

Information Wave of P53 Amplified Anti-Cancer Effect of Alkaline Reduced Water

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Abstract: It was revealed that every matter has its accompanying wave. The wave part of the matter contains information (called information wave in the paper), and can be transferred to water physically by shaking or tapping, and thus serially diluted water have been used to stimulate natural healing power in traditional homeopathy. This way of transferring the wave part of the matter to water has been already demonstrated by Benveniste and other researchers. In this study, instead of traditional homeopathic method a new electronic machine was devised to transfer the wave of matter to variety of medium including water. P53 functions as a potent tumor suppressor. However, there is no practical way to utilize the function of P53. If the information wave of P53 could be transferred to water or any medium contacting water, various strategies could be possible. In this study, information wave of P53 was first transferred to UM (mixture of ceramic balls which makes alkaline reduced water), and then UM produced alkaline reduced water with P53 information by contacting water. The water containing information wave of P53 inhibited cancer proliferation, showed anti-metastasis, and increased apoptosis. Water memory effect could be very useful for future cancer therapy.

Keywords: Alkaline reduced water, anti-cancer effect, anti-metastasis, anti-proliferation, apoptosis, homeopathy, P53, water memory.

INTRODUCTION

In 1988 Benveniste and colleagues published a controversial article showing a biological reaction of ultra high diluted solution, which could be called as 'water memory'. In the paper it was demonstrated that human basophil degranulation was triggered by extremely diluted antiserum against IgE [1]. Since then, they published many papers proving water memory effect under various experimental conditions [2-4]. As biological reaction in the absence of any effective molecules cannot be explained by conventional theory, the results of Benveniste and colleagues sparked many investigation of various seriousness. The most serious one was the research performed double blind by 4 independent European laboratories in 2004 [5]. They thoroughly investigated the possibility of water memory using basophil activation by extremely diluted histamine, and they all obtained the same results supporting Benveniste.

Benveniste first used homeopathic method to activate water by shaking with each dilution. Later he has developed a new technology as follows: An aqueous solution in which molecules were dissolved was put into a copper tub, then white noise was applied to one wall of the copper tub and received from a wall opposite to the one wall of the copper tub using a microphone, and a wave of the molecules was recorded in a sound blaster card that can record sound waves

of 20 to 20,000 Hz. Thereafter, Benveniste and colleagues confirmed through repeated experiments that, when the recorded sound wave was converted into a vibration signal to vibrate the water, a physiological reaction was also induced [6-11]. Benveniste automated the device to eliminate human influence, and obtained same results. These results suggest that there is inherent wave-like characteristic for each molecule, which could be transferred to water, modulated to sound, and could reproduce physiological reaction like the molecule itself. The inherent wave-like characteristic which interacts with environment was called as pilot wave by de Broglie and Bohm [12] and as information wave by Tiller [13], as it decrease thermodynamic entropy (called as information wave in this paper).

We also developed a new electronic device which could replace time-consuming homeopathy to activate water [14]. The device uses 7.8Hz frequency as a carrier which is the resonance frequency of the earth. Using the device information wave of hormones and other cytokines could be transferred to water and even to other medium like ceramic balls. Information wave of the hormone or cytokine transferred to ceramic balls could be passed to water indirectly by contacting water. Such water containing information wave of the matter functioned like hormone for human.

Water consists 70% of human body. Water reaches every tissue of human body within 30 minutes after drinking. It even flows through blood brain barrier and has almost no side effect. If water itself could work as a radical scavenger, it would be an ideal antioxidant. Recently, alkaline reduced water produced near cathode with high pH and negative

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Table 1. Composition of UM

Component	Mg	Coral	Tourmaline	Illite	Biotite	Magnetite
Wt %	50	10	10	10	10	10

oxidation reduction potential (ORP) was shown to have superoxide dismutase (SOD)-like activity and catalase-like activity, and thus, scavenge reactive oxygen species (ROS) which render oxidative damages to biological macromolecules and protect DNA from damage by oxygen radicals *in vitro* [15]. As ROS cause or aggravate variety of incurable diseases such as cancer, cardiovascular diseases, neuro-degenerative diseases as well as aging, if water could work as a radical scavenger, it is believed to prevent and/or cure variety of diseases due to oxidative damage including cancer.

Instead of using electrolyzed alkaline reduced water we developed a mineral combination to produce alkaline reduced water with high pH and low ORP (MRW, Mineral Reduced Water). Considering that alkaline reduced water can act as a scavenger of reactive oxygen species (ROS) and free radicals that contributes to cancer progression, we explored the possibility that MRW possess anti-cancer properties using animal model [16]. When B16-BL6 melanoma cells were inoculated subcutaneously and intraperitoneally into C56BL/6 mice, mice fed with MRW showed a significant delay in tumor growth and lengthened their survival compared to control group. MRW also showed the inhibition of metastasis by reducing the numbers of B16-BL6 melanoma induced colonies in the lung when these cells were injected through tail vein. The amount of ROS was significantly reduced in most of the organs in mice fed with MRW except for spleen, which is a major organ for immunity. In actuality MRW intake invoked systemic cytokines, such as, Th1 (IFN- γ , IL-12), cytokines for cellular immunity and Th2 (IL-4, IL-5), cytokines for humoral immunity. Both anti-oxidant effect and immune boosting effect could have contributed to anti-cancer effect of MRW.

P53, a transcription factor with molecular weight of 53 kilodalton, functions as a potent tumor suppressor [17]. P53 hold the cell at G1/S regulation point long enough for DNA repair proteins will have time to fix DNA damage and the cell will be allowed to continue cell cycle. P53 can also initiates apoptosis, the programmed cell death, if DNA damage proves to be irreparable. P53 is central to many of the cell's anti-cancer mechanisms. Thus, mutation in P53, could lose function as a tumor suppressor, which happens in most of the cancer.

Although P53 is so important for prevention of cancer, there is no way to utilize the function of P53. If we could transfer the information wave of P53 to water, various strategies could be possible. In this study we transfer the P53 information wave to UM which makes alkaline reduced water. Alkaline reduced water produced by UM (MRW) with P53 information wave was investigated for its anti-cancer effect.

MATERIALS AND METHODS

Generation of Mineral Reduced Water (MRW)

A special combination of ceramic balls plus magnesium (named as UM meaning healing mineral in Korean, patent

no: KR10-074860 and KR10-068 1409, summarized in Table 1) were devised to produce mineral alkaline reduced water (MRW). After overnight contact of 50g of UM with 2 liter of tap water, MRW with pH 9.8 and -290mv of ORP was prepared and used for the experiment.

Information waves of P53 was transferred to UM by home made electronic device which uses 7.8Hz frequency as a carrier [14]. Subtle magnetic field was generated with the frequency around the input container where coil is wrapped around to activate substance. Activated information wave of P53 was transferred to UM which is in the output container where coil was wrapped around. P53 information wave transferred to UM could be passed to water indirectly by contacting water.

ROS Assay

Quantitation of cytosolic ROS was measured by oxidation method of 2',7'-dichlorofluorescein-diacetate (DCF-DA) as described elsewhere [18]. 12.5 μ M DCF-DA was incubated with liver, spleen, lung, or brain homogenate. Change of fluorescence was measured at 490 nm of excitation wavelength and 526 nm of emission wavelength. For *in vitro* assay 0 – 50% MRW was added in the tube containing DCF-DA and Hypoxanthine/Xanthine oxidase (HX/XOD) system. After 30 minutes incubation of MRW with DCF-DA (12.5 μ M) with HX/XOD system at room temperature in dark place, fluorescence at 526 nm (excitation at 490 nm) was measured. 1.25 μ M ascorbic acid (AsA) was used for positive control.

Cell Culture

The MDA-MB-435 and MDA-MB-231 human cancer cells were obtained from the Lombardi Breast Cancer Depository at Georgetown University. They were grown in low glucose DMEM containing 10% FBS, 1% pen/strep, and 25 mM Hepes. For ARW condition, UM was incubated with DMEM for 3 hours before addition of FBS, pen/strep. pH of culture media was readjusted to 7.8 to generate MRW-DMEM. All cell lines were cultured in humidified incubators at 37°C in 5% CO₂. For the cell proliferation assay, cells were plated on 6 well culture plate at the density of 2×10^5 cells per well and their growth was measured every other day by using bright line counting chamber (Hausser Scientific, Horsham, PA).

Apoptosis Assay

The cells were seeded in 6-well culture dishes at a density of 1 to 3×10^5 cells per well in DMEM supplemented with 10% FBS and were grown overnight at 37°C in a humidified incubator with 5% CO₂. Cells were treated with MRW or control media for 3 days, followed by apoptosis assay using the Annexin V-PE Apoptosis Detection Kit I (BD Biosciences, San Diego, CA).

Cell Motility and Invasion Assay

For the cell motility assay, the upper and lower surface of the membrane in transwell inserts (Costar, Cambridge, MA)

Table 2. pH and ORP of MRW

Time(hr)	pH	ORP(mV)
0	6.86	+420.2
1	7.69	+71.6
2	8.78	-32.1
4	9.26	-150.9
8	9.68	-209.7
24	9.98	-290.6

were coated with collagen I at 4°C overnight. To prepare for the invasion assay, matrigel (0.5 µg, Collaborative Research, Bedford, MA) was diluted with cold water and dried onto each filter overnight at room temperature. On the following day, transwell membranes were blocked with DMEM for 1 hour at 37°C. Cells were trypsinized and resuspended in serum free DMEM/bovine serum albumin. A total of 10⁵ cells was added to upper chamber of each well. 100 ng/ml lysophosphatidic acid (LPA) was added to the lower chamber as a chemo-attractant. For MRW condition, cells were incubated with MRW-DMEM for 24 hours before the assay and during the assay. Inserts were incubated for 2-3 hours and non-migrating cells were mechanically removed using cotton swabs. The number of cells that were attached to the bottom side of the membrane were stained and counted using crystal violet. Assays were performed in triplicate and repeated several times.

RESULTS

Evaluation of Antioxidant Effect of MRW *In Vitro*

MRW was generated by contact with UM as mentioned in materials and methods. We monitored pH and ORP of

MRW up to 24 hours upon contact with UM (Table 2). MRW with pH around 10 and ORP of -290 mv was produced after overnight contact with UM. The pH of tissue culture media was re-adjusted into 7.8 for *in vitro* assays.

We then measured the ability of MRW to act as a scavenger of reactive oxygen species (Fig. 1) *in vitro*. Concentration of ROS was measured with MRW using DCFH-DA as described in materials and methods section. MRW showed a concentration dependent anti-oxidant effect as the reduction of ROS by 50% MRW solution (vs buffer) was similar to that of 1.25 µM ascorbic acid, a well known strong anti-oxidant.

Effect on Cancer Cell Proliferation and Survival

The data that MRW acts as an anti-oxidant suggest the possibility of its anti-cancer effect. To address this issue, we used MDA-MB-435 and MDA-MB-231 human cancer cell lines. These cell lines were well characterized for their malignant behaviors to induce tumorigenesis and metastasis [14-16]. To assess the effect of MRW on cancer cell proliferation, MDA-MB-435 and MDA-MB-231 cancer cells were maintained in either regular or MRW media for 1-5 days and their rate of proliferation was monitored. Control groups maintained a steady state growth as there is a 2 fold increase of proliferation in MDA-MB-435 cells (Fig. 2A) and a 1.5 fold increase in MDA-MB-231 cells (Fig. 2B) in every other day. In contrast, both MDA-MB-435 and MDA-MB-231 cells under MRW condition did show moderate declining in cell numbers (Fig. 2). However, MRW with information wave of P53 showed marked decrease in cell number. Both MRW and MRW with P53 information showed chemically same characteristic. Data showed that MRW effectively blocks cancer cell growth, but when P53 information wave was transferred to MRW, cancer cell growth was almost completely blocked suggesting that the tumor suppressing effect of P53 information wave worked against cancer cell growth.

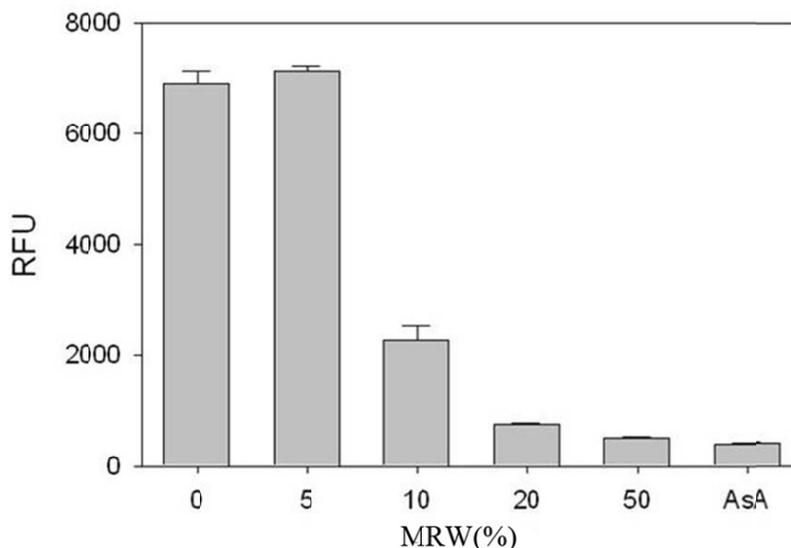


Fig. (1). Antioxidant effect of MRW *in vitro*. 0 – 50% MRW was added in the tube containing DCFDA and HX/XOD system. After 30 minutes at room temperature in dark place, fluorescence at 526 nm (excitation at 490 nm) was measured. 1.25 M ascorbic acid (AsA) was used for positive control. Data was expressed mean ± SD.

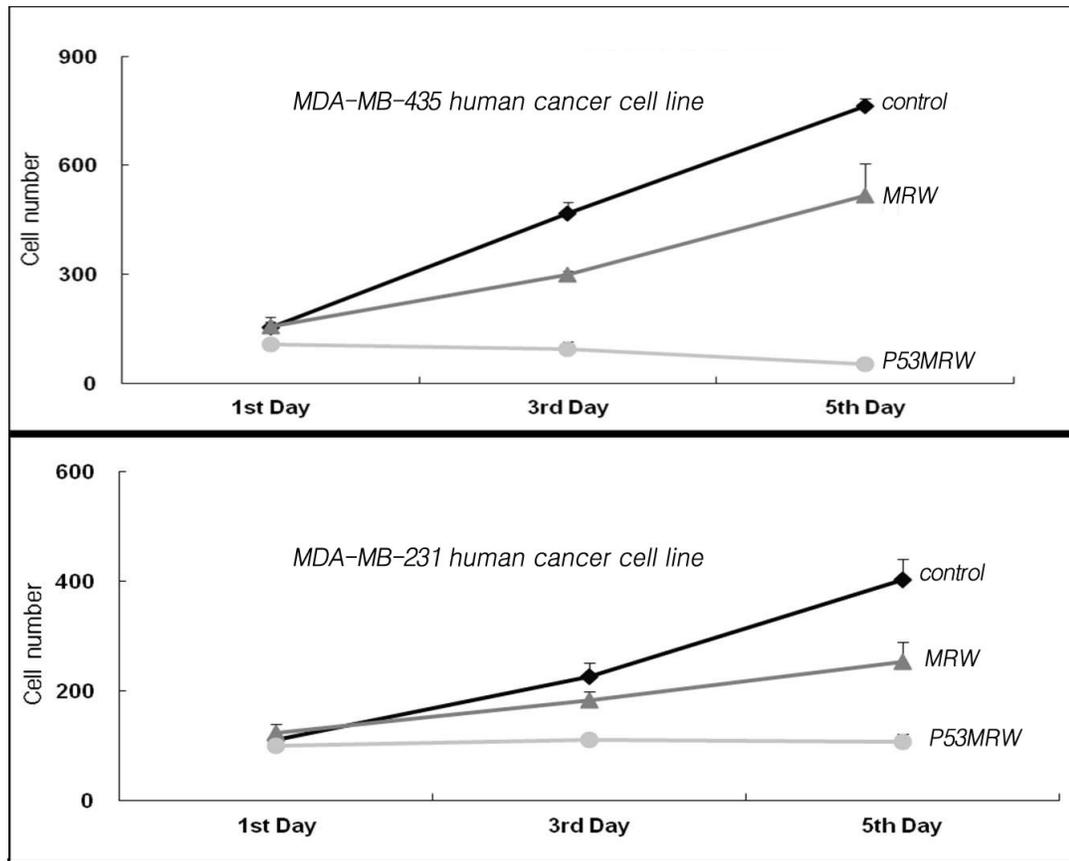


Fig. (2). Effect on the inhibition of tumor cell proliferation. The MDA-MB-435 and MDA-MB-231 human cancer cells were grown in low glucose DMEM containing 10% FBS, 1% penn/strep, and 25 mM Hepes. For MRW condition, UM was incubated with DMEM for 3 hours before addition of FBS, penn/strep. pH of culture media was readjusted to 7.8 to generate MRW-DMEM. For the cell proliferation assay, cells were plated on 6 well culture plate at the density of 2×10^5 cells per well and their growth was measured every other day. Data were expressed as mean \pm SD.

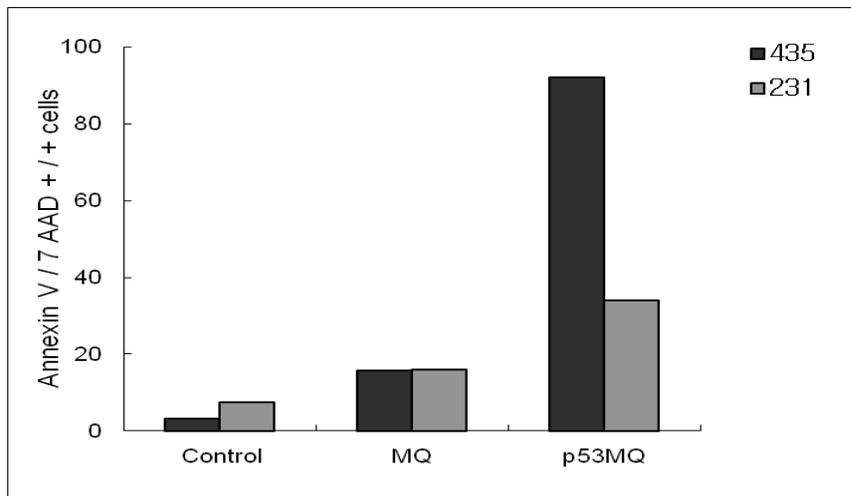


Fig. (3). Effect on apoptosis. The cells were seeded in 6-well culture dishes at a density of 1 to 3×10^5 cells per well in DMEM supplemented with 10% FBS and were grown overnight at 37°C in a humidified incubator with 5% CO_2 . Cells were treated with MRW, MRW with P53 information wave and control media for 3 days, followed by apoptosis assay.

Effect on Apoptosis

Declining of cancer cell growth by MRW suggests that the prolonged incubation of cancer cells with MRW may induce cancer cell death. To test the effect of MRW on apoptosis of cancer cells, we monitored the apoptotic index

of MDA-MB-231 and 435 cells by measuring annexin PE staining under regular and MRW tissue culture conditions (Fig. 3). Incubation of these cells with MRW for 3 days increases apoptosis of MDA-MB-435 cells about 5 fold and that of MDA-MB-231 cells about 2 fold (Fig. 3). We did not observe a significant increase of apoptosis of these cell lines

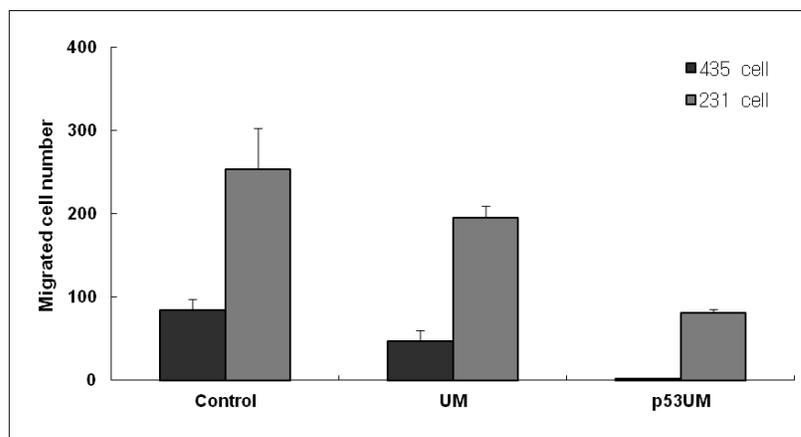


Fig. (4). Effect on the cell motility and invasion. For the cell motility assay, the upper and lower surface of the membrane in transwell inserts (Costar, Cambridge, MA) were coated with collagen I at 4°C overnight. For MRW condition, cells were incubated with MRW-DMEM for 24 hours before the assay and during the assay. Inserts were incubated for 2-3 hours and non-migrating cells were mechanically removed using cotton swabs. The number of cells that were attached to the bottom side of the membrane were stained and counted using crystal violet. Assays were performed in triplicate and repeated several times. Data were expressed as mean \pm SD.

up until 2 days of MRW incubation (data not shown) suggesting that long term incubation (3 days or longer) of MRW induces apoptosis of cancer cells.

When incubation was carried out with MRW with P53 information, apoptosis markedly increased almost 30 fold for MDA-MB-435 cells about 10 fold for MDA-MB-231 cells, suggesting that information wave of P53 contained in UM worked effectively to increase apoptosis.

Effect on Cancer Cell Motility and Invasion

We next assessed the effect of MRW on cancer cell functions important for late stages of progression such as cell motility and invasion. Cell motility and invasion are the essential characteristics of cancer cells for metastasis [19]. We monitored cell invasion and motility using Boyden chamber assay and used lysophosphatidic acid (LPA) as a chemoattractant as previously described [20]. We pre-incubated both MDA-MB-231 and MDA-MB-435 cells with MRW, MRW with P53 information wave, and control culture media for 24 hours prior to the cell motility and invasion assay. As mentioned previously, there was no significant increase in apoptosis during 24 hours of MRW incubation, which rule out the possibility that the effect on cell motility and invasion is not due to cell death. As shown in Fig. (4), MRW moderately reduced the ability of MDA-MB-435 and MDA-MB-231 cells to migrate towards LPA, while MRW with P53 information wave showed dramatic reduction by about 75% and 90% respectively. Invasive potential of these cells was reduced to similar extent. Based on our findings, MRW may prove to be a potent anti-migratory agent that potentially prevents the spread of the breast cancer from primary origin to distal organs. However, MRW with P53 information wave proved to be a much more potent anti-migratory agent. As both MRW and MRW with P53 information have same chemical characteristics, it could be suggested that anti-tumor suppressing effect of P53 information wave worked against the inhibition of migration.

DISCUSSION

Homeopathy views symptoms originated from the human body's natural healing process [21]. Homeopathy uses

potential toxins which induce symptoms similar to diseases to invoke natural healing process. As the substances which induce disease-symptoms are mostly toxins with serious side effects, homeopathy used the dilution method until there are no harmful effects to the human body, but by physically stimulating (either tapping or shaking) with each dilution. Homeopathy claims that the effects did not disappear even after levels of dilution were done until none of the toxin's molecules were left in the solution. Homeopathy has been ignored by the orthodox medical circles until now because current science could not explain how a substances' effect can be shown without the actual substance. However, it is not that no medical researches have been done on homeopathy. There have been almost there hundred verification experiments done on homeopathic effects in the past decade. Furthermore, about 80% of those showed homeopathy to have different effects from placebo effect. Even though not empathized, homeopathy uses water's memorizing ability [21, 22]. If homeopathy is effective therapy, it implies that water could store the information of toxins to boost natural healing power. If water could stores information of material, then this capacity does not need to be confined to homeopathy.

Benveniste was the first to show water memory effect in scientific way using human basophil degranulation [1]. According to Benveniste and colleagues, human basophil was still degranulated when anti-IgE was diluted extremely until there was no molecule left in the solution. Many debates were followed afterward, as water memory cannot be explained by conventional theory in which molecules should come in physical contact with a cell receptor to initiate signal transmission. In 2004 water memory effect was finally proved by double blind test using human basophil activation by extremely diluted histamine. This study was thoroughly investigated by 4 independent European laboratories [5]. They could not explain their findings and encourage others to investigate this phenomenon.

Benveniste suggested that when a wave propagated from molecule is transferred to a cell receptor through water, the wave can induce resonance of a receptor initiating intracellular signal transmission. The inherent wave of the

particle is not a new concept. In 1924 de Broglie proposed that every matter has accompanying wave (matter wave). The existence of the wave inherent to matter was experimentally confirmed, awarding a Noble prize for de Broglie. De Broglie further suggested that the wave inherent to matter is guiding the trajectory of particle (pilot wave) [12]. In 1952 Bohm redeveloped almost forgotten pilot wave theory. According to Bohm, pilot wave of particle is affected by the environment and also affects the environment, which is whole universe, giving non-locality [12]. In 1961 Eisberg showed by calculation that pilot wave is faster than the speed of light [23]. Calling such wave as 'information wave', Tiller suggested mass particle and pilot wave interacts so as to be experimentally operational [13]. According to Tiller, pilot wave inherent wave of the matter could be transferred to water, and the pilot wave transferred to water could affect receptor by resonance, and intracellular signal transmission could be initiated. In contrast, instead of inherent wave portion of matter, Meyl suggested that wave could be rolled to vortex which is carrying information and acting like particle [24]. According to Meyl, particle state and wave state does not coexist, although in equilibrium as a whole.

P53 is a transcription factor which interacts with specific position DNA. Our data demonstrated that information wave of P53 contained in water also could function as a tumor suppressor. Although P53 is very important for suppression of tumor, so far there is no way to utilize the function of P53. The information wave of P53 contained in water could be very useful for cancer therapy, especially for brain tumor where blood brain barrier blocks the flow of substances.

Water could reach to every organ of human body in 30 minutes without any obstacle. The information wave contained in water at least will not give unwanted side effects due to material decomposition. Information wave contained in water is not confined to P53 [14]. Many kinds of cytokine and hormone could be clinically utilized successfully [25]. Further researches regarding water memory are expected.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

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