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Targeting colon cancer via antimicrobial RT2 peptide: a system biology study

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ABSTRACT

Aim: This study aims to investigate the anticancer molecular mechanism of RT2 through protein-protein interaction (PPI) network analysis. For this aim, a bioinformatics evaluation of the proteome profile of colon cancer is carried out.
Background: Antimicrobial peptides such as RT2 showed anticancer properties against various tumors. The molecular mechanism of the anticancer effect of RT2 is a challenging subject.
Methods: By applying Cytoscape V.3.9.1 and integrated apps, the profile of the interaction network and related centrality is analyzed. An enrichment analysis of hub bottlenecks was also performed, and highlighted biological processes were visualized and determined.
Results: Several 207 differentially expressed proteins were retrieved by PPI network analysis, and 10 hub bottlenecks were introduced. Among these differentially expressed proteins (DEPs), only AKT1 is from the queried DEPs. Key biological processes contributing to RT2 targeting mechanism include "Regulation of fibroblast proliferation", "Positive regulation of cyclin-dependent protein serine/threonine kinase activity", "positive regulation of miRNA transcription", and "fungiform papilla formation".
Conclusion: In conclusion, central proteins Tp53, MYC, EGFR, AKT1, HDAC1, and SRC can be introduced as a targeted biomarker panel of bioactive peptide treatments. However, extensive research is required to establish this claim before clinical application.

Keywords: Bioactive peptides, RT2 peptide, Colon cancer, Network analysis, Treatment.

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Introduction

Antimicrobial peptides (AMPs) such as RT2 have been reported effective in cancer (1). These types of peptides are cationic active molecules against both gram-positive and gram-negative bacteria (2, 3). These natural peptides can be obtained from different sources, including animals, vegetables, and microorganisms (4). These bioactive peptides are generally less toxic to healthy tissues (4). On the other hand, the requirement for better treatment options for cancer promotes researchers to explore less invasive replacements (1). Colorectal cancer (CRC), as one of the important malignancies, remains a health issue around the world, especially for male populations. This is due to the elevated resistance of cancer cells to the common treatment options and their adverse effects on healthy cells (1). In this sense, alternative treatment options are seeking by researchers for better and less invasive therapeutic approaches such as application of bioactive peptides (5). Proteomics as a high-throughput study could assist in detecting biomarkers with a fundamental contribution to the anticancer treatment mechanism of bioactive peptides. Further evaluation of these biomarkers could be handled as protein-protein interaction network analysis (6). In this respect, more potential biomarkers and new ones could be introduced by applying diverse algorithms of interacting network

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software. Central proteins of a PPI network are vital for the network stability and effective function. Disruption of these central proteins' regulation largely affects the function of many other related proteins in the network (7). Therefore, malfunction of related biological processes of these proteins could alter the whole biological system. These proteins could be dysregulated due to different conditions, such as disease state (8). Uncovering the regulated proteins in the presence of applied treatment agents could accelerate the recognition of that specific treatment mechanism of regulation (9). In the main study, a number of 212 regulated proteins in the presence of RT2 in colon cancer (Caco-2) are reported that most of them are down-regulated (10). This study introduced some mechanisms by which RT2 eliminates cancer development that is through metabolism changes, apoptosis, and reduction of cancer cells proliferation. Complementary study by protein-protein interaction network analysis could indicate which protein markers are more efficient in these processes. In addition, perhaps more details regarding contributing biomarkers and their related processes could be identified. What is more, other central proteins in the PPI network could be detected as possible contributing markers in the antitumor mechanism of RT2 after extensive validating studies.

Methods

By applying label-free proteomics, different concentrations of RT2, a cationic peptide (0, 15, 30, 60, and 120 μM), were assessed on colon cancer (Caco-2). The Caco-2 cells are selected as the typical cancer cells. This active molecule is derived from Crocodylus siamensis leukocytes. Based on the MTT assay conclusion, the four first doses were chosen for further proteomics analysis considering the toxicity level of cancerous and healthy cells (11). The data from proteomics analysis was then applied for protein-protein interaction network exploration by Cytoscape V.3.9.1. (https://cytoscape.org/) (12). The software used the STRING database (http://string-db.org/) to retrieve the PPI network of the RT2 effectiveness on colon cancer profile. STRING App. Identifies interaction relationships from four sources, including protein query, PubMed query, Disease query, and Stitch (13). The protein query was handled with the aid of STRING App. and two networks of DEPs were obtained.

The first network is confirmed without any additional proteins, while the second network consists of DEPs and several 50 first neighbors. The statistical analysis used for the PPI network query was set to score confidence of 0.5 to gain more confident results. The second network was used for centrality analysis in terms of the detection of Degree (K) and betweenness centrality (BC) parameters via "NetworkAnalyzer," a well-established application in Cytoscape (14). The nodes with the highest K are hubs, and nodes with the biggest amount of BC are the bottlenecks. In addition, nodes with both features are hub bottlenecks. NetworkAnalyzer was applied to detect the central elements of the PPI network (9). After identifying hub-bottlenecks, annotating these elements was important to examine. ClueGO 2.5.9+CluePedia1.5.9 plug-ins aid the gene ontology of hub bottlenecks concerning biological processes (15). Term grouping was set to 0.5 for a more confident outcome, and the number of proteins and percentage per term was chosen as 2 and 3, respectively. Other statistical criteria were as default, including the p-value of grouping and the correction test were 0.05 and Bonferroni step-down, respectively. The kappa score cut-off for activation, expression, and inhibition determination was set to 0.5.

Results

Several 207 nodes with 139 links were identified by STRING through Cytoscape. By adding 50 neighbor proteins, the obtained network consists of 257 nodes and 1593 links (data are not shown). Some of the individual nodes in the first network joined the whole interaction network in the second query. Based on hub and bottleneck calculation, the interacting network was then designated for the centrality analysis and key nodes. In this analysis, 10% of top nodes with respect to high degree and betweenness values were chosen, and common nodes (hub-bottlenecks) were depicted in Table 1.

In Table 1, ten top nodes were determined as important keys of the PPI network. AKT1 is the only queried node (DEP) in this list. These proteins are hub bottlenecks that show high degree and betweenness centrality values. TP53 is the most highlighted hub bottleneck with a degree of 71 and betweenness centrality of 0.07. TP53 and ESR1 are the top bottlenecks with a BC value of 0.07. AKT1 shows the

high degree value, but it is not a significant bottleneck as its betweenness centrality value is 0.02.

Table 1. The hub-bottleneck nodes ranked based on greatest to smallest values in degree. The node with asterisk is from queried proteins.

Row	Display name	K	BC
1	TP53	71	0.07
2	MYC	63	0.03
3	GAPDH	63	0.03
4	CTNNB1	61	0.04
5	HSP90AA1	60	0.04
6	EGFR	56	0.03
7	ESR1	56	0.07
8	AKT1*	56	0.02
9	HDAC1	54	0.05
10	SRC	53	0.03

For further analysis, the biological terms related to the hub bottlenecks were considered to be evaluated, as indicated in Figure 1. The Terms were extracted from all default sources of the CluGO plug-in, such as KEGG.

Among 10 queried terms in ClueGO, 9 were detected considering designated statistical criteria. The most highlighted groups of terms include “Regulation of fibroblast proliferation”, “Positive regulation of cyclin-dependent protein serine/threonine kinase activity”, “positive regulation of miRNA transcription”, and “fungiform papilla formation”.

Table 2 presents the relationship between hub bottlenecks and bioactive peptides based on a literature review. The finding indicates that Tp53, MYC, EGFR, AKT1, HDAC1, and SRC among the 10 introduced critical DEPs are related to the bioactive peptides.

Discussion

Due to current treatment option limitations regarding their side effects and lack of personalization, natural sources have received great attention recently (9). Many different types of cancers are reported to be studied by treating antimicrobial peptides (AMPs) (31). The effectiveness of these bioactive molecules has been demonstrated by molecular studies such as a powerful tool known as proteomics (32, 33). One of these peptides is RT2; its anticancer properties on colon, cervical, brain, and melanoma are well-established according to the previous findings (34-36).

Based on the main study, cell viability was reduced as the dosage was increased according to the MTT assay. The cell viability was decreased from 100% to 8.55% as the doses were increased. This showed that the anticancer effect of RT2 is dose-dependent and should be considered in molecular studies. Our study considered the protein expression profile in the presence of RT2 dosage significantly changes protein expression for PPI analysis. The analysis showed that the network is scale-free, and some nodes are essential to the network's stability and strength. These nodes are

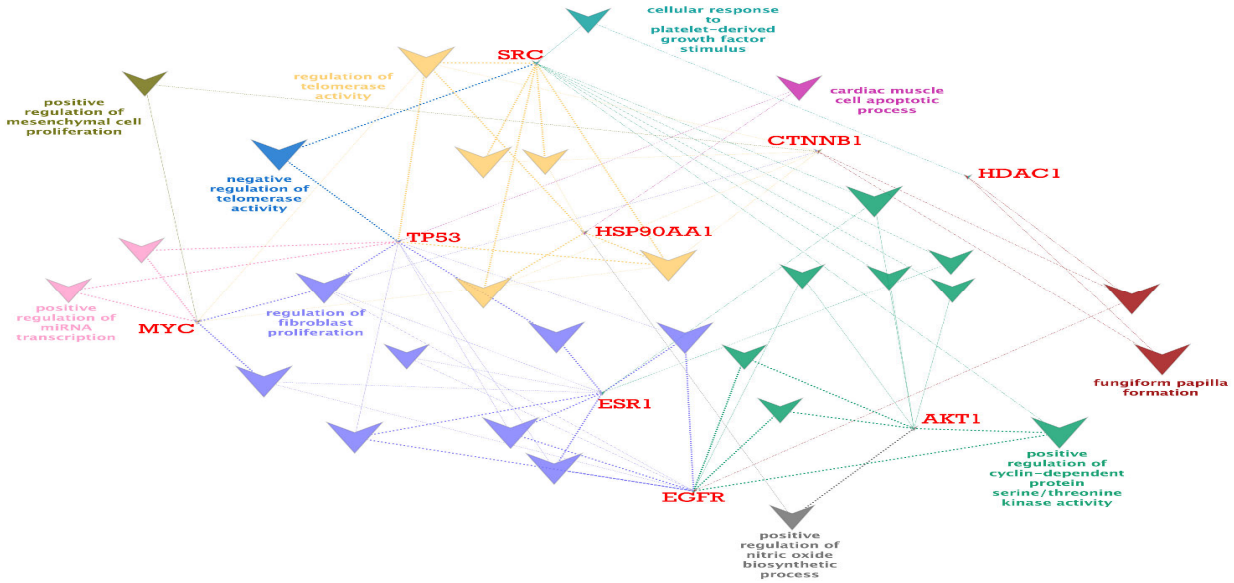

Figure 1. Biological processes related to top proteins in PPI network analyzed by ClueGO+CluePedia. The colored terms refer to the related groups of biological terms. The assessed proteins are presented in red color.

Table 2. Target hub-bottlenecks with previous reports on cancer therapy with bioactive peptides

Protein Name	Target Cancer	Literature References	Bioactive Peptide-Based Therapeutics
Tp53	Breast cancer, Colon cancer, Leukemia	(16-19)	RT2, CPe-III, P2, Gloriosa superba Peptides, Lunasin
MYC	Breast cancer, Neuroblastoma	(20, 21)	FPPa, Peptide FNIII14, (ACBP)s from goal spleen
EGFR	Colon cancer	(22-24)	TFF3, AMPs rich in tryptophan, proline, and arginine
AKT1	Colon cancer, Breast cancer	(25-27)	RT2, Lunasin, SCIP
HDAC1	Colon cancer, Gastric cancer, Breast cancer	(28, 29)	Lunasin, FHPFPR, NWFPLPR, and HYNPYFPG
SRC	Breast cancer	(27)	Lunasin

considered the central hub bottlenecks in the PPI map. Several ten proteins were recognized as hub bottlenecks; AKT1 is from DEPs. TP53 is the most highlighted central protein, and SRC is the value.

To better understand the role of the hub bottlenecks in the anticancer mechanism of RT2, a literature review of the top ones previously reported as bioactive peptide targets was conducted. TP53 tumor suppressor protein (TP53) is this study's first highlighted hub- bottleneck. Its extensive associations in cancer trigger and development are well-defined (37). Moreover, this protein level increment has been reported in treating RT2 in cancer therapy (38). Therefore, our finding indicates that not only is TP53 a central protein in the PPI network, but the presence of RT2 treatment also influences it. This shows that TP53 is a valuable therapy target by peptides such as RT2. This protein also targets similar bioactive peptides such as CPe-III, P2, Lunasin, and Gloriosa superba Peptides from natural sources (20). These peptides induce up-regulation in TP53, which is responsible for apoptosis. MYC, the next hub bottleneck, is a key proto-oncogene in cancer triggers and developments. (ACBP)s from goal spleen FPPa and Peptide FNIII14 are the popular bioactive peptides affecting MYC expression in a way that can cause the reduction in this protein expressions (4, 21, 22).

Histone deacetylases (HDAC) is the fifth reviewed protein. Some bioactive peptides from plant sources inhibit this protein's activity in different cancers.

Quinoa peptides, including FHPFPR, NWFPLPR, and HYNPYFPG, were effective against the HDAC gene (29) reported by our study as one of the key hub bottlenecks.

HDAC inhibition could trigger apoptosis processes in the anticancer activity of bioactive peptides (29).

The processes linked to these proteins could be novel in the RT2 mechanism of cancer-fighting. However, more studies are required to indicate the role of hub bottlenecks in the antitumor mechanisms of RT2. The literature review application in this study also indicated that Lunasin is one of the effective bioactive peptides in food sources.

Conclusion

In conclusion, RT2, as an antibacterial peptide, promotes biological processes that could be vital in cancer-fighting. In addition, some central proteins contribute to these biological processes, and their role in the RT2 anticancer mechanism could be important. Validation studies are required to identify this connection and possibly more effective cancer target therapy.

This PPI study suggests that antimicrobial peptides such as RT2, targeting key biomarkers in cancer, could be introduced as promising cancer treatment approaches. However, extensive research in this respect is required to validate the claim.

Conflict of interests

The authors declare no conflict of interest.

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