0.5 М сахарозу с целью удаления остатков цитоскелетных структур, которые могут оставаться связанными с ядрами после лизиса клеток. Поскольку количество ACTN4 в цитоплазме значительно превышает его содержание в ядре, этот этап критичен для корректного анализа ядерного ACTN4.

После получения и очистки ядер мы исследовали присутствие ACTN4 в растворимой фракции (нуклеоплазме) и в хроматине. Для расщепления геномной ДНК и экстракции белков хроматина ядра дополнительно обрабатывали бензоназой (Moreno et al., 1991). На рис. 5а, дорожка 3 видно, что в растворимой фракции ядерных белков гораздо слабее детектируются фракция гистонов (15 кДа). Иммуногибридизация показала, что количество белка ACTN4 в хроматиновой фракции значительно больше, чем в растворимой (рис. 5б).

Полученные нами данные согласуются с опубликованными сообщениями о том, что ACTN4 обнаруживается в комплексах ремоделирования хроматина, таких как INO80 (Kumeta et al., 2010). INO80 состоит из 15 белков (Shen et al., 2000), которые участвуют в регуляции транскрипции, репликации и репарации молекулы ДНК (Poli et al., 2017).

Таким образом, мы обнаружили, что в линиях H1299 с полным нокаутом гена *ACTN4* происходит подавление экспрессии некоторых, но не всех, NF-kB-зависимых генов. Тем не менее нам не удалось выявить зависимости между влиянием *ACTN4* на экспрессию генов и повышенной резистентностью нокаутных клеток к ДНК-повреждающим препаратам. Более того, мы не выявили какого-либо влияния активности NF-kB на устойчивость клеток H1299 к генотоксическому стрессу. Обнаруженное нами присутствие ACTN4 в хроматиновой фракции позволяет предположить его непосредственное влияние на сборку комплексов белков, участвующих в репарации ДНК.

БЛАГОДАРНОСТИ

В работе использовано оборудование Центра клеточных технологий Института цитологии РАН; авторы благодарны за предоставление доступа к его конфокальной платформе CQ1.

ФИНАНСИРОВАНИЕ РАБОТЫ

Работа выполнена при внутренней финансовой поддержке (ВФНД) Института цитологии РАН (проект “Участие альфа-актинина 4 (ACTN4) в репарации двухцепочечных разрывов ДНК”).

СОБЛЮДЕНИЕ ЭТИЧЕСКИХ СТАНДАРТОВ

В экспериментах работы животные и люди не участвовали.

КОНФЛИКТ ИНТЕРЕСОВ

Авторы заявляют об отсутствии конфликта интересов.

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ACTN4-DEPENDENT REGULATION OF DOUBLE-STRAND DNA BREAK REPAIR IS INDEPENDENT OF NF-KB ACTIVITY

D. V. Kriger[a,*], G. V. Vasilevaa, E. V. Lomerta, D. G.Tentlera

[a] *Institute of Cytology, Russian Academy of Sciences, St-Petersburg, 194064, Russia*
** E-mail: daryamalikova@gmail.com*

α-Actinin-4 is an actin-binding protein that is involved in a wide range of cellular processes. Along with actin and other proteins of the actin cytoskeleton, α-actinin-4 was found not only in the cytoplasm, but also in the nucleus of various cells. As a nuclear protein, it is involved in regulation of certain transcription factors. In particular, it can regulate transcriptional activity of NF-kB, which largely determines the resistance of cancer cells to apoptosis and anticancer therapy. During our previous studies, it was found that α-actinin-4 can influence resistance of cancer cells to topoisomerase II inhibitors and determine the efficiency of DNA double-strand break repair. We have demonstrated that α-actinin-4 interferes with the assembly of complexes involved in DNA repair via NHEJ and HRR, which in turn leads to an imbalance between these pathways. In this study, we were answering to the question of how α-actinin-4 is involved in the regulation of the DNA double-strand breaks repair following genotoxic stress. Our results indicate that the effect of α-actinin-4 on repair progression in H1299 non-small cell lung cancer cells does not depend on the transcription factor NF-kB activity. We found that in the nucleus of H1299 cells, α-actinin-4 is localized not only in the nucleoplasm, but also reveals close association with chromatin.

Keywords: ACTN4, NF-kB, DNA repair, Non-small cell lung cancer (NSCLC)