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Original Articles

Decreased expression of SIRT6 promotes tumor cell growth correlates closely with poor prognosis of ovarian cancer

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Summary

Introduction: Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Sirtunins belong to a protein family and it is present in all organisms. SIRT6 is downregulated in tumor and acts as tumor suppressor. These sirtunin proteins are linked to repair DNA and metabolism. *Material and Methods:* To measure the role of SIRT6 in tumor cell, 20 mice were used and European Collection of Cell Cultures (ECACC) cell lines were used for the analysis. A histopathological technique showed the level of tumor cells. *Results:* A recent study provided exceptional insight into the mechanism of SIRT6-related chromatin regulation. According to the histopathology of cancer, SIRT1 localizes to the promoters of several aberrantly silenced tumor suppressor genes whose DNA is hypermethylated. SIRT1 has a role associated with the epigenetic hallmarks of cancer. *Conclusion:* The link between SIRT6 and cancer provide new insight into the therapeutic potential of small molecule activators or specific targets of SIRT6 for the prevention and treatment of cancer. Further investigation into the specific mechanism of SIRT6 is required to realize this potential.

Key words: Ovarian cancer; SIRT6 expression; Sirtunins.

Introduction

Cancer cells are characterized by the attainment of several characteristics that enable them to become tumorigenic [1]. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is initiated by both external factors (tobacco, infectious organisms, chemicals, and radiation) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). These contributory factors may act collectively or in sequence to initiate or promote carcinogenesis [2].

Sirtunins belong to a protein family and it is present in all organisms. Acetylated lysines of various peptides and proteins are targeted by the sirtunin [3]. Sirtunins play important role in cellular stress and ageing and disease like Alzheimer's disease [4], Parkinson's disease [5], and cancer [6] also related to sirtunin. They have two binding sites i.e. NAD+ binding site and catalytic binding site [7]. Through the NAD+ dependent deacetylation reaction, cellular energy is produced. They also play a part in health promotion of several species [8]. It is activated through stress, caloric restriction, and pharmacological agents [9]. Metabolic state of cell and sirtunin are directly correlated with each other [10]. Deacetylation begins with the breakdown of amide from the NAD+ and formed nicotinamide and covalent ADP-ribose peptide imidate intermediate (ADPR) and it is NAD+ dependent reaction. There are seven classes of sirtunin present in humans. Each class has its own function, characteristic, and localization [11]. SIRT 1, 6, and 7 are present in the nucleus, while SIRT3, 4, and 5 are present in the mitochondria [12]. SIRT2 is found in the cytoplasm [13].

Nuclear localization signals (NLS) are also present in sirtunin. SIRT6 and SIRT7 have a single nuclear localization signal while SIRT1 has two nuclear localization signals. Besides nuclear localization signals, nuclear export signals are also present in sirtunin [14]. Because of their histone deacetylase activity, sirtunin is involved in the expression of gene regulation. The role of sirtunin in disease is identified by SIRT1 by emerging as tumor, either by providing tumor suppressor or tumor promoter as their function [15]. Redox regulation of mitochondria is carried out by SIRT3 in mitochondrial matrix, where SIRT5 is present, where n-terminus is cleaved. Carbamoyl phosphate synthase-1 (CPS-1) is a main target of the

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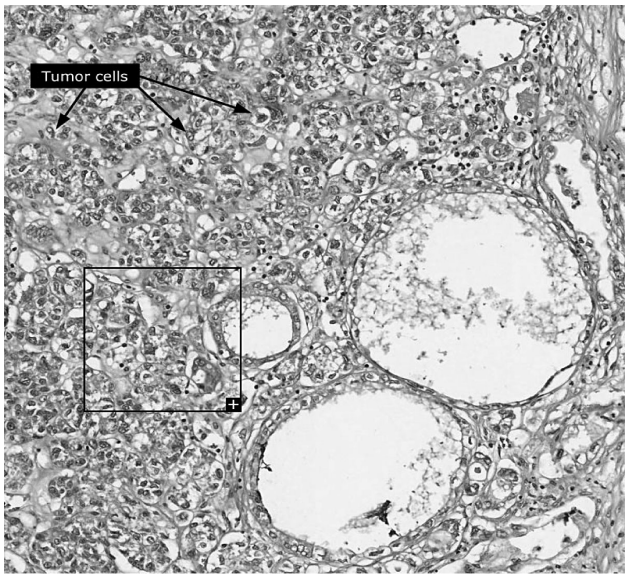

Figure 1. — Ovarian cancer cells in mice.

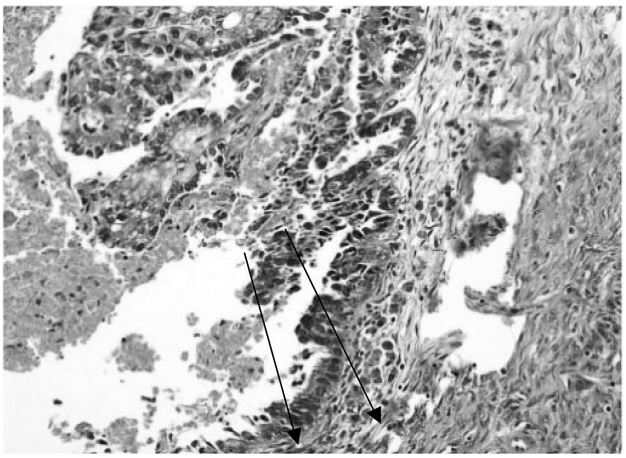

Figure 2. — Cells affected by SIRT6 in the ovarian carcinoma in mice.

SIRT5 [16]. SIRT6 has a key role in the base excision repair (BER). DNA-dependent protein kinase is stabilized directly by SIRT6 at dsDNA breaks site and DNA repair complex is formed [17]. It is also related to chromatin [18] and also present in the promoter region of NF-kB activated proteins. The deficiency of SIRT6 is related to the shortness of lifespan and increases ageing phenotypes. Maintenance of telomeres and telomeric function is accomplished by SIRT6. Due to the removal and reduction of SIRT6, telomere dysfunction and to Werner's syndrome is also caused by the absence of SIRT6. The cells that have low or deficient SIRT6 have increased possibility to genotoxic DNA damage. In the controlling of DNA damage and NF-kB function through SIRT6 also indicate a major role in tumorigenesis [19].

Under stress conditions, survival of the cell takes place or is promoted by SIRT1 by repressing p53 dependent apoptosis. Deacetylation of p53 which is mediated by SIRT1 has been confirmed by other groups [20]. P53 mediated tumor suppression role of SIRT1 remains unknown. In case of humans, several types of cancer, prostate cancer, acute myeloid leukemia, and colon cancer is highly expressed by SIRT1 [15]. Node-positive breast cancer versus non-malignant breast tissues is related to high level of SIRT3 [21]. As well as oral squamous cell carcinoma, SIRT1 and SIRT3 might be lost in late stages of tumor progression. This also suggests that SIRT6 is downregulated in tumor and acts as tumor suppressor. These sirtunin proteins are linked to repair of DNA and metabolism.

The authors investigated the role of SIRT6 in the progression of ovarian cancer in female albino rats.

Materials and Methods

The entire experimental work was conducted at the Shandong Academy of Medical Sciences. All experiments were performed according to the rules and regulations of authority. This study was also conducted according to the rules and regulations of authority of Shandong Academy of Medical Sciences.

For this experiment 20 albino rats of 200-250 grams were selected to study the role of SIRT6 in the progression of ovarian cancer. The rats were housed in cage and maintained in controlled temperature at 30^0C during the entire experimental work in animal cages.

The sample were processed and analyzed for the estimation of role of SIRT6 in the mice. The mice were divided into two groups: one included healthy and normal mice that constituted the control group and the other included mice suffering from ovarian cancer. The authors aimed to discover that role of SIRT6 in both groups and to assess the survival rate.

Tissue was drawn and immediately placed it on ice cold normal saline. Later, two-ml 0.15M Tris HCl and two-ml phosphate buffer was added and grinded into micro tube by micropestle. The mixture was centrifuged and stored in a cold environment.

All cells were grown in a 5% CO_2, 3% O_2 incubator at 37°C in Eagle's minimal essential medium supplemented with 15% FBS,100 units/ml penicillin, and 100 μg/ml streptomycin.

ECACC cells were transfected with 0.1 μg of HR construct linearized by NheI. Cells were maintained in media which contained one-mg/ml G418 for eight days to select colonies with integrated reporter cassettes. After colonies were formed, cells were trypsinized, reseeded, and then cultured in one plate until they reached confluence. Three separate transfections were proficient, giving rise to three independent pools. These cells lines were named and then consecutively passaged, with split every three to four days, until they reached senescence at PD71.

Results

The criteria used for tumor grading and to separate borderline tumors from carcinomas was based on histologic subtype. These results were based on modern criteria for histotyping ovarian carcinomas. Serous carcinomas showed a very broad spectrum of histologic appearances, that con-

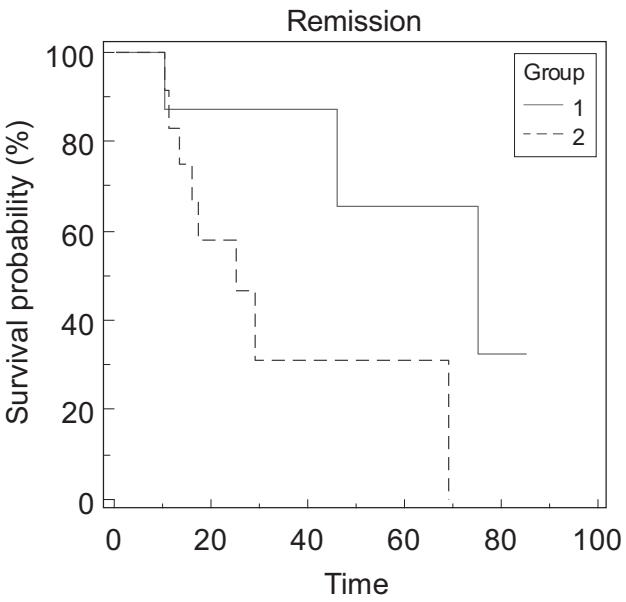

Figure 3. — Kaplan-Meier Survival curve which shows the survival rate of ovarian carcinoma (group 1) and with the effect of SIRT6 (group 2).

trast with most other primary ovarian carcinomas in which morphologic variation is considerably less. The morphologic heterogeneity of serous carcinomas is likely an expression of the genetic and heterogeneity of these tumors and suggests that some tumors currently diagnosed as serous carcinomas represent transformation or progression from other tumor type.

A recent study provided exceptional insight into the mechanism of SIRT6-related chromatin regulation. According to the histopathology of cancer, SIRT1 localizes to the promoters of several aberrantly silenced tumor suppressor genes whose DNA is hypermethylated. SIRT1 has a role associated with the epigenetic hallmarks of cancer (Figures 1 and 2). The Kaplan-Meier survival curve shows that the survival rate was slow in case of SIRT6, as shown in Figure 3. SIRT1 negatively regulates p53-dependent apoptosis by deacetylation of p53 in response to cellular damage.

Discussion

Important intracellular signal transduction pathways that are necessary for the action of some antineoplastic agents can also be affected by oxidative stress. There are two major pathways of drug-induced apoptosis following cellular damage by anti-neoplastic agents: (1) mitochondrial pathway, initiated by release of cytochrome c; and (2) CD95 death receptor pathway, initiated by CD95L binding to its death receptor [22-25].

Table 1. — *Comparison of survival curves (Log Rank Test).*

Chi-square	4.3031
Degree of freedom DF	1
Level of significance	$p = 0.03$

The sirtunin genes encode an important and complex family of proteins that participate in a wide spectrum of physiological processes. In several species, caloric restriction has been shown to increase lifespan and decrease spontaneous rates of illness, such as insulin resistance, neurodegenerative disease, and cancer. According to Table 1 the significant value is 0.03 and degree of freedom is 1. These results will be analyzed on the basis of Kaplan-Meier curve. To gain additional insight into the biochemical cascade that SIRT6 initiates to drive apoptosis in cancer cells, the present authors assessed whether SIRT6-mediated killing of cancer cells was dependent on any of the classic apoptotic pathways.

Conclusion

Over the past decade, SIRT1 has been the most investigated gene involved in diverse cellular functions. The link between SIRT6 and cancer provide new insight into the therapeutic potential of small molecule activators or specific targets of SIRT6 for the prevention and treatment of cancer. Further investigation into the specific mechanism of SIRT6 is required to realize this potential.

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