PDE5 inhibitor potentiates the 67 kDa Laminin Receptor-mediated anti-inflammatory and anticancer activity of Polyphenols

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Abstract

Background: 67-kDa laminin receptor is a cell surface receptor for laminin and polyphenols like EGCG. 67LR overexpressed in different cancers. Polyphenols-induced activation of 67LR activates Akt/eNOS/NO/cGMP/PKCδ pathways very well known for apoptotic signalling and inhibits cell growth via PKA/PP2A activation, ultimately triggering cancer cell death. The binding of EGCG to the Human laminin receptor (67LR) upregulates Tollip expression, inhibits TLR4 signaling, and prevents inflammation. Method: Protein-ligand docking was performed considering Human laminin receptor (67LR) precursor (PDB ID - 3BCH) as protein and 18 polyphenols as ligand using Autodock Vina 1.1.2. Result: combining EGCG or other potent polyphenols with PDE5 inhibitors may impart a rationale for the clinical efficiency of this formulation for cancer therapeutics and 67LR is conceivably an absolute novel target for cancer chemotherapy and treatment of inflammation. Discussion: Developing the anticancer formulation of the anticancer drug, 67LR inducer along with inhibitor of PDE5 and SET could exert strong antitumor effects via the 67LR. It will reduce the dose of anticancer drugs and adverse side effects.

Introduction:

The 67-kDa laminin receptor (67LR) is a nonintegrin cell surface receptor for laminin. 67LR trigger the apoptosis through Akt/eNOS/NO/cGMP/PKCδ signaling pathway [1]. Phosphodiesterases (PDEs) catalyze the breakdown of cGMP and regulate its homeostasis in the cells. Phosphodiesterase type 5 (PDE5) is an isoform of PDEs and overexpress in different cancer cell types. The 67LR play a vital role in tumor progression, invasion, metastasis, and drug resistance. The 67LR overexpressed in multiple myeloma (MM), in some prostate, gastric, pancreatic, and breast cancer samples [2]. The PDE5 act as a negative regulatory enzyme for EGCG-induced apoptosis, it is remarkably overexpressed in several types of cancer, which include MM, pancreatic, breast, gastric, and prostate cancers. So, the inhibitors of PDE5 could be used to elevate cGMP levels to induce 67LR-mediated, cancer-specific cell death.

Using a surface plasma resonance assay, Tachibana et al. first observed the binding of Tea polyphenols EGCG to the 67LR (Kd, 0.04 μM)[3]. Our preliminary result shows, that other polyphenols have a higher binding affinity to 67LR than EGCG (Table 1). PDE5 inhibitors are FDA-approved and widely used drugs for chronic use [4]. The coactions between EGCG or other polyphenols and other inhibitors such as [methoxyquinazoline (MQZ), zaprinast, and sildenafil (Viagra) selectively of PDE5 may significantly reduce the IC50 of EGCG and other potent polyphenols [1]. Milk exosome formulation of EGCG or other polyphenols and PDE5 Inhibitors for delivery, may increase the bioavailability of potent polyphenols and reduce their therapeutic dose. As all the study focuses on the 67 LR mediated anticancer activity of EGCG, there is almost no report on binding and 67kDa LR mediated anticancer activity of other polyphenols. Interestingly, our preliminary docking result shows polyphenols like Thearubigin, Theaflavin, Rutin, EGC,
and ECG also have a strong affinity for 67LR. So, these polyphenols may trigger 67 LR-mediated cancer cell death.

67LR is the novel Anticancer Target: Laminin interacts with the C-terminal domain of the 67LR. 67LR expression increases in cervical, breast, lung, colon, ovary, pancreatic, and prostate carcinomas. Inhibition of 67LR impedes invasion of fibrosarcoma cells and tumor metastasis. 67LR plays an important role in tumor progression and targeting 67LR will be a new approach to cancer therapeutics. Fujimura et al identified anticancer activity of EGCG is mediated by its cell surface receptor 67LR and 67LR silencing abrogates the apoptosis induced by EGCG in myeloma cells [5]. There is a good correlation between EGCG susceptibility and 67LR expression patterns in normal cells and myeloma patient samples [6]. 67LR expression increases in metastasis and enhances the responsiveness of MCF-7 cells treated with EGCG with low micromolar concentration [3].

EGCG anticancer activity mediated by 67LR: Green tea polyphenols inhibit the formation of tumors for cancers of the skin, esophagus, small intestine, liver, pancreas, colon, mammary glands, bladder, and prostate animal models [8]. EGCG is the amplest and most studied polyphenol among all green tea polyphenols. Several mechanisms underlying the anticancer activities of EGCG, - a. EGCG, chemically reduces ROS, and ROS accumulation is an indication of cancer [8]. b. EGCG signaling for cancer prevention through 67LR is mediated by eEF1A and MYPT1 [5]. c. The several phenolic groups of EGCG can donate hydrogen bonds to many biomolecules. d. 67LR is the exclusive cell surface receptor of EGCG and a mediator of its in vivo anticancer impact [3, 5]. The EGCG-mediated activation of 67LR in primary MM cells and in MM cellular traces (RPMI 8226, ARH-77, and U266) resulted in multiplied degrees of cGMP. The cGMP activates PKCδ, followed by the activation of acid sphingomyelinase in a unique death pathway and triggers apoptosis [1]. 67LR activates the distinct signaling pathway (Akt/eNOS/NO/cGMP/PKCδ) and triggers apoptosis. The up-regulation of cGMP may be a rate-determining manner of cell death due to 67LR [1]. Among all polyphenols, only EGCG′s 67LR-mediated anticancer activity was extensively studied.

Anticancer activity of EGCG depends on cellular expression of 67LR and PDE5: Interestingly, EGCG shows minimal anticancer activity in U266 multiple myeloma cells (IC50, 23.2 μM). U266 multiple myeloma cells overexpressed phosphodiesterase 5 (PDE5), which is a negative regulator of cGMP. Phosphodiesterases (PDEs), significant negative regulators of cGMP, act by degrading the phosphodiester bond. This impressive synergism was additionally proven in MPC-11 multiple myeloma cells and breast cancer MDA-MB-231 cells in a xenograft model in addition to in vitro in some pancreatic, prostate cancer, and gastric cell lines. Overexpression of both 67LR and PDE5 was noticed in these cell lines [1].

The overexpression of 67LR and PDE5 was also observed in some gastric, prostate, pancreatic, and breast cancer samples. In this cell, the inhibition of PDE5 can raise cGMP ranges to result in 67LR-mediated, cancer-specific cell death [1]. In this case, the combination of EGCG/other selected anticancer polyphenols and PDE5 inhibitors will show robust anticancer activity.

EGCG exclusively binds to the 67LR, which is widely expressed in many cells type, including hepatocyte and adipocyte [9, 10, 11]. EGCG binds with 67LR, and upregulates Tollip expression, through the activation of the src/Akt/eNOS/cGMP signaling pathway [12].

Tollip is an adaptor protein that is directly associated with the cytoplasmic domain of IL-1R, TLR 4, and TLR2 and acts as their inhibitory protein [13, 14]. EGCG is able to rapidly upregulate the expression of Tollip to inhibit TLR4 signaling [15]. The EGCG and cGMP inducers are attractive negative regulators and are capable of attenuating TLR4 signaling [16]. The cGMP is inactivated by Phosphodiesterase 5 (PDE5), which hydrolyzes the 3, 5’ Phosphodiesterase bond [17]. The synergistic effect of PDE5 inhibitor (e.g., sildenafil) may potentiate the EGCG-mediated upregulation of Tollip, consequently down-regulate TLR4 and TLR2, hence inhibiting LPS and Peptidoglycans (PGN) induced TLR4 and TLR 2 signaling respectively [18]. So, the 67 LR and its downstream molecules cGMP may be a potential target for the therapy of NAFLD and NASH. Currently, it is not clear to what extent an intact EGCG and PDE5 inhibitor combination will modulate the initiation and progression of NAFLD in vivo.
The low Bioavailability of EGCG limits its anticancer efficacy: EGCG is a potent antioxidant and has a sturdy binding affinity to different biological molecules. But its therapeutic application is restricted because of its low systemic bioavailability when ingested orally. After, the intake of 2-3 cups of green tea, plasma levels of EGCG peak become 0.2–0.3 μM. A 3.5 μM plasma level of EGCG was reported followed by a higher intake of green tea polyphenols (e.g., EGCG 800mg/day) [19]. The clinical application of EGCG and other polyphenols is limited by their low bioavailability.

Exosome formulation of Polyphenols and PDE5 inhibitor overcomes the limited bioavailability of polyphenols and reduced the required dose of polyphenols: In therapeutics, a synergistic effect of polyphenols (e.g., EGCG) and vardenafil (PDE 5 inhibitor) will additionally kill most 67LR and PDE5 expressing cancer cells [20]. Besides this, combining the synergistic anticancer activity of polyphenols (e.g., EGCG) and vardenafil will reduce the effective anticancer dose of EGCG. Developing the milk exosome formulation of selected polyphenol and PDE5 inhibitors will increase the bioavailability and delivery of polyphenols (e.g., EGCG) and PDE5 inhibitors and therefore increase their anticancer therapeutic efficacy.

Different active compounds including polyphenols can induce 67LR-mediated anticancer activity: The 67LR functions as a death receptor that mediates the apoptosis signaling pathway in cancer cells.

Grape seed extract contains a polyphenol called procyanidins (PC1). Melanoma cell surface 67LR is recognized by PC1, which then binds and inhibits cell development by activating PKA/PP2A and dephosphorylating MRLC at Thr18/Ser19. This anticancer activity of PC1 was abolished by 67LR knockdown. In addition to this, melanoma cell growth is also inhibited by activation of the 67LR/cAMP signaling pathway.

Rhizomacoptidis contain a natural alkaloid, Coptisine. Coptisine promotes apoptosis in liver cancer cells (SMMC7721 and HepG2) by increasing 67LR activity. Silencing of 67LR and treatment with an inhibitor of cGMP-NS2028, reduce apoptosis induced by coptisine significantly and inhibit cell viability. The anticancer activity of Coptisine is mediated by the 67LR/cGMP pathway [21].

Protein-ligand docking was performed considering Human laminin receptor (67LR) precursor (PDB ID - 3BCH) as protein and 18 polyphenols like - epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), genistin, engeletin, kaempferol, rutin, quercetin, resveratrol, chlorogenic, caffeic, gallic, ellagic, cyanidin, apigenin, theaflavin and thearubigin as ligand using Autodock Vina 1.1.2 [22]. Binding site residues were anticipated using CASTp (Computed Atlas of Surface Topography of proteins) webserver tool [23]. We performed rigid molecular docking of laminin protein with all 18 polyphenol ligands using a grid map of 42 x 70 x 50 Å and spacing of 0.38 Å on this predicted active site. A distance-dependent function of the dielectric constant was used for the calculation of the electrostatic map. Lamarckian Genetic Algorithm was employed for docking simulations.

Our preliminary result shows other polyphenols like Thearubigin, Theaflavin, Rutin, EGC, ECG, and EGCG have a high binding affinity toward 67LR (Table 1) (Fig. 1.).

**Table 1:** Shows the Binding affinity top 10 polyphenols among 18 polyphenols with 67LR.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ligand</th>
<th>Binding affinity (Kcal/mol)</th>
<th>Sr. No.</th>
<th>Ligand</th>
<th>Binding affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Thearubigin</td>
<td>-10.6</td>
<td>6.</td>
<td>Epigallocatechin</td>
<td>-6.5</td>
</tr>
<tr>
<td>2.</td>
<td>Theaflavin</td>
<td>-7.6</td>
<td>7.</td>
<td>Genistein</td>
<td>-6.5</td>
</tr>
<tr>
<td>3.</td>
<td>Rutin</td>
<td>-7.3</td>
<td>8.</td>
<td>Quercetin</td>
<td>-6.4</td>
</tr>
<tr>
<td>4.</td>
<td>Epigallocatechin-3- Gallate</td>
<td>-6.8</td>
<td>9.</td>
<td>Engeletin</td>
<td>-6.3</td>
</tr>
<tr>
<td>5.</td>
<td>Epicatechin-3-gallate</td>
<td>-6.7</td>
<td>10.</td>
<td>Epicatechin</td>
<td>-6.3</td>
</tr>
</tbody>
</table>
**Fig 1.** The model structure presentation of ligand (Polyphenols) and 67LR interaction shows the binding of the 67LR with ligands

**Discussion:** So, the clinical success of this formulation for cancer therapies may be explained by combining EGCG or other potent polyphenols with PDE5 inhibitors and 67LR is a potential target for cancer chemotherapeutics target.

The PDE5 and SET are the negative regulators of the 67LR/cAMP signaling pathway and 67LR/PKA/PP2A activation, consequently, inhibiting the 67LR-mediated anticancer activity. SET, a potent inhibitor of PP2A, is overexpressed in malignant melanoma. Both PDE5 inhibitors (e.g., sildenafil) and SET inhibitors (FTY720, OP449, and EMQA). FTY720, OP449, and EMQA target the SET-PP2Ac interface to limit tumor growth and overcome therapeutic resistance in many unique malignant diseases [24].
Fig. 2. Anticancer formulation: A combination of 67LR inducer (Polyphenols e.g., EGCG) and PDE5 Inhibitor and/or SET inhibitor could exert strong anti-cancer effects.

Sugiyama et al. develop the Doxorubicin (DOX)-loaded EGCG-PEG-modified liposome (EPL). EPL exerts strong antitumor effects in mice bearing high 67LR-high-expressing tumors. The ODX in EPL inhibits topoisomerase II and EGCG in EPL triggers apoptosis via a caspase-8 activity which is induced and followed by the binding of EGCG to 67LR [25]. So liposomal formulation 67LR activators (e.g., EGCG) and a low dose of the anticancer drug may have a superior effect against 67LR overexpressing tumor cells without causing adverse side effects.

So, screening the 67 LR-mediated anticancer efficacies of different polyphenols and their synergistic activity with a PDE5 and SET inhibitor may overcome the limited bioavailability of polyphenols by reducing the required dose and reducing toxicity (Fig. 2). PDE5 inhibitors e.g., vardenafil is widely used FDA approved drug for long term use. The novel approach of combining vardenafil with the potent polyphenols to specifically inhibit PDE5 will significantly decrease the dose of polyphenol for cancer cells and overcome the limitation due to low bioavailability. One well-known adverse effect of high-dose EGCG is Hepatotoxicity. Combining EGCG and vardenafil will lower the dose of EGCG and lessen the chances of EGCG side effects. Combining the polyphenols and PDE5 inhibitors will help to improve the clinical implication of polyphenols in spite of their low bioavailability. For example, the difficulty in the clinical use of EGCG is due to its poor bioavailability. Combining the EGCG and PDE5 inhibitors will reduce the dose of EGCG and overcome the above limitation. This therapeutic combination may replace established modalities and develop a new anticancer therapeutic strategy. After tumor resection, this formulation can kill the remaining cancer cells and inhibit a recurrence.

Conclusion: 67LR is the potent anticancer target for the tumor that overexpress the 67LR. Developing the anticancer formulation of the anticancer drug, 67LR inducer as well as an inhibitor of PDE5 and SET could exert strong antitumor effects via the 67LR. It will reduce the dose of anticancer drugs and adverse side effects. Besides this, it will increase bioavailability and targeted delivery of natural 67LR inducers like EGCG.
LIST OF ABBREVIATIONS

PC1, Procyanidins; DOX, Doxorubicin; 67LR, 67-kDa laminin receptor; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; cGMP, cyclic guanosine monophosphate; PDE5, phosphodiesterase type 5; MQZ, methoxyquinazoline; EC, Epicatechin; ECG, epicatechin-3-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate;

CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Dr. Amit Ghosh: study design, manuscript preparation, supervision. Dr. Dillip Kumar Samal, Biswanath Beherab, Dr. Sonali Mohapatra: Manuscript Preparation, Dr. Arnab Nayek, Abhilipsa Patra: Data Accumulation and analysis.

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