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WIF-1 might play additional role in carcinogenesis and cancer growth via EGFR signaling pathway

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Abstract

All protein-protein interaction is a well-known phenomenon in cancer cell signaling pathway. To study the potential docking between WIF-1 and EGFR molecules, initial protein-protein docking was performed using CAPRI-listed online servers, namely ZDOCK, GRAMM-X, HEX and PatchDock. The predicted docking conformation issued from various servers suggested the similar potential protein-protein conformational interaction. The lowest docking energy of WIF-1/EGFR binding calculated using HEX server was -616.40 kcal/mol. This was comparable to that of EGF/EGFR binding (-627.18 kcal/mol), indicating a possibility for WIF-1 to bind to EGFR through its EGF-like domain.

Keywords: WIF-1, EGFR, WNT, protein-protein interaction, cancer, cell signaling pathway

Introduction

Over 57 million global deaths in 2008, cancer was one of the main noncommunicable diseases that accounted for 7.6 million deaths. By 2030, the number of new cancer cases has been predicted to increase to 21.4 million with nearly two thirds of all cancer cases being diagnosed from low- and middle-income countries (WHO 2010). To reduce such global burden, cancer researches, ranging from physical treatment to new drug discovery, have intensively been conducted worldwide. In recent decades, scientists started to focus on cancer cell signaling pathways to understand better the mechanisms of cancer formation and development. With hope, such fundamental study will contribute to the current status in cancer treatments. The evolving of new technologies in nanoparticle and bioinformatics for instance, has enhanced the advancement in cancer marker identification and in drug targeting (Gundampati et al. 2011).

A general paradigm model for human carcinogenesis is viewed as a multi-stage disease due to the accumulation of tumor suppressor genes and oncogenes in the cell (Luu et al. 2004). By understanding the cancer cell signaling pathways with the key players identified, it is hoped that therapeutic agents could be developed. Among the signaling pathways related to cancer development, epidermal growth factor receptor (EGFR) and WNT signaling pathways play an important role as these pathways are involved in cell differentiation and proliferation. With the responsive effects of EGFR inhibitors (e.g. gefitinib, genistein) and WNT inhibitors (e.g. WIF-1, sFRP-1) in cancer treatment, clinical interest in EGFR and WNT signaling pathways have been highlighted (Bhargava et al. 2005; Tang et al. 2009).

Given the potential of WNT inhibitory factor 1 (WIF-1) to be used as natural inhibitor of WNT signaling pathway to prevent carcinogenesis and/or the progression of cancer resulted from the activity of WNT signaling pathway, it is crucial to ensure that WIF-1 would not contribute to any unwanted negative effects in the potential cancer-inhibition function. Nevertheless, contradictions were found in many cases wherein the expression of WIF-1 was increased with cancer formation (Suzuki et al. 2007a; Suzuki et al. 2007b). This type of observations implies that WIF-1 might play a cancer-promoting role via other mechanism(s) in addition to its endogenous inhibitory property in WNT signaling pathway.

Interestingly, WIF-1 (UniProt ID: Q9Y5W5) is made up of WIF domain and 5 repeated EGF-like domains whereby epithelial growth factor (EGF; UniProt ID: Q6QBS2) is one of the ligands that specifically bind to EGFR. The comparison of nucleic acid sequence between EGF and EGF-like domains of WIF-1 has been shown to be almost 50% identity. The respective amino acid sequence of these could be grouped into three conserved regions, namely C1-C3, C2-C4, C5-C6, when compared using BioEdit (Version 7.0.9.0) (Hall 1999) (Table 1). In most proteins, such EGF-like amino acids sequences would tend to form disulfide bonds within the domain by pairing up the six cysteine residues. As EGFR signaling pathway could be playing a cancer-promoting role if it is aberrantly activated, the possibility of a ligand other than EGF, such as WIF-1, to bind to the receptor might trigger the undesired cancer-promoting outcomes. Thus, the aim of this study is to investigate the potential of EGF-like domain of WIF-1 molecule to bind with EGFR extracellular domain using computational tools. Such a binding may affect the effectiveness of current cancer treatment given in both WNT and EGFR signaling pathways. Further investigation on such hypothesis should be performed at wet lab experimental level.

Methodology

To understand the protein-protein interaction of WIF-1 molecule and EGFR molecule, primary docking calculations were conducted to generate as many near-native complex structures as possible. The

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Table 1a: The identity at nucleic acid level between EGF gene sequence and EGF-like domains of WIF-1

EGF-like domains in WIF-1	% Identity in nucleic acid sequence compared to EGF gene sequence
EGF-like 1	46.46
EGF-like 2	41.67
EGF-like 3	41.38
EGF-like 4	47.47
EGF-like 5	48.96

Table 1b: The conserved cysteine residues (in red color) found in EGF and EGF-like domains of WIF-1

Conserved regions as compared to EGF protein sequences

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NSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELR-(EGF)
-QQAECPGG----CRNGGFCN-----ERRICECPDGFHGPHCE----------(EGF-like 1)
--KALCTPR----CMNGGLCV-----TPGFCICPPGFYGVNCD----------(EGF-like 2)
--KANCSTT----CFNGGTCF-----YPGKCICPPGLEGE-------------(EGF-like 3)
-EISKCPQP----CRNGGKCI-----GKSKCKCSKGYQGDLCS----------(EGF-like 4)
--KPVCEPG----CGAHGTCH-----EPNKCQCQEGWHGRHCN----------(EGF-like 5)
```

**Note: "---"denoted no amino acid in the sequence shown*

3-D structure of EGF molecule (PDB ID: 1EGF) (Montelione et al. 1992), EGF-like domain of WIF-1 molecule (PDB ID: 2YGQ)

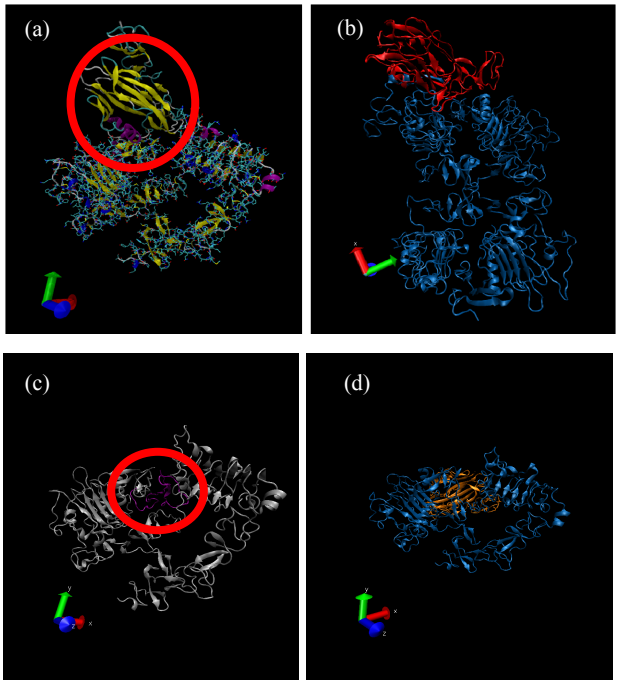

Figure 1: protein-protein docking simulation of WIF-1 and EGFR from (a) GRAMM-X: Yellow (in circle) = WIF-1 EGF-like domain (PDB: 2YGQ), the remaining molecule = EGFR Extracellular Domain (PDB: 1NQL); (b) ZDOCK: Red = WIF-1 EGF-like domain (PDB: 2YGQ), Blue = EGFR whole protein model (PDB: 1IVO); (c) HEX: Purple (in circle) = EGF molecule (PDB ID: 1EGF), Silver = EGFR extracellular domain (PDB ID: 1NQL); (d) PatchDock & HEX = Orange = EGF-like domain of WIF-1 molecule (PDB ID: 2YGQ), Blue = EGFR extracellular domain (PDB ID: 1NQL).

(Malinauskas et al. 2011) and extracellular domain of EGFR (PDB ID: 1NQL) (Ogiso et al 2002) were constructed using the templates issued from RCSB Protein Data Bank. Visual molecular dynamics (VMD) software was used to visualize the PDB files and text editor was used to delete any unwanted atoms or ligands in the PDB files before docking. Protein-protein docking simulation was performed using online server listed in Critical Assessment of PRediction of Interactions (CAPRI) such as ZDOCK (Chen et al. 2003), HEX (Ritchie 2002), GRAMM-X (Tovchigrechko and Vakser 2006), and PatchDock (Schneidman-Duhovny et al. 2005). EGF-like domain of WIF-1 molecule (PDB ID: 2YGQ) was set as ligand molecule and extracellular domain of EGFR (PDB ID: 1NQL) or whole protein model of EGFR (PDB: 1IVO) as receptor molecule. Default settings were used during all calculations to predict the interaction of these two target proteins.

The generated PDB files from different online servers were then compared for their ligand-bound complex conformations and these were analyzed based on the lowest docking energy.

Results and Discussion

From GRAMM-X and ZDOCK algorithms, the similar binding conformations were predicted with default settings. Results from both programs indicated that the EGF-like domain of WIF-1 molecule was docked at the outer binding cavity of EGFR protein model (Figure 1a and 1b). The conformation of the ligand-bound complex issued from HEX algorithm (Figure 1c and 1d) was deduced based on the evaluation of free docking energy of both EGF molecule (PDB ID: 1EGF) or EGF-like domain of WIF-1 molecule (PDB ID: 2YGQ) to extracellular domain of EGFR (PDB ID: 1NQL). E_{total} for EGF/EGFR docking was estimated to be -627.18 kcal/mol while that for WIF-1/EGFR docking was -616.40 kcal/mol. From PatchDock algorithm (Figure 1d), the 20 best solutions were re-ranked using FireDock. The simulated docking conformation was similar to the previous reported results in GRAMM-X and ZDOCK (Figure 1a and 1b). The results obtained from protein-protein docking programs were satisfactory and the predicted docking conformations were consistent, indicating a high reproducibility at least *in silico*.

The overall results indicated a possibility that EGF-like domain of WIF-1 might mimic EGF and bind to EGFR. The present results suggest that the EGF-like domain of WIF-1 molecule might interact with EGFR extracellular domain, initiating the EGFR signaling pathway and therefore interfering in cancer cell signaling pathways that are normally related to EGFR signaling pathway. This might trigger carcinogenesis via EGFR signaling pathway itself, or it might be done via the crosstalks between EGFR and other cell signaling pathways, including with WNT signaling pathway.

The capability of WIF-1 to act as a ligand to EGFR and trigger carcinogenesis or metastasis might explain the observations wherein the expression of WIF-1 was high in cancer cases (Suzuki et al. 2007a; Suzuki et al. 2007b). In these cases, WIF-1 might be playing dual roles by inhibiting WNT signaling pathway on one hand, but initiate EGFR signaling pathway on the other hand. This latter would engrave the situation by promoting cancer cell growth, while researchers are expecting WIF-1 being playing a cancer-cell-growth inhibiting role. The confirmation of the "bad guy" role of WIF-1 under the camouflage of it as inhibitor of WNT signaling pathway would pave new insights to the cancer formation and eventually assist in cancer treatment research.

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