Plasma-induced oxidation of the cancer – natural killer cell inhibitory axis: a computational-experimental approach

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Abstract: Plasma-based immunotherapy aims to activate the immune system against cancer through treatment with cold atmospheric plasma (CAP). NK-cells form an interesting target for this, but the effects of plasma treatment on their interaction with cancer cells at the atomic level remain unexplored. We used molecular dynamics simulations to computationally investigate the effect of CAP-induced oxidation on NK-cell inhibiting ligands and the interaction between NK-cells and cancer cells.

Keywords: Non-thermal plasma, natural killer cells, cancer treatment, molecular dynamics

1. Introduction

Our body naturally produces an immune response against emerging tumours. However, cancer cells can learn to evade this immune response. Plasma-based immunotherapy, a recent addition to the field of plasma-oncology, aims to harness and improve the natural immune response against cancer through treatment with cold atmospheric plasma (CAP) [1]. Studies have been conducted to induce immunogenic cell death in cancer cells via CAP treatment to stimulate the immune system against these cancer cells [2-4]. Positive effects on immune cells themselves, like macrophages, have also been reported [5, 6].

Natural killer (NK) cells, lymphocytes of the innate immune system that have the ability to directly kill malignant cells, play a major role in the body's cancer immunosurveillance. For this reason, they form an attractive target in immunotherapy [7]. We previously showed that treatment of skin cancer cells with CAP augmented NK-cell mediated toxicity [8]. This effect was attributed to the observed change in expression of surface ligands on the treated cancer cells, induced by the reactive oxygen and nitrogen species (RONS) produced by the plasma. Indeed, whether or not an NK-cell will attack a certain cell is determined by the balance between activating and inhibiting signals received through surface receptors, binding to relevant ligands on the target cell membrane [9]. However, apart from intracellular effects, which can change the expression of ligands on the cancer cell surface, RONS can also interact with the ligands already expressed on the cells. It is known that plasma treatment can cause oxidation and conformational changes in proteins expressed on treated cells, which in turn can influence their ability to bind their receptors [10].

Here, we computationally investigate the effect of oxidation, as would be induced by plasma treatment, of the cell surface ligands HLA-Cw4 and HLA-E, on their ability to bind their NK-cell expressed receptors, respectively KIR2DL1 and the heterodimer NKG2A/CD94. These two complexes are part of the cancer – NK-cell inhibitory axis, and prevent NK-cell activation and toxicity upon binding to its target cell. Indeed, to NK-cells, expression of both ligand types is a sign that the target cell is healthy [11]. By employing molecular dynamics simulations, we determine the free binding energy of the two investigated complexes in both their native and oxidized state.

2. Computational details

The simulations were performed using the GROMACS software (2020.2), with the GROMOS 54A7 force field [12]. Each model system was prepared by placing the simulated protein complex in a simulation box with periodic boundary conditions and solvating it in water containing 150 mM NaCl. The system was then energy minimized using the steepest decent algorithm, followed by a 2 ns equilibration in the NVT ensemble (i.e., constant number of particles (N), volume (V) and temperature (T)), and a series of equilibrations totalling 10 ns in the NPT ensemble (i.e., constant number of particles (N), pressure (P) and temperature (T)) with decreasingly strong position restraints enforced on the heavy atoms of the proteins. The equilibrations were done at 310 K and 1.0 bar to mimic the conditions the proteins would experience in the body, employing the v-rescale thermostat and the Parrinello-Rahman barostat. A final equilibration, without any enforced position restraints, was performed for up to 310 ns.

To investigate the binding affinity of the protein ligand-receptor complex, the fully equilibrated complex was pulled apart by subjecting the NK-cell receptor to a harmonic potential while position restraining four residues buried inside the cancer cell ligand, in this way increasing the distance between both by 100 pm/ns for 40 ns. During the pulling, the receptor was position restrained only in the plane perpendicular to the pulling direction with so-called flat-bottomed position restraints. Along the reaction coordinate of this pulling simulation, 40 frames (later supplemented to up to 53 frames to assure adequate sampling) separated by 100 pm were isolated to serve as the initial structure in so-called umbrella sampling (US) simulations to sample conformational space at that pulled distance. Finally, the weighted histogram analysis method (WHAM) [13] was used to extract the free energy profile

along the pulling coordinate. The US simulations were performed for up to 75 ns, of which the final 60 ns were used for data collection.

The above explained simulation method was performed for both ligand-receptor complexes in their native as well as their oxidized state. Each model system was prepared in triplicate, meaning a total of 12 systems was simulated and investigated. The oxidized proteins were created with the Vienna-PTM web server [14] by replacing the relevant native amino acids with their oxidized form. The amino acids that would be oxidized were determined based on two factors. First, the amino acid types were chosen based on the work of Takai *et al.* [15], who investigated the susceptibility of amino acid oxidation by plasma treatment. The five amino acids most susceptible to oxidation were chosen. They are listed in Table 1, together with their oxidized form. Second, a solvent accessible surface (SAS) analysis was performed to investigate which of these amino acids would be reachable by the solvent and thus by the RONS produced by the plasma. As, in reality, only the cancer cells would be plasma-treated, only the ligand of both complexes was replaced by its oxidized state.

Table 1. Amino acid types oxidized in the simulations.

Native amino acid	**Oxidized state**
Methionine	Methionine sulfoxide
Cysteine	Cysteic acid
Tryptophan	6-hydroxytryptophan
Phenylanaline	Tyrosine
Tyrosine	3,4-dihydroxyphenylanaline

3. Results and discussion

Figure 1 shows the calculated free energy profiles of both simulated complexes in their native and oxidized form. It can be seen that the oxidation of the ligands has only very little effect on the profiles. The free binding energy, i.e. the depth of the potential well, of the HLA-E – NKG2A/CD94 complex was calculated to be (-138 ± 12) kJ/mol in its native form, changing to (-122 ± 19) kJ/mol in its oxidized form. For the HLA-C – KIR2DL1 complex, the calculated native and oxidized binding energies are (-82 ± 4) kJ/mol and (-89 ± 5) kJ/mol, respectively. This means that both investigated cancer cell ligands respectively have similar binding affinity to their NK-cell receptor regardless of being subjected to oxidation through plasma treatment.

The lack of a significant effect on the binding affinity can be explained by the fact that the amino acids affected most by the oxidation are not the main contributors to the interaction between the ligand and the receptor. Additionally, although both investigated ligands contain cysteine-mediated disulphide bridges, important for protein structure, our SAS analysis revealed that these are inaccessible to the solvent and thus the oxidizing species produced by the plasma. Indeed, no significant conformational change was observed in the oxidized proteins compared to their native form.

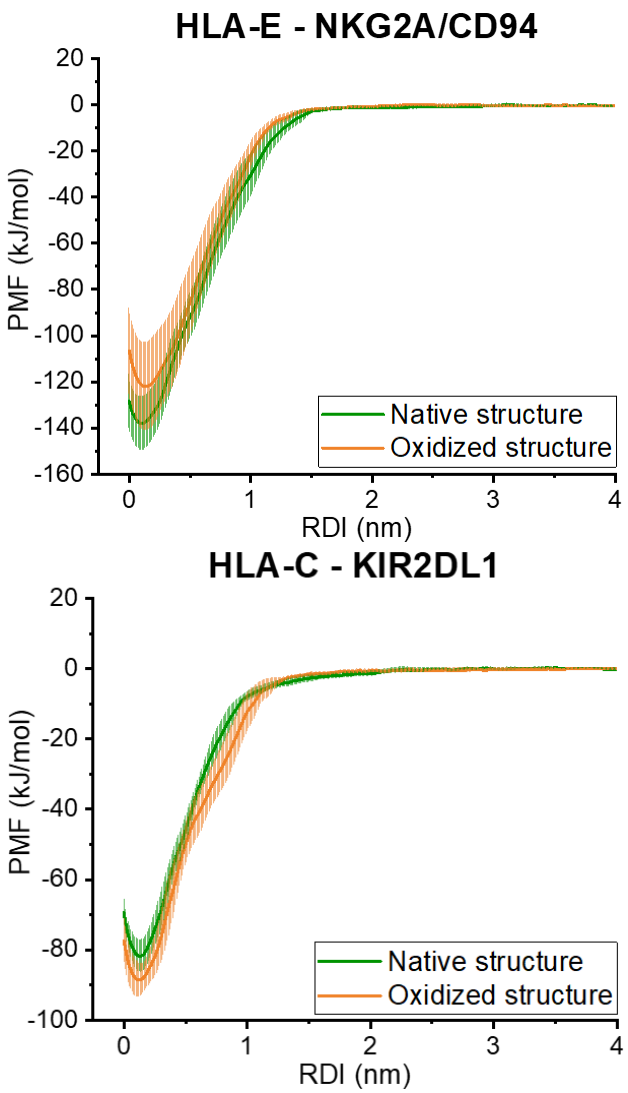

Fig. 1. Calculated free energy profiles of the simulated complexes in their native and plasma-oxidized forms. The potential of mean force (PMF) is shown as a function of the relative distance increase (RDI) between ligand and receptor.

Our previous, experimental results [8] indicated augmented NK-cell mediated toxicity after CAP-treatment of skin cancer cells. Hence, the combination of these experiments with our computational results shown here indicates that the improved NK-cell interaction after exposure to plasma is likely attributed to the effects of the treatment on the expression of the ligands important for the interaction between both cell types, as opposed to oxidative effects on the ligands already expressed on the cancer cell surface.

Our computational results will additionally be compared with new experiments (results in progress), to validate our model predictions. The experimental design consists of measuring the basal and post-treatment inhibitory ligand expression and evaluating the NK cell receptor-ligand

binding, followed by assessing the cytotoxic capacity of NK cells in a cancer – immune cell co-culture setting.

4. Conclusion

Molecular dynamics simulations were used to investigate the effect of oxidation, as would be induced by plasma treatment, of two important NK-cell inhibiting cell surface ligands. Our simulation results show that the oxidation has a negligible effect on the binding affinity of both complexes. Combined with our previous experiments, showing improved NK-cell toxicity against plasma-treated skin cancer cells [8], our computational results indicate that the improved interaction must be attributed to the changed ligand expression after treatment, and not to oxidative changes in the already expressed ligands.

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6. References

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