Влияние эпигенетических модуляторов (BRD, KDM5, SMARCA, DOT1L) на пролиферацию нормальных и опухолевых клеток с инактивацией генов *PTEN u RB1*

Epigenetic modulators (BRD, KDM5, SMARCA, DOT1L) inhibition and its effect on proliferation of normal and cancer cells with inactivated *PTEN and RB1*

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INTRODUCTION

Опухолевые заболевания – одна из ведущих причин смертности в мире. Разработка лекарств и методов лечения злокачественных новообразований является важной и не до конца решенной научной задачей. Одной из основных причин развития опухолей являются мутации в генах супрессорах опухолевого роста. В нашей работе мы создали модель опухолевого роста с помощью инактивации двух наиболее часто мутированных опухолевых супрессоров, *PTEN* и *RB1.* С помощью этой модели мы оценивали влияние различных препаратов и генов на рост опухолевых клеток по сравнению с нормальными клетками. Мы применили два разных подхода: 1) с использованием различных химических препаратов - ингибиторов определенных белков (фармакологический подход); 2) с помощью выключения соответствующих генов посредством РНК-интерференции (генетический подход). Для ингибирования мы выбрали регуляторы эпигенетического состояния клетки: DOT1L, KDM5C, KDM5D, BRD2, BRD3, BRD4, SMARCA2 и SMARCA4. На основании полученных результатов из этого списка генов мы выбрали потенциальные мишени для дальнейшей разработки противоопухолевых препаратов.

Cancer is one of the leading causes of mortality in the world. Anti-cancer drug and therapeutics development is still an important and not fully solved problem. Mutations of tumor suppressor genes are one of the major mechanisms of tumor development and growth. Here, we created a model of tumor growth by inactivating two tumor suppressor genes most frequently mutated in human cancers – *PTEN* and *RB1*. Using this model, we assessed how different chemical compounds and human genes affect the growth of tumor cells compared to normal cells. We used two methods: 1) treating cells with chemical inhibitors that selectively modulate the function of proteins (pharmacological approach); 2) silencing corresponding genes by using RNA interference (genetic approach). For our studies, we chose the following epigenetic regulator genes to inhibit: DOT1L, KDM5C, KDM5D, BRD2, BRD3, BRD4, SMARCA2 and SMARCA4. Based on our results, we selected potential targets for further investigation and anti-cancer therapeutics development.

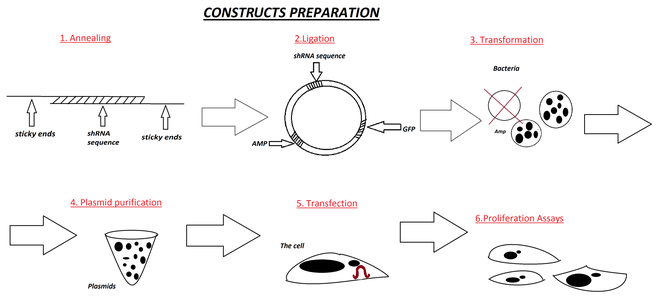

Figure 1. ShRNA cloning scheme.

MATERIALS AND METHODS

Drugs (химические препараты**):**

JIB_04 is KDM1A, KDM4C, KDM6B, KDM5A, KDM5C and KDM5D inhibitor

EPZ004777 is DOT1L inhibitor.

JQ1 is forceful inhibitor of BET bromodomains proteins BRD2, BRD4, BRD3, BRDT

PFI3 is selective inhibitor of bromodomain in SMARCA4 and SMARCA2

Cell culture and transfections

HEK293 cells were cultured in DMEM (high-glucose, glutamine, 10% FBS, 1% pen/strep). shRNAs for transfections were cloned into pTRC8 vector, purified using Qiagen miniprep kit. Lipofectamine 2000 (Invitrogen) was used as transfection reagent according to manufacturer's protocol. Transfection efficiency was assessed on Axiovert200 with excitation at 495 nm, emission at 517 nm.

Proliferation assays (измерение пролиферации**):**

Quantification of cell proliferation

Crystal violet (CV).	**ATP.**	
Cells were fixed with 4 % formalin solution and dyed with 0.05 % CV. Optical density was measured in Microplate Absorbance Reader (540 nm).	Cells were quantified by ATP concentration, using ATPlite kit. Luminescence reaction : O_2 + Luciferin + ATP + Luciferase→ Light + CO_2 + PPi + AMP + Oxyluciferin. Luminiscence was measured in luminometer.	**Confluency measurement** was made using the automatic camera in Clone Select Imager Genetix.

RESULTS

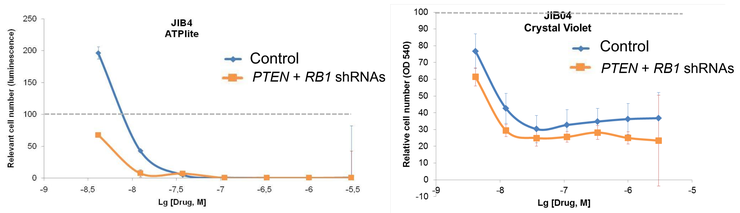

Figure 2. Cell proliferation measured with ATPlite (A) and Crystal Violet (B).

HEK293 cells transfected with control or *PTEN* + *RB1* shRNAs and treated with JIB04 for 72 hr.

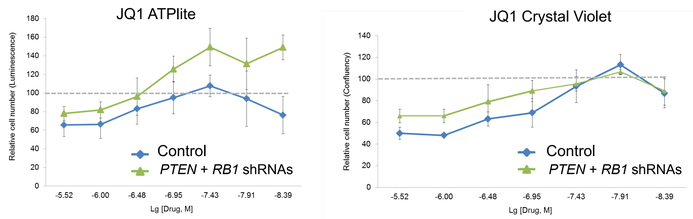

Figure 3 . Cell proliferation measured with ATPlite (A) and Crystal Violet (B)

HEK293 cells transfected with control or *PTEN* + *RB1* shRNAs and treated with JQ1 for 72 hr.

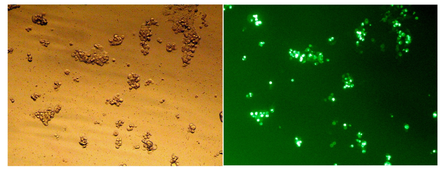

Figure 4. GFP expression in transfected cells.

Wide-field (left) and fluorescent microscopy (right) images of HEK293 cells, transfected with *PTEN* + *RB1* shRNA. GFP expression confirms successful transfection.

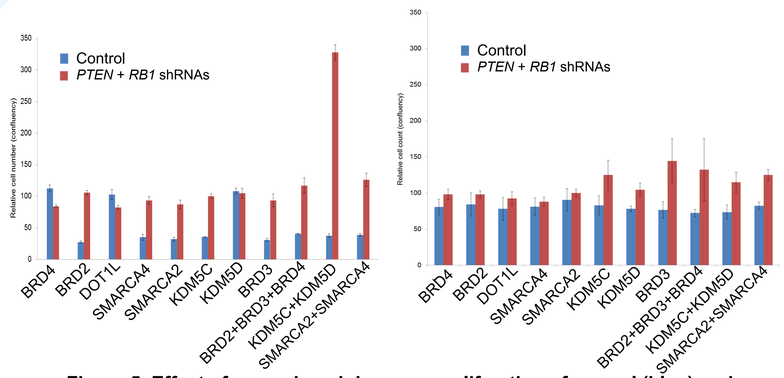

Figure 5. Effect of genes knockdown on proliferation of normal (blue) and *PTEN+RB1* shRNA-treated cancer (red) cells.

HEK293 cells were transfected with control or *PTEN* + *RB1* shRNAs for 72 hr, and their proliferation was assessed by confluency measurement.

CONCLUSIONS

1) Drug JQ1 (BRD inhibitor) increases tumor cells proliferation in comparison to normal cells. This result was confirmed by BRD2, BRD3, BRD4 shRNAs.

Препарат JQ1 (ингибитор BRD) усиливает пролиферацию опухолевых, но не нормальных клеток. Эти данные подтве ржда ются генетическим методом при использовании shРНК против генов семейства BRD.

2) Drug JIB4 (KDM5 inhibitor) inhibits tumor cells proliferation significantly more than proliferation of normal cells.

При использовании препарата JIB04 пролиферация опухолевых клеток значительно уменьшается по сравнению с нормальными клетками.

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