In Silico Analysis of Silver and Selenium Nanoparticles Derived from *Sinapis nigra* (Mustard) Seeds Targeting Ovarian Cancer: A Molecular Docking Study

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Abstract

Ovarian cancer is one of the common types of cancer in women that lead to death and it is largely due to the fact that this type of cancer is diagnosed late and it cannot be treated effectively by chemotherapy. In this study, the focus was on the use of AgNPs and SeNPs as anticancer agents due to their high reactivity and toxicity. Multitude of plant extracts (phytochemicals) has the potential to function as reducing and capping agents during the synthesis of nanoparticles. In the present study, the researchers investigated the in-silico docking reports of the nanoparticles being analyzed to determine how the nanomaterial interacts with the receptors over-expressed in ovarian cancer. Firstly, ligand configurations were produced and energy-optimized in Avogadro, the target molecules were downloaded from the Protein Data Bank and cleared of water and heteroatoms, and rigid docking was done using PatchDock. The complexes resulting from docking were classified depending on PatchDock score and area of interaction, interface size, and atomic contact energy (ACE) of the complexes, and visualized in ChimeraX. All three targets demonstrated the favorable geometric compatibility with both types of nanoparticles. EpCAM displayed the highest score of docking results for AgNPs (2322) and the strongest binding energy of SeNPs (ACE of -407.46 kcal/mol), ceruloplasmin had the highest AgNP score (2378) confirming the fact that it is highly metal-binding agent, while FOLR1 achieved the largest docking area together with the highest docking score of 3194 for SeNP (the area was 358.0 Å²). According to the study, Se-nanoparticles had the highest efficiency in terms of binding energy over all three targets. These results show that EpCAM is the most preferable target and give evidence that it is necessary to study mustard-seed derived Se-nanoparticles in vitro and in vivo as targeted low-toxicity agents in treatment of the ovarian cancer.

Keywords: Ovarian cancer; Silver nanoparticles; Selenium nanoparticles; *Sinapis nigra*; Molecular docking; PatchDock; EPCAM; FOLR1; Ceruloplasmin

1. Introduction

1.1 Ovarian Cancer: Burden and Treatment Gap

Ovarian cancer remains one of the top ten causes of cancer mortality among women in the world. One of the reasons is the suboptimal prognosis due to non-specific symptomatology in the early stages of the disease, which results in patients visiting their doctors at a later stage [1]. In addition, the emergence of chemoresistance after platinum/taxane therapy entails a strong necessity to develop new treatment methods, ensuring tumor specificity, reduced toxicity and evading the developed resistance [2], [3].

1.2 Metal and Metalloid Nanoparticles as Anticancer Agents

Two of the most frequently researched inorganic nanomaterials for application in oncology are silver nanoparticles (AgNPs) and selenium nanoparticles (SeNPs). AgNPs have a high surface area-to-volume ratio and reactive capacity, allowing them to produce reactive oxygen species (ROS), disrupt mitochondrial membrane potential and induce caspase-dependent apoptosis in cancer cells [4], [5].

AgNPs produced using green chemistry have proven capable of triggering ROS generation, caspase-3/9 activation and nuclear condensation in several strains of cancer cells, resulting in their death [6].

In contrast, selenium is an essential trace element that retains excellent antioxidant properties in its nanoparticle form and allows the living cells to get rid of free radicals at the same time exerting pro-apoptotic, cell cycle-violating and redox modifying effects on tumours usually with lower toxicity level compared to silver[7], [8].

1.3 *Sinapis nigra* as a Green Nanoparticle Precursor

The seeds of *Sinapis nigra* (mustard) contain a high concentration of glucosinolates, flavonoids, and phenolic compounds. These phytochemicals have a dual function in green synthesis: they reduce the metal/metalloid ions to nanoparticles, while also capping and stabilizing the nanoparticles [9]. This allows for the final nanomaterial to be more bioactive and biocompatible than the equivalent chemically synthesized counterparts. The green synthesis way is an attractive and cost-effective method and can easily be scaled to any level [10].

1.4 Molecular Targets in Ovarian Cancer

Numerous genes have been described as over-expressed in ovarian tumours, but because the panel identified varies depending on study cohorts, histological type and analytical methods, the genes that are defined differ as well. Out of that pool, EPCAM, FOLR1 and ceruloplasmin were chosen as three of the most robust and reliable over-expressed markers across studies and used as docking

targets for the current research. EPCAM is found to be over-expressed in the majority of cases of epithelial ovarian carcinomas and it is proposed that over-expression of EPCAM in epithelial cancers is in correlation with acquired chemoresistance, through decreased sensitivity to chemotherapeutic agents [11]. FOLR1 (folate receptor alpha) is over-expressed in a large proportion of epithelial ovarian cancers, particularly the serous histotype, and is now clinically validated as a companion-diagnostic and antibody–drug-conjugate target (e.g., mirvetuximab soravtansine) in platinum-resistant disease [12].

The glycoprotein of plasma ceruloplasmin (CP), which binds copper, possesses a promoter that has been evidenced to be strongly activated in cancer cells in comparison to normal cells with CP expression levels increased by about 30 times. Several other genes, including GPX3, CLDN3, CLDN4, and KLK7, have also been found to be deregulated in ovarian tumor tissues whereas EPCAM, FOLR1, and CP are discussed in this study due to more frequent appearance in gene expression studies [13], [14].

2. Materials and Methods

2.1 Ligand Preparation

The three-dimensional structure models corresponding to silver (Ag) and selenium (Se) nanoparticles/ions were generated in Avogadro. Thereafter, all the structures went through energy minimisation through force-field optimisation in order to achieve stable and low energy geometry before the docking process. The generated structure was then converted into the file required for use with docking programs (PDB/PDBQT compatible input file) [15].

2.2 Selection and preparation of target proteins

The chosen proteins for docking experiments are associated with the changes and growth of ovarian epithelial cells including EPCAM, FOLR1, and ceruloplasmin that were obtained from the RCSB Protein Data Bank. In order to allow docking, the structures of the proteins were processed to eliminate the presence of water from the crystallograms, binded heteroatoms and unnecessary polar hydrogen atoms so that the structure generated should give a better view of the actual surface of the protein [16].

2.3 Docking Protocol

Molecular docking of the ligands (AgNP, SeNP) to each of the three proteins was done through rigid body docking using PatchDock (a geometry-based algorithm based on Connolly's definition of the molecular surface) to include the concave, and convex and flat patches from the target and the ligand molecule to match. PatchDock proved to be very efficient in the docking studies. PatchDock module could generate several potential conformations for any ligand-target complex

and separately rank them based on their shape complementarity and estimated energy of interaction [17].

2.4 Assessment of Docked Complexes

Through three complementary parameters indicated by PatchDock, the evaluation of each docked complex was established; here are the parameters:

PatchDock score — basically means the geometric shape complementarity of ligand and receptor surface; more PatchDock scores signify better geometric fitting since it is a cumulative score of the geometric shape complementarity between ligand and receptor interaction. Contact area (Å²) — this is the area of the buried contact surface between the ligand and receptor, which is expected to be higher when there is more of the contact surface area showing. Atomic Contact Energy (ACE, kcal/mol) — it demonstrates the energy of desolation/binding of the complex in question; a lesser value predicts a better binding action of the complex [16,17]

3. Results

3.1 Silver Nanoparticles (AgNPs) against Ovarian Cancer Targets

Table 1 summarises the best docking metrics obtained for AgNP complexes with each of the three target proteins.

Target Protein	Best PatchDock Score	Contact Area (Å²)	ACE (kcal/mol)
FOLR1	2320	~229.8 – 281.5	0.0
EpCAM	2322 (best of the three)	~234.7 – 274.6	0.0
Ceruloplasmin	2378 (highest overall)	~242.9 – 271.3	0.0

With respect to all three AgNP complexes, the ACE value was 0.0, which most likely indicates the previously reported inadequacy of the atomic-contact-energy factor of PatchDock in regards to metals/ionic ligands. Due to the fact that atomic desolvation parameters are not well-established for metal ions, it is therefore presumed that the binding energy exists but is undetected. Hence, the parameters of binding score and area were used to compare the silver complexes.

The best score for FOLR1-silver interaction (Figure 1) achieved was 2320 along with contact area values on the order of 229.8–281.5 Å². The moderately high score and interface area values imply

relative goodness of fit of the structures tested in the study and confirm that nanoparticles can potentially utilize folate receptor system for internalization in ovarian cancer.

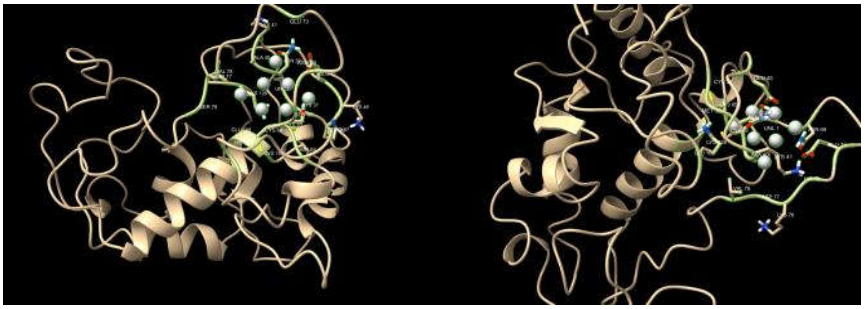

Figure 1. FOLR1–AgNP docked complex: (a) top conformation selected by PatchDock score; (b) top conformation selected by interface area.

Amongst the three proteins analyzed, the docking score of EpCAM with silver (Figure 2) turned out to be the highest (2322) along with a favorable interaction region of about 234.7-274.6 Å² and steady scores across conformations showing the stability of the binding site.

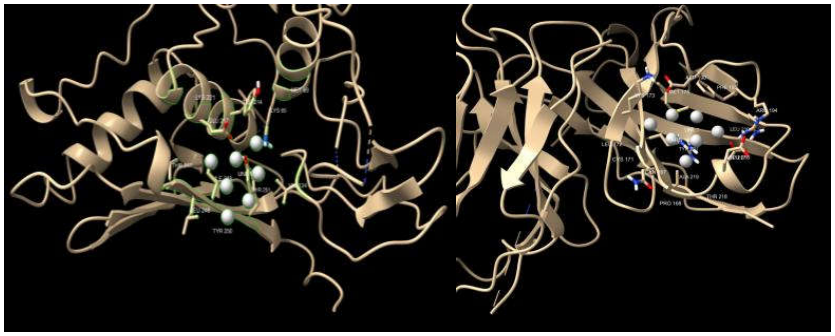

Figure 2. EpCAM–AgNP docked complex: (a) top conformation selected by PatchDock score; (b) top conformation selected by interface area.

Ceruloplasmin and silver (Figure 3) has shown the greatest absolute PatchDock score of 2378 among all AgNP complexes along with a good contact area of ~242.9–271.3 Å². This strong geometrical fit arises from ceruloplasmin being a naturally existing metal-binding protein.

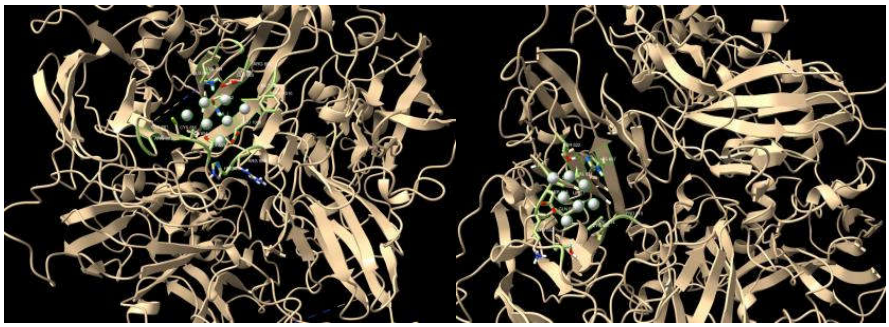

Figure 3. Ceruloplasmin–AgNP docked complex: (a) top conformation selected by PatchDock score; (b) top conformation selected by interface area.

In summary, out of the three proteins studied for ovarian cancer, binding to silver particles was observed. EpCAM protein showed best docking scoring and interface stability, ceruloplasmin was successfully to give highest raw docking score of all the three proteins while FOLR1 protein gave acceptable but moderate interaction.

3.2 Selenium Nanoparticles (SeNPs) against Ovarian Cancer Targets

Table 2 summarises the best docking metrics for SeNP complexes, selected on the basis of atomic contact energy (ACE).

Target Protein	**Best PatchDock Score**	**Max Contact Area (Å²)**	**Best ACE (kcal/mol)**
FOLR1	3194 (highest of all complexes)	358.0 (largest)	−331.77
EpCAM	2792	314.5	−407.46 (most negative)
Ceruloplasmin	2992	351.7	−363.65

Unlike AgNP complexes, the SeNP complexes had non-zero and very negative ACE values for all the three targets which provided an opportunity to use binding energy as a criterion for ranking. The binding energy of the complexes with SeNP was predicted to be more negative than for AgNP complexes in all cases.

For interaction of the receptor FOLR1 and selenium (Figure 4), the PatchDock score achieved the highest value compared to all other studied complexes (3194) in addition to the maximal interaction area (358.0 Å²) and ACE of −331.77 kcal/mol that signifies excellent fitting and energetically favorable interface.

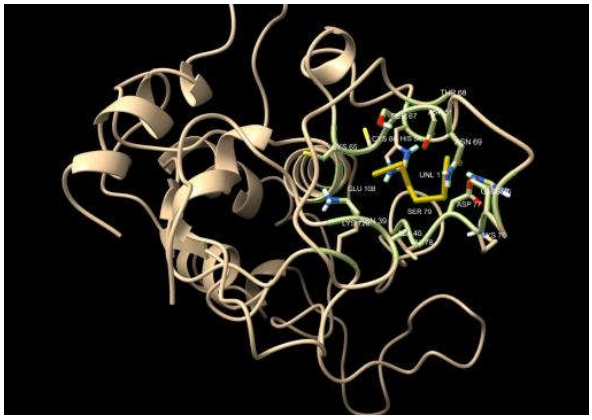

Figure 4. FOLR1–SeNP docked complex, top conformation selected by ACE score.

EpCAM combined with selenium (Figure 5) has a moderate-to-high docking score (2792) and an acceptable interaction surface area of 314.5 Å². However, it has the lowest ACE value recorded in the entire study (−407.46 kcal/mol), which means that while EpCAM does not have the best geometric fit, it has the best energetic complementarity with the selenium nanoparticles, making EpCAM the most stable and specific complex.

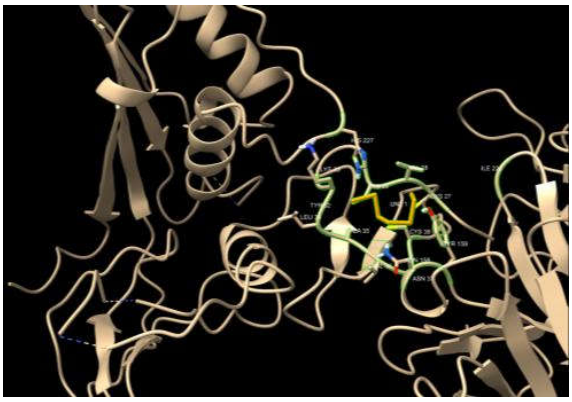

Figure 5. EpCAM–SeNP docked complex, top conformation selected by ACE score.

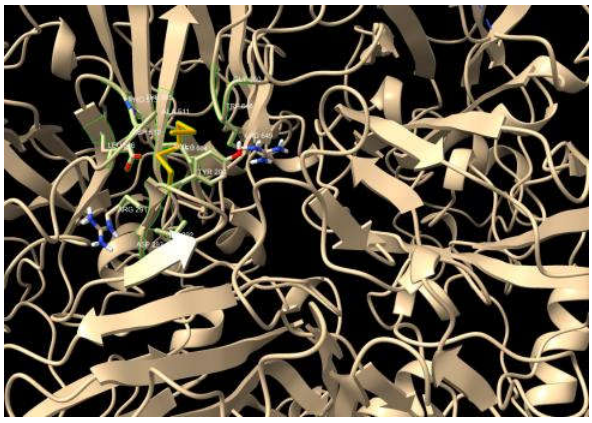

Figure 6. Ceruloplasmin–SeNP docked complex, top conformation selected by ACE score.

In the case of the protein complex ceruloplasmin when interacting with selenium (shown in Figure 6), it had the best overall docking score of 2992, larger interactions area of 351.7 Å² and a very good calculated ACE of -363.65 kcal/mol which proves how strongly this ligand binds to metal/metalloid compounds. Overall, we can see that in case of binding energies selenium nanoparticles have exhibited greater binding affinities and energies as compared to silver nanoparticles on all three ovarian cancer markers where EpCAM had the lowest binding energy among all.

3.3 Comparative Analysis: AgNPs versus SeNPs

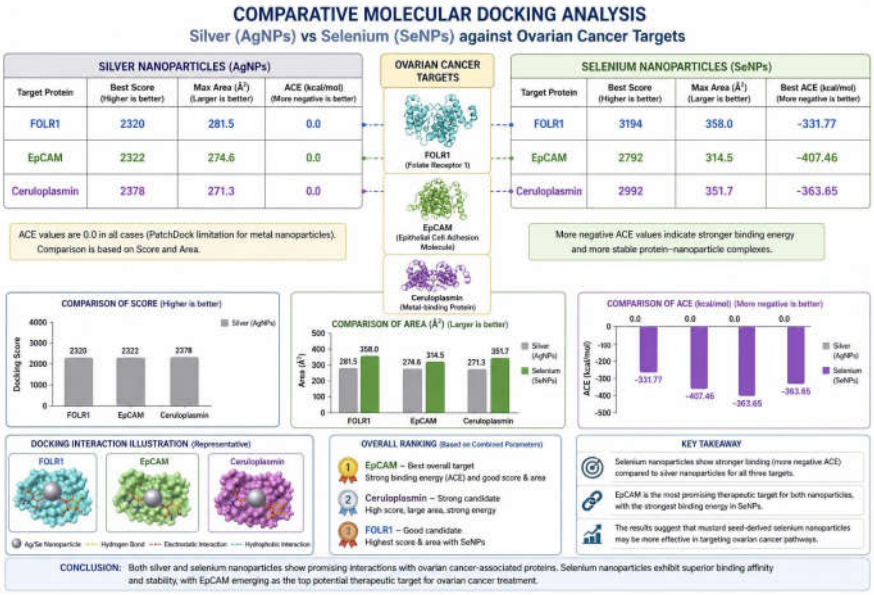

Figure 7. Comparative molecular docking analysis of silver (AgNPs) versus selenium (SeNPs) nanoparticles against the three ovarian cancer target proteins, with combined ranking based on score, area and ACE.

All in all, the comparative data show the following ranking according to docking score, interface area and binding energy: (1) EpCAM, which ranks highest, has excellent AgNP docking score and area values, and it also has the best binding energy with respect to SeNP; (2) Ceruloplasmin ranks second as it has high scores and area values in case of binding with both types of nanoparticles adhering to its natural ability of binding metals; (3) FOLR1 is third, having the highest docking score and interface area in regard to SeNP, along with satisfactory value of AgNP binding energy.

4. Discussion

This in silico docking study suggests that all of the three proteins EPCAM, FOLR1 and ceruloplasmin, which are always over-expressed in case of ovarian cancer, are able to create favorable complexes from both energetical and geometrical points of view with silver and selenium nanoparticles obtained from *Sinapis nigra* seed phytochemicals. There are three things to be mentioned in this respect.

The first one is that selenium nanoparticles showed better results in the analysis when compared to silver nanoparticles with regard to the binding energy values for all of the examined targets. Given that PatchDock's ACE term gave 0.0 in every case of AgNP complexes, this means that the method had serious limitations in terms of defining interaction energy in the case of metal/ionic ligands, and thus, it would be very difficult to make direct comparisons between AgNP and SeNP related scores [18] Second, ceruloplasmin's consistently strong docking performance with both nanoparticle types is readily rationalised by its native biological function as a copper-binding, multi-copper oxidase plasma glycoprotein [19].

As ceruloplasmin promotes activity in ovarian cancer cells, so its presence along with its metal binding capability, makes it realistic to postulate that the substance could interact with silver and selenium nanoparticles. Finally, EpCAM has been identified as the most promising target that displays both an appropriate binding ability to AgNP and a very favourable interaction with selenium-based nanoparticles [19], [20]

This is especially important as EpCAM overproduction has been associated with chemoresistance in ovarian cancer and chemotherapeutical response to platinum agents is limited. Thus, the use of the nanoparticles might localise the materials inside the affected cells and consequently overcome the resistance, although this is only speculative [21].

FOLR1 has a low binding potential to silver but has the highest interaction area in the interaction with selenium, which means that FOLR1/folate receptor alpha, which is already used for treatment in platinum-resistant high-grade serous ovarian cancer. Thus, this finding provides further evidence for the consequent development of folate receptor-targeted formulations of nanodrugs by attaching folate or mimetics on the formulation's surface and inducing endocytosis via folate receptors.[22]

The present study has some limitations, in particular, PatchDock program used for the analysis does not incorporate flexibility of ligands and receptors, nor does it account for any influence of the solvent and the nanoparticles' behaviour inside living organisms; also, nanoparticles were considered as separated rigid ligands rather than as nonuniform particles that would be formed in natural ways due to the use of Sinapis nigra phytochemicals [23], [24]. In conclusion, predictions using the methods discussed above should be seen only as computational; since no empirical binding or any other test data has been produced; hence experimental verification will be needed.

5. Conclusion

In this in silico molecular docking paper, the study aims to assess how silver and selenium nanoparticles, derived from Sinapis nigra mustard seed phytochemicals, interact with three protein targets that are overexpressed in ovarian cancer, EPCAM, FOLR1 and ceruloplasmin. The authors report that all their three targets had good geometry and/or energetics with the nanoparticles. Overall, selenium nanoparticles were more successful than silver nanoparticles in terms of binding energy. Among these three, the most promising target was EpCAM, even though ceruloplasmin and FOLR1 were also ranked higher than certain other molecular targets. The authors conclude, therefore, that the use of nanoparticles derived from mustard seeds should be further explored in clinical trials.

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