Synthetic lethality approach: the search for genetic, pharmacological and metabolic targets for *TSC2*-deficient cells

Принцип синтетической летальности: поиск гентических, фармакологических и метаболических мишеней для *TSC2*-дефицитных клеток

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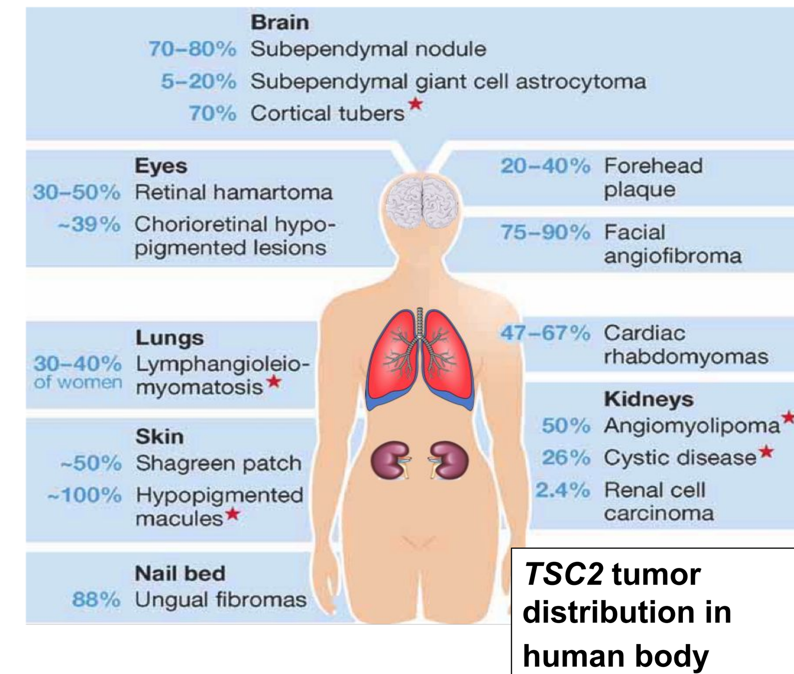
Introduction

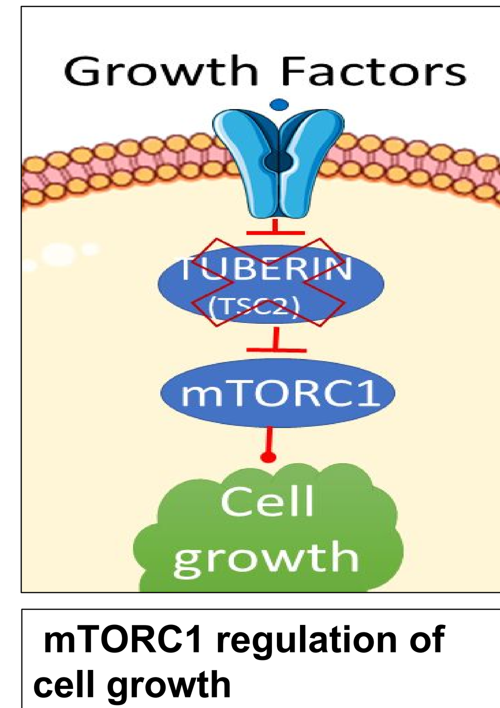
Oncological diseases cause every sixth death in the world. Mutations in the gene *TSC2* lead to activation of the kinase complex mTORC1 and development of tuberous sclerosis, characterized by formation of tumors in lungs, brain, kidneys, skin and heart. Functional activation of the kinase complex mTORC1 is observed in 80% of cancer cases. In our project we use synthetic lethality approach to search for new targets, which could be used to selectively kill tumor cells with inactivated *TSC2*.



TSC2 tumor distribution in human body

Онкологические заболевания являются причиной каждой 6-й смерти в мире. Мутации в гене *TSC2* приводят к активации киназного комплекса mTORC1 и развитию туберозного склероза, который характеризуется образованием опухолей в легких, мозге, почках, коже и сердце. Функциональная активация киназного комплекса mTORC1 наблюдается в 80% случаев всех опухолевых заболеваний.

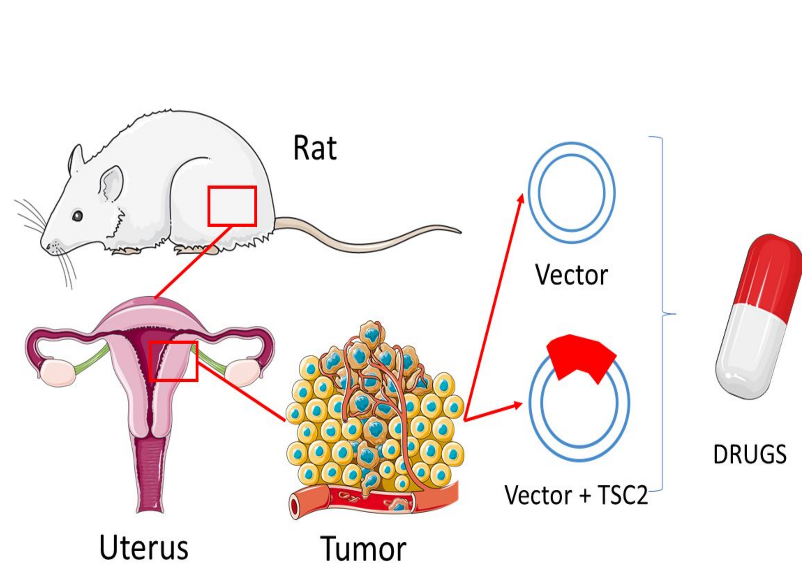
В нашем проекте мы использовали принцип синтетической летальности для поиска новых мишеней, воздействие на которые вызывает селективную гибель опухолевых клеток с инактивацией гена *TSC2*.



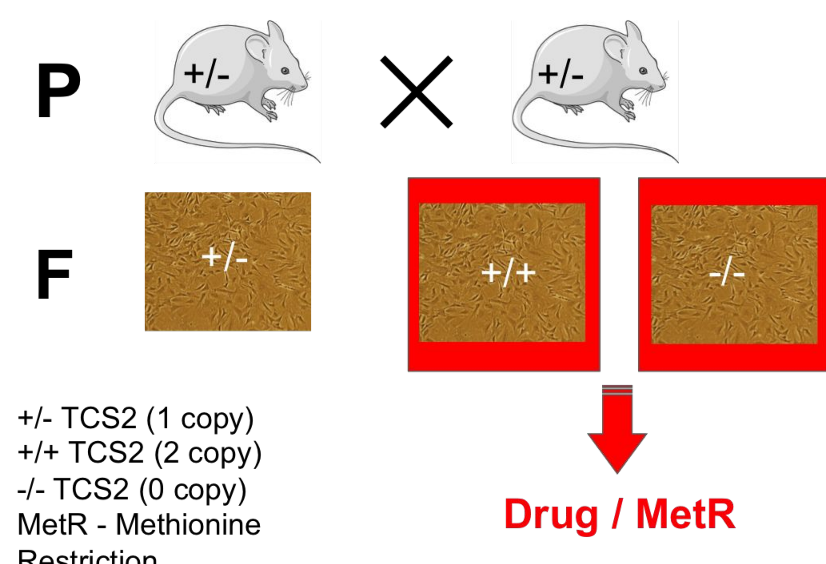
mTORC1 regulation of cell growth

Materials

Cell culture and transfection: HEK293T cells, Elt3 cells, MEFs

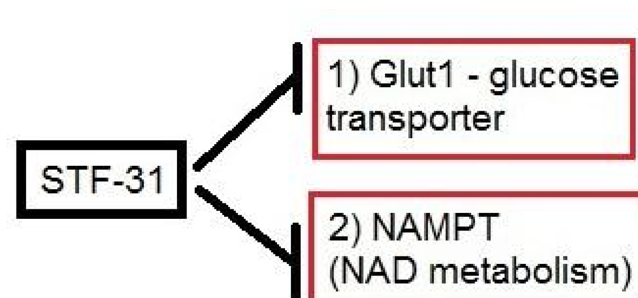


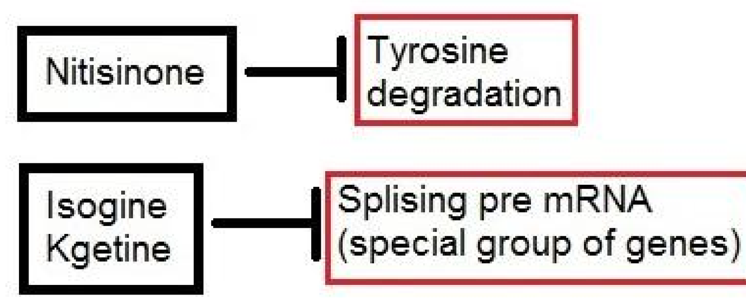
ELT3 (Eker Rat-derived TSC2-null cells)



Mouse Embryonic Fibroblasts (MEFs)

Drugs: STF31, Nitisinon and Isoginkgetine

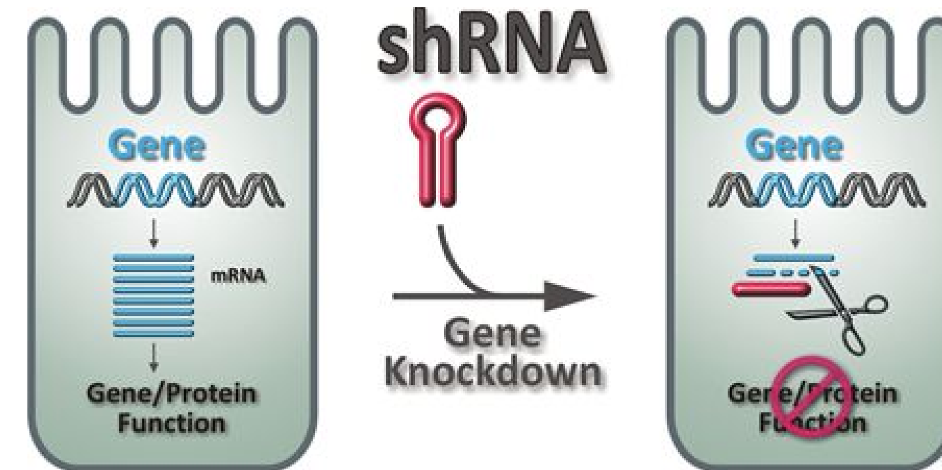




Methods

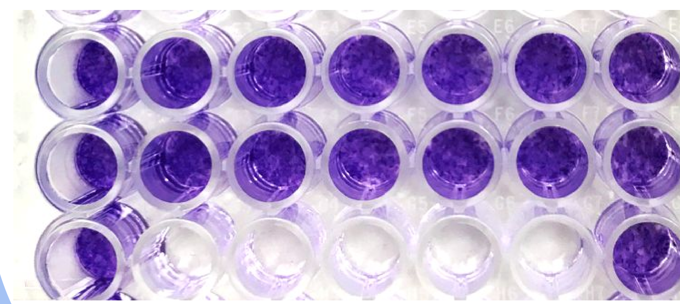
shRNA cloning protocol (rotations):

1) shRNA sequences were chosen based on complementarity to the target genes
2) Oligonucleotides were annealed and ligated into pre-made shRNA plasmid vector
3) The ligation mixes were transformed into bacteria and selected for ampicillin resistance
4) Single-cell colonies were grown in liquid culture
5) Plasmid DNA were purified using mini-prep kit
6) Plasmid DNA concentration was measured with Nanodrop
7) Plasmid DNA was transfected into HEK293T cells using Lipofectamine2000

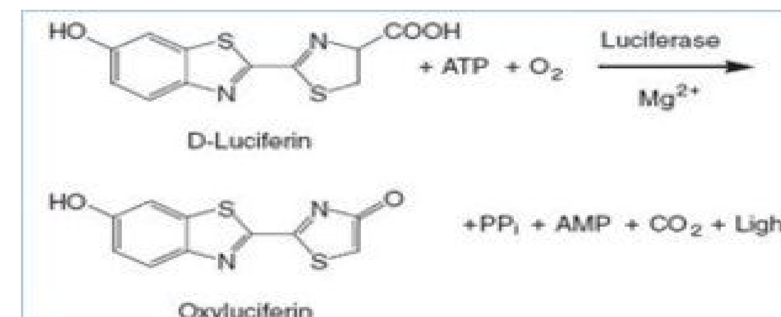


Proliferation assays:
Cell proliferation was measured using ATPlite and Crystal Violet.

Crystal Violet:

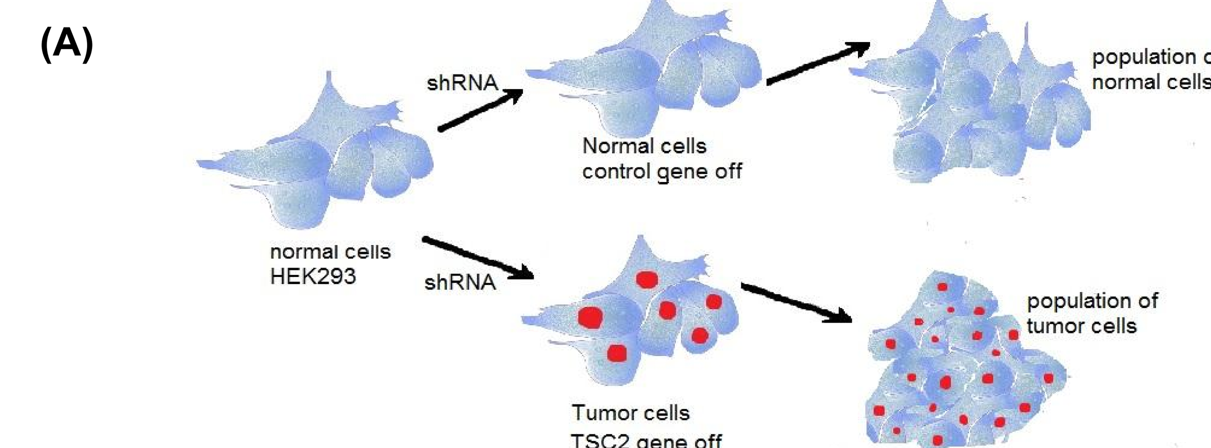


ATPlite:

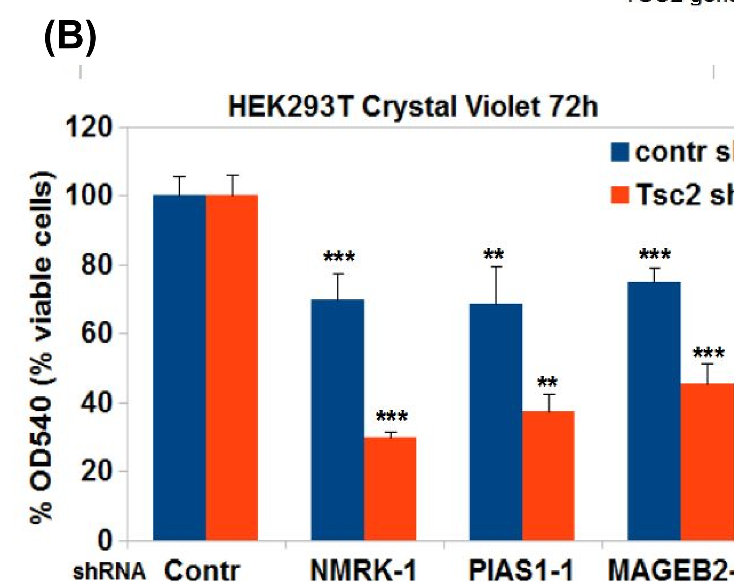


Genetic Approach

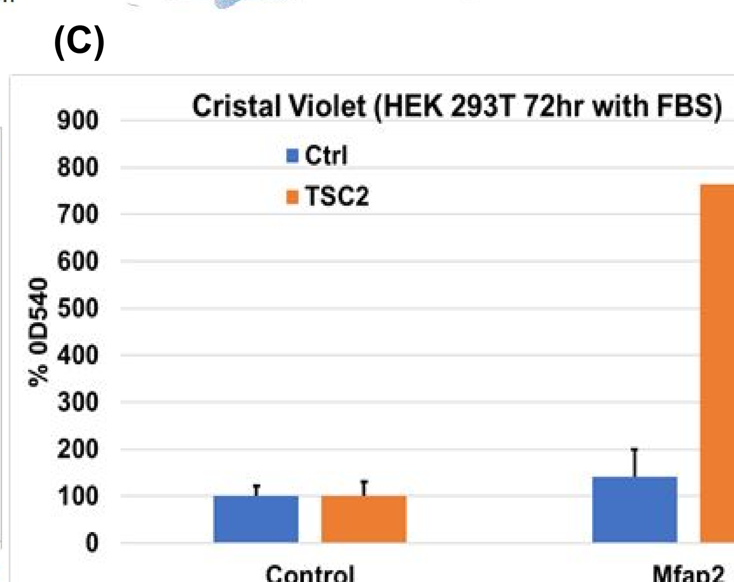
(A)



(B)



(C)



(A) HEK293T cells were co-transfected with control or *TSC2* shRNA together with shRNA for gene of interest for 72 hours.

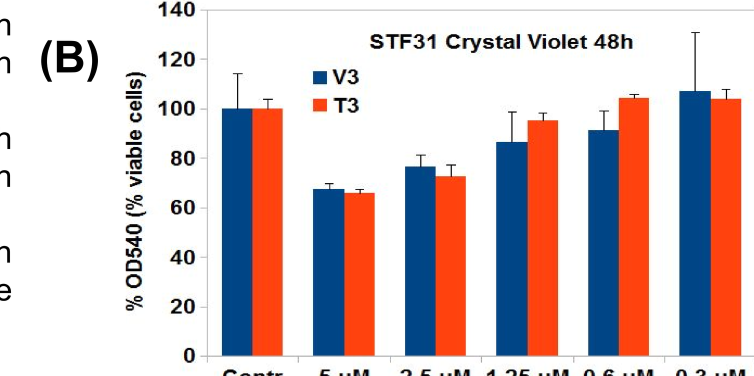
(B) shRNAs NMRK-1, PIAS1-1 and MAGEB2-2 selectively decrease proliferation of *TSC2*-deficient cells as measured by Crystal Violet assay

(C) shRNA (Mfap2) selectively increases proliferation of *TSC2*-deficient cells

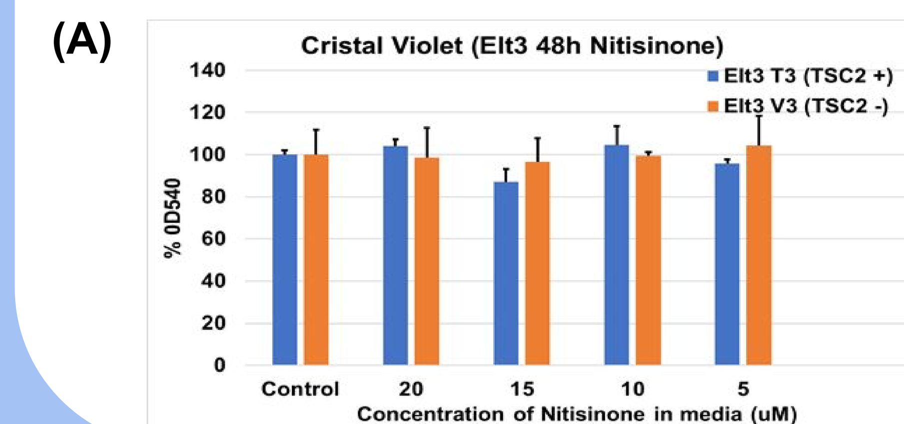
Pharmacological Approach

(A) Elt3 cells (Tsc2+ (T3) and Tsc2- (V3)) were incubated with Nitisinone for 48hr with concentrations 5-20 uM. The proliferation was measured using Crystal Violet staining.
(B) Elt3 cells (Tsc2+ (T3) and Tsc2- (V3)) were incubated with STF-31 for 48hr with concentrations 0.31-5 uM. The proliferation was measured using Crystal Violet staining.
(C) MEFs cells (Tsc2 +/+ and Tsc2 -/-) were incubated with Isoginkgetin for 48hr with concentrations 0.3125-5 uM. The proliferation was measured using ATP lite.

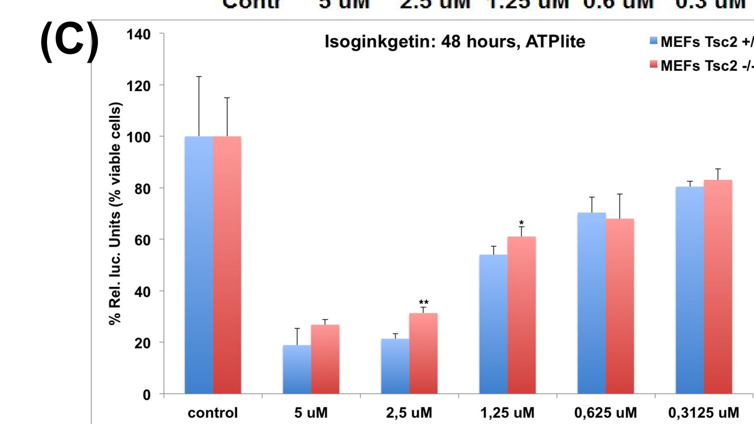
(B)



(A)

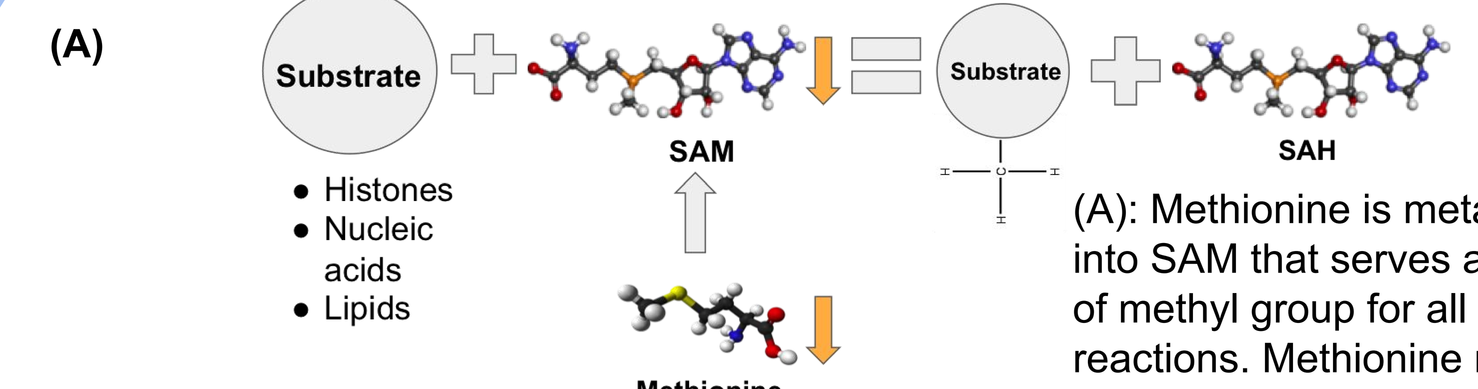


(C)



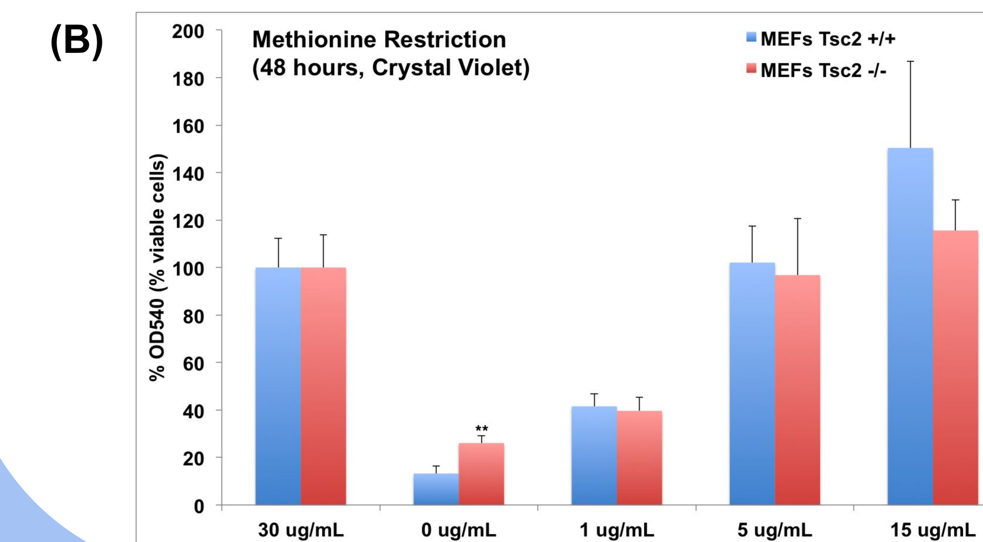
Metabolic Approach

(A)



(A): Methionine is metabolized into SAM that serves as a donor of methyl group for all methylation reactions. Methionine restriction limits SAM production and inhibits all methyltransferases.

(B)



(B): Methionine restriction: impact of methionine concentration reduction on the proliferation of MEFs with *TSC2* +/+ and *TSC2* -/- gene.

Conclusions

1. Inactivation of *NMRK1*, *PIAS1*, and *MAGEB2* genes using shRNA approach selectively suppresses proliferation of *TSC2*-deficient (tumor) cells

Инактивация генов *NMRK1*, *PIAS1* и *MAGEB2* с помощью РНК-интерференции селективно подавила пролиферацию *TSC2*-дефицитных (опухолевых) клеток

2. Nitisinone, tyrosine degradation inhibitor, doesn't influence proliferation of control or *TSC2*-deficient cells. *TSC2*-deficient cells are more resistant than wild-type cells to both STF-31 (glucose and NAD metabolism inhibitor) and to Isogingketin (splicing inhibitor).

Нитизинон, ингибирующий деградацию тирозина, не оказывает влияния на пролиферацию контрольных и *TSC2*-дефицитных клеток. *TSC2*-дефицитные клетки более устойчивы к действию STF-31 (ингибитор метаболизма глюкозы и НАД) и к действию Isoginkgetin (ингибитор сплайсинга), чем клетки дикого типа.

3. Methionine Restriction non-selectively suppresses proliferation of control and *TSC2*-deficient cells.

Метиониновое голодание неселективно подавляет пролиферацию *TSC2*-дефицитных и контрольных клеток.

Acknowledgments