Секция «Биоинженерия»

KRT16 is a novel participant of the Integrated Stress Response

Научный руководитель – Евстафьева Александра Георгиевна

Шошинова Мария Сергеевна
Студент (специалист)
Московский государственный университет имени М.В.Ломоносова, Факультет биоинженерии и биоинформатики, Москва, Россия
E-mail: littlecrazydevil@yandex.ru

An Integrated Stress Response (ISR) is a gene expression program regulated by activating transcription factor 4 (ATF4). The program aims to improve cell survival during different kinds of stress. In our laboratory it has been revealed that increased levels of ATF4 induce KRT16 transcripts, and conversely, ATF4-specific RNA-interference suppresses the induction of KRT16 mRNA caused by mitochondrial respiratory chain inhibition. These results suggest that KRT16 may be a novel participant of ISR.

KRT16 encodes a cytokeratin, associated with activation and hyperproliferation of keratinocytes synthesized in mammalian cells during epidermal barrier breach or innate immunity response. Mutations in this gene are involved in several types of squamous cell carcinoma and cause a congenital disease Pachyonychia congenita. ATF4-dependent regulation of KRT16 hasn't been described yet in the scientific literature.

The mechanism of ATF4-dependent regulation of KRT16 was studied by the method of reporter constructs where transcription of the firefly luciferase gene was driven by KRT16 promoter. KRT16 promoter region containing two putative ATF4-binding sites was amplified from total human DNA by PCR and after sequencing inserted into pGL3-Basic expression vector upstream of luciferase reporter gene. In response to ectopic expression of ATF4 there was a strong increase in luciferase activity in transfected human cells indicating that ATF4 is able to induce KRT16 promoter. Then the distal putative ATF4-binding site was deleted. This deletion had no effect on the induction of luciferase activity implying that the distal site does not contribute significantly to induction of KRT16 promoter.

To localize the ATF4-responsive element on KRT16 promoter, the proximal site was inactivated by site-directed mutagenesis. Mutations completely prevented the induction of the KRT16 promoter-driven luciferase expression indicating that the proximal site is a true ATF4-responsive element. The ATF4-driven induction of KRT16 was tested in the cells exposed to several stresses that cause ISR.

Work was supported by Russian Foundation for Basic Research grant 15-04-04945