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Role of an Atomic-Level-Based Approach for Improving Cancer Therapy

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Additional information is available at the end of the chapter

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Abstract

Looking at the atomic level of biological activity, the electron spin may be considered a key parameter, governing fundamental biological processes. Spin states have a major role in defining the structure, reactivity, magnetic and spectroscopic properties of a molecule. In the last decades, there has been a growing interest in the use of magnetic fields (MF) to study their influence on different biological systems, considering their effect on electron spin energy levels and consequently on redox-related cellular changes. Different authors have studied the use of magnetic fields as potential antitumor agent as well as an adjuvant agent to chemotherapy and radiotherapy with promising results. Overall, the published data support the presence, in laboratory animals, of antitumor efficacy in many types of cancer including adenocarcinoma, breast cancer, melanoma and neuroblastoma. Those antitumor effects seem to be associated with no observable side effects or toxicity in animals or in humans. More studies are necessary, mainly at the clinical level, to understand the real potential of this atomic approach in improving availability of cancer therapy. In addition, this approach may contribute to fulfill a knowledge gap facing biomedical science today, the one between the atomic level and the cellular level.

Keywords: magnetic fields, quantum biology, free radicals, cancer treatment, apoptosis, atomic biology, spin state in biological systems, antitumor effects, electromagnetic fields, biological effects, electron spin, p53

1. Introduction

Only 25 years ago, at the beginning of the 1990s, chemical biology was just an idea. Since then, that idea evolved into a global community of scientists, dedicated to understanding science at the interactions of chemistry and biology. The work of this community has greatly contributed to the development of medical science.

Among these studies, the molecular studies (molecular biology branch) have traditionally played a very important role in chemical biology research. From a panel of experts, the Nature Chemical Biology editors [1], the need for covering the remaining greatest knowledge gap facing biology today has been underlined: the one between the atomic level and the cellular level.

In fact, since molecules are made of atoms, the new frontier should be to study the influence of atomic structure on biological activity. This may be considered a natural evolution of medical science development. This development has made impressive progresses through centuries, moving from considering humans as an entire entity to considering organ system, organ, cell, cell components and, nowadays, molecules (molecular biology). In this trend, the missing additional part is the atom, and as a consequence, this new branch of medicine should be based on what we could call “atomic biology” (**Figure 1**).

This opens a fascinating scientific adventure: we strongly believe that Physics has to play an important role for the reasons better explained below. The development of a new branch of medical science that, in analogy with Chemical Biology, we can call Physical Biology, can have the main objective to study the influence of atomic structure on the characteristics of the biochemical reactions influencing cell life. A most important contribution is requested to elucidate phenomena at the base of biomolecular activity, influencing the genetic pathways that regulate cell life in genetically based illnesses, like cancer.

For this typology of illnesses, in fact, Chemical Biology and its branch Molecular Biology have been unable to help Medicine to obtain completely satisfactory therapeutic results. More selective treatments to avoid important adverse effects are needed.

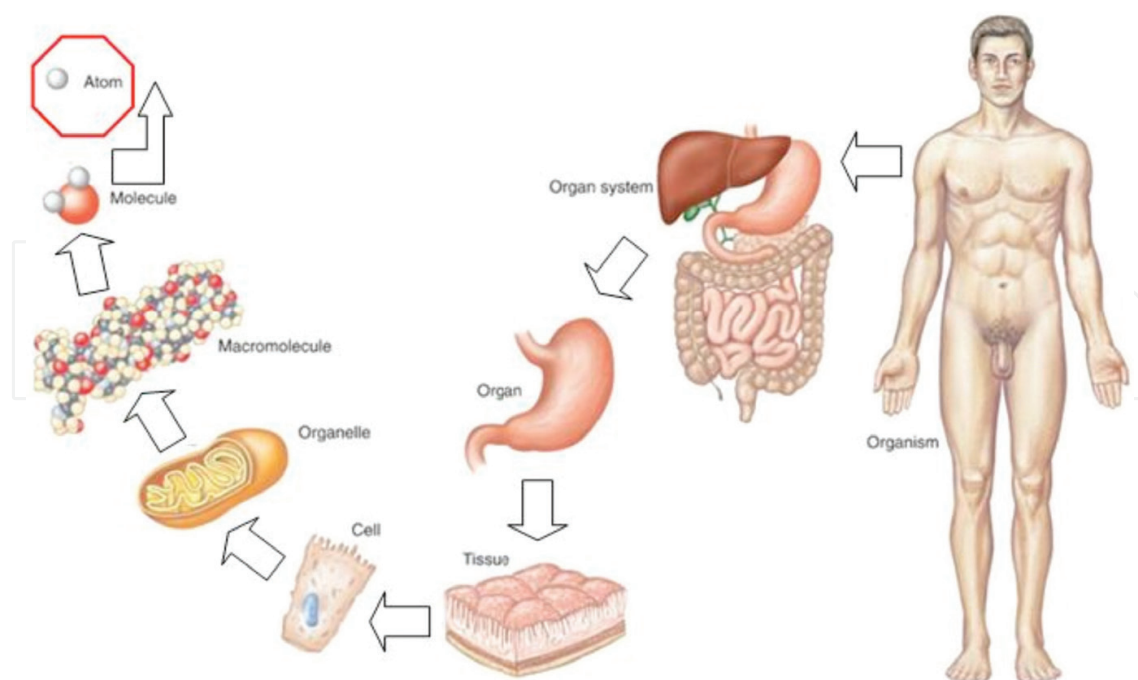


Figure 1. Trend of medical science from its beginning to now. With time (see from right to left), medicine has been developing considering first humans as unity and then made by organs, tissues, cells, until arriving to the present time of molecular biology. Since molecules are made of atoms, the next frontier should be the connection between atomic and biological levels.

The fundamental laws of physics have the capability of describing the very tiny processes that are at the core of life in matter. Because of this, we know for example that using magnetic fields with specific characteristics we can influence spin energy levels. This, for example, gives the possibility to medical doctors to use magnetic resonance imaging to have a very powerful clue of what happens at molecular/atomic levels of the biological structure and function, having consequently the possibility of making diagnosis with an accuracy not possible before.

We know from chemistry that electron spin state has a pivotal role in all the reduction-oxidation reactions that are at the core of the cellular metabolic pathway, governing the behavior of the biological system, influencing genetic stability. The synthesis of many complex molecules often requires the oxidation of their precursor, via the use of molecular oxygen. Availability of electrons to transfer changes when electron spins assume specific energy levels. These energy levels are known from quantum physics to be easily influenced by magnetic fields. Physics allows the use of specific static magnetic fields like those used in magnetic resonance imaging, but almost two/three order of magnitude (100–1000 times) less intense, not thermal, to influence electron spin state.

Aim of this chapter is to analyze the scientific reasons why a more physics standpoint approach to biological processes, or in other words an atomic-level-based approach to biological processes, may contribute to cancer therapy. We will consider biological processes from an atomic-level prospective, analyzing first the correlation between the atomic structure, its influence on availability of electrons, and key biological functions connected to cancer genetics and consequently the available literature reporting results obtained in different laboratories on the use of magnetic fields to influence cancer biology.

2. Atomic structure and fundamental role played by electron spin in key biological processes

Considering the three particles (electron, proton and neutron) constituting the atomic structure, the electron is the only elementary particle and it plays a pivotal role in chemical/biochemical reactions. Electron exchange allows chemical reactions to take place and electron transfer reactions are critical steps in diverse arrays of biological transformations, ranging from photosynthesis to aerobic respiration. Electrons are classified inside all the elementary particles according to their spin value that, being half integer, collocates them inside one of the two families of elementary particles, the fermions [2]. So, the spin has a fundamental role in the nature of matter's structure. Spin is an intrinsic property (form of angular momentum) of particles connected to their behavior in the presence of magnetic fields, where they seem to act as small magnets. In classical physics, a charged spinning object has magnetic properties that are very much like those exhibited by these elementary particles (**Figure 2**). Similarly, physics describes elementary particles in terms of their "spin." Despite this, spin is a purely quantum-mechanical phenomenon; it does not have a counterpart in classical mechanics and obeys quantum physics laws.

Fermions and then electrons obey the Pauli exclusion principle, which states that two identical electrons cannot exist in the same state, that is, electrons paired in the atomic structure in a way to have opposite spin value. Without Pauli exclusion principle, chemistry would not

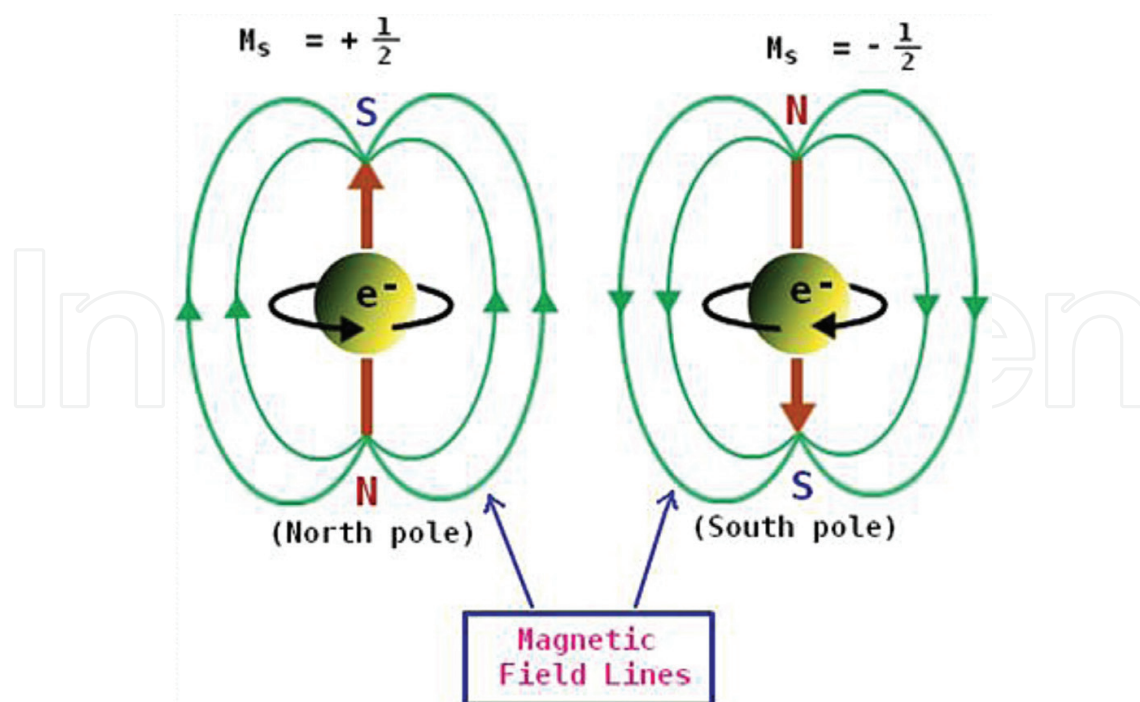


Figure 2. Electron while traveling around nuclei or between atoms/molecule rotates about its axis producing a magnetic field (from physics in fact we know that any moving charge produces a magnetic field) called angular momentum or spin. The electron spin value is $\pm\frac{1}{2}$ depending on the direction of its rotation.

have the Periodic Table. For these reasons, spin is an essential property influencing the order of electrons and nuclei in atoms and molecules, thus having great physical significance in chemistry. Therefore, we may consider the spin a very important physical entity when studying biomolecular processes. The spin, although being purely quantum-physical, has profound implications for real-world, large-scale systems like, for example, living tissue. In most cases, according to Pauli principle, electrons need to pair up to allow the system, to which they belong, to reach a lower level of energy. This process of electron pairing up facilitates stability in the system (i.e., chemical stability in a biological environment), but it is possible only when, as above states, electron spins have opposite values. This favorable situation depends on the electron origin as well as on the conditions of the chemical environment. When the electron pairing up is not possible, the system accumulates an excess of unpaired electrons. This process leads to the formation of different spin states of individual electrons as well as of molecular species containing unpaired electrons.

Chemically, any molecule containing a single, unpaired electron is defined as free radical. Free radicals are often highly unstable elements which in fact are chemically highly reactive (**Figure 3**). In many cases, the spin state has been found to be a key factor governing the behavior of the biological system since their influence in chemistry and bioinorganic chemistry [3].

The spin state has a pivotal role in all the reduction-oxidation (or redox) reactions that are at the core of our metabolic machinery. Redox reactions involve the transfer of electrons from one reactant to another. This kind of reactions is so important that our life depends on them. The synthesis of many complex molecules often requires the oxidation of their precursor, via the use of molecular oxygen. The utilization of molecular oxygen is vital in many biological

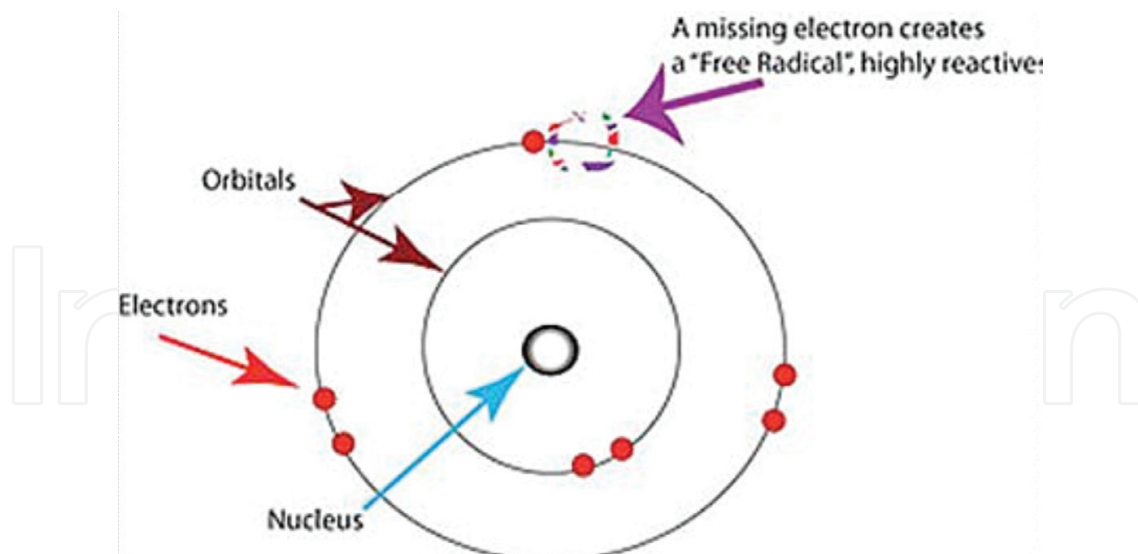


Figure 3. Atoms that do have an unpaired electron, like the atom here presented with atomic number of 7 (nitrogen), are called free radical. Because of their uncoupled spin, they have a not nulled magnetic moment and, in agreement with Pauli principle, they look for a counterpart to reach physical/chemical stability. For these reasons, free radicals are highly chemically reactive.

pathways. The ability of aerobic organisms to harness the power of molecular oxygen as a terminal electron acceptor in their respiratory cycles has revolutionized the evolution of life. Oxygen itself is a diradical: Oxygen-based radicals are also often referred to as radical reactive oxygen species (ROS), such as superoxide anion (O_2^-) and hydroxyl radical (OH \cdot). Electrons constituting molecules generally have opposite spins. These electrons stay in different orbitals and may take part to the bond formation. A peculiar characteristic of the oxygen structure is that it has two electrons that are not spin-paired, lying in different orbitals, and each of them is looking for an additional electron to pair up. In this case, the process of electron pairing-up, making two pair per atoms or four pairs per molecule, is oriented to produce water molecules to reach a state of lower free energy. Redox reactions are facilitated by enzymes since they have binding sites that can keep oxygen in contact with oxidizable substrates for a long time. This contact time, longer than that made possible only by collision, substantially improves the chance of spin reversal and allows the two electrons to pair up, process that otherwise would be not possible due to limited energy, below the kinetic barrier that would be provided by only collisions. Another important aspect is that enzymes may have such characteristics to catch and withhold available energy from oxidation processes, activity very useful and important in ATP high-energy-related compounds. In addition to this, we know that redox reactions affect signaling between molecules bound to DNA with potentially key effect on cell cycle. It has been shown that oxidative stress conditions may control the very important tumor suppressor protein p53 activity on different promoters using DNA-mediated electron transport [4–6]. One of the most challenging endeavors from both experimental and theoretical point-of-view is to elucidate the role and effect of different spin states on the properties of a biological system, even deciding which spin-state occurs naturally. There is in fact a growing interest in spin states in biochemistry from a chemical standpoint [3]. It can be supposed that a more physics-dependent approach may contribute significantly to this elucidation. In fact, being the spin a purely physical entity (localized magnetic moment/field), its energy states

can be theoretically better influenced by physical means than by chemical reactions. In fact, starting from the 1980s, magnetic fields have been widely used to influence the free radical chemistry, thanks to their influence on electron spin state energy levels [7].

3. Redox balance and metabolic processes in cancer

The maintenance of redox balance is considered a key factor for the cancer cell metabolism [8–11]. Adaption requires for these cells to be able to respond to the proliferative signals that are delivered by oncogenic signaling pathways. After malignant transformation, many cancer cells show a sustained increase in intrinsic generation of ROS which maintains the oncogenic phenotype and drives tumor progression throughout chemical reactions that are electron spin state dependent. Redox adaption through upregulation of antiapoptotic and antioxidant molecules allows cancer cells to promote survival and to develop resistance to anticancer drugs [12].

The dependence of tumor cells and cancer stem cells on their antioxidant capacity makes them vulnerable to agents that dampen antioxidant systems. There is a realistic prospect for treatments aimed to dramatically increase intracellular ROS to kill cancer cells by decreasing their antioxidant capacity. This may be obtained using compounds that inhibit antioxidant systems or through inhibition of specific signaling pathways that upregulate antioxidants in cancer cells. The resulting increase in reactive oxygen species may then induce tumor cell death either through random damaging functions of ROS or by specific induction of apoptosis via death signaling pathways. The advantage of such a strategy is that normal cells are not significantly affected since they have lower basal ROS levels and therefore are less dependent on up-/downregulation of oxidative stress [13]. A supposedly more simpler strategy would be to selectively influence the ROS concentration and then redox activity in cancer cell by directly influencing the spin state energy levels by using appropriate magnetic fields.

It is important to note that dealing with ROS concentration we have to consider the double-edge sword of ROS action. In fact, for example, a chemopreventive and an antitumor action have been reported by the use of nutraceuticals derived from fruits, vegetables, spices, and other natural products used in traditional medicine that show antioxidant efficacy. This lasts in agreement with observations [14, 15], suggesting that antioxidant enzymes like the mitochondrial antioxidant manganese superoxide dismutase (MnSOD) may function as new type of tumor-suppressor gene. The above suggest both “upside” (cancer-suppressing) and “downside” (cancer-promoting) actions of the ROS. Thus, similar to tumor necrosis factor- α , inflammation, and NF- κ B, ROS act as a double-edged sword.

4. How magnetic fields can influence biological chemistry through spin state

The fascinating aspect of spin states is that the formation of spin-correlated radical pair states between enzyme and oxygen radicals is magnetic field sensitive. In fact, the use of

appropriate magnetic fields is capable of changing the spin states of radical pairs modifying the rate of conversion between singlet (S) and triplet (T) and consequently influencing the free radical recombination rate and finally their concentration with downstream biological consequences [16].

Let us now see more in detail the mechanism through which magnetic fields influence spin states and consequently free radical chemistry. Radical pairs are typically formed by electron transfer or photolytic bond cleavage from a molecular precursor. The radical characteristics and then their chemical reactivity depend on their spin states of their unpaired electrons that, like all electrons, present an intrinsic spin angular momentum (quantum number $s = \frac{1}{2}$) characterized by two states, known as spin up and spin down. These two states are labeled by the magnetic quantum numbers $m_s = \pm\frac{1}{2}$ (m_s specifies the projection of the spin angular momentum on a fixed axis). Spin-correlated radical pairs are generated according to the physical law that implies the conservation of total spin angular momentum, a singlet molecular precursor leads to a singlet born radical pair, while a triplet precursor leads to a triplet born radical pair. The correspondent two spins of the born radicals will be aligned antiparallel and parallel for the singlet and triplet born, respectively. S_0 is generally used for the singlet state, its total spin quantum number S is zero as well as its overall spin angular momentum, so their energy is independent of any applied magnetic fields. Vice versa, the three triplet states T_0 , T_+ and T_- are defined by a total spin $S = 1$ and spin projection numbers equal to 0, +1 and -1, respectively. In the presence of an applied static magnetic field, the energy of the T_0 state is therefore also field independent, whereas how the energies of the T_+ and T_- states are shifted by the Zeeman interaction is proportional to the strength of the external applied static magnetic field (**Figure 4**). In the absence of an external static magnetic field, interconversion between the singlet and triplet states of the radical pair is driven by internal electron-nuclear hyperfine interactions.

The use of an external static magnetic field influences the interconversion between singlet and triplet states (see circle in **Figure 4**), influencing the rate of recombination of radical pair and consequently influencing free radical concentration. Even if the energy released by the magnetic field to the electron spin is significantly less than the thermal energy, the interesting aspect is that the spin-correlated radical pair, being in a nonequilibrium state, allows a kinetic effect if subsequent radical reactions are spin selective.

The frequency, amplitude and orientation of the magnetic field that perturbs the radical pair dynamics depend significantly on the local enzymatic chemical environment [17]. Specifically, the use of magnetic fields of moderate intensity (milli Tesla, or mT, range), static and/or at extremely low frequency, (frequency up to 300 Hz, down to 3.3 milliseconds) acts through the Zeeman effect removing the degenerative triplet energy levels. Considering the very short life span of a free radical (from microseconds to nanoseconds), it sees the extremely low-frequency magnetic fields as static, which intensity is time dependent.

It results, as discussed above, in the splitting of three levels of different energies, decreasing the probability of transaction from triplet to singlet spin states. The rate of free radical recombination, at a frequency from low to zero, depends on the intensity of magnetic fields that regulates the conversion from triplet to singlet state (**Figure 4** crossing between T_- and S_0).

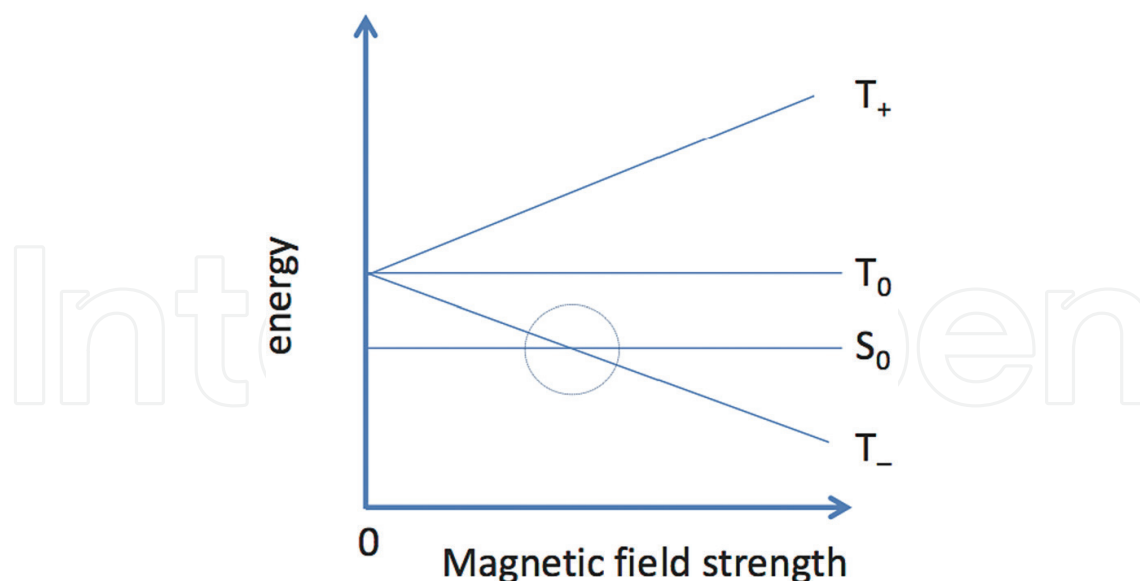


Figure 4. Effect of the Zeeman splitting on the singlet (S_0) and triplet (T_0 , T_+ and T_-) states. As we can see, increasing magnetic field intensity, the degenerative energy levels of triplet separate to reach a value (circle) at which we observe the crossing between T_- and S_0 .

Magnetic fields at much higher frequency (a mega Hz, or MHz, order of magnitude) used in combination with static magnetic fields can also have importance in influencing the spin states and then the correspondent chemical reactions. There are in fact multiple interactions in which weak magnetic fields (intensity on the order micro Tesla, or μT) at higher frequency can change the population distribution in the various spin states, among them the electron-nuclear hyperfine interaction. External static in combination with high frequency magnetic fields can alter radical pair spin dynamics by Zeeman and HFI resonance effects and thereby change the relative yields of reaction products that derive, alternatively, from singlet and triplet radical pair states [18–20]. Many biological molecules exhibit hyperfine splitting constant that ranges from 0.1 to 35 MHz [20–22], so fields of this frequency may be used to influence hyperfine coupling resonance. Magnetic fields at this higher frequency and very low intensity have been used, together with static magnetic fields, to influence hyperfine resonance, decreasing the intracellular superoxide concentration to selectively increase rat pulmonary arterial smooth muscle cell proliferation [23].

One can then suppose that appropriate magnetic fields may be used to perturb spin energy levels influencing the singlet-triplet and vice versa transaction probability and therefore controlling specific biochemical reactions and metabolic pathways. Different experimental data support the above hypothesis that magnetic fields, mainly static and extremely low-frequency fields, influence the ROS chemistry. This hypothesis is in agreement with the conclusion of a survey conducted, considering 41 scientific original publications showing that the use of this type of fields is capable of influencing the ROS chemistry in biology [24].

In addition, data coming from one multiannual, multicenter, multidisciplinary cancer research project have been reviewed [25]. In this project, static and extremely low-frequency magnetic fields having intensity on the mT range were used to influence electron spin energy levels and

consequently ROS chemistry and related redox cellular signals showing promising results. In fact, important anticancer effects have been reported in vitro as well as in vivo through an influence on the genetic pathway that increases apoptosis via the p53 protein with no adverse effects in different human cancer models.

5. Magnetic fields as a new potential effective antitumor agent acting through the electron spin

In the last decades, there has been a growing interest in the use of magnetic fields, in studying their influence on different biological systems, considering their effect on electron spin energy levels and consequently on redox-related cellular changes and on genetic instability [25].

Different authors have studied the use of static and extremely low-frequency magnetic fields as a potential antitumor agent as well as an adjuvant agent to chemotherapy and radiotherapy with promising results. Overall, the published data support the presence of antitumor efficacy in many types of cancers including adenocarcinoma, breast cancer, melanoma and neuroblastoma.

Different Italian Health Institutions and Universities have realized the importance of this potential new approach to cancer treatment by starting, years ago, a multiannual, multidisciplinary, multicenter research project, conducted, for the laboratory part, mainly in a GLP-certified laboratory. The project aim was to validate the hypothesis that an atomic-level-based approach to biological processes may help to improve cancer therapy. The project has produced a variety of results published in different Journals. Later, the research activity, which can be considered a continuation of the project, restarted in the Medical School of a Chinese University.

Different from other projects, the considered one provides an entire set of data obtained from a series of multiple in vitro and in vivo laboratory trials, as well as a pilot study conducted on humans. Project carried out with this logic have yielded a corpus of data, organized and linked together in a logical fashion to allow organic and more complete, articulated analysis than with those coming from a single study. These characteristics are even more important in the fields of bioelectromagnetisms, where different authors have been using different magnetic fields and different biological means, providing a huge set of data difficult to correlate and then to interpret. These are the reasons why in analyzing the published data, supporting the efficacy of magnetic fields to induce antitumor effects, in agreement with the hypothesis that an atomic-level-based approach to biological processes may contribute to improve cancer therapy, we start with those coming from the above-cited project. Analysis of data coming from other project will follow (see the paragraph “Scientific consensus on the anticancer efficacy of magnetic fields”).

5.1. Connecting atomic structure to biological activity

The multicenter project first objective was to find a connection between the fundamental law regulating the structure of matter and key biological function(s) regulating the stability of

genetic machinery and the conservation of the species. First the atomic structure was analyzed, realizing that the key parameter governing the formation of matter as well as its stability is the (electron) spin state as above reported.

In fact, let us consider the biological processes at the base of our life. The human body is constituted by almost 37,000 billion cells, and as an average, billion cells replicate every day; for each replica, almost a thousand billion of DNA bases replicate for every cell division. These incredible numbers tell us how complex and at the same time fascinating is the biological life as we know it. Which incredible organization Nature has set up to avoid as much as possible diseases due to the inevitable mistake during such huge number of copies of DNA basis. This organization allows that most of these errors will remain silent, but also minor errors can have a serious impact. In addition, despite its essential role in storing genetic information, the DNA molecule has limited chemical stability and is subject to spontaneous decay [26].

Processes such as hydrolysis and oxidation occur at significant levels *in vivo*, in part due to reactive metabolites continuously generated in various physiological processes. In addition, external factors like radiation and genotoxic chemicals will further stimulate DNA damage formation. The inherent instability of DNA constitutes both an opportunity and a threat. DNA lesions can block important cellular processes such as DNA replication and transcription, cause genome instability and impair gene expression. Lesions can also be mutagenic and change the coding capacity of the genome, which can lead to devastating diseases and conditions associated with genome instability, including cancer, neurodegenerative disorders and biological aging. At the same time, without mutations, Darwinian evolution would not be possible.

Cells use different biological processes like DNA repair, apoptosis and others like autophagy working in a well-defined and coordinated manner to contrast genome instability and prevent much as possible the onset of serious diseases.

Analyzing these processes, apoptosis appeared the most interesting one to be connected with the key physical parameter governing the stability of matter, that is, electron spin. In fact, apoptosis is the process set by cells to control genetic machinery in order to avoid replication of cell having an altered DNA. Thousands of proteins take part in a well-organized manner into this process that will send to death the cell with altered DNA before replication. In case of cancer, among thousands of proteins, the p53 protein, called the “DNA guardian,” seems to have a key role since, in mutated form, it is present in most cases of human cancers. This protein is considered so important that its encoding genes taken together are the most studied protein and genes in literature, with a total of more than 80,000 entries in PubMed [27].

5.2. Magnetic field characteristics, cell culture results and tumor growth inhibition

As seen before, different papers relate to p53 activity with redox machinery and ROS formation [4–6]. Accordingly, an intriguing hypothesis is to consider the possibility of selectively affected tumor cell growth using appropriate magnetic fields such as to influence redox signaling via an effect on electron spin state energy levels of ROS/enzymes that are connected with p53 activity/status (scheme of **Figure 5**) [28].

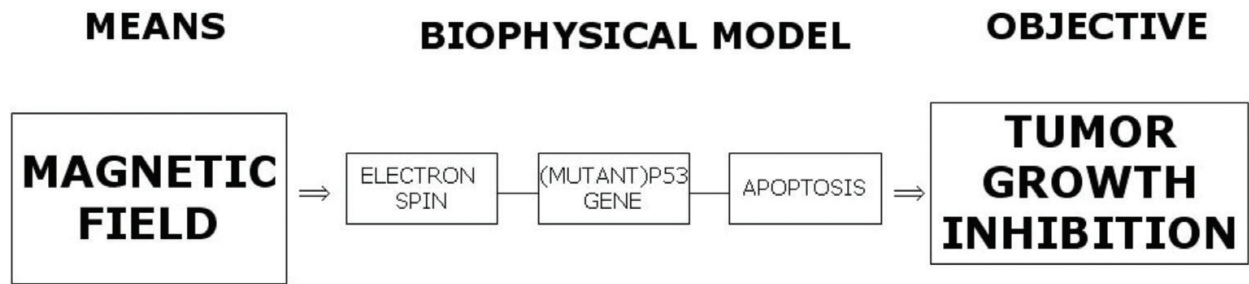


Figure 5. Biophysical model used to produce antitumor effects. Magnetic fields' effect on free radical recombination rate, activating redox signaling that influence mutant p53 activity and inhibit tumor growth via apoptosis.

According to the above-reported effect on the influence of magnetic fields on electron spin state, characteristics of the fields that are more suitable to influence cancer cell redox activity and p53-dependent apoptosis should result in agreement with known theory [7, 16, 28–30]. A series of about 100 in vitro trials exposing three different cell lines (MCF-7 human breast adenocarcinoma, WiDr human colon adenocarcinoma and MRC-5 human embryonal lung fibroblast) have been performed and apoptosis as a function of magnetic field exposure characteristics (intensity and frequency) has been assessed [31]. The magnetic field characteristics that gave best results were constituted by a combination of static and extremely low frequency, to form an extremely low-frequency modulated static magnetic field with intensity varying between 1 and 8 mT, as shown in **Figure 6**. The total time average intensity of this magnetic field was 5.5 mT. The characteristics of this field, experimentally selected, were in agreement with what was predicted by theory. The efficacy of this field has been confirmed in an animal trial exposing different group of nude mice bearing a WiDr human colon adenocarcinoma, each group with different magnetic field exposure regime, assessing tumor growth inhibition and apoptosis [31].

The in vitro results, obtained using different magnetic field frequency as well as intensity, show that magnetic fields are able to induce apoptosis like death, only in the considered

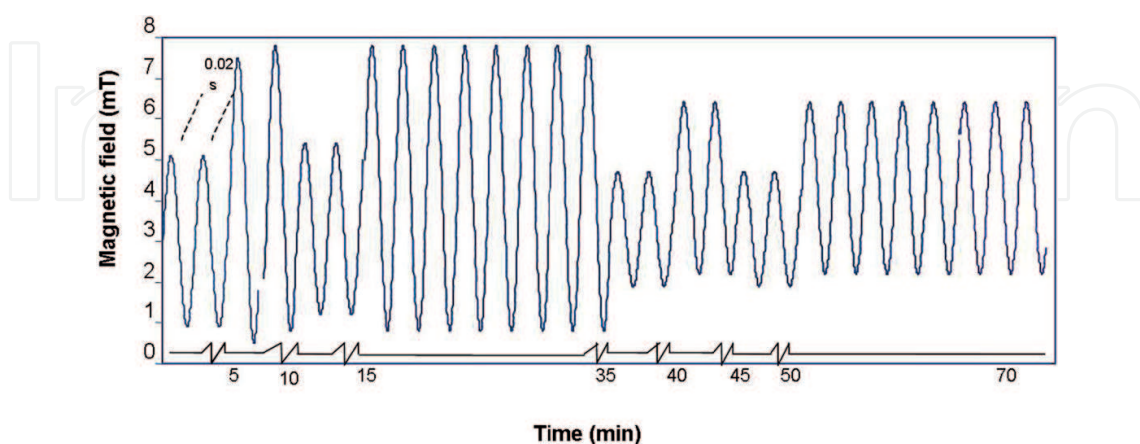


Figure 6. Magnetic field treatment characteristics. The magnetic fields were obtained with superimposition of a static magnetic field with an alternating 50-Hz (0.02 s) magnetic field forming an intensity-modulated magnetic fields which total intensity ranges from 1 to 8 mT with a time total average of 5.5 mT. The total treatment time for daily session is 70 min.

tumor cells, when their intensity is higher than 1 mT and this does not depend upon magnetic field frequency in the studied frequency range (0–300 Hz). This suggested to the authors that, in agreement with the theory at the base of the entire project, the biophysical mechanism connected to the apoptosis like death induction may be more related to free radical recombination processes than to ion resonance like mechanisms. Free radical recombination processes are activated by a direct action of magnetic fields on electron spin energy state levels of atoms and molecules with unpaired electrons. It was in fact known that free radical recombination processes occur in a timescale of nanoseconds to microseconds, and in this timescale, the extremely low-frequency (0–300 Hz) magnetic fields are seen as static [32, 33]. In addition, the authors noted that the need for amplitude-modulated fields (the one that gave the best tumor growth inhibition) to increase the effect otherwise obtained using only static or extremely low-frequency magnetic fields observed *in vitro* and *in vivo* [31] is in agreement with the need for establishing optimal condition(s) for the singlet-triplet spin state conversion required for the free radical recombination processes [34]. Safety analysis, in agreement with the theoretical biophysical mechanism, shows no toxic morphological changes induced by the magnetic field exposure in renewing, slowly proliferating, or static normal cells.

Treatment time may exert also an important role: a 70 min per day treatment for 5 days a week for 4 weeks has shown an inhibition of tumor growth of about 50%. The same 70-min treatment used two times a day gave a tumor growth inhibition of almost 70%, suggesting that in analogy with a chemical treatment this type of physical treatment exerts a form of dose-response efficacy, considering the time treatment connected to dose response.

5.3. Survival, apoptosis and p53 studies

In another animal trial using the same tumor model, nude mice were exposed, once a day, 5 days a week for the entire life to study survival, tumor growth inhibition and immune-reactive p53 [35]. After almost 1 year of treatment, the treated mice improved significantly their life span and the correspondent Survival Index was 1.31, that is, 31% survival time increase (**Figure 7**).

Specimens from each experimental mouse (magnetic fields exposed and not magnetic fields exposed) after weighted underwent histopathology, immunohistochemistry and transmission electron microscopy analysis. The results show that exposure to magnetic fields inhibits tumor growth of mice bearing a subcutaneous WiDr human colon adenocarcinoma, in agreement with the previous study. In addition, significant variation (by about 50%) in mitotic index (decrease), apoptosis (increase) and mutant p53 protein (decrease) (**Figure 8**) in tumor tissue is analyzed at the end of exposure time.

The observed tumor growth inhibition appears to be associated with morphological changes only in transformed cells. No morphological changes in renewing (i.e., bone marrow cells), slowly proliferating (i.e., hepatocytes) and static (i.e., terminally differentiated neurons) normal cells were observed. In addition, no significant differences in the number and morphology of blood corpuscular elements, excretory function of liver and kidney, and bone metabolism were detected, between the exposed and not-exposed animals. Authors' comments were

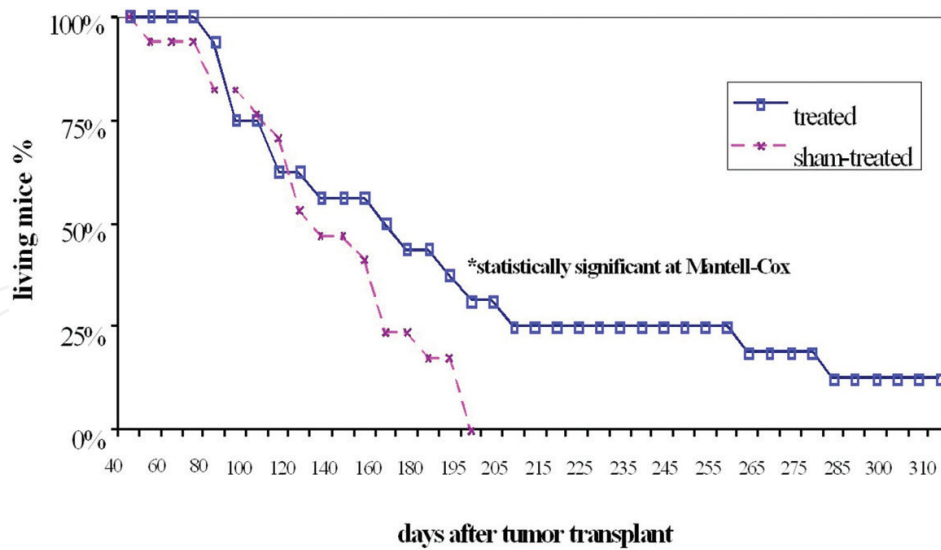


Figure 7. Survival time (days) of nude mice bearing human adenocarcinoma cancer and magnetic field treated (continue line) versus survival time (days) of nude mice bearing human tumor and not magnetic fields treated (dotted line). The magnetic field increases significantly the life span of nude mice bearing human cancer.

that the lack of adverse responses in normal cells and tissues suggests that the safety of this physical treatment may be related to its ability to interfere preferentially selectively with transformed cells. About p53 results they commented that from literature it is known that a loss of p53 functional status, due to either lack of gene expression or overexpression of its mutant form, leads to genomic instability and cancer [36]. The most frequently encountered mutations of p53 reduce its thermodynamic stability, determining the loss of the DNA binding conformation indispensable to the transcription regulation and tumor suppressor activity [37]. Pharmacological rescue of mutant p53 conformation and function has been also reported [38]. Others demonstrated that metal ions play a regulatory role in the control of p53 folding and DNA binding activity [39]. Specific DNA binding is influenced by redox regulation of p53, and binding of metal ions may directly affect p53 redox potential, either at the zinc binding cysteine residues or at other cysteine residue on the protein surface [40]. Thus, based on these data, authors suggest that the observed decrease of mutant p53 after magnetic field exposure, together with the increased apoptotic index and the slower growth of experimental tumors, could be explained by a rescue of wild-type p53. This phenomenon could be related to the effect of magnetic field exposure redox chemistry connected with metal ions.

5.4. Inhibition of metastatic spread and growth

Another important parameter to be evaluated in the assessment of potential antitumor efficacy of a treatment is its capability to inhibit the metastatic process. For this reason, a subsequent animal trial was conducted to evaluate the influence of the magnetic field treatment in the inhibition of metastatic spread and growth in a breast cancer model [41]. More specifically, a highly metastatic (in the lung) human cancer (MDA-MB-435) model, transplanted in nude mice, was used. Mice were exposed at the same magnetic field treatment regime (70 min a day for 5 days a

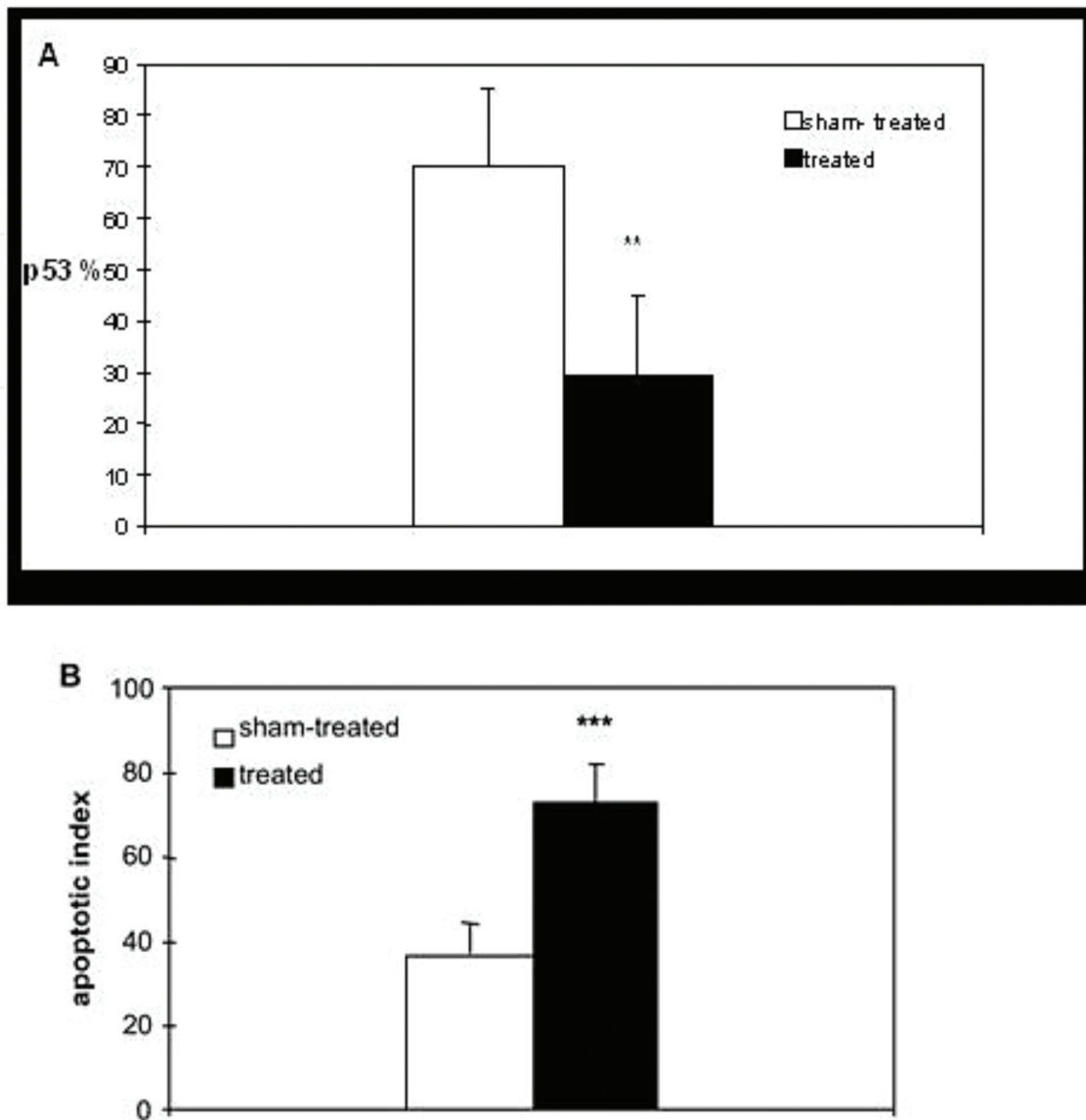


Figure 8. Influence of the magnetic field treatment on mutant p53 concentration (A) that markedly decreases, and on apoptosis (B) that markedly increases.

week) for 6 consecutive weeks. To allow a more complete evaluation of the potential antitumor efficacy of the magnetic field treatment, a positive control group treated with a chemotherapeutic agent (cyclophosphamide) was also used. At the end of the experiment, separate sections from each lung were examined at the microscope to determine the incidence of the different treatments (magnetic fields and cyclophosphamide) on number and sizes of metastases. Lung metastases were histologically counted, and each one was scored on the basis of the number of tumor cells. The size of each metastasis was evaluated by classifying the metastases in three categories (<10, 10–100, and >100) according to the total number of cells contained. As shown in **Figure 9**, both magnetic fields and cyclophosphamide treatments significantly decreased the number of lung metastases, classified according to the number of cell contained. In addition, the magnetic field treatment performed significantly better than cyclophosphamide.

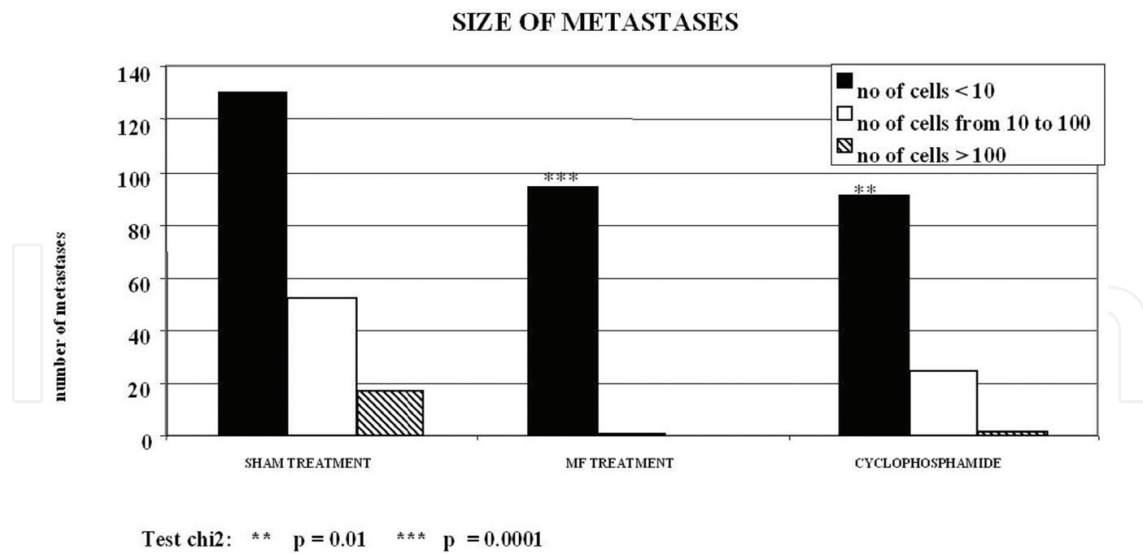


Figure 9. Effect of magnetic field (MF) in spread and growth of lung metastasis in nude mice bearing human metastatic breast cancer. Results are compared with those coming from the same experiment where the other group, of the same nude mice bearing the same human cancer, was treated with cyclophosphamide, a known chemotherapeutic agent. Results, in terms of small-size metastasis (black bars), medium-size metastasis (white bars) and large-size metastasis (black and white bars), show that the magnetic field treatment is more efficient than cyclophosphamide in inhibiting spread and growth of metastasis.

In fact, while magnetic field treatment and cyclophosphamide-treated mice reported almost the same number of metastases in the lowest cell content category (<10 cells), magnetic field-treated and cyclophosphamide-treated mice in the medium cell-content category (10–100 cells) reported 98% and 50% reduction in the number of metastases, respectively, while in the high cell-content category (>100 cells) was 100% and 90% for the magnetic field treated and cyclophosphamide-treated mice, respectively, compared to the control mice. Safety analysis was performed in all experimental animals. Results were in agreement with those observed in the previous trials confirming the safety of the treatment. In fact, gross pathology at necropsy, hematoclinical/hematological, and histological examination did not show any toxic or abnormal effects.

5.5. Synergism with chemotherapeutic agent atomic biology

The following trial was conducted to enquire about the possible synergism between magnetic field treatment and chemotherapeutic agents in terms of their influence on survival time [42]. Two animal models were tested, and immune-competent mice bearing murine Lewis Lung Carcinoma (LLCs) or B16 melanotic melanoma were exposed to magnetic fields treated with two commonly used anticancer drugs. The chemotherapeutic agents under investigation were cis-platin and cyclophosphamide, for the first and second models, respectively. The mice were exposed to the same magnetic fields used in the previous trials (static with the superimposition of extremely low-frequency magnetic field having a total time average intensity of 5.5 mT), provided daily (7 days a week) for the entire life. Synergistic activity was found only with cis-platin. In fact, the cis-platin antitumor efficacy was increased by magnetic field exposure,

leading to significantly prolonged animal survival. The magnetic field treatment almost tripled the efficacy of cis-platin since the effect of cis-platin low dose (3 mg/Kg) used in combination with magnetic field exposure was similar to that of cis-platin high dose (10 mg/Kg) alone. Unfortunately, it is not possible to make a direct comparison between the presence/absence of synergism between magnetic fields and the anticancer activity of cis-platin and cyclophosphamide because the two drugs were tested on two completely different animal models (different mouse strains and tumors). The authors' comments were that the synergistic activity observed between magnetic field exposure and cis-platin can be explained by the hypothesized ability to influence free radical chemistry exerted by the magnetic field treatment [28]. Two mechanisms, alone or combined, may be at the base of the observed results. First the platinum ion stimulates superoxide radical production [43, 44], and the magnetic field exposure enhances active oxygen production. When this production occurs at the cell membranes, the respective permeability changes, influencing the cell drug intake [44]. Second, it has been shown that the rate of conversion of cis-platin to reactive species, able to bind to DNA, is increased by localized production (in our case possibly due to the magnetic field exposure) of free radicals [45].

5.6. Safety of the treatment and considerations on efficacy

This magnetic fields treatment was then used in a pilot study where, according with the authorization of the Ethical Committee instituted by law, patients with advanced neoplasm were exposed to magnetic fields to assess safety and acute toxicity [46]. Eleven patients were treated with the same magnetic field characteristics we used in animal trials (static with the superimposition of extremely low-frequency magnetic field having a total time average intensity of 5–5 mT). Treatment included neck, thoracic and abdomen areas. Two treatment protocols that differed in the length of daily exposure to magnetic fields were set. In the first, patients were treated for 20 min/day, 5 days a week, over 4 weeks; in the second, patients were treated for 70 min/day, 5 days/week, over 4 weeks. A minimum of two patients was introduced in each treatment plan; if intolerable toxicity was not observed, two to five additional were treated. The reported results show that human exposure to the used magnetic fields treatment is not associated with important toxic and adverse side effects. Different exposure regimes, exposing 20–70 min daily, respectively, appear to be associated only to small changes in some laboratory parameters. Authors of the study conclude that the overall data of this clinical study on safety in humans seem to be in agreement with safety and toxicity data from animal trials, showing no toxic or abnormal effects when gross pathology at necropsy, blood and histological examination were performed [28, 31, 35, 41, 42]. In conclusion, the findings of this pilot study carried out in a small number of cancer patients support the possibility that the human exposure to magnetic fields with specific physical characteristics is associated with a favorable safety profile and good tolerability.

Based on all above-reported laboratory studies, it has been possible to confirm the antitumor efficacy of this new physical treatment that uses specific magnetic field characteristics. In fact, the reported data confirm the capability by magnetic fields to exert significant antitumor effects in different laboratory animal models as well as synergistic activity with chemotherapy without significant adverse effects. This may support the validity of this new approach

to biological processes. More studies are necessary, mainly at the clinical level, to understand the real potential of this atomic approach in improving availability of cancer therapy. In addition, this approach may contribute to fulfill a knowledge gap facing biomedical science today, the one between the atomic level and the cellular level.

5.7. Result confirmation

The antitumor efficacy reported in the above illustrated papers was confirmed, years later, in a different laboratory, located within the Medical School of the Zhejiang University, China. This replica performed in a different laboratory located in a different continent, using the same exposure machine as well as the same magnetic field characteristics, gives to the old/previous project results the necessary scientific validity, scientific validity that is confirmed when the same results are reported in different laboratory using the same methodology. In this university, the antitumor efficacy of magnetic fields treatment has been studied in two pediatric tumors, nephroblastoma and neuroblastoma [47]. The antitumor efficacy exerted by this magnetic field treatment as well as its combined effect with cis-platin was studied *in vitro* and *in vivo*. In this Chinese study, the time-average intensity of the magnetic fields was slightly different from the previous studies, 5.1 mT instead of 5.5 mT. This is due to the modification of the time duration of each of the eight rounds constituting, as in the old project, one magnetic fields treatment session. In the old project, each round lasted different times [48]. Now, each round lasted 3.5 min, and consequently each exposure session of treatment lasted 28 min. One or more treatment sessions (up to 4) were administered daily. In addition to the use of the standard static with the superimposition of extremely low-frequency magnetic field having a total time average intensity of 5.1 mT, alternatively, only static magnetic fields were used, while the total time-average field intensity was kept to 5.1 mT to help understanding the biophysical mechanisms.

For the *in vivo* part of the study in China, mice magnetic field exposure was based on the same exposure system and with the same protocol except that each round lasted 10 min; thus, each session lasted 80 min instead of 70 min as in the old project. Mice received one session of treatment daily for 15 consecutive days. *In vitro* results show that after daily exposure of 2 h the cell number of nephroblastoma and neuroblastoma cell lines (G401, CHLA255, N2a) decreased significantly from day 2, and the inhibition rate reached to about 20% after 3 days of exposure. The inhibitory effect was positively associated with exposure time, and subtraction of the AC field decreased the inhibition rate. Furthermore, it was found that the field decreased cell proliferation and induced apoptosis. Combining of the field with chemotherapeutic cisplatin further increased the inhibition rate compared with single use of either cisplatin or MF. In G401 nephroblastoma tumor model in nude mice, daily exposure of 80 min per day combined with cisplatin resulted in significant decrease of the tumor mass. The side effect of combinational treatment was limited to mild liver injury (an increase in aminotransferase levels), while magnetic field exposure did not hamper liver and kidney functions by itself. In conclusion, this 50 Hz, static modulated magnetic field exhibited antitumor effect on neuroblastoma and nephroblastoma and had the potential to be used in combination with cis-platin for increased efficacy and reduced side effects in these two childhood malignancies.

These results from Zhejiang University are completely in agreement with the previous results of the multiannual, multi-disciplinary, multicenter research project, confirming the antitumor efficacy of the magnetic field treatment exerted in two new human cancers (nephroblastoma and neuroblastoma), its synergistic activity with the studied chemotherapeutic agent cis-platin, with no induction or trivial induction of adverse effects. This agreement confirms the scientific validity of the potential antitumor efficacy of this new physical treatment that uses magnetic fields (electromagnetic energy) and that comes from a new approach of biological processes based on quantum physics, and such approach considers the atomic structure as a key aspect in studying the biological activity possibly introducing to a new additional branch of medical science that might be called atomic biology in analogy with molecular biology.

5.8. Scientific consensus on the anticancer efficacy of magnetic fields

There has been a growing scientific consensus on the anticancer activity of static and extremely low-frequency magnetic fields. In the last decade, many authors have published different papers, reporting results that are in agreement with those analyzed above. We now will shortly analyze the content of these papers.

Specifically, tumor growth inhibition has been studied on nude mice bearing metastatic mouse breast tumor cells exposed to 100 mT, 1 Hz magnetic fields for different times a day (60, 180, and 360 min/day) for 4 weeks, observing a tumor growth inhibition as a function of the exposure time reaching the suppression of tumor growth when exposure was 360 min/day [49]. Tumor growth inhibition as well as metastasis inhibition was observed in mice bearing hepatocarcinoma cells exposed to 400 mT, 7.5 Hz magnetic fields, 120 min/day for 30 days, observing an inhibitory effect on tumor growth [50]. In another study, the application of 4.5 mT, 120 Hz magnetic fields, 50 min/day for 32 days inhibited preneoplastic lesions chemically induced in the liver of male rats by reducing cell proliferation [51]. The synergistic effect with anticancer drugs has been studied *in vivo* and *in vitro* by different authors. *In vivo*, El-Bialy *et al.* [52] studied female mice bearing an ascites carcinoma treated with 3 mg/Kg i.p. cis-platin and exposed to 10 mT, 50 Hz magnetic fields 60 min/day for 2 weeks, showing that extremely low-frequency magnetic fields enhanced the cytotoxic activity of cisplatin and potentiate the benefit of using a combination of low-dose cisplatin and extremely low-frequency magnetic fields in the treatment of ascites carcinoma. Chen W.F. *et al.* [53] studied human leukemic cells (K562) exposed *in vitro* to 8.8 mT static magnetic fields, treated with cis-platin at concentrations from 20 to 10 microg/ml, and the results suggest that the mechanism is correlated with the DNA damage model. Hao Q. *et al.* [54] reported results showing that an 8.8 mT static magnetic fields enhanced the cytotoxic potency of adriamycin (25 ng/ml) on K562 cells, and a decrease in P-gp expression may be one reason underlying this effect. Kakikawa M. *et al.* [55] reported results showing that 50 mT, 60 Hz magnetic fields enhanced the cytotoxicity both of mitomycin C and of cis-platin on *E. coli* bacterium; these results suggest that magnetic fields change the permeability of the cell membrane and affect drug intake. Results of a clinical trial devoted to studying the effects on palliation of general symptoms as well as survival were reported by C. Sun *et al.* [56] in 13 advanced nonsmall cell lung cancer (NSCLC) patients treated with 400 mT, 0–50 Hz magnetic fields 120 min/day for 6–10 weeks.

The authors observed prolonged survival and moderately improved general symptoms without any severe toxicity or side effects. More recently other three studies have been published enlarging the above scenario. Two hours of treatment with 50 Hz, 20 mT magnetic fields makes resistant cells of human ovarian carcinoma sensitive to cisplatin via p53 activation [57]. A metastatic melanoma mouse model exposed 400 mT, 7.5 Hz magnetic fields, 120 min/day for 27 days reported a significant growth inhibition of metastatic tumor burden of lung, showing that extremely low-frequency magnetic field exposure promoted the inhibitory effect of ROS on AKT pathway and decreased Foxp3 expression [58]. Three-hour exposure to 1 mT 50 Hz magnetic fields induces apoptosis on osteosarcoma cells via oxidative stress [59].

Part of the above-cited studies has been also considered in two reviews published in 2013, one devoted to analyze if radiotherapy could be enhanced by electromagnetic field treatment [60]. The first review concludes that the analyzed studies reflect encouraging results and corroborate the hypothesis that combined exposure to some chemical agents ionizing radiation should be used to increase DNA damage and help cancer treatment. The other review covers three areas of investigation connected to the use of magnetic fields, in particular free radical generation and oxidative stress, apoptosis, genotoxicity and cancer [61], concluding that magnetic field causes oxidative stress and, as a result, damages ion channels, leading to changes in cell morphology and expression of different genes and proteins and also changes in apoptosis and proliferation. In addition, about the use of magnetic fields in combination with other external factors, such as ionizing radiation and some chemicals, there is evidence strongly suggesting that magnetic fields modify their effects, improving cancer treatment. Finally, the authors stated that the analyzed studies provide valuable insight into the phenomenon of biomagnetism and open new avenues for the development of new medical applications. More recently, L. Montagnier, the 2008 Nobel Prize for Medicine assignee, has stressed, also on the base of the above scientific scenario, the importance of the use of magnetic fields in cancer treatment [62].

6. Comments and conclusions

All the reported reviews conclude that additional studies are necessary to better clarifying the biomolecular mechanism(s) and understand the real potential of this new possible medical treatment. The call for additional studies, included clinical ones, has been also suggested by the more recent review dealing with the capability of magnetic fields to influence genetic stability and the potentiality of their use in cancer treatment [25]. This last concludes that a number of papers reports on the correlation between static and extremely low-frequency magnetic fields and genetic instability. This correlation has been found in studies on gene expression and DNA damage due to oxidative stress, including double-strand breaks, chromosomal aberrations and micronucleus induction. This review also underlines that the analyzed literature makes it plausible to apply an atomic-level approach to biological processes (atomic biology approach) using electromagnetic energy as a bridge between the atomic level (spin energy levels) and the cellular level (oxidative stress, DNA damage, genetic instability, p53 status and apoptosis).

The content of present chapter, together with the consensus among the analyzed literature, supports the capability by magnetic fields to exert significant antitumor effects in different laboratory animal models as well as synergistic activity with chemotherapy. This, without significant adverse effects observed in the laboratory animal trials as well as in the limited human studies, highlights the potential validity of this new atomic based approach to biological processes.

We are only at the beginning of a scientific adventure of this new potential branch on biological/medical research that may be called atomic biology. The atomic (electron spin) based approach to cancer treatment has given promising results to foresee great potentiality of this approach to open new frontiers of biomolecular research and medical application, since magnetic fields, different from chemical products, have the capability of influencing in a very selective way only the desired spin state of a given biomolecule. The expected clinical results for this type of approach would hopefully be more selective, that is, with less adverse effects. More studies are necessary, mainly at the clinical level, to understand the real potential of this atomic approach in improving availability of cancer therapy.

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References

- [1] Bucci M, Goodman C, Sheppard TL. A decade of chemical biology. *Nature Chemical Biology*. 2010;6:847-854. DOI: 10.1038/nchembio.489

- [2] Braibant S, Giacomelli G, Spurio M. *Particles and Fundamental Interactions: An Introduction to Particle Physics*. 2nd ed. New York & Frankfurt: Springer; 2012. DOI: 10.1007/978-94-007-2464-8
- [3] Swart M, Costas M (Eds): *Spin States in Biochemistry and Inorganic Chemistry: Influence on Structure and Reactivity*. Ltd.: John Wiley & Sons; 2016. DOI: 10.1002/9781118898277
- [4] Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein BA. Model for p53-induced apoptosis. *Nature*. 1997;**389**:300-305. DOI: 10.1038/38525
- [5] Liu B, Chen Y, St Clair DK. ROS and p53: A versatile partnership. *Free Radical Biology and Medicine*. 2008;**44**(8):1529-1535. DOI: 10.1016/j.freeradbiomed.2008.01.011
- [6] Augustyn KE, Merino EJ, Barton JK. A role for DNA-mediated charge transport in regulating p53: Oxidation of the DNA-bound protein from a distance. *PNAS*. 2007;**104**(48):18907-18912. DOI: 10.1073/pnas.0709326104
- [7] Steiner UE, Ulrich T. Magnetic fields effects in chemical kinetics and related phenomena. *Chemical Reviews*. 1989;**89**:51-147. DOI: 10.1021/cr00091a003
- [8] Bhat AV, Hora S, Pal A, Jha S, Taneja R. Stressing the (epi)genome: Dealing with ROS in cancer. *Antioxidants & Redox Signaling*. 2017 Aug 17. DOI: 10.1089/ars.2017.7158
- [9] Ray PD, Huang B-W, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular Signalling*. 2012 May;**24**(5):981-990. DOI: 10.1016/j.cellsig.2012.01.008
- [10] Che M, Wang R, Wang H-Y, Steven Zheng XF. Expanding roles of superoxide dismutases in cell regulation and cancer. *Drug Discovery Today*. 2016 January;**21**(1):143-149. DOI: 10.1016/j.drudis.2015.10.001
- [11] Liu H, Liu X, Zhang C, Zhu H, Qian X, Youquan B, Lei Y. Redox imbalance in the development of colorectal cancer. *Journal of Cancer*. 2017;**8**(9):1586-1597. DOI: 10.7150/jca.18735
- [12] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nature Reviews Cancer*. 2010;**11**:85-95. DOI: 10.1038/nrc2981
- [13] Lio G-Y, Storz P. Reactive oxygen species in cancer. *Free Radical Research*. 2010;**44**(5). DOI: 10.3109/10715761003667554
- [14] Bravard A, Sabatier L, Hoffschir F, Ricoul M, Luccioni C, Dutrillaux B. SOD2: A new type of tumor-suppressor gene? *International Journal of Cancer*. 1992;**51**:476-480. DOI: 10.1002/ijc.2910510323
- [15] Hitchler MJ, Oberley LJ, Domann FJ. Epigenetic silencing of SOD2 by histone modifications in human breast cancer cells. *Free Radical Biology and Medicine*. 2008;**45**:1573-1580. DOI: 10.1016/j.freeradbiomed.2008.09.005
- [16] Barnes FS, Greenebaum B. The effects of weak magnetic fields on radical pairs. *Bioelectromagnetics*. 2015;**36**:45-54. DOI: 10.1002/bem.21883

- [17] Woodward JR, Foster TJ, Jones AR, Salauru AT, Scrutton NS. Time-resolved studies of radical pairs. *Biochemical Society Transactions*. 2009;**37**:358-362. DOI: 10.1042/BST0370358
- [18] Canfield JM, Belford RL, Debrunner PG, Schulten K. A perturbation-theory treatment of oscillating magnetic-fields in the radical pair mechanism. *Chemical Physics*. 1994;**182**: 1-18. DOI: 10.1016/0301-0104(93)E0442-X
- [19] Schulten K, Staerk H, Weller A, Werner HJ, Nickel B. Magnetic-field dependence of geminate recombination of radical ion-pairs in polar-solvents. *Zeitschrift für Physikalische Chemie*. 1976;**101**:371-390. DOI: <https://doi.org/10.1524/zpch.1976.101.1-6.371>
- [20] Usselman RJ, Chavarriaga C, Castello PR, Procopio M, Ritz T, Dratz EA, Singel DJ, Martino CF. The quantum biology of reactive oxygen species partitioning impacts cellular bioenergetics. *Scientific Reports*. 2016;**6**:38543. DOI: 10.1038/srep38543
- [21] Schleicher E, Wenzel R, Ahmad M, Batschauer A, Essen LO, Hitomi K, Getzoff ED, Bittl R, Weber S, Okafuji A. The electronic state of flavoproteins: Investigations with proton electron-nuclear double resonance. *Applied Magnetic Resonance*. 2010;**37**:339-352. DOI: <https://doi.org/10.1007/s00723-009-0101-8>
- [22] Ritz T, Thalau P, Phillips JB, Wiltschko R, Wiltschko W. Resonance effects indicate a radical-pair mechanism for avian magnetic compass. *Nature*. 2004;**429**:177-180. DOI: 10.1038/nature02534
- [23] Usselman RJ, Hill L, Singel DJ, Martino C. Spin biochemistry modulate reactive oxygen species (ROS) production by radio frequency magnetic fields. *PLoS One*. 2014;**9**(3). DOI: 10.1371/journal.pone.0093065
- [24] Mattsson MO, Simkò M. Grouping of experimental conditions as an approach to evaluate effects of extremely low-frequency magnetic fields on oxidative response in in vitro studies. *Frontiers in Public Health*. 2014;**2**:132. DOI: 10.3389/fpubh.2014.00132
- [25] Tofani S. Electromagnetic Energy as a Bridge Between Atomic and Cellular Levels in the Genetic Approach to Cancer Treatment. *Current Topics in Medicinal Chemistry*. 2015;**15**(6):572-578. DOI: 10.2174/1568026615666150225104217
- [26] Lindahl T. Instability and decay of the primary structure of DNA. *Nature*. 1993;**362** (6422): 709-715. DOI:10.1038/362709a0
- [27] Fischer M. Census and evaluation of p53 target genes. *Oncogene*. 2017 Jul 13;**36**(28):3943-3956. DOI: 10.1038/onc.2016.502
- [28] Tofani S. Physics may help chemistry to improve medicine: a possible mechanism for anticancer activity of static and ELF magnetic fields. *Physica Medica*. 1999;**15**(4):291-294 <https://www.researchgate.net/publication/262487109>
- [29] Grissom CB. Magnetic field effects in biology: A survey of possible mechanisms with emphasis on radical-pair recombination. *Chemical Reviews*. 1995;**95**:3-24. DOI: 10.1021/cr00033a001

- [30] Kattinig DR, Evans EW, Dejean V, Dodson CA, Wallace MI, Mackenzie SR, Timmel CR, Hore PJ. Chemical amplification of magnetic field effects relevant to avian magnetoreception. *Nature Chemistry*. 2016;**8**:384-390. DOI: 10.1038/nchem.2447
- [31] Tofani S, Barone D, Cintorino M, de Santi M, Ferrara A, Orlassino R, Ossola P, Peroglio F, Rolfo K, Ronchetto F. Static and ELF magnetic fields induce tumor growth inhibition and apoptosis. *Bioelectromagnetics*. 2001;**22**(6):419-428. PMID: 11536283
- [32] Scaiano JC, Cozens FL, McLean J. Model for the rationalization of magnetic field effects *in vivo*: application of the radical-pair mechanism to biological systems. *Photochemistry and Photobiology*. 1994;**59**:585-589. DOI: 10.1016/S0006-3495(96)79263-9
- [33] Engström S. In: Barnes FS, Greenebaum B, editors. *Handbook of Biological Effects of Electromagnetic Fields: Bioengineering and Biophysical Aspects of Electromagnetic Fields*. Third ed. Boca Raton: CRC Press; 2007. pp. 157-168. ISBN: 9780849329524
- [34] Polk C. Dosimetry of extremely-low-frequency magnetic fields. *Bioelectromagnetics*. 1992;**1**:209-235. DOI: 10.1002/bem.2250130720
- [35] Tofani S, Cintorino M, Barone D, Berardelli M, De Santi MM, Ferrara A, Orlassino R, Ossola P, Rolfo K, Ronchetto F, Tripodi SA, Tosi P. Increased mouse survival, tumor growth inhibition and decreased immunoreactive p53 after exposure to magnetic fields. *Bioelectromagnetics*. 2002;**23**(3):230-238. PMID:11891753
- [36] Sherr CJ. Cancer cell Cycle. *Science*. 1996;**274**:1672-1677. PMID: 8939849
- [37] Bullock AN, Henckel J, DeDecker BS, Johnson CM, Nikolova PV, Proctor MR, Lane DP, Fersht AR. Thermodynamic stability of wild-type and mutant p53 core domain. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**:14338-14342. PMCID: PMC24967
- [38] Foster BA, Coffey HA, Morin MJ, Rastinejad F. Pharmacological rescue of mutant p53 conformation and function. *Science*. 1999;**286**:2507-2510. PMID: 10617466
- [39] Meplan C, Richard MJ, Hainaut P. Metalloregulation of the tumor suppressor protein p53: zinc mediates the renaturation of p53 after exposure to metal chelators *in vitro* and in intact cells. *Oncogene*. 2000;**19**:5227-5236. DOI: 10.1038/sj.onc.1203907
- [40] Wu HH, Sherman M, Yuan YC, Momand J. Direct redox modulation of p53 protein: potential sources of redox control and potential outcomes. *Gene Therapy and Molecular Biology*. 1999;**4**:119-132. http://gtmb.org/volumes/Vol4/_11FC%20%20%20Momand%20p53%20119-132.pdf
- [41] Tofani S, Barone D, Peano S, Ossola P, Ronchetto F, Cintorino M. Anticancer activity by magnetic fields: inhibition of metastatic spread and growth in a breast cancer model. *IEEE Plasma Science*. 2002;**30**(4):1552-1557. DOI: 10.1109/TPS.2002.804209
- [42] Tofani S, Barone D, Berardelli M, Berno E, Cintorino M, Foglia L, Ossola P, Ronchetto F, Toso E, Eandi M. Static and ELF magnetic fields enhance the *in vivo* anti-tumor efficacy

- of cis- platin against Lewis lung carcinoma, but not of cyclophosphamide against B 16 melanotic melanoma. *Pharmacological Research*. 2003;**48**(1):83-90. PMID: 12770519
- [43] Masuda H, Tanaka T, Takahama U. Cisplatin generates superoxide anion by interaction with DNA in a cell-free system. *Biochemical and Biophysical Research Communications*. 1994;**203**:1175-1180. DOI: 10.1006/bbrc.1994.2306
- [44] Maruyama M, Asano T, Nakagohri T, Uematsu T, Hasegawa M, Miyauchi H, et al. Application of high energy shock waves to cancer treatment in combination with cisplatin and ATX-70. *Anticancer Research*. 1999;**19**:1377-1383. PMID: 10470144
- [45] Tonetti M, Giovine M, Gasparini A, Benatti U, De Flora A. Enhanced formation of reactive species from cis-diammine- (1,1-cyclobutanedicarboxylato)-platinum(II) (carbo-plati) in the presence of oxygen free radicals. *Biochemical Pharmacology*. 1993;**46**:1377-1383. PMID: 8240386
- [46] Ronchetto F, Barone D, Cintorino M, Berardelli M, Lissolo S, Orlassino R, Ossola P, Tofani S. Extremely low frequency- modulated static magnetic fields to treat cancer: a pilot study on patients with advanced neoplasm to assess safety and acute toxicity. *Bioelectromagnetics*. 2004;**25**:563-571. DOI: 10.1002/bem.20029
- [47] Yuan Lin-Qing, Wang Can, Zhu Kun, Li Hua-Mei, Gu Wei-Zhong, Zhou Dong-Ming, Lai Jia-Qi, Zhou Duo, Lv Yao. Tofani S, Chen Xi: The Antitumor Effect of 50 Hertz Modulated Static Magnetic Fields against Nephroblastoma and Neuroblastoma. Submitted to *Bioelectromagnetics*, June 2017
- [48] Tofani S. Electromagnetic field exposure system for the study of possible anti-cancer activity. *IEEE Transactions on Electromagnetic Compatibility*. 2002;**44**(1):148-151. DOI: 10.1109/15.990721
- [49] Tatarov I, Panda A, Petkov D, Kolappaswamy K, Thompson K, Kavirayani A, Lipsky MM, Elson E, Davis CC, Martin SS, DeTolla LJ. Effect of magnetic fields on tumour growth and viability. *Comparative Medicine*. 2011;**61**(4):339-345. PMID: PMC3155400
- [50] Nie Y, Du L, Mou Y, Xu Z, Weng L, Du Y, Zhu Y, Hou Y, Wang T. Effect of low frequency magnetic fields on melanoma: tumor inhibition and immune modulation. *BMC Cancer*. 2013;**13**:582. DOI: 10.1186/1471-2407-13-582
- [51] Jiménez-García MN, Arellanes-Robledo J, Aparicio-Bautista DI, Rodríguez Segura MA, Villa-Treviño S, Godina-Nava JJ. Anti-proliferative effect of extremely low frequency electromagnetic field on preneoplastic lesions formation in the rat liver. *BMC Cancer*. 2010;**10**:159. DOI: <https://doi.org/10.1186/1471-2407-10-159>
- [52] El-Bialy NS, Rageh MM. Extremely low-frequency magnetic fields enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. *BioMed Research International*. 2013;**2013**:189-352. <http://dx.doi.org/10.1155/2013/189352>
- [53] Chen WF, Qi H, Sun RG, Liu Y, Zhang K, Liu JQ. Static magnetic fields enhanced the potency of cisplatin on k562 cells. *Cancer Biotherapy & Radiopharmaceuticals*. 2010;**25**(4): 401-408. DOI: 10.1089/cbr.2009.0743

- [54] Hao Q, Wenfang C, Xia A, Qiang W, Ying L, Kun Z, Run-guang S. Effects of moderate-intensity static magnetic field and adriamycin on k562 cells. *Bioelectromagnetics*. 2011;**32**(3):191-199. DOI: 10.1002/bem.20625
- [55] Kakikawa M, Yamada S. Effect of extremely low-frequency (ELF) magnetic field on anti-cancer drugs potency. *IEEE Transactions on Magnetics*. 2012;**48**:2869-2872. DOI: 10.1109/TMAG.2012.2200881
- [56] Sun C, Yu H, Wang X, Han J. A pilot study of extremely low- frequency magnetic fields in advanced non-small cell lung cancer: Effects on survival and palliation of general symptoms. *Oncology Letters*. 2012;**4**:1130-1134. DOI: <https://doi.org/10.3892/ol.2012.867>
- [57] Baharara J, Hosseini N, Farzin TR. Extremely low frequency electromagnetic field sensitizes cisplatin-resistant human ovarian adenocarcinoma cells via P53 activation. *Cytotechnology*. 2016 Aug;**68**(4):1403-1413. DOI: 10.1007/s10616-015-9900-y
- [58] Tang R, Xu Y, Ma F, Ren J, Shen S, Du Y, Hou Y, Wang T. Extremely low frequency magnetic fields regulate differentiation of regulatory T cells: Potential role for ROS-mediated inhibition on AKT. *Bioelectromagnetics*. 2016 Feb;**37**(2):89-98. DOI: 10.1002/bem.21954
- [59] Yang ML, Ye ZM. Extremely low frequency electromagnetic field induces apoptosis of osteosarcoma cells via oxidative stress. *Zhejiang Da Xue Xue Bao. Yi Xue Ban*. 2015;**44**:323-328. PMID: 26350014
- [60] Artacho-Cordon F, Salinas-Asensio Mdel M, Calvente I, Rios-Arrabal S, Leon J, Roman-Marinetto E, Olea N, Nunez MI. Could radiotherapy effectiveness be enhanced by electromagnetic field treatment? *International Journal of Molecular Sciences*. 2013;**14**: 14974-14995. DOI: 10.3390/ijms140714974
- [61] Ghodbane S, Lahbib A, Sakly M, Abdelmelek H. Bioeffects of static magnetic fields: Oxidative stress, genotoxic effects, and cancer studies. *BioMed Research International*. 2013:602987. DOI: 10.1155/2013/602987
- [62] Montagnier L. Method for Digital Transduction of DNA in Living Cells, US Patent Application 20160002620, January 7, 2016. <https://www.google.com/patents/US20160002620>

