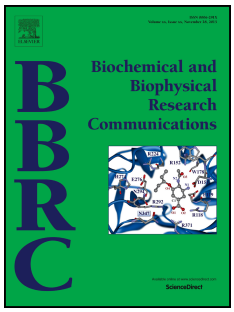
Accepted Manuscript



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PII: S0006-291X(16)31890-3

DOI: 10.1016/j.bbrc.2016.11.035

Reference: YBBRC 36737

To appear in: *Biochemical and Biophysical Research Communications*

Received Date: 31 October 2016

Accepted Date: 7 November 2016

Please cite this article as: K. Sakamoto, S. Sogabe, Y. Kamada, S.-i. Matsumoto, A. Kadotani, J.-i. Sakamoto, A. Tani, Discovery of GPX4 inhibitory peptides from random peptide T7 phage display and subsequent structural analysis, *Biochemical and Biophysical Research Communications* (2016), doi: 10.1016/j.bbrc.2016.11.035.

Discovery of GPX4 Inhibitory Peptides from Random Peptide T7 Phage Display and Subsequent Structural Analysis

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Abstract

The phospholipid hydroperoxidase glutathione peroxidase (GPX4) is an enzyme that reduces lipid hydroperoxides in lipid membranes. Recently, GPX4 has been investigated as a target molecule that induces iron-dependent cell death (ferroptosis) selectively in cancer cells that express mutant Ras. GPX4 inhibitors have the potential to become novel anti-cancer drugs. However, there are no druggable pockets for conventional small molecules on the molecular surface of GPX4. To generate GPX4 inhibitors, we examined the use of peptides as an alternative to small molecules. By screening peptide libraries displayed on T7 phages, and analyzing the X-ray crystal structures of the peptides, we successfully identified one peptide that binds to near Sec73 of catalytic site and two peptides that bind to another site on GPX4. To our knowledge, this is the first study reporting GPX4 inhibitory peptides and their structural information.

Keywords

Peptide, Inhibitor, GPX4, Crystal structure, Phage display

Introduction

GPX4 is a member of the glutathione peroxidase protein family, including GPX1–8, and is classified as a selenoenzyme that has a selenocysteine (Sec) in the catalytic pocket [1]. GPX4 catalyzes the reduction of lipid hydroperoxides to the corresponding alcohols using glutathione (GSH) as a reductant [2]. In 2014, Wan SY et al. reported that GPX4 regulates ferroptosis, an iron-dependent form of nonapoptotic cell death in cancer cells [3, 4]. Ferroptosis is induced by increased reactive oxygen species and the accumulation of peroxidized lipids in cells [5]. Therefore, GPX4 inhibitors are expected to be useful as novel anti-cancer drugs.

The crystal structure of GPX4 has been resolved [6]. Unfortunately, the molecular surface is round and has no druggable pockets, in contrast to conventional targets like enzymes or GPCRs that have deep cavities for binding of small molecules [7–9]. Although there is catalytic site surrounding Sec73, it is too small to bind small molecules. Furthermore, allosteric sites of GPX-isoforms have not been reported. Considering these points, GPX4 is not an ideal druggable target, and small molecule inhibitors of GPX4 are considered to be much weaker than inhibitors for other target classes, even if an inhibitor is obtained.

To overcome this hurdle, we examined the feasibility of using peptides as drug molecules. Since peptides are larger than small molecules, they are expected to have higher affinity and selectivity to target proteins. In this study, we used phage display technology which makes it possible to display a variety of foreign amino acid sequences on the outer surfaces of bacteriophages by fusing peptides to coat proteins [10]. GPX4-binding phages were isolated from the libraries through biopanning selection, and the displayed amino acid sequences can be easily deduced by sequencing the DNA of bound phages. Thus, this system provides a powerful tool for generating bioactive peptides, and the binding ligands obtained from phage display are expected to accelerate drug discovery and development [11–13].

As a result of phage display screening for GPX4-binding peptides, we successfully