Kaempferol Inhibits the DNA Binding of Zeb1 by Targeting Asn13 in its Homeobox Binding Domain: An *in silico* Exploration

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***Abstract*- Epithelial-Mesenchymal Transition (EMT) is a cell biological program that aids in the conversion of epithelial cells to mesenchymal phenotype normally observed during embryonic development but also represents a characteristic feature of metastatic cells. The phenotypic switch observed during EMT is controlled by a group of transcription factors called EMT TF's that include Zeb1, Snail, Slug and Twist. These EMT-TF's constitute significant pharmacological targets for the development of effective therapeutic agents to ablate EMT and thus malignant progression in tumors. In this study we used a structure based drug designing approach to target the homeobox binding domain of Zeb1 by a bioactive phytocompound, Kaempferol. The interaction between Zeb1 and Kaempferol was studied by molecular docking simulations. AutoDock tool was used for molecular docking simulations and it was observed that Kaempferol forms a single non-covalent interaction with homeobox binding domain of Zeb1. The best binding pose based on ΔG was used for further analysis. The Kaempferol-Zeb1 complex was observed to have a binding energy of -5.34 k/cal/mol and includes a conventional hydrogen bond involving Asn13. These data thus point at the inhibition of DNA binding of Zeb1 by Kaempferol as a potential mechanism of limiting EMT but warrants further *in vitro* and *in vivo* investigations.**

***Keywords*- Epithelial-Mesenchymal Transition, Zeb1, Kaempferol, Molecular Docking Simulations.**

I. Introduction

Cancer metastasis is the major cause of cancer related deaths. This process is responsible for approximately 90% of cancer deaths. Epithelial-mesenchymal transition (EMT) is a characteristic feature of the majority of metastatic cells. It is basically a cell biological program, originally described in the context of cellular differentiation and migration during early development (1). This program is also integral to metastasis and has been found to be associated with the progression of various cancer types (2). EMT is accompanied by the decreased expression of epithelial markers like E-cadherin and over-expression of mesenchymal markers like vimentin and N-cadherin (6). Thus cells lose their cell-cell adhesion molecules and normal polarization, thus becoming highly motile (7). At molecular level, EMT is orchestrated by a set of transcription factors, such as Snail, Twist, and Zeb families (4, 5). EMT-TF Zeb1 is a key factor for the formation of precursor lesions, invasion and notably metastasis. Depletion of Zeb1 has been found to inhibit stemness, colonization capacity and plasticity of tumor cells, thus ablating the metastatic capacity (8). Hence Zeb1 can be defined as an attractive target to overcome the metastatic process in cancer cells.

Natural products have always been considered as better chemopreventive agents being safe, efficacious and having lesser side as compared to conventional chemotherapeutics (9-11). Kaempferol is such a natural phytochemical of flavonoid family with various bioactive properties including anti-metastatic activities. Kaempferol has been shown to be involved in the regulation of cell cycle, metastasis, angiogenesis and apoptosis in various cancer cell types (12-14). In particular Kaempferol has been shown to modulate the metastasis of human non-small cell lung cancer cells by inhibiting epithelial-mesenchymal transition (15). However the mechanistic action of kaempferol in overcoming EMT is still undetermined. In the present study, we therefore investigated the effect of Kaempferol on the action of Zeb1 and studied their interaction using molecular docking simulations.

II. Material and Methods

Molecular Docking Analysis

The x-ray crystallographic structure of Zeb1 homeobox binding domain (PDBID: 2E19) was used for molecular docking studies against kaempferol. The binding mode of Kaempferol with Zeb1 homeobox binding domain was analyzed using AutoDock 4.2 (16). The tool was used to dock and calculate the binding energy to confirm the binding mode with the zinc finger of Zeb1. The docking energy was obtained from the summation of van der Walls energy and hydrogen bonding energy, while as binding energy was built up from van der Walls energy and desolvation energy. Lamarckian Genetic Algorithm (GA) was considered for the run and for each ligand 10 GA runs, with 27,000 maximum generations, 0.02 rate of gene mutation and 0.8 as rate of crossover were set. A grid of 60×60×60 points in x, y, and z direction was built centered around zing finger. Top five binding poses were studied.

Molecular visualization

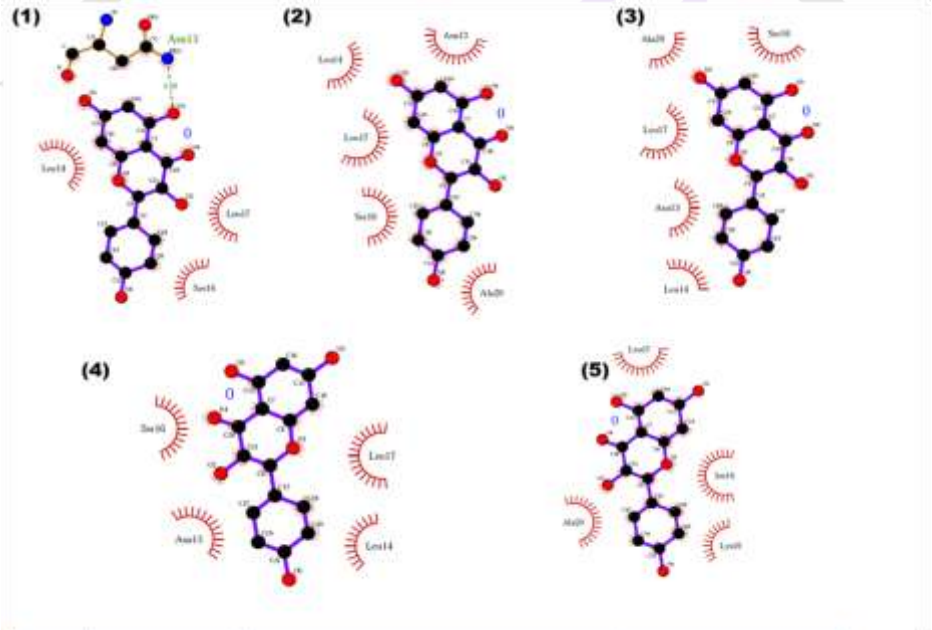
All the visualizations were carried out using Pymol (17), Ligplus (18) and Discovery Studio (19).

III. Results and Discussion

Molecular docking is a tool most widely used in modern day drug design and discovery. It is a computer-assisted program which involves studying the interaction of two or more molecules forming a stable adduct. This approach can be used to model the interaction between a small molecule and a protein at the atomic level, thus allowing to study the behavior of a molecule in the binding site of target protein which in turn aids in elucidating the underlying biochemical process. The process involves predicting the leading binding mode(s) of a ligand with a protein of interest with known three-dimensional structure. The procedure involves two basic steps including prediction of the ligand conformation as well as its position and orientation within these sites and evaluation of the binding affinity. In the context of our study we used three dimensional structure of homeobox binding domain of Zeb1 (Fig. 1) to study its interaction with kaempferol using molecular docking. It is this domain of the Zeb1 that is required for its binding to specific regulatory regions of the target genes such as CDH1, Crumbs3, HUGL2 etc.

Figure 1. Three dimensional structure of homeobox domain of Zeb1 under consideration for drug discovery.

Molecular docking simulations were carried out to analyze the binding mode of kaempferol with the homeobox binding domain present in Zeb1. The top five binding poses are depicted in Fig. 2, and only one pose of kaempferol was observed to form hydrogen bond interaction (Fig. 3). The top binding pose has a Delta G of -5.34 k/cal/mol and forms a hydrogen bond with Asn13 of Zeb1. The other amino acids that are present in its binding pocket include Leu17, Ser16 and Leu14.



Rank	Free Energy (KJ/Mol)	Pocket	Hydrogen Bonds
1	-5.34	ASN13, LEU17, SER16, LEU14	1
2	-4.22	ASN13, LEU17, SER16, LEU14, ALA20	0
3	-2.93	ASN13, LEU17, SER16, LEU14, ALA20	0
4	-2.43	ASN13, LEU17, SER16, LEU14	0
5	-2.22	LEU17, SER16, LYS19, ALA20	0

Figure 2: Two dimensional representation of the top five binding poses of kaempferol with the zinc finger in the homeobox domain in Zeb1.

Kaempferol, 3, 4′, 5, 7-tetrahydroxyflavone, a natural flavonoid, is known for its anti-oxidant, anti-inflammatory, anti-microbial, anti-diabetic, neuroprotective, and cardioprotective activities (20). Furthermore, it has been reported that kaempferol has anti-

proliferation activity and can induce apoptosis in several human cancer cell lines (12-14). Kaempferol has been shown to exhibit anti-invasive activity by reducing the expression of MMP-2 and MMP-9 through down regulation of ERK1/2 and the AP-1 signaling pathways (21). In a recent study, Kaempferol has been shown to inhibit the metastasis of human non-small cell lung cancer cells by suppressing EMT (15). It was demonstrated to cause a dose-dependent increase in expression of E-cadherin, an epithelial marker while concomitantly decreasing the expression of a mesenchymal marker, vimentin but the underlying mechanism of action has not been delineated. In this study, we therefore used kaempferol to target a transcription factor, Zeb1. Zeb1is a zinc finger homeobox transcription factor which plays a pivotal role in EMT. The HTH motif of homeobox transcription factors is characterised by two alpha-helices which are required to establish the contacts with DNA and are joined by a short turn. We used a structure based drug designing approach to target the homeobox binding domain of Zeb1 by Kaempferol and observed that Kaempferol interacts with zeb1 homeobox binding domain through its Asn13 involving a hydrogen bond. Thus it can be argued that the interaction of kaempferol with Zeb1 compromises its ability to regulate the expression of its target genes, one of the ramifications of which may be the ablation of EMT. This may also influence its interaction with other proteins in the interactome (Fig 4).

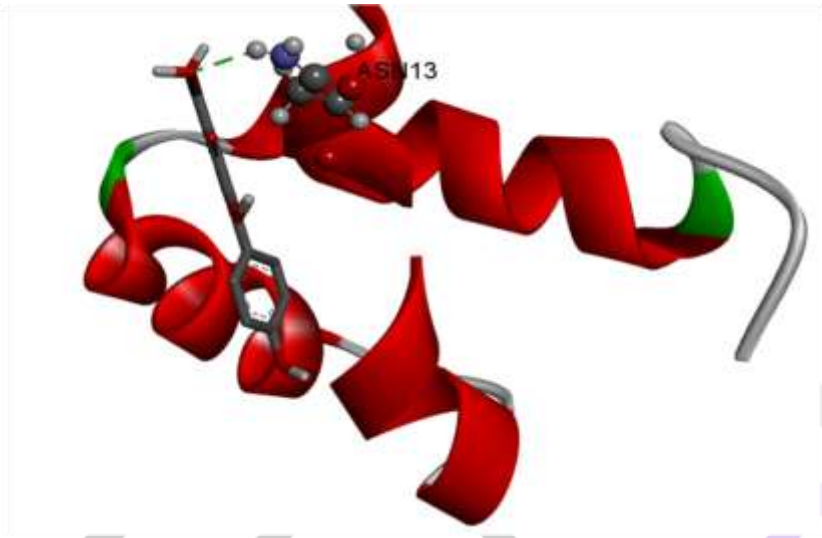


Figure 3. The hydrogen bond interaction at the atomic level

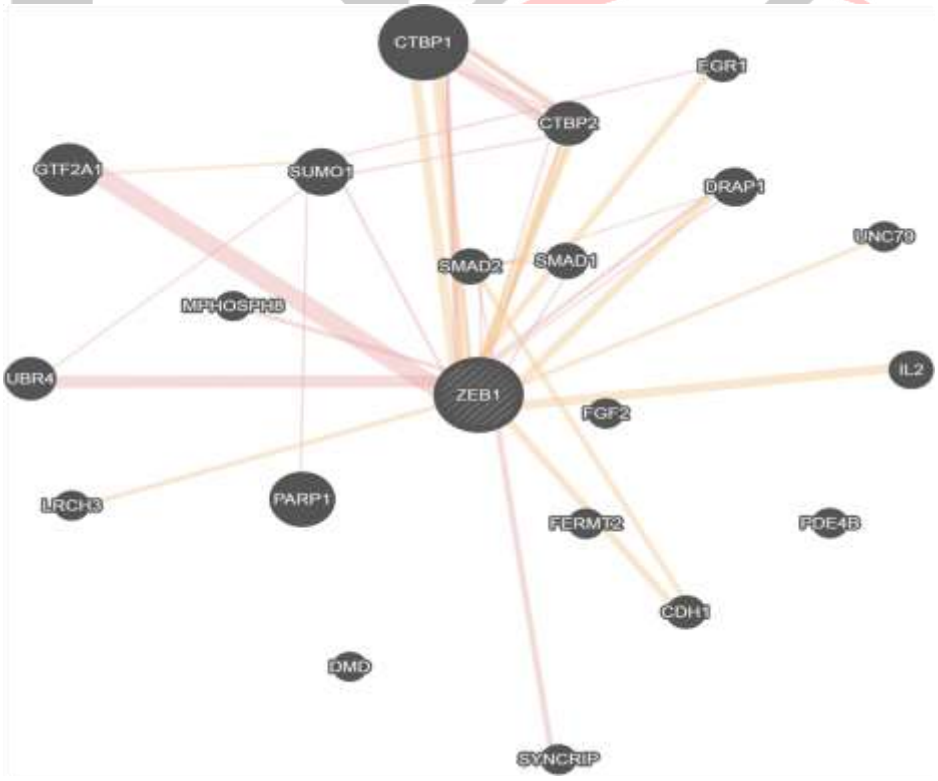


Figure 4. The protein network frame work of Zeb1 obtained from GeneMANIA

IV. Conclusion

The data generated points at the physical inhibition of DNA binding of Zeb1 by Kaempferol as a potential mechanism of limiting EMT but warrants further *in vitro* and *in vivo* investigations.

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