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Genome editing of LKB1 (STK11) mutation in A549 lung cancer cells using CRISPR/Cas9 system

Ana Paula Morelli*, Isadora C. B. Pavan, Mariana R. Tavares, Luiz G. Salvino, Nathalie F.P. Pereira, Fernando R. Silva, Tharcísio C. Tortelli Jr., Roger Chammas, Fernando Moreira Simabuco.

Abstract

Several gene alterations are known to contribute to the development of lung cancer. Some of these alterations are known to be specific to lung tumor histopathology and probably indicate differences in carcinogenesis and cell type of origin. Mutations and deletions of the tumor suppressor LKB1 (STK11) are common in several malignancies, especially in non-small lung cancer cells (NSCLC). In that cell type, LKB1 inactivation coexists with mutations at other important cancer genes, including KRAS and TP53. The mutant LKB1 indirectly increases transcription of genes involved in metastasis, including COX-2. LKB1 directly activates AMPK decreasing mTOR activity. Furthermore, LKB1 plays roles in several other processes such as cell metabolism, cell polarity, apoptosis and DNA damage via p53 interaction. Cisplatin is the first base treatment for lung cancer, but is strongly associated to chemoresistance. Metformin, an antidiabetic drug, have shown antitumor effects and sensitizes cells in coadjuvant cisplatin treatment. Metformin acts in AMPK independent and dependent mechanisms. Thus, we propose the application of CRISPR/Cas9 system as genome editing tool to correct the LKB1 nonsense mutation and restore protein expression and normal function, providing new insights regarding cisplatin resistance, survival and metformin response in A549 lung cancer cells.

Key words:
CRISPR/Cas9, LKB1, Lung cancer.

Introduction

LKB1 (STK11) regulates cell energy homeostasis, cell polarization, apoptosis and DNA damage via p53 regulation. The most studied substrate of LKB1 is AMPK, a sensor of cellular energy status that is phosphorylated at Thr172 in the presence of high levels of AMP. LKB1-dependent and AMPK-dependent suppression of the mTOR pathway is possibly the most potent antineoplastic effect of metformin, an antidiabetic drug, but several studies shows positive effects in cancer cells by LKB1-AMPK-independent pathway[1]. Our previous data show that metformin can sensitizes A549 lung cancer cells after treatment with cisplatin (CDDP) while suppresses mTOR signaling.

A549 has a nonsense mutation at aminoacid 37 leading to loss of LKB1 expression[2]. Loss of LKB1 is frequent in lung cancer cells, appearing in 20-30% of non-small cell lung cancers (NSCLCs) and ranks as the 3rd most frequent mutated gene in lung adenocarcinoma, after p53 and Ras, characterized as a critical barrier to pulmonary tumorigenesis, controlling initiation, differentiation and metastasis[3].

Given the successful application of CRISPR/Cas-9 for gene editing and correction in cell cultures, the present study aims to employ the system to correct LKB1 gene and restore protein expression, improving metformin effects in LKB1-dependent suppression of mTOR pathway.

Results and Discussion

The specificity of the Cas9 nuclease is determined by the 20-nt guide sequence within the sgRNA. Thus, we designed LKB1 sgRNA (single guide-RNA) to insert in PX459 CRISPR plasmid and checked the specificity by the online CRISPOR software, that assesses the likelihood of a given guide sequence to have off-target sites. Basically, that sgRNA recognizes the mutated gene region, which will be cleaved by Cas9. After, we designed the repair template (donor DNA) containing a codon substitution (c.109T>C; LKB1 mutation: c109C>T)/, p.Q37*) and homology arms flanking the site of alteration. The sgRNA insertion in px459 plasmid was confirmed by sequencing of PX459-LKB1 clones.

To functional validate the plasmid, HEK293 cells (LKB1 WT) were transfected with sequence-verified CRISPR plasmid (PX459-LKB1), empty plasmid and transfection control (GFP) and selected with puromycin (1 µg/ml) for 48 hours. Cas9 activity was confirmed by western blotting. Furthermore, the plasmid was validated by the T7 endonuclease assay in HEK293 (data not shown).

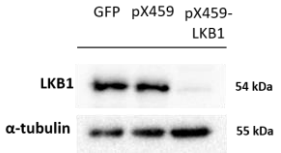

Image 1. CRISPR/Cas9 induces LKB1 knockout in HEK293 by specific genomic cleavage. HEK 293 cells were transfected with 1000 ng of pBABE-GFP, PX459 or PX459-LKB1 using lipofectamine and extracts were collected 2 weeks after puromycin selection at 1 µg/ul.

The next step will be application of the plasmid and the donor DNA in A549 to specific correct the LKB1 gene.

Conclusions

In this study we constructed a CRISPR/Cas9 plasmid with an LKB1 sgRNA able to cut the genomic mutation region, confirmed by transfection in HEK293 cells. Restore of LKB1 in A549 lung cancer cells will provide new highlights in metformin treatment, especially in CDDP-resistant cells.

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