Systemic lupus erythematosus: PKCA is an inhibition pathway for mTOR by the active ingredient of green tea

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Abstract. This study aims to evaluate the interaction of green tea active compounds with proteins related to mTOR signals. The in silico study uses SEA protein target software, DB strings, and AUTODOCK PYRX 9.5. There are twenty target proteins that can interact with the active compounds of green tea. Of the twenty proteins, only six proteins are connected to the mTOR pathway. Of the six proteins, one that is a regulator of mTOR inhibitors is PKCA. Epigallocatechin has the strongest interaction with PKCA 4ARA (-8 kcal/mol). Cianidanol has the strongest interaction with PKCA 3lW4 (-9.3 kcal/mol). To analyze the involvement of autophagy, a docking between ULK1 and AMPK was conducted, and there was an interaction between ULK1 and AMPK (bond energy of −1446.11 kcal). For the interaction between mTOR and ULK1, the bond energy is −624.5 kcal. For active green tea compounds, the bonding energy is more positive than the mTOR bond with ULK1. It was concluded that the green tea active ingredient as an inhibitor control against mTOR through PKCA and ULK1-AMPK (autophagy pathway).

Keywords: autophagy, green tea, SLE, mTOR, inhibitor

1. Introduction
Systemic lupus erythematosus is a multisystem autoimmune-inflammatory disease, characterized by T and B cell dysfunction and antinuclear antibody production. In 100,000 population, 20-150 people with SLE are found [1]. Factors that cause SLE come from genetic factors and environmental exposure [2]. The spectrum of clinical manifestations is very broad, in the form of mild skin lesions to serious organ damage. Although the etiology of this disease is still unclear, several mechanisms are thought to be involved in the pathogenesis of this disease, including the Toll-like receptor pathway, apoptotic defects, and abnormal activation of the interferon pathway [3].

The mammalian target of rapamycin (mTOR) is a protein complex as a member of phosphoinositide 3-kinase (PI3K)-related protein kinase family [4]. mTOR integrates signal growth factor and nutrition to support cell survival, growth and proliferation [5, 6]. The mTOR mRNA levels in SLE patients
were not significantly different compared to controls [7]. MTOR activation contributes to the pathogenesis of SLE. The contribution of mTOR activation is found in various SLE cell cases, including T cells, B cells, mesenchymal stem cells, and hepatocytes [8].

Autophagy is a catabolic process mediated by lysosomes to eliminate long-lived misfolded proteins and damaged organelles. Autophagy plays a role in maintaining cellular homeostasis and survival under stressful conditions [9, 10]. This process takes place in basal conditions, but increases in stress response and disease [11]. Autophagy dysregulation was found in SLE, in the form of an increase in basal autophagy levels in T cells that have the potential to increase autophagy cell death. In addition, the lack of autophagy response due to induction by certain stimuli causes a decrease in cell survival and an increase in apoptosis which triggers an increase in the release of autoantigen [12].

The chemical composition of green tea varies according to weather, season, horticultural practice, and leaf position. The main composition of the active ingredient is polyphenols. The main polyphenols in green tea are flavonoids. The four main green tea flavonoids include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Epigallocatechin gallate is the most significant component [13]. The use of green tea in autoimmune diseases is still very little. EGCG can suppress RNA and autoantigen proteins in normal cells in the salivary glands and skin [14]. In addition, EGCG is also a competitive inhibitor of active ATP against mTOR [15]. To the knowledge of the researchers, there are still rare studies that analyze the mechanism of green tea active compounds against the mTOR pathway in the autophagy target. Therefore this study aims to investigate the mechanism of green tea active compounds against the mTOR pathway for autophagy as an effort to inhibit the development of SLE.

2. Material and Methods

2.1. Protein target prediction analysis
The green tea compound content was obtained from Dr. Duke's database (https://phytochem.nal.usda.gov/phytochem/search/list). One of the main compounds of green tea is known as EGCG, so further exploration is needed. To find out the target of the EGCG compound protein from green tea, analysis was carried out using the SEA Target webserver (http://sea.bkslab.org/). The parameter used in SEA Target is the Max TC value that reflects the accuracy of predictions if it approaches a score of 1.

2.2. Network analysis
To determine the interaction of the relationship between the target of EGCG protein and mTOR pathway, a network analysis was performed using STRING webserver (https://string-db.org). The STRING webserver is able to show the interaction of several proteins taken from several study pathways namely Biocarta, BioCyc, GO, KEGG, and Reactome (Szklarczyk et al. 2015). Some proteins obtained from the target SEA and have a value of less than 1 are considered potential and play a role in finding new pathways, such as PRKCA, PRKCE, PRKCH also included as input at STRING.

2.3. Molecular docking
To determine the relationship between PRKCA and bioactive compounds in green tea, molecular docking analysis was performed using PyRxD v0.9.5. 3D samples of PRKCA proteins (ID 4ARA and 31W4) were obtained from the GDP database (https://www.rcsb.org), while the green tea bioactive compounds were theoflavin (ID 1147), epigallocatechin (ID 65064), and cianidanol (ID 73160) obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). When docking, validation using two 3D structures with different controls to show that the potential possessed by bioactive Green Tea has a target MTOR protein through PRKCA. Furthermore, the docking protein is visualized using PyMol software.
3. Results
First, we analyze the content of green tea compounds with Dr. Duke Phytochemical. The analysis shows that green tea contains EGCG. Then the target protein analysis of EGCG was carried out with the SEA Target Protein badger device. From the target analysis the accuracy values are obtained as in Table 1.

| Target Name | Description | Max TC |
|-------------|-------------|--------|
| FUTP4       | Alpha-(1,3)-fucosyltransferase 4 | 1      |
| PGD         | 6-phosphogluconate dehyrogenase, decarboxylating | 1      |
| ST3GAL3     | CMP-N-acetylneuraminate-beta-1,4-galactoside alpha-2,3-sialyltransferase | 1      |
| FUT7        | Alpha-(1,3)-fucosyltransferase 7 | 1      |
| BCL2        | Apoptosis regulator Bcl-2 | 1      |
| MMP14       | Matrix metalloproteinase-14 | 1      |
| MAPT        | Microtubule-associated protein tau | 1      |
| STAT1       | Signal transducer and activator of transcription 1-alpha/beta | 1      |
| DNMT1       | DNA (cytosine-5)-methyltransferase 1 | 1      |
| TERT        | Telomerase reverse transcriptase | 1      |
| MMP2        | 72 kDa type IV collagenase | 1      |
| MET         | Hepatocyte growth factor receptor | 1      |
| DYRK1A      | Dual specificity tyrosine-phosphorylation-regulated kinase 1A | 1      |
| MAPK14      | Mitogen-activated protein kinase 14 | 1      |
| KCNH2       | Potassium voltage-gated channel subfamily H member 2 | 1      |
| PGF         | Placenta growth factor | 0.32   |
| PRKCA       | Camp dependent protein kinase catalytic subunit alpha | 0.31   |
| PRKCH       | Protein kinase c eta type | 0.31   |
| PRKCE       | Protein kinase c epsilon type | 0.31   |
| SERPINE1    | Plasminogen activator inhibitor 1 | 0.48   |

Table 2. Docking With PKCA 4ARA.

| Receptor | Ligand                      | Binding Affinity (kkal/mol) |
|----------|-----------------------------|-----------------------------|
| PKCA 4ARA| Theoflavin 11477            | -7.4                        |
| PKCA 4ARA| Epifalocatechin 65064       | -8                          |
| PKCA 4ARA| Cianidanol 73160            | -7.3                        |
| PKCA 4ARA| Control inhibitor 4ara (1R)-9-{(3S,4S)-1,3-dimethylpiperidin-4-yl}-8-(2-fluorophenyl)-1-methyl-3,5-dihydro[1,2,4]triazino[3,4-c][1,4]benzoxazin-2(1H)-one | -9.8 |

Table 3. Docking With PKCA 3IW4.

| Receptor | Ligand | Binding Affinity (kkal/mol) |
|----------|--------|-----------------------------|
| PKCA 3IW4| Theoflavin 11477    | -7.4                        |
| PKCA 3IW4| Epifalocatechin 65064 | -9.0                        |
PKCA 3IW4 | Cianidanol 73160 | -9.3
PKCA 3IW4 | Control inhibitor 3iw4 | -11.3
-((1H-indol-3-yl)-4-[2-(4-methylpiperazin-1-y1)]quinazolin-4-yl)-1H-pyrrole-2,5-dione

All proteins presented in table 1 are then analyzed by STRING DB to find out their interactions with mTOR. Some proteins that have interactions with mTOR include PRKCA, PRKCE, TERT, BCL2, MAPK14, and PRKCH, as presented in Figure 1.

**Figure 1. Prediction of Interaction of Compounds with Interest Proteins.**

PKCA 4ARA is docking with EGCG and the comparative compounds include theaflavin, cianidanol, and control inhibitor compounds, namely (3S, 4S)-1,3-dimethylpiperidin-4-yl)-8-(2-fluorophenyl)-1-methyl-3,5-dihydro [1,2,4] triazino [3,4] [1,4] benzo-xazin-2 (1H) -one. Bond affinity can be seen in Table 2.

PKCA 3IW4 is docking with EGCG and the comparative compounds include theaflavin, cianidanol, and control inhibitor compounds, namely (1H-indol-3-yl) -4-[2-(4-methylpiperazin-1-y1)]quinazolin-4-yl]-1H-pyrrole-2,5-dione. Bond affinity can be seen in Table 3.

Furthermore, to analyze the involvement of the autophagy pathway, a docking between ULK1 and AMPK was carried out, there was an interaction between ULK1 and AMPK (bond energy of 41,446.11 kcal. For interactions between mTOR and ULK1, bonding energy was −624.5 kcal. (+)-Epigallocatechin has docking energy with mTOR of −242.8 kcal. (+)-catechin has docking energy with mTOR of −245.8 kcal. (-)-Epicatechin gallate has docking energy with mTOR of −279.7 kcal. -
catechin has docking energy with mTOR of −246.9 kcal. (-)-gallocatechin has docking energy with mTOR of −253.1 kcal. Epicatechin it has docking energy with mTOR of −247.3 kcal. Epigallocatechin gallate has docking energy with mTOR of 272.2 kcal (as shown in Table 4).

Table 4. Docking With mTOR.

| Docking                          | Energy Docking |
|----------------------------------|----------------|
| 1. ULK1 with AMPK                | 41.446,11kkal  |
| 2. ULK1 with mTOR                | -624,5 kcal    |
| 3. Epigallocatechin_CID_104      | -242,8 kcal    |
|                                  | 25234 with bmTOR complexes |
4. catechin_CID_9064 with mTOR complexes  
-245.0 kcal/mol

5. Epicatechin gallate_CID_107905 with mTOR complexes  
-279.7 kcal/mol

6. -catechinCID_73160 with mTOR complexes  
-246.9 kcal/mol

7. -galocatechinCID_9882981 with mTOR complexes  
-253.1 kcal/mol
8. EpicatechinCID_72276 with mTOR complexes -247.3 kkal/mol

9. (-)-epigallocatechin gallate (EGCG) with mTOR complexes -272.7 kkal

4. Discussion

mTOR is a master regulator of cellular metabolism and plays an important role in autophagy. When there is energy deficiency, mTOR activity is low, autophagy is upregulated to carry out nutrient recycling. When nutrients and energy are available, mTOR is active and downregulated. In SLE mTOR levels were found to be comparable to controls. This indicates that in SLE the formation of phagolysosomes is obtained but there is a degradation blockade [7]. In this study it was proven that EGCG as the active ingredient of green tea has the ability with proteins in the mTOR pathway, namely PKCA. Regarding PKCA 4ARA it was found that EGCG was easier to interact than theaflavin and cianidinol. Meanwhile, against PKCA 31W4 it was found that EGCG was easier to interact with theaflavin. This indicates that the effect of EGCG as one of the active ingredients of green tea is at least through the PKCA 4ARA pathway. PKC is one of the regulators that is involved in pathway transduction that is related to various cell functions [16]. This study is consistent with previous findings that EGCG can inhibit PKC activity [17, 18].

Against the autophagy pathway, this study proved that the interaction between ULK1 and AMPK has more negative docking energy compared to the docking energy between ULK1 and mTOR. This indicates that in basal conditions there is an easier interaction between ULK1 and AMPK than ULK1 with mTOR. Interestingly, of the various green tea active compounds, the docking energy formed in the interaction with mTOR was more positive than that between mTOR and ULK1. This indicates that there is an easier interaction between mTOR and ULK1 compared to the interaction of green tea active ingredients with mTOR. This study extends previous findings that EGCG is able to modulate and inhibit mTOR [19, 20].

We conclude that the green tea active ingredient which is an inhibitor control against mTOR through PKCA and ULK1-AMPK pathway. Thus, in the pathomechanism of SLE, EGCG can be a candidate in SLE treatment through the PKCA-mTOR pathway and ULK1-AMPK (autophagy).
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