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ORIGINAL ARTICLE

Sirt3 regulates the level of mitochondrial DNA repair activity through deacetylation of NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 in colorectal cancer

Authors' Contribution:
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

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ABSTRACT: Colorectal cancer (CRC) is one of the most common malignant tumors. One of the factors increasing the risk of its occurrence may be the reduced efficiency of repairing DNA damage, both nuclear and mitochondrial. The main mechanism for repairing oxidative damage is the BER system (in mitochondria mtBER), whose key proteins NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 obtain full efficiency only at the appropriate level of acetylation. Sirtuin 3 is a key protein for mitochondrial homeostasis, regulating a number of metabolic processes related mainly to the control of the level of reactive oxygen species. Because Sirt3 possesses acetylase activity, it can modulate the level of activity of mtBER proteins by their deacetylation. The conducted study showed that the tested proteins NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 are the substrate for the enzymatic deacetylation activity of Sirt3, which may lead to modulation of the risk of CRC, and in cancer cells may be a potential therapeutic target enhancing the action of cytostatic drugs.

KEYWORDS: cancer, colorectal cancer, DNA damage/repair, genetics, proteins

ABBREVIATIONS

APE1 – Apurinic/apyrimidinic endonuclease
BER – Base Excision Repair
CRC – colorectal cancer
LIG3 – DNA ligase 3
MUTYH – mutY DNA glycosylase
NEIL1 – Endonuclease VIII-like 1
NEIL2 – Endonuclease VIII-like 2
OGG1 – 8-Oxoguanine glycosylase
Sirt3 – Sirtuin 3

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy in the world. In 2018, more than 1.8 million new cases were diagnosed, and the CRC itself was responsible for over 10% of all cancer cases. Importantly, CRC is one of the cancers for which the incidence is systematically growing, despite the use of extensive preventive programs. At the same time, colorectal cancer accounts for approximately 700.000 deaths annually, showing a downward trend (despite the increase in incidence), which should be attributed to significant advances in its treatment. However, the therapeutic success depends mainly on the stage of CRC in which the diagnosis will be made, which confirms a 5-year survival rate of over 90% for cases diagnosed at an early stage and 13% for the late stage. However, despite intensive research, the unequivocal reasons for CRC remain unknown.

The subject of particular interest is genetic factors that, by modifying proteins that take part in a number of different metabolic pathways, can interfere with cellular processes leading to the neoplastic process. An important role in the process of cellular protection against cancer are DNA repair systems, including mitochondrial DNA, since if they work with reduced efficiency they may not be able to remove damage of the genetic material and as a consequence lead to malignant transformation [1, 2, 3, 4]. Base Excision Repair (BER) is a DNA repair mechanism that is responsible for removing damage that arises primarily as a result of oxidative stress, and its main role is cutting out oxidation-damaged bases. It affects both nuclear DNA (BER) and mitochondrial DNA (mtBER). Among the proteins of this system, important functions can be assigned to NEIL1 (Endonuclease VIII-like 1), NEIL2 (Endonuclease VIII-like 2), OGG1 (8-Oxoguanine glycosylase), MUTYH (mutY DNA glycosylase), APE1 (Apurinic / apyrimidinic endonuclease) and LIG3 (DNA ligase 3). All these proteins make up the proper functioning of the mtBER system, and the dysfunction of any of them disturbs the integrity of the repair path, consequently leading to the process of neoplastic formation, including CRC formation [5, 6, 7]. At the same time, one of the key elements required for the proper functioning of mtBER proteins is their proper level of acetylation, as deacetylated proteins show a significant decrease in the efficiency of DNA damage removal [8, 9, 10, 11].

Sirtuin3 (Sirt3) is a representative of the sirtuin class, performing a number of roles in the cell, with particular emphasis on the regulation of metabolic processes and the response to stress conditions such as oxidative stress [12, 13]. The potential contribution of Sirt3 to carcinoma processes seems to be very complex and depends on many additional factors, and thus is not fully understood. However, because Sirt3 has a deacetylase function, it can affect the activity of metabolic pathways and repair systems through deacetylation of proteins involved in them.

The aim of this study was to assess the potential of Sirt3 to affect the efficiency of the mtBER DNA repair mechanism through deacetylation of NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 in colorectal cancer.

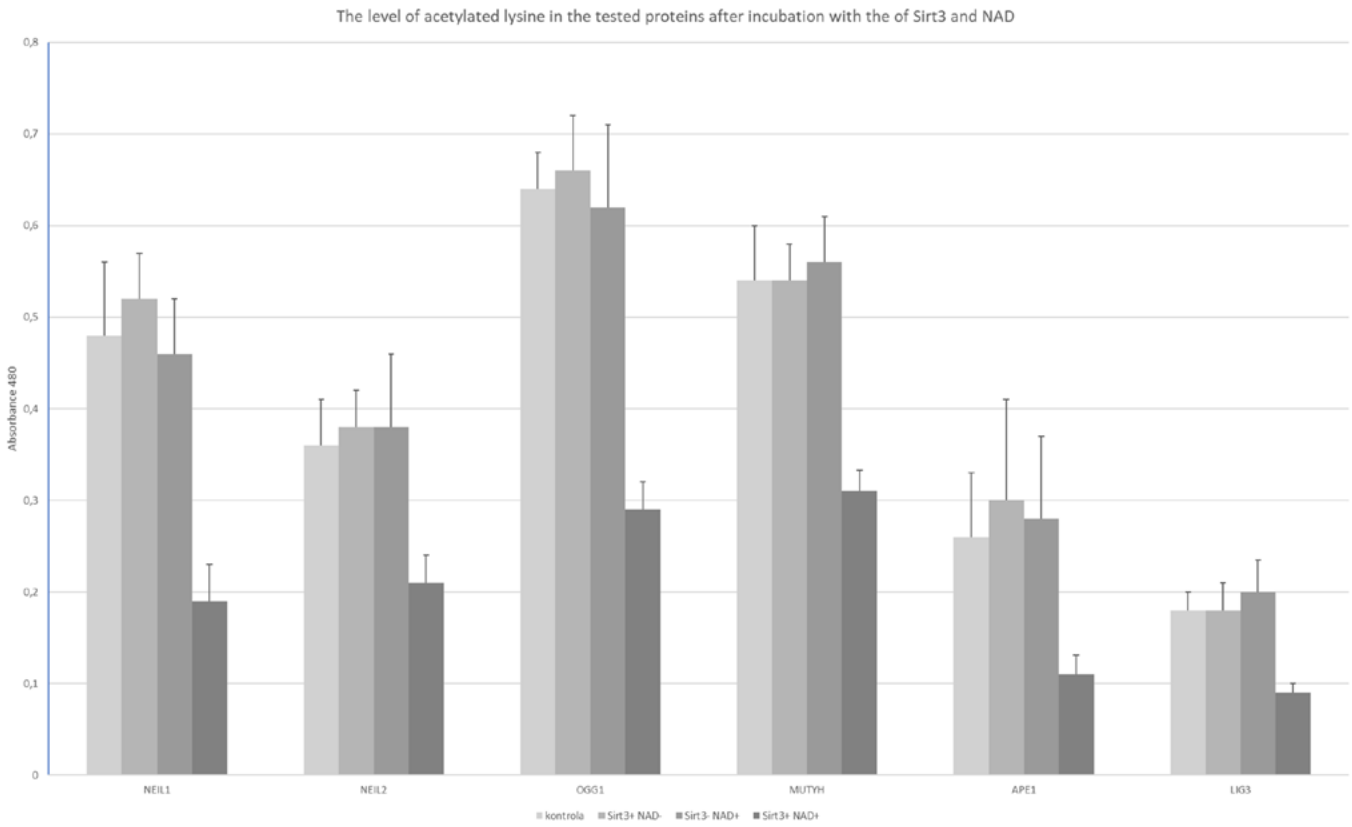

Fig. 1. The level of acetylated lysine in the tested proteins after incubation with the mixture of Sirt3 and NAD.

MATERIALS AND METHODS

Cell culture

The experiment utilized the HT-29 cell line (human colon cancer cells). The culture was carried out at a concentration of 5% CO2 at 37°C using EMEM Medium (ATCC) with FBS to a final concentration of 10% and penicillin and streptomycin (100 units/mL).

Deacetylation test

Control cells were transfected with the Flag-Sirt3 plasmid and the tested cells with Flag-NEIL1, Flag-NEIL2, Flag-OGG1, Flag-MUTYH, Flag-APE1 and Flag-LIG3. Cells were then lysed in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) with the addition of a protease inhibitor at room temperature for 20 minutes and then centrifuged at room temperature, 12.000 xg for 20 minutes. The test proteins were purified using immunopurification (ANTI-FLAG M2 Gel, Sigma). In the deacetylation test, purified mtBER proteins were incubated with Sirt3-Flag protein in the presence or absence of 1 mM NAD + in deacetylase buffer (50 mM Tris-HCl [pH 9.0], 4 mM MgCl2, 50 mM NaCl, 0.5 mM dithiothreitol) at 30°C for 3 hours, and then the levels of acetylated lysine residues in the tested proteins were measured using ELISA and antibodies Acetyl-Lysine Antibody LS-C71873 (LifeSpan BioSciences) treating it as a measure of deacetylation compared to the control.

RESULTS

The results of the experiment are shown in Figure 1. For all tested proteins, the level of acetylated lysine after incubation with Sirt3 and its cofactor NAD was significantly lower than in the case of controls. Additionally, in order to confirm the necessity of both Sirt3 showing the enzymatic activity of deacetylase and its cofactor NAD, incubations of the tested proteins were performed in the option: only Sirt3, only NAD and Sirt3 + NAD. A significant decrease in acetyl lysine was observed only in the case of NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 incubation with both Sirt3 and NAD.

Because lysine is an excellent target for acetylases, its amount in the reaction mixture is a derivative of the activity of Sirt3 as deacetylase. A reduced level of acetylated lysine indicates that the tested proteins may be a substrate for Sirt3 in the presence of NAD.

DISCUSSION

The role of Sirtuin 3 is extremely complex, mainly due to the very large number of cellular mechanisms it interacts with. The widely studied potential of Sirt3 in the neoplastic process and its impact on the risk of cancer give ambiguous results allowing to qualify Sirt3 simultaneously as an oncogene and a tumor suppressor, depending on the circumstances [14]. On the one hand, the basic function of Sirt3 is to regulate the level of reactive oxygen species and protect the cell from oxidative stress and resulting DNA damage, on the other hand, such an action in cancer cells can be considered as cancer promotion due to the fact that it protects the cell from entering the path of apoptosis [15, 16]. In any case, it should be recognized that Sirt3 is crucial for the maintenance of mitochondrial homeostasis and the proper functioning of metabolic pathways. At the same time, however, more and more often attention is paid to the fact that sirtuin's deacetylation possibilities can lead not only to proper regulation of cell

processes, but also to dysfunctions in the effective action of a number of enzymes [17] and regulatory proteins like p53 called "genome guard" [18].

Because the mtBER system proteins are the basic tool of the cell in the field of protection against DNA damage and, consequently, against neoplastic transformation, any modulation of their effectiveness is a serious threat [19, 20, 21]. The necessity of an adequate level of acetylation of these proteins has been confirmed by the decrease in their efficiency in the case of deacetylation [22], which is a known regulatory mechanism of most mitochondrial proteins [23]. Considering the necessity of acetylation of mitochondrial DNA repair proteins and the deacetylation activity of Sirt3 – the basic protein regulating a number of mitochondrial processes, the subject of the study was to investigate the deacetylation ability of NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3 proteins by Sirt3. The results confirmed that all tested proteins can be a substrate for enzymatic deacetylation, which means that Sirt3 can affect the efficiency of DNA repair systems. Excessive exposure of mtBER proteins to Sirt3 activity can lead to a reduced repair efficiency and consequently to an increased risk of CRC. At the same time, however, attention should be paid to the potential role of such a process in cancer cells. Many CRC therapies are based on inducing lethal lesions in tumor cells, and thus a reduction in the efficiency of repairing such induced lesions by a cancer cell would be desirable from the therapeutic point of view. For some time, Sirt3 has been considered as a potential target in anti-cancer therapy [24, 25, 26]. One of the paths of such an action may potentially be to reduce the efficiency of DNA repair in tumor cells during the treatment process resulting in increased cytostatic efficacy of drugs as a result of decreased cell proliferation and promotion of apoptosis. However, further research in this area is necessary to show a complete image of the interaction between Sirt3 and NEIL1, NEIL2, OGG1, MUTYH, APE1 and LIG3.

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