Staphylococcal Nuclease as a Model System for Investigating the Interaction between SND1 and MTDH
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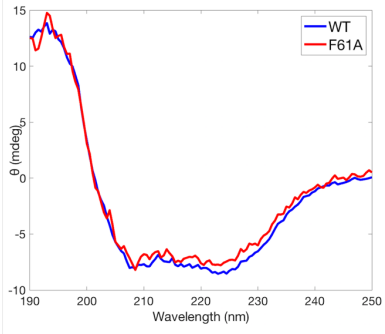
Introduction: Studies have shown that the Metadherin (MTDH) and Staphylococcal nuclease domain containing 1 (SND1) interaction plays a role in the tumorigenic effects of tumor-initiating cells (TICs). MTDH is overexpressed in over 40% of human primary breast tumors with connections to metastasis and poor prognosis. SND1 was found to be a requirement in various means of survival of TICs under stress conditions, such as DNA replication stress. Research has shown that the binding complex is necessary to prevent SND1 degradation and to support the function of both MTDH and SND1, making SND1 a suitable target for the suppression of their tumor-initiating effects. A crystal structure of the MTDH-SND1 complex has been reported, indicating the binding site of SND1 and suggesting that an F250A mutation at this site of SND1 plays a role in abolishing the MTDH-SND1 binding. Further understanding the mechanism of this interaction site can provide insight into possible drug targeting methods.

Materials and Methods: SNase Δ+PHS was the Staph Nuclease variant used as a model for the SN domains in SND1 due to its stability relative to SND1. On the SNase Δ+PHS variant, the equivalent mutation was at F61. The primers to make the mutation during site-directed mutagenesis were designed with Agilent QuikChange primer design tool. Site-directed mutagenesis was performed using the Lightning QuikChange Site-Directed Mutagenesis Kit. Circular dichroism was used to determine the changes in secondary structure from the mutation. The vibrational Stark effect (VSE) was used to determine the changes in the electrostatic field near the binding site. Protein Data Bank accession code for the MTDH-SND1 complex and SNase Δ+PHS are 4QMG and 3BDC respectively.

Results and Discussion: We used circular dichroism to analyze the structural differences between the WT SNase and mutated SNase. With circular dichroism, the structural elements of proteins, α-helixes and β-sheets, have specific circular dichroism signatures at specific wavelengths. Comparing the resulting bands, θ, of the WT SNase structure and the F61A SNase structure indicates any changes in the structural percentages of α-helixes and β-sheets present, and thus, if the mutation changed the protein structure in any manner. Figure 1 displays the results of the average of five WT SNase CD runs and F61A SNase CD runs. The two negative troughs at ~222nm and ~208nm and peak in the 190-200nm range indicates the proteins folded correctly. Analytical estimates of the data suggest slight differences in the component percentages of α-helixes and parallel and anti-parallel β-sheets.



The Vibrational Stark Effect (VSE) is used to measure the electrostatic field around a probe placed in a protein. We inserted a vibrational probe at location A109 on the SNase variant approximately 10Å from the F61A mutation site in order to analyze the environment around the binding site for MTDH. The measured change in absorption frequency is related to the electrostatic field through the equation, $\Delta E = hc\Delta\nu = -\Delta\vec{\mu}\cdot\Delta\vec{F}$. Mapping the change from the amino acid elucidates the binding mechanism and the type of inhibitor needed to prevent their binding and halt their tumorigenic functions.

Figure 1. *CD spectra of the WT SNase and F61A mutated SNase.*

Conclusions: An F61A mutation was made on SNase Δ+PHS to analyze its properties as a potential drug target. Preliminary results suggest F61A may alter the electrostatic field without largely disrupting the structure of SNase, this suggests further experiments to investigate the role of the electrostatics in binding to MTDH.

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