RESEARCH HIGHLIGHT

Baby steps toward modelling the full human programmed Death-1 (PD-1) pathway

Marawan Ahmed[1], Khaled Barakat[1,2,3]

[1]*Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada*
[2]*Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada*
[3]*Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, Alberta, Canada*

Correspondence: Khaled Barakat
E-mail: kbarakat@ualberta.ca
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Immune checkpoints are vital elements in regulating the immune system. They preserve the immunological balance between preventing continuous activated immune responses and defending against chronic infections and cancer. Blocking the immune inhibitory checkpoints pathways recently emerged as a 'game changer' approach in cancer and antiviral immunotherapy. Modeling these pathways at the atomic level provides a key step toward rationally designing selective blockers for these pathways. Current crystal structures for the immune checkpoints are mainly not for human and are very limited in their scope of interactions. Our team has been focused on building atomistic models for these proteins, characterizing their protein-protein interactions and designing new inhibitory drugs for their activity. This article highlights our recent study on modelling the human Programmed Death-1 (hPD-1) pathway by characterizing the interactions between hPD-1 and its two human ligands. In this study, we showed that hPD1 binds differently to its two ligands. We also showed that the modes of binding for each ligand are different between mouse and human, emphasizing the limited information in current mouse crystal structures. Our findings enhanced the understanding of the receptor-ligand(s) interactions and formed a significant step toward building a full model for the whole PD1 pathway. This undoubtedly will foster the ongoing efforts to develop antibodies and small molecule drugs against this important T cell immune-regulatory mechanism.

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The immunological symphony relies on the existence of a critical balance between different stimulatory and inhibitory signals [1]. These signals are generated from the binding of the T cell surface receptors with either stimulatory or inhibitory ligands [2]. The different inhibitory T cell receptors together with their corresponding ligands are termed as the immune checkpoint pathways[3, 4]. From a physiological point of view, these inhibitory pathways inhibit the attack of immune cells to self-organs [4]. Binding of the inhibitory ligands to their receptors deactivates T cells' cytotoxic effects and induces an immune tolerance. For example, binding of the CD80 or CD86 ligands to the Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), a T cells surface receptor, results in decreased T cell proliferation and impairment of cytokine production [5]. Recently, blocking the immune checkpoints pathways through the use of monoclonal antibodies (mAbs) directed against immune checkpoint receptors/ligands has been envisaged as a very successful therapeutic approach against several chronic infections and malignant tumours [6]. Augmented with their unique pharmacological properties, mABs directed against immune checkpoint pathways have established a new era of cancer treatment [7] with some reports claiming a 3 years or longer survival rates for patients with refractory tumours, such as metastatic melanoma [8].

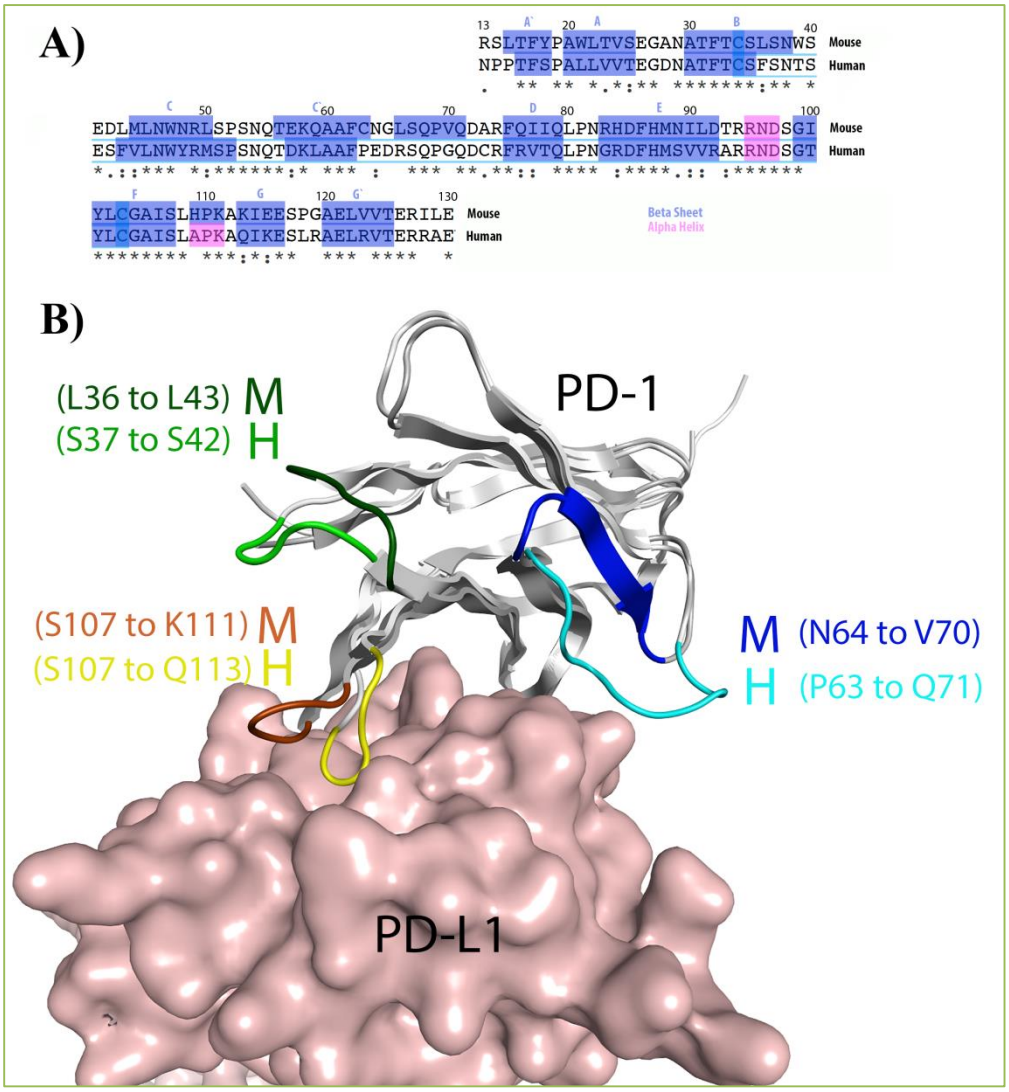

Figure 1. Sequence and structural alignments between human and mouse PD-1. (A) The two proteins have ~65% sequence identities, with their major differences are imposing more flexibility to the human PD-1. (B) PD-L1 is shown in a surface representation and colored in pink. Regions that are not forming the binding interface with PD-L1 are quite similar in both human and mouse PD-1 (colored in white). The major differences between the two proteins are at the binding interface with the ligand. The most important variations are the lack of a beta strand (dark blue) present in the mouse structure that is replaced with a long flexible loop (light blue), the more flexibility in the loop formed by residues S107 to Q113 (yellow) and the loop between residues S37 to S42 in human PD-1 (light green) [30]. Reused with permission from Elsevier science, license number: 3617961067094.

Of particular interest is the PD-1 pathway for which a number of monoclonal antibodies (mAbs) have been clinically approved and several others are currently undergoing clinical development [7]. PD1 is a T cell receptor that belongs to type I trans-membrane glycoproteins. A full-length human PD1 receptor is a 288 amino acid protein and is organized into three major topological domains, namely, an Ig Variable-type (V-type) extracellular domain, a trans-membrane α-helix, and a cytoplasmic domain [9]. The cytoplasmic domain of PD1 is responsible for delivering the inhibitory signal through its interactions with other cellular signalling molecules, such as the Src homology 2 tyrosine phosphatases SHP-2 [10]. In contrast to CTLA-4 that regulates T cell functions at the initial stages of T cell activation, PD-1 blocks the function of already activated T cells.

A distinct advantage for targeting PD-1 over CTLA-4 in tumour diseases is that the two known PD-1 ligands, PD-L1 and PD-L2, are expressed in abundance in the vicinity of resistant tumour cells [11]. This relative abundance is a hallmark for the involvement of these ligands in attenuating the innate immunity of T cells against cancer [12]. Most recently, FDA granted an accelerated approval to Keytruda (pembrolizumab; MK-3475), an anti-PD-1 mAB for advanced

melanoma patients who are not responsive to traditional chemotherapy [13]. Nivolumab is another anti-PD-1 mAb that has been recently approved for the treatment of melanoma. Nivolumab has been also shown to be safe and very effective in patients with advanced, refractory squamous non-small-cell lung cancer [14].

A major obstacle against developing other therapeutic approaches against the PD1 immune checkpoint pathway is the lack of high-resolution crystal structures for the human PD-1 (hPD-1) receptor in complex with either human PD-L1 (hPD-L1) or human PD-L2 (hPD-L2)[3,15]. With this limitation, molecular modelling and computer simulations can offer a comprehensive and alternative approach to understand these interactions [16-35]. Although there have been previous attempts to model the hPD-1/hPD-L1 and hPD-1/hPD-L2 complexes, none of these models correlated well with available experimental data. For example, Cheng *et al*. used an NMR structure for the extracellular domain of hPD-1 to predict the binding mode with hPD-L1 and hPD-L2 [36]. The perturbation of the hPD-1 protein backbone NMR signals (1HN, 15N and 13C′) was used as a metric to analyze these interactions. To elucidate potential binding modes of the human bound complexes they have used mouse complexes as references. They have superimposed the human proteins on the resolved crystal structures for mPD-1/hPD-L1 and mPD-1/mPD-L2. The superimposed models did not correlate with the NMR data and mutational analyses for many residue interactions.

In our recent work we addressed the issue of lacking accurate structural models for hPD1 bound to its ligands by generating highly precise models for the hPD1-hPD-L1 and hPD-1-hPD-L2 complexes [30]. We have carried out a comprehensive protein-protein docking simulations for hPD-1 against hPD-L1 and hPD-L2, followed by careful analysis and binding energy calculations. First; we started by extracting dominant protein conformations for hPD-1, hPD-L1 and hPD-L2 through clustering an exceptionally long molecular dynamics (MD) trajectory for the extracellular/interacting domains of each protein. These starting protein conformations were subjected to extensive protein-protein docking simulation using the Fast Fourier Transform (FFT) protein-protein docking algorithm Z-dock [37]. The enormous numbers of docking solutions generated by Z-dock were filtered in several stages. First, complexes that do not satisfy the NMR observed residue-residue contacts were excluded. Second, the remaining complexes (239 complexes for hPD-1-hPD-L1 and 50 complexes for hPD-1-hPD-L2) were analyzed by visual inspection and only complexes that show proper interactions of the V domains were selected (29 complexes). Selected complexes were subjected to additional MD simulation and free binding energy calculations using the MMPBSA [38] module available within AMBER12. Only models with the best binding energies are selected for further structural analysis.

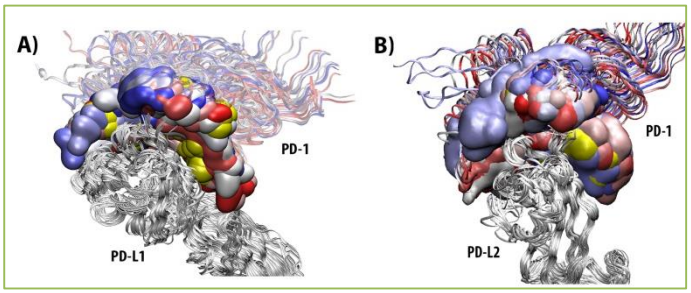

Figure 2. Top hits from protein–protein docking for (A) PD-1/PD-L1 and (B) PD-1/PD-L2. The top hits are superimposed on each other and the ensemble-based docking simulations allowed the V domains of the three proteins to extensively explore all possible conformations. Residues from PD-1 at the binding interface are shown in surface representation and colored by a distinctive color with the best hit is shown in yellow. Residues outside the range of the binding interface are shown in ribbon representations [30]. Reused with permission from Elsevier science, license number: 3617961067094.

Figure 1 represents the potential binding mode of hPD-1 to hPD-L1, revealing a major finding from our study. For the purpose of comparison, the structure and sequence of mouse PD1 (mPD1) were included and superimposed on their hPD-1 counterparts. As can be seen in the figure, hPD-1 and mPD-1 share approximately 65% sequence identity; nevertheless, certain regions at the binding interface with PD-L1 show notable discrepancies. The most obvious difference between hPD-1 and mPD1 is the replacement of the N64-V70 beta strand in mPD1 with a flexible loop (P63-Q71) in hPD1. This flexible loop at the binding interface resulted in different binding modes in the human complexes from the mouse complexes. It is noteworthy to mention that hPD-L1 and mPD-L1 share approximately 34% sequence identity, whereas their PD-L2 counterparts share more than 73% sequence identity. From the above data it is clear that it is difficult to generalize data drawn from mouse complexes to their human counterparts.

The differences in the binding poses of hPD-1 to hPD-L1 and hPD-L2 are illustrated in Figure 2. In this context, hPD-1 interacts in a different manner with the two ligands. The binding interface of hPD-1 with PD-L2 is larger than that for hPD1 binding to PD-L1, which may explain the observed greater affinity of hPD-1 to PD-L2 than its affinity for PD-L1 [39]. Furthermore, certain amino acid residues that exist only in PD-L2 contribute very favourably to the overall binding energy. For example, we have shown that Trp110 in PD-L2 contributes by almost -12 kcal/mol to the binding energy.

Our current focus is to address other related interactions to

fully model the whole hPD-1 pathway. For example, although previous studies have shown that glycosylation is not mandatory for PD1 binding [9], it will be interesting to study this phenomenon at the atomic level of details. Moreover, none of the available experimental crystal structures of murine PD-1 complexes with its ligands, mPD-L1 or mPD-L2 exhibited a glycosylation dependent interaction [40]. Also, the interaction of the intracellular domain of hPD-1 with other cytoplasmic signalling systems, such as SHP-2 [41], will enhance our understanding for the regulation mechanism by which T cell controls the duration and amplitude for a given immunological response. Ultimately this will create a new paradigm in the treatment of cancerous as well as other immune-related or infectious diseases.

In a nutshell; we have presented the first two precise models for hPD1 bound to its two known human ligands, hPD-L1 and hPD-L2. Our data suggests that the human complexes are remarkably different from that previously hypothesised based on the mouse-based models. Our ultimate goal is to build all the different protein-protein interactions involved in the PD-1 pathway, taking the PD-1 interactions with its two ligands as a baby step toward this goal. Understanding all these interactions will not only offer a way to construct the full picture of this mechanism, but also will highlight novel hotspots to target these interactions with new therapeutic modalities (work in progress).

Conflicting interests

The authors have declared that no competing interests exist.

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