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Anticancer action by natural ingredient NMN and its mechanism: Focusing on the molecular mechanism of NMN on breast cancer cells.

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Abstract:

It was confirmed that NMN causes a decrease in breast cancer cells. Breast cancer is now a major problem for women because it is sensitive to estrogen. The standard treatments for breast cancer are generally surgery, anticancer drugs, and radiation therapy. In addition, as a complementary / alternative therapy, it is said that stress management, relaxation for stress re-duction, vitamins, and dietary fiber, which are said to have antioxidant effects as nutritional components, are used. However, although there is potential from an antioxidant perspective at this stage, no evidence is available at this time. Therefore, this time, this research group investi-gated the effect on breast cancer cells at the in vitro level using the component “NMN”, which is currently attracting attention. When NMN was added to breast cancer cells, a significant reduc-tion in breast cancer cells was observed in a concentration-dependent manner. Therefore, this time, we investigated α-ketoglutaric acid, NAD +, and AMPK, which are thought to be related to the anticancer effect. It was found that NMN has the effect of activating these, suggesting that NMN may be used as a complementary / alternative therapy for breast cancer patients in the fu-ture

Keywords:

NMN, Functional Foods, nutritional therapy, complementary / alternative, nutrients, α-ketoglutaric acid, NAD +, AMPK

1. Introduction

In recent years, the number of deaths from breast cancer has been increasing world-wide, and countermeasures are urgently needed.[1] Therefore, we have conducted basic research using MCF-7 cells derived from human breast cancer. As a result, when NMN (nicotinamide mononucleotide) was allowed to act on MCF-7, a growth inhibitory effect was confirmed. Since MCF-7 cells have an estrogen receptor[2], they are sensitive to estro-gen and are known to be associated with the development of breast cancer in women. Current standard treatments for breast cancer are resection, antineoplastic treatment, hormone therapy, and radiation therapy.[3]In addition, since the existence of “active oxy-gen” is known as one of the factors promoting the growth of cancer cells[4], ingestion of nutritional components having antioxidant power has been attempted as a complemen-tary / alternative therapy.[5] At present, although the possibility of a cancer cell-reducing effect focusing on such an antioxidant effect is fully considered[6], detailed research re-sults such as the mechanism have not been obtained. Therefore, this time, we examined the mechanism at the in vitro level using the nutritional component “NMN (nicotinamide mononucleotide)”, which is currently attracting attention. NMN is a substance contained in nicotinic acid (niacin), a coenzyme present in the cells of all living organisms, and is produced in the body.[7] NMN is also known as a precursor of NAD +.[7] In addition, NMN is a coenzyme present in all species and is found in various nutrient sources such as milk and broccoli. It has been reported that when NMN decreases due to aging, the amount of NAD + produced also decreases, and cell damage and mitochondrial activity also decrease.[8]It is believed that this causes cell damage and causes cancer.[9]There are no reports on the antioxidant activity of NMN, and there are reports that it improves mi-tochondrial function and increases metabolism.[10]NMN is an ingredient that is currently attracting attention in various fields as an “anti-aging substance” and is a substance made from vitamin B3.[11]In addition, NMN is said to

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be deeply involved in maintaining and promoting health by acting on the sirtuin gene.[12]If the mechanism is elucidated in this study, the use of NMN as a complementary / alternative therapy will be extremely valua-ble not only for breast cancer but also for various cancers in the future. Therefore, in this study, we confirmed the AMPK activity, α-ketoglutaric acid expression, and NAD + ex-pression level by NMN as one of the clarifications of the above-mentioned contents. The purpose of this study was to verify how NMN changes these indicators, which is the first data in the world to be searched by myself as a pilot study using breast cancer cells.

2. Materials and Methods

2.1. Cell viability evaluation

MCF-7 cells are cultured according to the standard method[13], NMN purchased from Wellness-One Co., Ltd. (Iwate, Japan) is adjusted to a final concentration of 1 mg / ml, allowed to act for 24 hours, and then Cell Counting Kit-8. Using (DOJINDO LABORATO-RIES, Japan), the absorbance was measured at a wavelength of 450 nm with a microplate reader and compared with the control (PBS) group. The obtained absorbance was set to 100% in the control group, and at that time, the cell viability was evaluated by the per-centage of the NMN-added group. The absorbance was statistically evaluated by the Mann-Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26).

2.2. α-Ketoglutaric acid measurement

In this experiment, α-Ketoglutarate Assay Kit-Fluorometric (DOJINDO LABORATORIES (Kumamoto, Japan)) was purchased and measured according to the operation of the kit. Compared with the control group (PBS group), the NMN-added group (final concentra-tion) The expression level of α-ketoglutaric acid (1 mg / ml) was measured by fluorescence, and the evaluation was statistically evaluated by Mann– Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26).

2.3. NAD + measurement

In this experiment, NAD / NADH (DOJINDO LABORATORIES (Kumamoto, Japan) was purchased and measured according to the operation contents of the kit. Compared with the control group (PBS group), the NMN-added group (final concentration 1 mg / ml)) NADH amount and total NAD + / NADH were measured at a wavelength of 450 nm with an absorptiometer, and the expression level of NAD + was measured by subtracting the NADH amount from the total NAD / NAD amount. The evaluation was performed by sta-tistical processing software (IBM SPSS Statistics). Statistical evaluation was performed by Mann– Whitney U test using Ver.26).

2.4. AMPK activity measurement

In this experiment, the CycLex® AMPK Kinase Assay Kit (MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. Tokyo, Japan) was used as per the standard method, and the PBS-added group was used for the AMPK activity in the NMN-added group (final con-centration 1 mg / ml). It was compared and evaluated as a control. In this kit, in order to measure the current amount of AMPK activity, the activity of AMPK was confirmed 1 hour, 12 hours, and 24 hours after the addition. In addition, the evaluation was performed sta-tistically by the Mann-Whitney U test using statistical processing software (IBM SPSS Sta-tistics Ver.26).

3. Results

3.1. Cell viability evaluation.(Table.1, Fig.1)

When NMN was allowed to act on MCF-7 cells for 24 hours, a significant decreasing tendency was observed as compared with the control group. The OD values of each group are as follows, and the reduction rate of MCF-7 cells in the NMN-added group was as follows when converted to% from this and the control group was taken as 100%.

Assuming that the OD value of the control group at this time was 100%, the OD value of the NMN-added group was converted to%.

Both of n=60	Average of OD value
Control	0.375±0.042 (100%)
Addition group of NMN(1mg/ml)	0.256±0.026 (68.4%)

Table.1 OD value in MCF-7 cells by control and NMN.

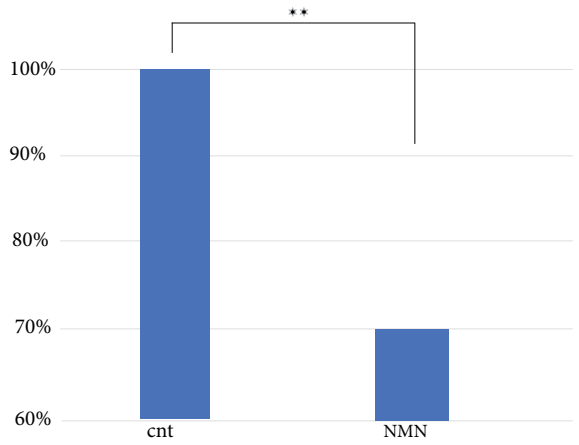

Fig.1 NMN's on reducing MCF-7 cells. When the Mann-Whitney U test was performed, adecreasing tendency was observed at P <0.01.

3.2. α-ketoglutaric acid measurement(Table.2, Fig.2)

In this experiment, α-Ketoglutarate Assay Kit-Fluorometric (DOJINDO LABORATORIE (Kumamoto, Japan)) was purchased and measured according to the operation of the kit. Compared with the control group (PBS group), the NMN-added group (final concentration) The expression level of α-ketoglu-

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taric acid (1 mg / ml) was measured by fluorescence, and the evaluation was statistically evaluated by Mann– Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26).

Both of n=3	Average of Fluorescence
Control	1.026±0.008 (100%)
Addition group of NMN(1mg/ml)	2.767±0.065(269.8%)

Table.2 Fluorescence of α-ketoglutaric acid in the control group and the NMN-added group.

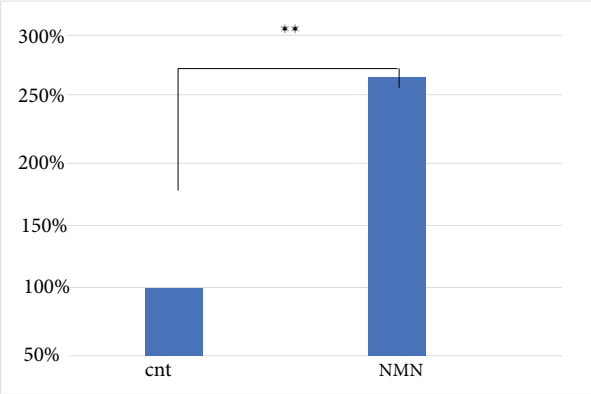

Fig.2 When the Mann-Whitney U test was performed, the expression level of α-ketoglutaric acid in the NMN-addedgroup increased at P <0.01.

3.3. NAD + measurement (Table.3, Fig.3)

In this experiment, NAD / NADH (DOJINDO LABORATORIES (Kumamoto, Japan) was purchased and measured according to the operation contents of the kit. Compared with the control group (PBS group), the NMN-added group (final concentration 1 mg / ml)) NADH amount and total NAD + / NADH were measured at a wavelength of 450 nm with an absorptiometer, and the expression level of NAD + was measured by subtracting the NADH amount from the total NAD / NAD amount. The evaluation was performed by statistical processing software (IBM SPSS Statistics). Statistical evaluation was performed by Mann– Whitney U test using Ver.26).

3.4. In this experiment, the CycLex® AMPK Kinase Assay Kit (MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. Tokyo, Japan) was used as per the standard method, and the PBS-added group was used for the AMPK activity in the NMN-added group (final concentration 1 mg / ml). It was compared and evaluated as a control. In this kit, in order to measure the current amount of AMPK activity, the activity of AMPK was confirmed 1 hour, 12 hours, and 24 hours after the addition. In addition, the evaluation was performed statistically by the Mann-Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26).

Both of n=3	Average of OD value
Control	0.177±0.005 (100%)
Addition group of NMN(1mg/ml)	0.544±0.008 (307.3%)

Table.3 Absorbance of NAD + in the control group and the NMN-added group.

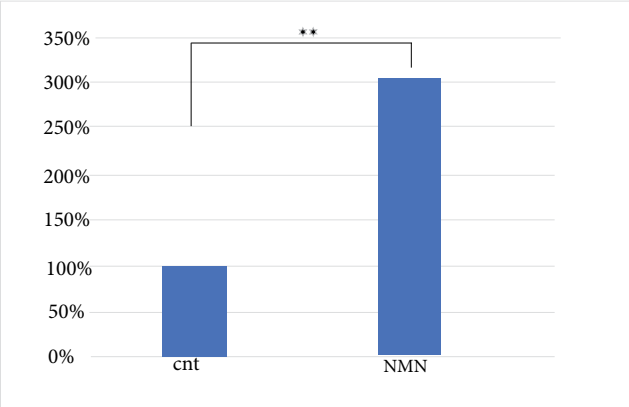

Fig.3 When the Mann-Whitney U test was performed, the expression level of NAD + in the NMN-added groupincreased at P <0.01.

4. Discussion

Currently, the standard treatments for breast cancer are resection, antineoplastic treat-ment, hormone therapy, and radiation therapy.[3] However, not all therapies give good results to these, and there are many cases in which they do not remit. In this study, the ef-fect of suppressing cancer cells was observed, suggesting that NMN may be a comple-mentary / alternative therapy in addition to the standard therapy in the future. Active ox-ygen is one of the factors considered as the mechanism of breast cancer growth.[14]In ad-dition, since cancer cells actively take up glucose even in the presence of oxygen, the gly-colysis system acts predominantly, so there is a tendency for cancer cells to proliferate rapidly due to the Warburg effect.[15]It is suggested that cancer cells are caused by muta-tions in genes, which in turn may cause abnormalities in genes and signals in their met-abolic processes.[16]It has been clarified that an abnormality of the mTOR complex exists as one of the keywords.[17]The mTOR complex consists of mTOR1, Mtorc2, mLST8 / GβL (mammalian LST8 / G-protein β-subunit like protein), Raptor (regulatory associated pro-tein of mTOR) and PRAS40 and DEPTOR.[18]This mTOR complex is involved in the reg-ulation of protein biosynthesis with information on nutrient, energy and redox states.[19]Of the mTOR complex, mTORC1 is suppressed by stimuli such as malnutri-

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tion, deficiency of growth factors, and reductive stress. Mutation / activation of PI3K and muta-tion / inactivation of p53, Tsc1 / 2, Lkb1, Pten, and Nf1 found in many cancer cells lead to activation of mTORC1.[19]Although α-ketoglutaric acid in this study is an intermediate metabolic product of the TCA cycle, it is said to show a suppressive effect on cancer cells via the p53 protein, which is a tumor suppressor gene product, in cancer cells.[20]In this study, it is considered that activation of p53 was observed by measuring α-ketoglutaric acid. Furthermore, NMN was found to activate AMPK. AMP-activated protein kinase (AMPK) is a cell energy sensor found in almost all eukaryotes.[21] Eukaryotic genetic studies suggest that the role of AMPK's ancestors responds to carbon source starvation and that AMPK is involved in lifespan extension in response to calorie restriction.[21] The major upstream kinase required for AMPK activation is LKB1, and LKB1 activates AMPK in response to metabolic stress. Since LKB1 is said to have an antitumor effect, it is con-sidered that activation of AMPK has an antitumor effect.[22] In the results of this study, it was found that AMPK was significantly increased, which may lead to the activity of Lkb1. Therefore, it was suggested that the action of mTORC was inhibited from various view-points, and that NMN may have an anticancer effect. The mTOR (mammalian target of rapamycin) inhibitor is also used in chemotherapy in cancer,[23] and it is possible that a causal relationship can be found with the results of this study. NAD + is also an im-portant coenzyme involved in the redox reaction of major metabolic pathways in cells. NAD exists as oxidized NAD + and reduced NADH in the cell, and the balance between these two is essential for maintaining cell function.[24]In recent years, a causal relation-ship between a decrease in NAD + level and aging-related diseases has also been pointed out.[25]In addition, NAD + is considered to be an important index because the suppres-sive effect on cancer is recognized by increasing the amount of NAD +[26]. Since a signifi-cant increase in NAD + was confirmed, it is considered that a growth inhibitory effect on breast cancer cells can be expected as a synergistic or additive effect of α-ketoglutaric acid, NAD +, and AMPK. It was suggested that it could also be used as a therapy. In the future, we plan to screen other cancer cells and conduct in vivo studies after undergoing their toxicity tests.

1)Absorbance

1hr		12hr		24hr	
Cnt	NMN	Cnt	NMN	Cnt	NMN
0.285±0.013	3.507±0.136	0.352±0.019	1.783±0.311	0.264±0.034	2.242±0.182

2)AMPK activity increase rate when cnt is 100%

1hr		12hrs		24hrs	
Cnt	NMN	Cnt	NMN	Cnt	NMN
100%	1230.50%	100%	506.50%	100%	849.20%

Table.4 Absorbance of hourly control group and NMN-added group

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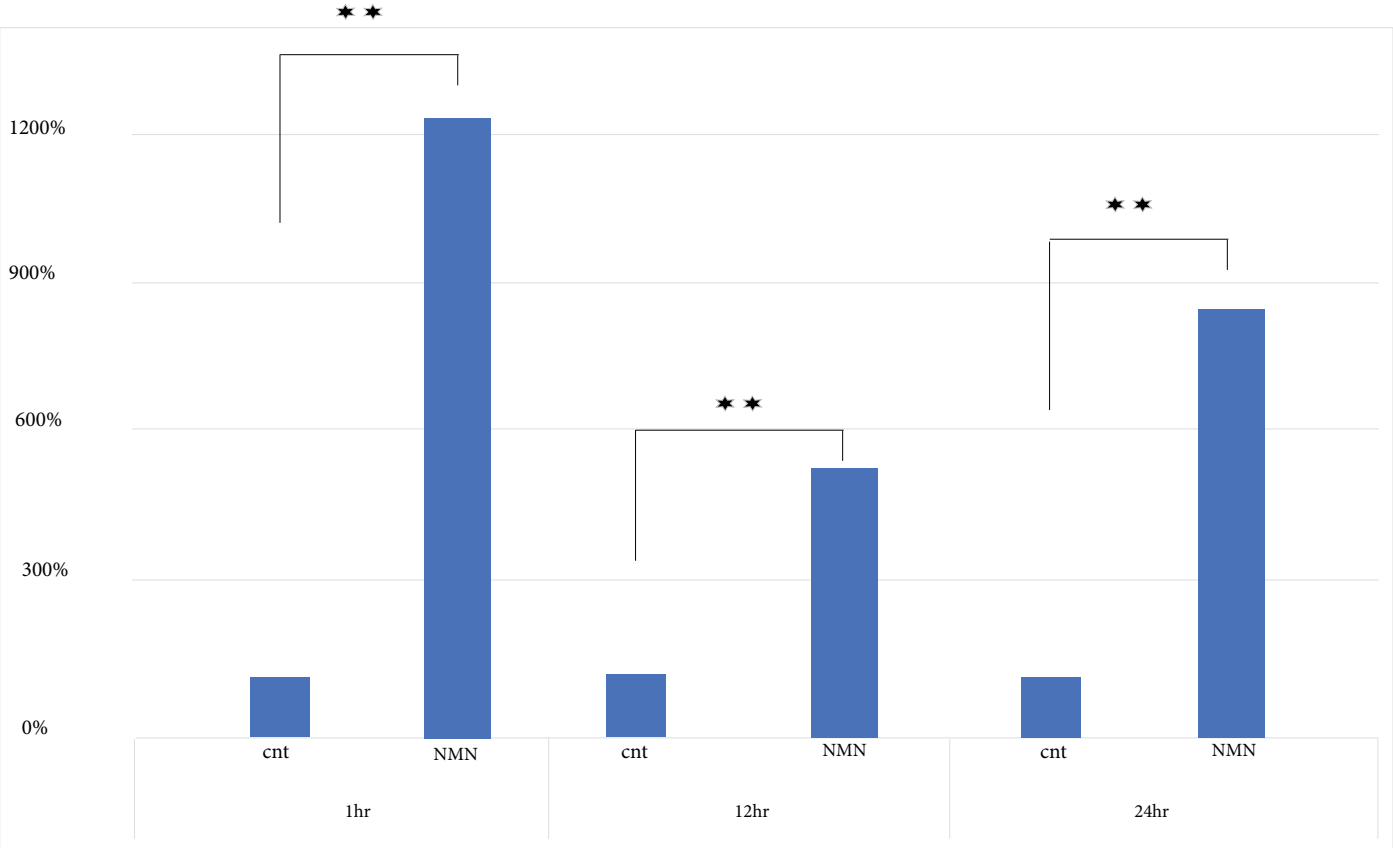

Fig.4 Rate of increase in AMPK activity in the control group and NMN-added group at each time.
(Mann-Whitney U test. P<0.01)

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6.Conflicts of Interest

The authors declare no conflict of interest.

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