et al.,2021).

One of the traditional Chinese herbs, Cymbopogon citratus (DC) Stapf, sometimes known as lemongrass, was employed in a recent study. (Larissa Vezon et al., 2018) C. citratus is native to southwest Asia, but it now grows naturally all over the world, primarily in tropical and savannah areas (Laboratório OIKOS et al., 2007). It was used to treat stomach cancer in Brazilian folk medicine (Larissa Vezon et al., 2018). Lemongrass may be effective against gastric ulcers, according to a 2012 rodent study published by the source trusted by National Institute of Health (https://www.ncbi.nlm.nih.gov,2:30pm, 14/10/22).

The characteristics of traditional Chinese medicine include its wide variety of elements and goals, a clear curative impact, comparatively few negative side effects, and a reasonable cost. Pharmacists are becoming more and more interested in it. Traditional Chinese medicine (TCM) has unique characteristics, which have hampered research into how it works and prevented it from being widely used and promoted.

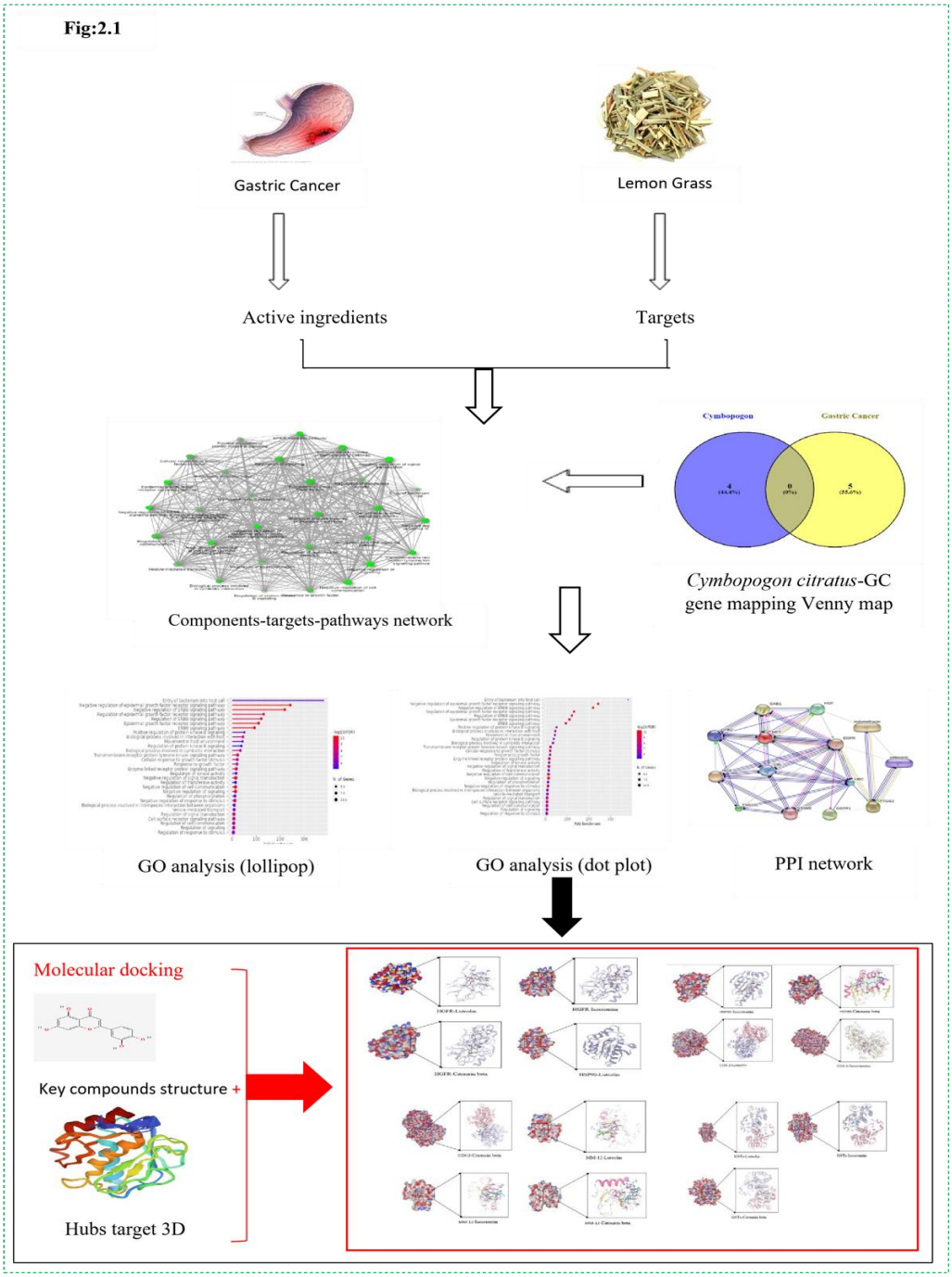
The basic concept of “network pharmacology” which is based on network biology was first presented in the year 2007 by a British researcher named Professor Hopkins. By considering the sites of interaction between genes, proteins, and metabolites in a multidimensional, multipath, and multi-target manner, it aims to both drive drug molecular design and discover the mechanism of therapeutic action. Network pharmacology and molecular docking are the computer simulation techniques which can accurately predict the traditional Chinese Medicine active compounds, possible targets and methods as demonstrated by many studies. These techniques can also help to reduce the cost of developing new medicines and also finding the structure and function of TCM. (Zao Hui Li et al.,2021).

The aim of this article is to clearly describe the exact mechanism of action of Cymbopogon citratus in the treatment of stomach cancer by using different methods like molecular docking and network pharmacology, they can also provide insight knowledge about Cymbopogon citratus effects and mechanisms for the application in healthcare system.

Methodology

Discovery Studio is a graphical interface that is unified and easy to use for protein modelling research and powerful drug design.

CB-Dock is a technique of docking proteins with ligands that customizes the docking box size based on the query ligands before performing molecular docking with Auto Dock Vina and calculates center and size, automatically detects binding sites.



Assessing Cymbopogon citratus active ingredients

All of the chemical components of Cymbopogon citratus that were used in this process were identified by the Conventional Chinese Medicine database and evaluation platform (https://tcmspw.com/tcmsp.php). Conventional Chinese Medicine database is a distinctive program which was designed for Plants based medicines used by Chinese for centuries. It can provide complete information about the compounds present in plants, their site of action in the body and the mode of action of basic constituents present in them. The OB maximum value was

set at 20% and DL screening threshold was set to 0.1 in order to identify the active ingredients in Cymbopogon citratus that were most potent.

Prediction of the possible sites for the constituents selected

STITCH and Conventional Chinese Medicine, two commonly utilized databases, were used to discover Cymbopogon citratus chemical targets. Search Tool for Interacting Chemicals commonly called STITCH is an archive which is used to know about the possible connections and reactions between different enzymes and compounds, and it based on the techniques like text analytics and molecular docking.

Protein-Protein Interaction Network Construction

The STITCH database then processed the targets to generate the PPI Network. STITCH was utilized to study possible active components and describe TCM's molecular mechanism. The STITCH database's entries for all the collected compounds were imported one at a time, the compound target gene names collected and with the species restricted to humans (Jialin Li et al.,2020).

GO Network Construction

All compounds with targets were assigned numbers based on their molecular ID numbers, and these numbers along with the pertinent targets were loaded into Shiny GO v0.741. With the use of this website, we were able to build Gene Id and Enrichment Network Analysis using the intuitive, graphical web software known as Shiny GO (Steven Xijin Ge et al.,2020).

Docking simulation of molecules

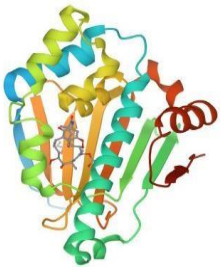
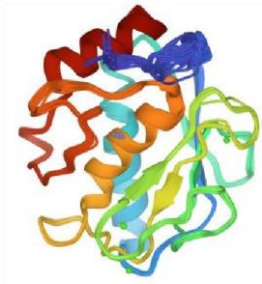
CB-Dock is a method for docking proteins and ligands that automatically discovers binding sites. To forecast the binding affinity between two molecules, molecular docking technique is used which gives us information about the remodeling of molecules and interaction of small protein molecules on the large binding sites. We processed ligands and receptors using CBDock, and we utilised it to dock molecules and analyse the results of that docking. Before the process of molecular docking, the energy of both the ligand and receptor molecule must be reduced, add magnetic field and charge, add polar hydrogen atoms, remove water molecules from small acceptor molecules (PDB files). Discovery Studio was used to show the data, and the hydrogen bonds and the locations where they were bound were examined. The value of docking energy can be calculated through mathematical function of the binding capacity of receptor with ligand. During this in-silico work, the binding capacity of chemical with its possible binding site was calculated by using the free binding energy of both. There is inverse relationship between the free binding energy and higher affinity of the molecules for successful docking. (Jialin Li et al.,2020).

Result:

Essential compounds and results of molecular docking for finding primary target

The molecular docking of five-degree targets of the PPI network with their corresponding active compounds was done. The data of the proteins used for docking like HGFR, HSP90, MM-12, GSTs and COX-2 was retrieved from the database called Protein Data Bank. The docking of 3D structure with active compound is done by using CB dock software. The docking of six prominent sites present in protein-protein interaction network with their relevant small sized molecules of drug ligands is done by using CB dock and discovery studio software as shown in table no. 3. When the binding energy is low, the ability of ligand to bind with protein increases. Hydrogen bonding and - stacking were the primary modes of interaction. This finding suggested that their combination could be beneficial in the treatment of gastric cancer with Cymbopogon citratus.

Table: 3.1

No	Proteins	PDBID	Protein Structure	Test Compound	Affinity (kcal\mol)
1	HGFR	2ASU			
				Luteolin	-8.6
				Citraurin beta	-8
				Isoorientin	-8.2
2	HSP90	4XDM			
				Luteolin	-7.9
				Citraurin beta	-7.4
				Isoorientin	-8.2
3	MMP-12	2POJ			
				Luteolin	-9.9
				Citraurin beta	-10.1
				Isoorientin	-9.6

4	COX-2	1CVU	
Luteolin	-9.5		
Citraurin beta	-9.7		
Isoorientin	-11		
5	GSTs	10GS	
Luteolin	-7.2		
Citraurin beta	-8.6		
Isoorientin	-8.2		

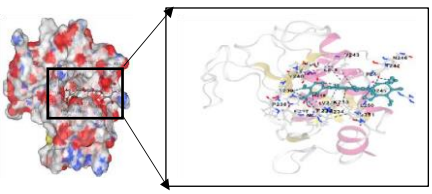
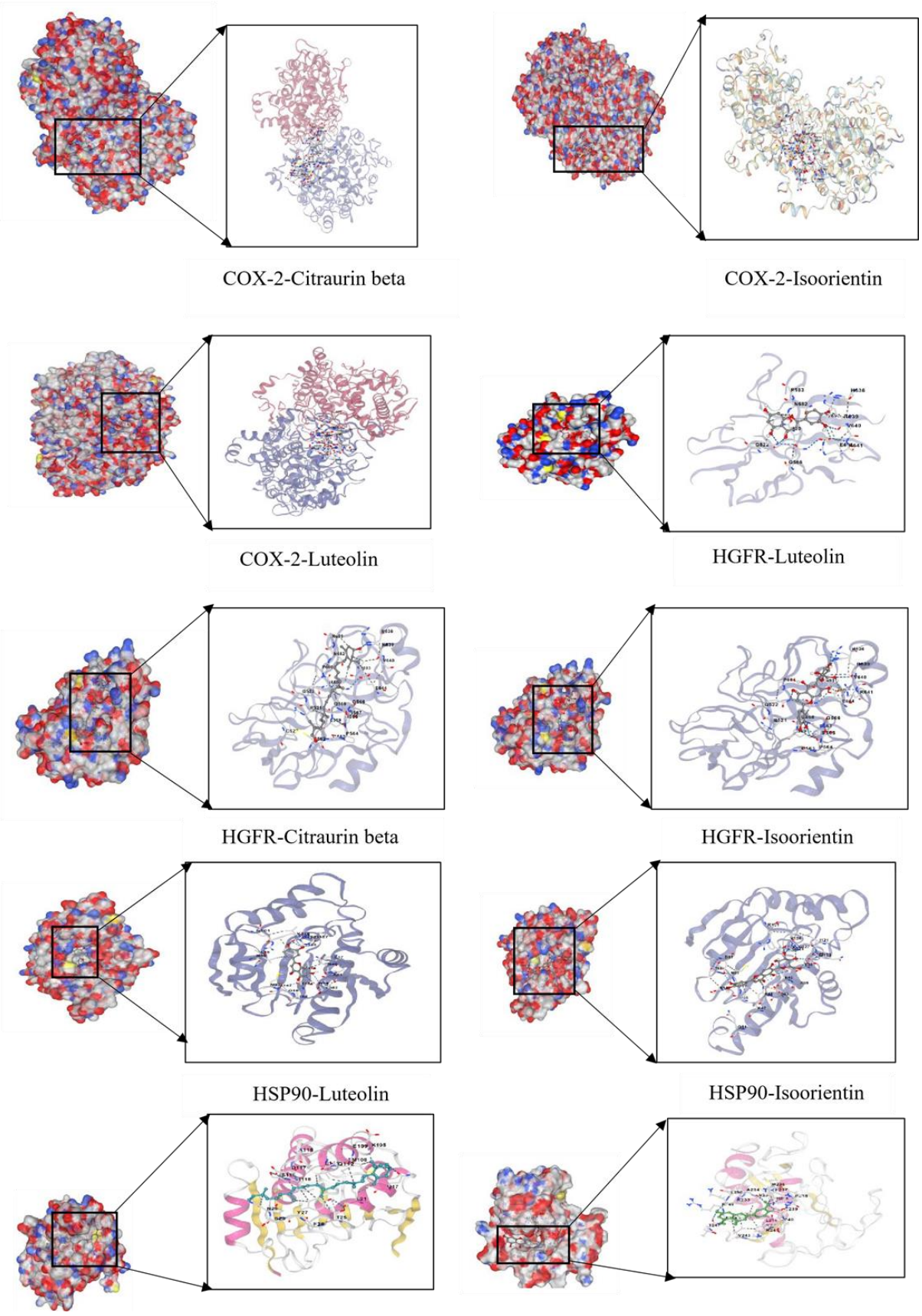


Fig:3.1


COX-2-Citraurin beta
COX-2-Isoorientin
COX-2-Luteolin
HGFR-Luteolin
HGFR-Citraurin beta
HGFR-Isoorientin
HSP90-Luteolin
HSP90-Isoorientin

Discussion

Gastric cancer can be a serious threat to the world's population be being among the most common cancers nowadays and also it has poor prognosis and high death rate. TCM works against cancer by inducing apoptosis, inhibiting proliferation, suppressing metastasis, reversing multidrug resistance, and regulating immunological function. TCM can also improve patients' quality of life. Lemongrass may help prevent gastrointestinal ulcers and boost immunity, according to a 2012 the National Institutes of Health Trusted Source published a rodent study.

(https://www.ncbi.nlm.nih.gov, 2:30pm, 14/10/22).

The action mechanism of gastric cancer from the standpoint of the primary active ingredients of lemon grass used in this study were obtained via TCMSP. Network pharmacology investigated the mechanism via which Cymbopogon citratus inhibits gastric cancer. When the active ingredients of Cymbopogon citratus overlap with illnesses, four composite targets of the herb, including stomach cancer, are the same as intersecting targets in the Venn diagram. The findings indicated that immunological control could account for 40% of the effectiveness of Cymbopogon citratus treatment for stomach cancer (Zao Hui Li et al.,2021).

According to the network pharmacology study, Cymbopogon citratus active components are more likely to affect HSP90, Macrophage Metalloelastase, Prostaglandin G/H synthase 2, Glutathione S-transferase P, and Hepatocyte Growth Factor Receptor in the immunological regulation of Gastric cancer.Expression of human macrophage metalloelastase (HME) mRNA and protein plays role in evaluation of their role in gastric cancer development, gastric cancer cell lines and correlation with patient prognosis. (H Zhang et al., 2007) Cyclooxygenase also known as Prostaglandin endoperoxide synthase, expression was upregulated in gastric cancer and its molecular mechanisms are being studied. The over expression of cyclooxygenase-2 in gastric cancer can be due to Nuclear factor kappa B activation H. pylori infection, and tumor suppressor gene mutations. (Jiancheng et al., 2013) Molecular chaperone heat shock protein 90 (Hsp90) has attracted attention as a promising target for anticancer drugs because it is important for maintaining the stability, integrity, conformation, and function of major carcinogenic proteins. Hsp90 is often upregulated in gastric cancer (MOSER et al., 2009). Glutathione S transferase placental morphology (GST) in cancerous tissues of the colon and esophagus was determined by a single radial immunodiffusion or activity inhibition test (K Satoet al., 1989). Cell proliferation and infiltration is caused by Hepatocyte growth factor (HGF) which in turn causes malignant cancer e.g.; Gastric cancer (Ae Koh., 2020).

The cure of stomach cancer essentially lies in the network of component-target-pathway which includes four functional compounds and five possible sites of action, found by this in-silico study. The results of molecular docking revealed the parts having effective free binding energy against important possible targets. The results obtained by this in-silico study revealed the reliability of

interactions between active compounds and the possible targets of gastric cancer for finding the alternative treatment option of stomach cancer. The strongest affinity is between prostaglandin and isoorientin. Additionally, ISO inhibited the growth of the majority of bacteria in the gut microbiota as well as the pathogenic genera of Helicobacter that can induce inflammation (Li Yuan et al., 2018). However, its mode of action is still unknown. On the basis of this discovery, more research will be done in the future.

The primary components of lemon grass have features that are peculiar to multi-component and multi-target processes in preventing stomach cancer, according to this paper, which integrates data from numerous databases and employs molecular docking for preliminary verification. The vast data already available is the foundation on which Network Pharmacology bases its predictions on the mechanism by which Lemon Grass inhibits gastric cancer. For the creation and use of Cymbopogon citratus and clinical research, it is later necessary to empirically verify the putative therapeutic components, target points, and mechanism of action indicated above.

Conclusion

We utilized a network pharmacology approach to establish a network, combine module analysis, target prediction, enrichment analysis and molecular docking and predict, clarify, and confirm likely mechanisms of Cymbopogon citratus on gastric cancer. In summary, our findings suggest that the active ccompounds luteolin and Isoorientin in the Cymbopogon citratus herb may have a therapeutic role in the treatment of stomach cancer. They mostly affect HSP90, Macrophage Metalloelastase, Prostaglandin G/H synthase 2, Glutathione S-transferase P, and Hepatocyte Growth Factor Receptor. They might be involved with the control of the βcatenin signalling pathway. We have a solid understanding of the traditional Chinese medicine's multiple targets, multiple compounds, and several pathways as a result of our research. Our studies have produced proof, and we now have a clear understanding of how traditional Chinese medicine works synergistically to treat gastric cancer using multiple targets, multiple compounds, and several pathways. However, in vivo research is needed to confirm the mechanism of the Cymbopogon citratus herb's anti-gastric cancer activity. **Funding**

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