AIM

Diseases such as cancer may be caused by the accumulation of persistent organic pollutants (POPs) in organisms; however, available studies are limited investigating interaction between cancer-linked proteins and POPs. Our previous studies showed that 2,2',4,4',5,5'- Hexachlorobiphenyl (PCB153), 2,2',4,4'-Tetrabromodiphenyl ether (PBDE47) and 4,4' – dichlorodiphenyl dichloroethylene (4,4'-DDE) were accumulated in the tissue of organisms in Laguna de Bay and proteomic analysis in cane toads collected from the riparian zones of the bay showed that some cancer-linked proteins, such as angiopoietin-related protein 7 (angptl7), annexin a2 (anxa2), and SWI/SNF- related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2 (smarca2), were differentially expressed. Hence, in this study, *in silico* molecular docking between POPs (PCB153, PBDE47 and 4'4DDE) and cancer-linked proteins (angptl7), (anxa2), (smarca2), and tumor protein (p53) was performed.

PROCEDURE

Target Protein Sequences and Ligand Structure Retrieval from Databases

Amino acid (FASTA) sequences of angptl7, anxa2, p53 and smarca2 of Xenopus tropicalis were obtained from UNIPROT (www.uniport.org) while 2,2',4,4',5,5'- hexachlorobiphenyl (PCB153), 2,2',4,4' tetrabromodiphenyl ether (PBDE47) and 4,4' – dichlorodiphenyl dichloroethylene (4,4'DDE) 3D structures (.Mol) were obtained from PubChem (www.pubchem.ncbi.nih.gov).

Generation of 3D Homology Models and Validation of Models

The 3D structure homology model of cancer-linked proteins was constructed using an automated web-based homology modeling tool (www.swissmodel.expasy.org). The amino acid sequences obtained from UNIPROT was submitted to predict the 3D structure of cancer-linked proteins. Five template structures were selected for model building. SWISS-MODEL assess the quality of the model through the GMQE (Global model quality estimation) and the QMEAN (Qualitative model energy analysis) score.

The quality of the generated 3D models was assessed to verify its reliability for further molecular docking simulation. One model of good quality was selected for each protein. The validation of the selected models was done by utilizing the online quality structure assessment tools, ERRAT available at Structural Analysis and Verification Server, (www.saves.mbi.ucla.edu) and Molprobity (www.swissmodel.expasy.org).

Molecular Docking Analysis and Visualization

The docking simulation was prepared using UCSF Chimera 1.15 (Pettersen et. al, 2004), a program designed for the visualization and analysis of molecular structures by adding hydrogen atoms in both POPs and cancer-linked protein models. The computation of the binding modes of ligands and cancer-linked proteins were performed using the web-based interface SwissDock (www.swissdock.ch/docking). SwissDock is based on the docking software EADock DSS docking algorithm which utilizes CHARMM (Chemistry at Harvard Macromolecular Mechanics) forcefield (Grosdidier et al. 2011). The prepared structures from UCSF Chimera were submitted to SwissDock web-based interface that shows an interactive table with the calculated binding affinity of each pose. The minimum energy docked conformers are ranked in terms of full fitness score.

The molecular visualization was carried out using UCSF Chimera. Each conformer was inspected to determine the number of binding pockets. The cluster that has the least full fitness score from each binding pocket was used to inspect the interactions between POPs and cancer-linked proteins.

RESULTS

Protein Homology Models and Validation

Based on ERRAT and MolProbity assessments (Table 1), angptl7 model 1, anxa2 model 1, p53 model 1 and smarca2 model 2 were selected as the best quality model to use in molecular docking analysis. ERRAT is an algorithm that analysis the statistics of non-bonded interactions between different atom

types (Colovos et al. 1993). The score is expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. The ERRAT score should be greater than 50% for considering a model of good quality. The overall quality factors (OQF) of all protein models were observed above 80% suggested that the models are good quality.

On the other hand, MolProbity is a structure-validation web service that provides broad-spectrum solidly based evaluation of model quality at both the global and local levels for both proteins and nucleic acids. It relies on the power and sensitivity provided by optimized hydrogen placement and all-atom contact analysis, complemented by updated versions of covalent-geometry and torsion-angle criteria (Chen et. al, 2010). The Molprobity score provides a single number that represents the central Molprobity protein quality statistics. It is obtained from log-weighted combination of the clash score, percentage Ramachandran not favored and percentage bad side-chain rotamers.

Table 1. MolProbity scores of cancer-linked protein models angptl7, anxa2, p53 and smarca2.

Parameters	**Cancer-linked Proteins**							
	Angptl7		Anxa2		P53		Smarca2	
	M1	M2	M1	M2	M1	M2	M2	M3
MolProbity Score	1.43	1.62	1.90	1.85	1.58	1.87	0.89	1.01
Clash Score	1.73	1.45	4.09	4.84	1.47	2.21	0.54	0.0
Ramachandran favored (%)	92.13	88.43	95.20	97.30	91.15	91.15	98.17	91.45
Ramachandran outlier	0.93%	1.85%	2.10%	1.50%	2.69%	1.92%	0.0%	0.85%
Rotamer outlier	1.06%	1.59%	3.05%	4.07%	1.72%	3.00%	1.89 %	0
C-Beta deviation	1	2	6	6	2	6	1	0
Bad bonds	0/1843	0/1835	0/2682	0/2685	0/2082	0/2083	0/935	0/1005
Bad angles	18/2487	20/2480	46/3615	32/3615	23/2816	20/2018	2/1259	6/1344
Cis Non-proline	2/213	2/213		1/328				
Twisted Non-proline	1/213	1/213	2/328					

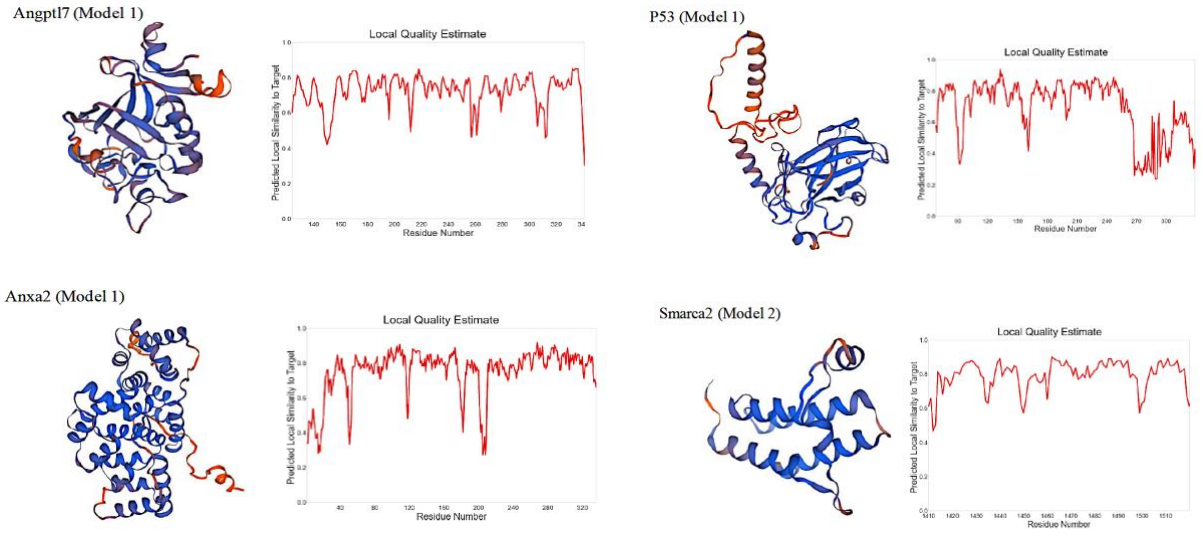

Figure 1. Three-dimensional (3D) structures and local quality plots of the cancer-linked protein homology models.

Molecular Interaction of POPs to Cancer-linked Protein Models

The docking results obtained using SwissDock (Figure 2) shows the most favorable binding pocket (BP) of PCB153, PBDE47 and 4,4'DDE with the 3D models of cancer-linked proteins were at the hydrophobic regions. The blue and red colored region indicates the hydrophilic and the hydrophobic portion of the model, respectively. The BPs of PCB153 and PBDE47 against the models of cancer-linked proteins were bound to the same BP. However, in the case of the 4,4'DDE, it was bound from different BP in anxa2 and p53 models. These difference in BPs may depend on the shape complementarity between the binding site and the POPs. Also, the comparison of the physicochemical properties of the protein binding site and the POPs represents an additional information layer for pocket detection which can be used to improve the estimation of the ligand binding affinity and specificity (Norel et al. 1999; Andersson et al. 2009).

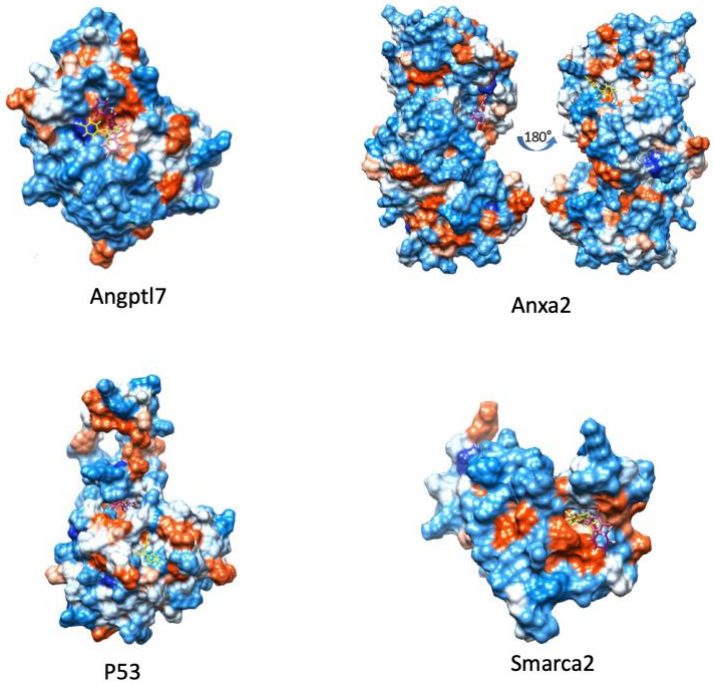

Figure 2. Docking poses of persistent organic pollutants (POPs) in stick representation (magenta: PCB153, purple: PBDE47, and yellow: 4'4'DDE) against the 3D models of cancer-linked proteins in ball representation.

The binding pocket of angptl7 with the three selected POPs is shown in Figure 2A. One of the aromatic rings of each POPs was interacting in the inner part of the BP. Further analysis in BP reveals several nonpolar amino acid residues such as Phe192, Phe199, Leu201, Leu195 and Val141. These amino acids are part of the C-terminal Fibrinogen-related domain (FRED) in angptl7 was believed to be a pro-angiogenic factor (Parri et al. 2014). In contrary, the study of Peek et al. 2002 and Toyono et al. 2015 showed that angptl7 act as anti-angiogenic protein in reducing the tumor growth and aberrant blood vessel formation in the avascular corneal stromal layer.

The hydrophobic interaction plays an important role in the association of POPs against angptl7. This result was in good agreement in the study of Cheng et al. 1996 from the interaction of the fibrinogen recognition exosite of thrombin with its naturally occurring inhibitor (hirudin). In addition, the nonpolar amino acid residues may be associated with stronger Van der Waals interactions. Furthermore, in the study of Nanchari et al. 2020, they demonstrated the interaction of phenol compounds from rhizomes of ginger with proteins involved in angiogenesis including the relative

protein of angptl7, the angiopoietin 2. Result suggests a strong binding affinity between the ligand and the protein as a factor in angiogenesis and lymphangiogenesis. POPs might change the angiogenic potential of angptl7.

Figure 2B shows the binding pockets of anxa2 to the three POPs. Like in angptl7, the POPs interact with the hydrophobic region of the model. This interaction of POPs to anxa2 includes the two aromatic rings. Further analysis in BP revealed several nonpolar amino acid residues such as Ile295, Trp20, Ala19, Thr136, Thr22, Leu23, Thr17, Ile26, Ala105, Thr103, Ile144 for BP in PCB153 and PBDE47 bound BP and Leu29, Thr59, Ile26, Ala334, Thr25, Tyr313, Leu329, Leu331, Ile55 in 4,4'DDe bound. These amino acids are part of the annexin repeats that binds to phospholipids in a calcium- dependent manner. In the study of Hakobyan et al. 2017 they revealed that binding residues of human anxa2 close to the membrane are Glu189, Asp192, Asp230, Asp239, Asp322, Asp338 and Asp339. These amino acids are different to the residues found in the binding pockets between POPs and anxa2. However, given that POPs interact to anxa2 via hydrophobic effects, POPs might affect the potential function of anxa2.

In Figure 2C, the 4,4' DDE was bound to different BPs in p53 protein like in anxa2. Analysis revealed several nonpolar amino acids residues such as Thr74, Leu227, Val143, Val71, Ile229, Leu278 in PCB153 and PBDE47, and Met181, Thr134, Thr231, Ile229, Thr186 in 4,4'DDE were bound to BPs These amino acid residues are part of the DNA binding domain which stimulates another gene to produce a protein that interacts with a cell division-stimulating protein (cdk2). P53 is a metalloprotein which contains zinc ion. Weakening the binding of zinc to p53 may lead to misfolding and loss of function (Kogan et al. 2018). This interaction of POPs might affect the binding of zinc to p53.

In Figure 2D, the POPs interact with smarca2 model at the same binding pocket. One of the aromatic rings of each one POPs was interacting in the inner part of BP. Further analysis in BP reveals several nonpolar amino acid residues such as Ile1504, Phe1497, Leu1452, Val1442, Leu1446, Phe1443, Tyr1455. These amino acid residues are part of bromodomain that regulates gene expression. These amino acid residues were also observed from the study of Prieto-Martinez et al. in 2018 where interacting flavonoids suggests an inhibitory potential against bromodomain and extra-terminal bromodomains (BET). This also suggests that POPs may exhibit an inhibitory potential against bromodomain and BET.

Binding Affinities of POPs to Cancer-linked Protein Models

Molecular interaction has an important contribution to ligand receptor binding affinities (Motiejunas and Wade, 2007). Table 2 shows the binding affinities from the interaction between POPs and the 3D models of cancer-linked proteins. According to full fitness score, PCB153 had the most favorable binding mode as indicated by negative scores -1191.81, -2216.42, -1891.27 and -798.01kcal/mol against angptl7 model 1, anxa2 model 1, p53 model 1, and smarca2 model
2, respectively.

Table 2. Binding affinities of persistent organic pollutants (POPs) to the homology models of cancer-linked proteins.

Models	PCB153		PBDE47		4,4'DDE	
	Fullfitness (kcal/mol)	ΔG (kcal/mol)	Fullfitness (kcal/mol)	ΔG (kcal/mol)	Fullfitness (kcal/mol)	ΔG (kcal/mol)
Angptl7-Model 1	-1191.81	-6.79	-1179.73	-6.98	-1176.09	-6.75
Anxa2-Model 1	-2216.42	-7.12	-2203.37	-7.32	-2199.02	-6.69
P53-Model 1	-1891.27	-7.09	-1877.07	-7.03	-1875.71	-7.12
Smarca2 – Model 2	-798.01	-6.78	-785.52	-7.01	-782.96	-7.08

PBDE47 had the best energy of binding against angptl7 (-6.98kcal/mol) and anxa2 (-7.32 kcal/mol) while 4,4'DDE had the best energy of binding against p53 (- 7.12kcal/mol) and smarca2 (-7.08kcal/mol) as indicated by more negative to the change in Gibbs fee energy (ΔG) score. These difference on binding energies may be due to the physicochemical properties of the protein BP and the POPs. Moreover, the difference in binding energies may be attributed to the orientation of the pollutants as the shape complementarity and the physicochemical property of the binding site is the main factor to improve ligand affinity.

The size and polarizability, hydrophilicity, Gibbs free of solvation, inductive and mesomeric effects are also factors influencing the binding affinity (Luthe et al., 2008). The change of Van der Waals (ΔGvdw) energy was then investigated (Table 3). Result suggests that the change of binding energies of 4,4'DDE to smarca2 is affected by the Van der Waals (vdw) forces as it shows the lowest energy like in ΔG. Interestingly, in angptl7 and anxa2, the ΔG value of PBDE47 was indirectly proportional to the ΔGvdw. This may be because of some residues/elements involved in the interaction.

Table 3. Change in Van der Waals energy of persistent organic pollutants (POPs) to homology models of cancer-linked proteins.

Model	**ΔGvdw (kcal/mol)**		
	PCB153	PBDE47	4,4'DDE
Angptl7 Model 1	-30.86	-27.82	-30.39
Anxa2 Model 1	-36.28	-34.93	-26.61
P53 Model 1	-22.51	-29.57	-34.09
Smarca2 Model 2	-25.51	-25.70	-29.59

Results of this study suggest that the direct interaction of POPs may impair the functions of the cancer-linked proteins. The interaction of POPs to angpt7 may inhibit the pro- angiogenic factor of the cancer-linked protein. This binding of POPs to angptl7 via fibrinogen C- terminal as molecular recognition unit may affect the identity of the protein to be recognize by the receptor from the cell. However, angptl7 acts like a hormone (Parri et al., 2014). This scenario may probably allow the cell to stimulate the production of angptl7 protein that can lead to high angptl7 expression.

Meanwhile, the binding of POPs to anxa2 via annexin repeats as binding site to phospholipids may inhibit the interaction of anxa2 protein to phospholipids. This inhibition of interaction may stimulate the production of anxa2 protein that can lead to high anxa2 expression. Increase in anxa2 may promote metastasis. In the study of Yang et. (2017), the overexpression of anxa2 was determined in rat with liver fibrosis which eventually can lead to cirrhosis and hepatocellular carcinoma.

The binding of POPs to p53 via DNA binding domain as tumor suppressor may inhibit the expression of a cyclin-dependent kinase inhibitor p21. This inhibition of p21 allows the expression of cyclin-dependent kinases such as cdk2 which can promote tumor growth (Wierod et al. 2007).

The binding of POPs to smarca2 via bromodomain for chromatin remodeling may inhibit the expression of regulatory proteins. Smarca2 is one of the proteins the comprises the switch/sucrose nonfermenting (SWI/SNF) chromatin remodeling complex that plays an important role in tumor suppression. In addition, SWI/SNF complex are important transcription regulator and DNA repair (Santen et a. 2012). This inactivation of smarca2 as component of SWI/SNF complex may lead to genomic instability and could fuels cancer development.

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