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Affinity enhancement of nanobody binding to EGFR: in silico site-directed mutagenesis and molecular dynamics simulation approaches

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Epidermal growth factor receptor (EGFR), a transmembrane glycoprotein, is overexpressed in many cancers such as head-neck, breast, prostate, and skin cancers for this reason it is a good target in cancer therapy and diagnosis. In nanobody-based cancer diagnosis and treatment, nanobodies with high affinity toward receptor (e.g. EGFR) results in effective treatment or diagnosis of cancer. In this regard, the main aim of this study is to develop a method based on molecular dynamic (MD) simulations for designing of 7D12 based nanobody with high affinity compared with wild-type nanobody. By surveying electrostatic and desolvation interactions between different residues of 7D12 and EGFR, the critical residues of 7D12 that play the main role in the binding of 7D12 to EGFR were elucidated and based on these residues, five logical variants were designed. Following the 50 ns MD simulations, pull and umbrella sampling simulation were performed for 7D12 and all its variants in complex with EGFR. Binding free energy of 7D12 (and all its variants) with EGFR was obtained by weighted histogram analysis method. According to binding free energy results, GLY101 to GLU mutation showed the highest binding affinity but this variant is unstable after 50 ns MD simulations. ALA100 to GLU mutation shows suitable binding enhancement with acceptable structural stability. Suitable position and orientation of GLU in residue 100 of 7D12 against related amino acids of EGFR formed some extra hydrogen and electrostatic interactions which resulted in binding enhancement.

Keywords: EGFR; nanobody 7D12; molecular dynamic; affinity enhancement

1. Introduction

Epidermal growth factor receptor (EGFR) (Downward et al., 1984; Ullrich & Schlessinger, 1990) is a transmembrane protein from the tyrosine kinase receptor family. This family has four members which are collectively referred to as the HER1 (or EGFR), HER2, HER3, and HER4 (Jura et al., 2009). Four sub-domains in the extracellular domain of EGFR have been identified. One of these sub-domains is involved in homo- and heterodimerization, and the ligand binding is done by two of the other three sub-domains (Garrett et al., 2002; Ogiso et al., 2002) (Supplementary Figure s1). Growth and proliferation of human cells is stimulated by signal proteins such as epidermal growth factors (EGF), binding of these proteins to EGFR induces EGFR dimerization and subsequent signaling pathways. Integration of signals by EGFR binding to various extracellular ligands leads to different intracellular responses (Lin, Chang, & Fischer, 2015). Several roles for EGFR in cell growth, differentiation, survival (Stamos, Sliwkowski, & Eigenbrot, 2002), and repair have been reported. Several functions such as apoptosis suppression, motility increasing, cell division, and angiogenesis have also been reported for over expressed EGFR (Ding et al., 2015; Gschwind, Fischer, & Ullrich, 2004). The over expression of EGFR has been seen in many epithelial cancers such as colorectal, breast, head, neck, lung, prostate, and skin cancers. Over expression of EGFR in epithelial cancers in one type of these cancers could be used as a key target for anticancer drug delivery and cancer treatment such as specific drug delivery to these cancer cells by the EGF-bonded cancer drugs (Downward et al., 1984; Rungnim, Rungrotmongkol, Kungwan, & Hannongbua, 2016).

In order to interfere with EGFR signaling, two strategies have been applied. The first one is in the intracellular level and this occurs by blocking the receptor using the inhibitors of tyrosine kinase. The second strategy focuses on the receptor blocking from extracellular part using monoclonal antibodies (mAbs) to prevent the binding of the ligand (e.g. EGF). However presence of some

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