Poster Number:

Engineered human L-Methioninase for therapeutic purposes.

Wei-Cheng Lu[1,3], Wupeng Yan[2], Olga Paley[1], Kendra Garrison[1], Andrew Ellington[2], Yan Zhang[2], Everett Stone[1,3], and George Georgiou[*,1,3]

[1]Department of Chemical Engineering, University of Texas at Austin, Austin, TX, [2]Department of Biochemistry, University of Texas at Austin, Austin, TX, [3]Aeglea BioTherapeutics, Austin, TX

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In cancer biology research, it has been found that cancer cells exhibit different metabolism compared to normal cells and it has been shown that some types of cancer cells, such as glioblastomas, medulloblastomas and neuroblastomas are much more sensitive than normal cells to methionine starvation. Past studies have shown that methionine-dependent tumor cells are not able to survive if the serum methionine is decreased to ≤ 5uM. Systemic depletion of serum methionine can be achieved by *Pseudomonas putida* methionine gamma-lyase (pMGL) but it has proven to be rapidly inactivated *in vitro* and be highly immunogenic in primate models. In order to apply systemic methionine depletion to human cancer therapy, we engineered human cystathionine γ-lyase to accept methionine as a substrate and have isolated several human Methioninase (hMETase) variants with high activity. Several active hMETase variants isolated from a phylogenetic analysis library and the best variant, hMETase V8.4 showed a 10-fold improved K_M (12.2 vs. 1.8mM) and 10-fold better k_{cat}/K_M values (0.59 to 5.3 1/s.1/mM) in degrading methionine compared to our previous version of variant, hMETase V3.1. Furthermore, hMETase V8.4 showed greater stability in thermal melting analyses (melting temperature: 63.2 vs. 70.2°C) and also in serum stability (half-life: 75 vs. >100 hours) compared to hMETase V3.1. In pharmacodynamic analyses, hMETase V8.4 efficiently lowered serum methionine concentration from 75 μM to ~15 μM in 48 hours without the requirement of a methionine restricted diet (one dose: 50 mg/ kg). In addition, we tested the efficacy of hMETase V8.4 on C57L/6 mice bearing A375 melanoma xenografts and it significantly improved the median survival from 35 – 43 days compared to the control group. The hMETase V8.4 efficiently lowered serum methionine concentration in pharmacodynamic analyses. Also, it significantly improved the survival time of C57L/6 mice bearing A375 melanoma xenografts. The hMETase V8.4 is a promising therapeutic enzyme candidate for systemic methionine depletion in cancer therapy.