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Screening E3 Substrates Using a Live Phage Display Library

Youhe Gao
Beijing Normal University, China

Ubiquitin ligases (E3s) determine specificity of ubiquitination by recognizing target substrates. However, most of their substrates are unknown. Most known substrates have been identified using distinct approaches in different laboratories. We developed a high-throughput strategy using a live phage display library as E3 substrates in in vitro screening. His-ubiquitinated phage, enriched with Ni-beads, could effectively infect E. coli for amplification. Sixteen natural potential substrates and many unnatural potential substrates of E3 MDM2 were identified through 4 independent screenings. Some substrates were identified in different independent experiments. Additionally, 10 of 12 selected candidates were ubiquitinated by MDM2 in vitro, and 3 novel substrates, DDX42, TP53RK and RPL36a were confirmed ex vivo. The whole strategy is rather simple and efficient. Non-degradation substrates can be discovered. This strategy can be extended to any E3s as long as the E3 does not ubiquitinate the empty phage.

Biography

Youhe Gao Professor Beijing Normal University. He received his MD from Peking Union Medical College, his Ph.D from University of Connecticut and postdoctoral training from Beth Israel Deaconess Medical Center Harvard Medical School. He was the professor of Department of Pathophysiology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences/ Peking Union Medical College from 2001-2014. His research interests include biomarker discovery in urine proteome, protein interaction and related bioinformatics.

gaoyouhe@bnu.edu.cn

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