

Site-Directed Mutagenesis of SOD3: Inactivation of an Extracellular Antioxidant Enzyme

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Extracellular superoxide dismutase (EcSOD) is one of three enzymes in the superoxide dismutase family, which are responsible for the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen. EcSOD (SOD3) is secreted from cells into the surrounding plasma and often bound to the extracellular matrix, whereas SOD1 & SOD2 are responsible for the removal of intracellular superoxide. The proteinaceous milieu of the ECM plays an important role in cellular signaling pathways and cell migratory properties. One example is the cleavage of heparan sulfate (HS) by heparanase, which may cause the release of growth factors and change the migratory potential of a cell. EcSOD, has been shown to decrease the metastatic potential of cancer cells by interacting with heparanase and HS. EcSOD has a natural variant that lacks the heparin-binding domain of the wild-type plasmid (EcSOD Δ HBD), allowing it to circulate more readily in the blood, a feature that may explain EcSODs potency as an anti-cancer agent. However, it has yet to be determined if EcSOD decreases heparanase activity through competition for HS binding or by inhibiting heparanase activity via the enzymatic reduction of extracellular superoxide molecules. **In this experiment, we performed site-directed mutagenesis on the SOD3 cDNA plasmid to create several enzymatically inactive EcSOD enzymes of both natural forms (EcSOD, EcSOD Δ HBD).** For each form, plasmids were mutated at the Arg-205 active site residue and the His-96/His-98 copper-binding domain residues, both of which would be predicted to eliminate the enzymatic activity of EcSOD when mutated to the amino acid alanine. The SOD3/H96A,H98A plasmid was successfully created as verified by DNA sequencing. Transfected HEK-293 cells expressed the plasmid vector to make the expected EcSOD/H96A,H98A protein. The activity of the resultant protein is under further study. The SOD3 R205A mutant was not detectably expressed as protein in transfected cells; further attempts are underway. Transient expression of the inactive EcSOD enzymes will allow us to determine whether the enzymatic activity of EcSOD or its binding to HS is responsible for the observed decrease in metastatic potential of cancer cells. This work is supported by the Interdisciplinary Graduate Program Research Program Summer Undergraduate Research Program at the University of Iowa, funded by the NIH and by R01 CA115438 (FED).