

RESEARCH ARTICLE

***In Silico* Interaction and Docking Studies Indicate a New Mechanism for PML Dysfunction in Gastric Cancer and Suggest Imatinib as a Drug to Restore Function**

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Abstract

Gastric cancer as one of the most common cancers worldwide has various genetic and environmental risk factors including *Helicobacter pylori* (*H.pylori*) infection. Recently, loss of a tumor suppressor gene named promyelocytic leukemia (*PML*) has been identified in gastric cancer. However, no mutation has been found in this gene in gastric cancer samples. Cag A *H.pylori* protein has been shown to exert post transcriptional regulation of some tumor suppressor genes. In order to assess such a mechanism for *PML* degradation, we performed *in silico* analyses to establish any interaction between *PML* and Cag A proteins. *In silico* interaction and docking studies showed that these two proteins may have stable interactions. In addition, we showed that imatinib kinase inhibitor can restore *PML* function by inhibition of casein kinase 2.

Keywords: *PML* - cag A - *Helicobacter pylori* - casein kinase - imatinib - docking study

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Introduction

Gastric cancer is the fifth most common cancer and the third leading cause of cancer death globally. Although several genetic and environmental risk factors have been identified, *Helicobacter pylori* (*H.pylori*) accounts for 60-70% of gastric cancer cases worldwide (Fock, 2014). In addition, Epstein-Barr virus (EBV) infection has been implicated in about 10% of gastric carcinomas (Sivachandran et al., 2012). Recent studies have shown decreased or loss of expression of the tumor suppressor, promyelocytic leukemia (*PML*) protein in gastric cancer. This event has been associated with an increased level of lymphatic invasion, higher pTNM staging, and poor prognosis (Lee et al., 2007; Kim et al., 2011). We recently have screened gastric cancer samples for *PML* mutations. As no deleterious mutations have been found in this gene in gastric cancer samples, it has been suggested that post-translational modifications or protein degradation could be mechanisms by which *PML* is not expressed in gastric tumors (Imani-Saber et al., 2015). EBNA1 as the only EBV nuclear protein expressed in gastric cancer has been shown to disrupt *PML* function and consequently result in impaired responses to DNA damage and promote cell survival (Sivachandran et al., 2012). Such EBNA1 role has been demonstrated to be performed via recruiting casein kinase 2 (CK2) to *PML* nuclear bodies and consequent increase in CK2-mediated phosphorylation of *PML* proteins which triggers *PML* degradation (Sivachandran et al., 2010). However, the possible interaction of *H.pylori*

proteins with *PML* has not been elucidated yet. Cag A *H.pylori* protein has been shown to induce proteasomal degradation of p53 in gastric epithelial cells infected with *H.pylori* (Wei et al., 2010). To evaluate the possible interaction of Cag A protein with *PML*, we performed *In silico* analyses using bioinformatics tools. In addition, we performed a docking study to assess if imatinib as a kinase inhibitor drug can influence casein kinase 2 functions.

Materials and Methods

In order to perform interaction study, pdb files corresponding to *PML* and Cag A proteins were retrieved from the protein data bank (PDB; <http://www.rcsb.org/pdb/>). Hex 8.0.0 software was used for protein docking predictions. SDF format of imatinib molecule was retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and changed to PDB format using open babel 2.3.2a tool. AutoDockTools 1.5-6rc3 and AutoDock Vina were used for prediction of imatinib binding to CK2. Lysine rich residues of 71-77 in A chain of CK2 was defined as the active binding site.

Results

The total energy of interaction between Cag A and *PML* molecules determined by Hex 8.0.0 software in the best conformation was -466.66 which implies that such interaction would be stable (Figure 1).

The interaction energy between imatinib and CK2

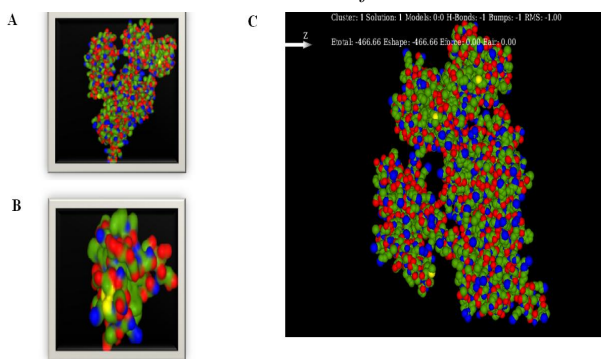


Figure 1. A. Cag A Protein Structure, B. PML Protein Structure, C. Docking Study of Cag A and PML Proteins

mode	affinity (kcal/mol)	dist from best mode rmsd l.b. rmsd u.b.
1	-4.3	0.000 0.000
2	-3.9	2.815 4.440
3	-3.3	2.403 3.501
4	-2.8	2.356 11.572
5	-2.2	2.370 3.848
6	0.8	3.165 5.140

Figure 2. Affinity Values for CK2 and Imatinib Interactions

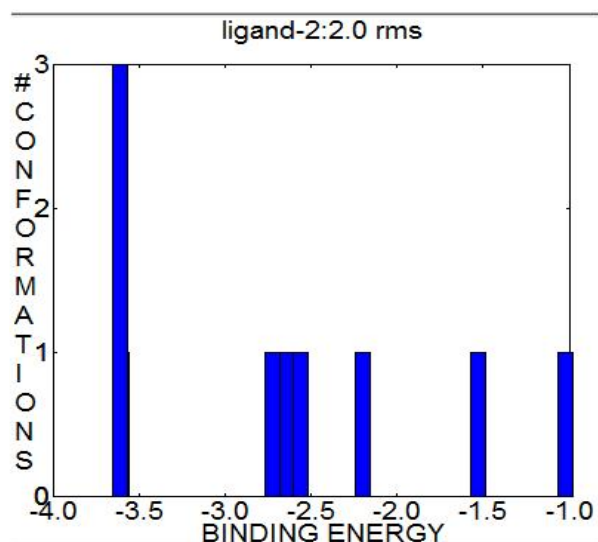


Figure 3. Clustering of Various Interactions between CK2 and Imatinib

was calculated for the Lysine 75 in the binding site. AutoDock Vina showed the values of affinity of such interactions were between -4 and 0 kcal/mol (Figure 2). Figure 3 shows the clustering of various dockings between CK2 and imatinib which has been performed by AutoDockTools. The negative values for binding energy of these conformations indicate effective binding of these proteins at that defined binding site.

Discussion

PML has multiple functions in growth inhibition, apoptosis induction, replicative senescence, inhibition of oncogenic transformation, and suppression of migration and angiogenesis. These roles have implied that it could be a suitable therapeutic target for cancer therapy

(Imani-Saber and Ghafouri-Fard, 2014). Although multiple studies have indicated loss of PML in gastric cancer, the exact mechanism of such loss of function is not clear. Among various risk factors for gastric cancer, the role of two infections in this malignancy has been highlighted. EBV nuclear protein has been shown to interact with PML protein and degrade it. However, the data regarding interaction of *H.pylori* proteins with PML is missing. We have performed *In silico* interaction study to find whether Cag A protein could interact with PML. Such studies showed that these two proteins have stable interactions. Furthermore, to find an effective molecule to restore PML function, we analyzed the interaction of the famous kinase inhibitor imatinib with CK2 which has a fundamental role in PML degradation. Our *In silico* studies suggest that imatinib can be served as a drug to inhibit CK2 function and maintain PML nuclear bodies. However, further researches are needed to confirm the results of such studies.

References

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