A novel fluorophosphonate probe for the identification of new valacyclovir-activating enzymes

Vikram P. Shenoy (Advisor: Gordon L. Amidon)

Medicinal Chemistry Third-Year Seminar

Thursday, March 16, 2017 at 4:00 PM

As pharmaceutical companies increasingly turn to the prodrug strategy to address shortcomings in bioactive molecules, identifying prodrug-activating enzymes (PAEs) will become ever more crucial. Identifying the PAEs for a prodrug allows for further characterization of enzymatic properties, such as tissue distribution and catalytic efficiency, which permit better prediction of the pharmacokinetic and pharmacodynamic profiles of the prodrug. If the PAEs have homologs, interspecies differences can be evaluated, facilitating preclinical studies. Furthermore, knowledge of PAEs elucidates potential prodrug-drug interactions that could have harmful consequences. Finally, a PAE with the appropriate combination of properties for a given therapeutic application can serve as a target for rational prodrug design such that the prodrug would act as its substrate.

In this vein, our lab has adapted a modern chemoproteomic technique, known as activity-based protein profiling (ABPP), for the discovery of serine hydrolase PAEs. ABPP relies on chemical probes that selectively and irreversibly bind the catalytic residue of mechanistically related classes of enzymes (e.g. serine hydrolases). The probes are linked to a “reporter” group, such as a fluorophore or a biotin group, for downstream visualization or enrichment, respectively. Given that only native, unobstructed enzymes are able to react with the probe, an ABPP assay provides a readout of both enzyme activity and active site occupancy state. When carried out in a competitive format using a prodrug, this technique can detect enzymes whose active sites are occupied by the prodrug and are thus its putative PAEs.

The current study focuses on valacyclovir (VACV), the 5’-valyl ester prodrug of acyclovir, whose primary PAE in humans, a serine hydrolase known as BPHL, was discovered in our lab 15 years ago. Attempts to use the widely employed fluorophosphonate ABPP probe FP-biotin to find new VACV PAEs failed. Consequently, a novel fluorophosphonate probe was synthesized by introducing a moiety based on the only known inhibitor of human BPHL, AEBSF. In vitro assays and subsequent ABPP data gathered with this new probe indicate the presence of other enzymes in the human intestinal epithelium that activate VACV, which the present study aims to identify and validate.