Tumour induction pathway and HF-EMF irradiation.

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Abstract

The response of cells to different types of electromagnetic fields can be induced by short exposures to electromagnetic irradiation (900 MHz) associated to mobile phone technologies. There are many examples of biological effects resulting from low-level (athermal) high frequency (HF) electromagnetic field (EMF) exposure. EMFcould trigger protein activation mediated by ligands, such as Ca^{2+}, that alter the conformation of binding proteins, especially plasmic membrane oxidase, and hence control their function. The classical signaling mechanisms are commonly found activated in human malignancies, and are involved in inflammation that characterize the oncogenic pathway responsible for tumors. The microenvironment that exists during chronic inflammation can contribute to cancer progression. The data support the proposition that cell phones may have a potential to cause hazardous effects on cells.

1. **Introduction**

Global system for mobile communication (GSM), which being used in most of the countries, has a frequency of either 900 or 1800 MHz. This low-energy (non-thermal) radiation is emitted not only by cell phones themselves, but also by the base stations. There are many examples of biological effects resulting from low-level (athermal) EMF exposure [1-4].

The environment is a well-established source of damaging or disrupting influences on cellular function. In the past, studies of the mechanisms by which such disruptions occur have focused largely on either direct toxic effects on cellular function at the protein or cell signaling level, or mutagenic effects that impact the genome. In recent years there has been a growing appreciation for the potential for environmental influences to disrupt the epigenome and mechanisms of epigenetic regulation within the cell. Indeed, because of the inherent lability of the epigenome, this represents a primary target for environmentally induced disruption [5].

Chronic inflammation has long been associated with the development of cancer [6-8]. Although inflammation is part of the defense response, paradoxically it can lead to important health disorders. Since it was discovered in the 19th century, chronic inflammation has been proven to be a risk factor for cancer both at the onset and as a prognostic marker [9]. Inflammatory response is followed by a proliferative response (basal cell hyperplasia, papillary elongation) [10].

An office workers cohort with high EMF exposure showed a cancer increase, with a high risk of malignant melanoma. School teachers exposed to a type of RFR had increased incidence of malignant melanoma and thyroid Cancer [11]. Pooling of data from Nordic countries and part of the UK yielded a significantly increased risk of

glioma and a significantly increased risk of acoustic neurinoma related to use of mobile phones for a period of 10 years or more on the side of the head where the tumour developed [12, 13]. In an Israeli study, where study subjects tended to report substantially heavier use of mobile phones, results suggest a possible relation between heavy mobile phone use and risk of parotid gland tumours [14, 15]. Several reports have indicated that EMFs enhance free radical activity in cells particularly via the Fenton reaction [16].

2. Cell responses

Duckweed growth and peroxidase activity was evaluated after exposure in a Gigahertz Transversal Electromagnetic (GTEM) cell to electric fields of frequencies 400, 900, and 1900 MHz. The growth of plants exposed for 2 h to the 23 V/m electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. At both frequencies a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics. Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. Therefore, the effects of EMFs strongly depended on the characteristics of the field exposure [17].

Exposure of tomato plants to non-thermal HF-EMF (900 MHz, 5 V m^{-1}) for 10 min led to show a direct relationship between HF-EMF exposure and responses at

the level of gene expression [1]. There was no direct link between the amplitude of the stimulation and the amplitude of the plant response, which suggested the concept of an 'all-or-nothing' response with a threshold of induction [18]. The energy used in the electromagnetic exposure (900MHz, 5 V.m^{-1}, 10 min) was very low (close to 0.1W dissipated in 200 m^3) and did not produce any thermal effect [1, 18]. The genesis and/or transmission of the informative signal were rapid and strongly dependent upon calcium. Expression of Hsp70 [19], and tumor suppressor p53 [20] are also affected by microwave irradiation.

Electromagnetic irradiation emitted from mobile phones (frequency 900 MHz) induces expression of proteins in various cells [21-23]. The specific absorption rate level to which cells were exposed was 2.8 W/kg. The effects observed were not caused by bulk heating of the cells [22]. Among the proteins whose expression is induced by mobile phone irradiation are transcription factors [21]. The proteomes of the same cell type (endothelium) but derived from different vascular beds (umbilical vein and brain microvasculature) express very different proteomes. The effect is frequency-dependent. When cells were exposed for 1 h at an average SAR of 2.0 W/kg and examined immediately after that. 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation [24]. Mobile phone radiation might alter protein expression in human skin, with a SAR induced in the skin, 1.3 W/kg, below the ICNIRP safety guidelines (2.0 W/ kg), for one hour [25].

Paulraj and Behari [26] indicated DNA single strand break and decrease in level of protein kinase at 2.45 GHz [27]. More recently, Kesari and Behari [28] reported that microwave (2.45GHz) exposure cause infertility by decreasing sperm count and an increase in apoptosis.

Tkalec *et al.* [29] showed that non-thermal exposure to the radiofrequency fields can induce mitotic aberrations in root meristematic cells of *A. cepa*. The observed effects were markedly dependent on the field frequencies applied as well as on field strength and modulation. They also indicate that mitotic effects of RF-EMF could be due to impairment of the mitotic spindle.

Microwave (MW) exposure at the water resonance frequency was able to induce alteration of the mitotic apparatus and apoptosis as a function of the applied power densities (5 and 10 mW/cm^2), together with a moderate reduction in the rate of cell division. After an exposure time of 15 min the proportion of aberrant spindles and of apoptotic cells was significantly increased, while the mitotic index decreased as well, as compared to the untreated V79 cells. Ballardin *et al.* [30] hypothesised that short-time MW exposures at the water resonance frequency cause, in V79 cells, reversible alterations of the mitotic spindle, this representing, in turn, a pro-apoptotic signal for the cell line.

Another mechanism that could be involved in the formation of aberrant spindles concerns the polar protein structures. Microtubule fibers represent extremely dynamic structure which functioning dependent on dynamical instability that is continuously binding and releasing of free tubule proteins. It is reasonably to assume that the external EM radiation might interact with polar cytoskeletal structure. This structure greatly contributes to the balance between internal physiological electrostatic forces. Depending on the dipole moment, an external electric field could theoretically disturb the equilibrium [31] and increase the number of free cytoplasmatic tubulin proteins following a mechanism of depolarization of tubulins involved in the formation of the mitotic apparatus. Recently, it has been shown that

electromagnetic field (90 V/m at 835 MHz) exposure can induce significant increases of mitotic disturbances in human–hamster hybrid cells [32].

3. EMF-target molecule interactions

Energy transfer of Microwave (MW) is conducted through two mechanisms, dipole rotation and ionic conduction [33]. Blank and Soo [34] examined the EM field effects on electron transfer and concluded that EM fields accelerate all linked redox reactions. Blank [35] was considered examples of direct effects of electric and magnetic fields on charge transfer in biological systems, and reported the structural changes driven by such effects, then concluded that conformational changes arising from alterations in charge distribution play a key role in membrane transport proteins, including ion channels. Thus, weak EMFs can control and amplify biological processes through their effects on charge distribution [4].

The EMF could interact with moving charges or charged species [36] which are implicated in various biological processes. Moreover, HF-EMF may lead to ion movement, directly or indirectly, and particularly near the plasma membrane [37] and this could initiate the biological response. Ligands, such as Ca^{2+}, alter the conformation of proteins and hence control their receptor function. EMF exposure produces changes in binding probability of the ligand. Occupation of one protein site by a ligand changes its conformation. It would be suggested that EMF could trigger a transition from a random array to an ordered array of phase transition in proteins [38]. Calcium has been well recognised as a signaling factor involved in the regulation of a wide range of cellular processes involving cell proliferation.

Panagopoulos *et al.* [39] have suggested that oscillating ions during forced vibration will also exert mechanical forces–pressure on the plasma membrane able to upset the membrane's electrochemical balance, under certain conditions, by opening or closing mechanically gated channel proteins, like some Ca influx channels. In any case, the irregular gating of ion channels caused by the forced vibration of the free ions, caused by external oscillating electromagnetic fields, is a fact that could upset the electrochemical balance of the plasma membrane and, consequently, the whole cell function.

4. Transduction signal and oncogene expression

Currently, cancer is recognized as a disease associated with both genetic and epigenetic alterations, and both of these components cooperate and complement each other to promote cancer progression. The carcinogenic process can be associated to a modification of gene expression regulation; the modification of the signal cascade into the cell can induce the dysregulation of transcription factor activities (overexpression, activation, uninhibition….) through the activation of specific cellular messengers. Free radicals play an essential role in the activation of certain signaling pathways [6]. The effects of reactive oxygen species (ROS) on cell proliferation occurred exclusively at low or transient concentrations of radicals that can stimulate proliferation and enhanced survival in a wide variety of cell types [40]. ROS can play a very important physiological role as secondary messengers including regulation of the cytosolic calcium concentration, which itself regulates biological activities, regulation of protein phosphorylation and activation of certain transcription factors such as NF-κB and the AP-1 family factors [40-42].

In general, the balance between the production and scavenging of ROS leads to homeostasis. Disturbance of this equilibrium can alter normal cellular processes; it often occurs in tumour cells [43]. The cumulative production of ROS through either endogenous or exogenous insults is termed oxidative stress and is common for many types of cancer cell that are linked with altered redox regulation of cellular signaling pathways [44]. A sustained oxidative stress has been associated with several steps of carcinogenesis, including transformation and achievement of a motile mesenchymal phenotype [45]. Thornber *et al.* [46] have demonstrated that NPM-ALK induces the production of reactive oxygen species (ROS) by a pathway involving the arachidonic acid-metabolizing enzymes of the lipoxygenase (LOX) family.

Several MAPkinases, signal transducers and activators of transcription, Akt/protein kinase B and phospholipase D-signaling pathways are all activated by ROS [47, 48] but in some cases, activation is indirect [49].

Cadmium is a toxic metal causing nephrotoxicity, immunotoxicity, osteotoxicity and tumors after prolonged exposures. Cd-generated ROS are often accompagnied by activation of redox sensitive transcription factors, such as for instance NF-κB, and alteration of ROS-related gene expression. Acquired Cd tolerance with aberrant gene expression plays important roles in chronic Cd toxicity and carcinogenesis [50]. Chronic inflammation induced by biological, chemical and physical factors, is associated with increased risk of tumors development in various organs and tissues. Association of cancer with chronic inflammation has been described, in particular, in pathological processes in the organs of the digestive system, where the risk of carcinogenesis increases in chronic inflammation (esophagitis, gastritis, hepatitis etc). Mediators of inflammation, to a large extent contributing the process of transformation, affect many aspects of malignant growth, including proliferation,

differentiation and apoptosis. Inflammation contributes to initiation, progression of malignant transformation of normal cells and tumor growth due to production of both proinflammatory cytokines and various forms of reactive oxygen and nitrogen. Significant activation of NF-κB can stimulate malignant transformation, providing antiapoptotic and proliferative signals.

Developmental signaling pathways that regulate normal stem cell self-renewal have been shown to be active in numerous human cancers [51]. Inflammatory breast cancer (IBC) is an uncommon disease accounting for 1–5% of all breast cancers [52, 53]. An abundance of experimental evidence now solidly supports the HER2 oncogene hypothesis and etiologically links amplification of the HER2 gene locus with human cancer pathogenesis [54]. HER2 protein overproduction is also seen uncommonly in cancers of the esophagus, stomach, ovaries and endometrium. Mutations in the HER2 gene are found rarely in these cancers and overexpression appears to be the principal mechanism by which HER2 mediates tumorigenesis in these cancers [55].

5. Signal transduction and EMF signaling pathways

Mitogen-activated-phospho-kinase (MAPK) family pathway control cell proliferation, metabolism and cell survival in response to tissue injury, infection, malignancy and other diseases. Activation of the MAPK cascades leads to phosphorylation of transcription factors that bind to the upstream regulatory elements on the gene promoter, relative to the transcription initiation site. Three out of four MAPK cascades are differentially activated by ELF EM fields [56].

The signaling pathways and the stress response are activated by EMF. Friedman *et al.* [57] have demonstrated that the initial step in transmitting extracellular EMF information from the plasma membrane to the nucleus of the cell occurs when NADH oxidase rapidly generates reactive oxygen species (ROS). These ROS stimulate matrix metalloproteinases, and then activate the extracellular signal regulated kinase 1\2 (ERK) cascade. These changes in activity of the protein kinases pathways can consequently regulate the physiological response of the exposed cells and organisms, and therefore are major regulators of the effects of electromagnetic fields at mobile phone frequencies.

Data indicate that, prior to the stress response, mobile phone irradiation induces an immediate effect in the cytoplasm which activates ERK signalling to induce further transcription of a variety of genes [58]. The fact that the stress-related cascades, which are known to be activated by heat or other related stresses, were not activated, in the time course of Friedman *et al.* [57] experiments, indicates that the activation of ERKs was induced by other mechanisms. It has been reported [59] that within 10 min of cell phone exposures, two MAPKinase cascades, p38 and ERK1\2, are activated.

Both ELF and RF activate the upregulation of the HSP70 gene and induction of elevated levels of the hsp70 protein. This effect on RNA transcription and protein stability is controlled by specific protein transcription factors that are elements of the mitogen MAPK cascade. EMF also stimulates serum response factor which binds to the serum response element (SRE) through ERK MAPK activation and is associated with injury and repair *in vivo* and *in vitro*. The SRE site is on the promoter of an early response gene, c-fos, which under specific cellular circumstances has oncogenic properties. The c-fos promoter is EMF-sensitive [60, 61].

Activation of NF-κB and AP-1, the downstream of the MAPK or PI3K-Akt pathway, are involved in many pathological processes, such as inflammation, cancer cell adhesion, invasion, migration, and angiogenesis [62, 63]. NF-κB transcription factors function as crucial regulators of inflammatory and immune responses as well as of cell survival. They have also been implicated in cellular transformation and tumorigenesis. The NF-κB pathway is required for the development of tumours in a mouse model of lung adenocarcinoma [64]. Elevated P13K/PDK/Akt or NF-κB signaling (or both) have been correlated with advanced tumor progression and poor prognosis in patients with breast cancer [65, 66].

NF-κB is maintained in the cytoplasm through interactions with an inhibitor of NF-κB (IκB), but upon dissociation, it moves into the nucleus and promotes cancer cells proliferation, angiogenesis, and metastasis. Carcinogens and tumour promoters involving toxic metals, UV radiation, phobol esters, asbestos, alcohol and benzo(α)pyrene are among the external stimuli that activate NF-κB [67]. Hepatitis B viral proteins (HBx) have oncogenic properties by activation of different promoter elements and then trigger activation of transcription factors such as AP-1 and NF-κB [44, 68].

Nuclear factor κB (NF-κB) is a transcriptional factor that regulates a battery of genes that are critical to innate and adaptive immunity, cell proliferation, inflammation, and tumor development [69]. Increasing evidence suggests that NF-κB-associated pathways are dysregulated in numerous malignancies [65, 70-73]. The activation of NF-κB-dependent anti-apoptotic genes may promote IBC (Inflammatory Breast cancer) tumorigenesis, as it has been shown in other inflammation-associated tumor types [74]. The NF-κB pathway appears to play a major role in IBC, possibly contributing to the unusual phenotype and aggressiveness of this form of breast

cancer [75]. DNA microarrays studies of IBC have shown abnormal expression of some NF-κB target genes [76, 77]. Activated NF-κB translocates to the nucleus and binds to the DNA to initiate expression of genes that enhances cell proliferation and survival [66, 72, 78].

Activation of nuclear factor (NF)-κB, one of the most investigated transcription factors, has been found to control multiple cellular processes in cancer including inflammation, transformation, proliferation, angiogenesis, invasion, metastasis, chemoresistance and radioresistance. NF-κB is constitutively active in most tumor cells, and its suppression inhibits the growth of tumor cells, leading to the concept of 'NF-κB addiction' in cancer cells. As NF-κB is the key transcription factor involved in the inflammatory pathway, NF-κB is constitutively active in most cancers, and many of the signaling pathways implicated in cancer are likely to be networked to the activation of NF-κB. NF-κB interacts with many transcription factors and transcriptional regulators [79].

Chronic inflammation has been associated with the development of cancer. Molecular biology studies have shown that there are genes, which when "activated" (oncogenes) or "inactivated" (tumour suppressor genes) contribute to the clonal expansion of an initiated stem cell. The cancer thus would be a disease of homeostasis [44]. Any exposure, including prolonged low-intensity ELF and RF exposures that result in increased free radical production may be considered a plausible biological mechanism for carcinogenesis [6].

6. Conclusions

Oncogenesis is mediated by a sustained and not a transient ERK activation [57] that could be related for base station antenna risk explanation. RF field studies

have similarly shown the stimulation of cell proliferation and stress proteins following exposure [56]. Production of ROS by mobile phone irradiation, a process that has already been demonstrated in previous studies [80, 81], is suggested in the present study to be induced by the activation of NADH oxidase, which appears to be an early acceptor of mobile phone irradiation. These events appear to occur within seconds and initiate the subsequent activation of the pathway that leads to the phosphorylation of ERKs. Several components of the proposed model (Fig. 1) have been implicated in the activation of the ERK cascade in other cellular systems.

The strongest microwave effects were always observed in stem cells. This result may suggest both significant misbalance in DSB repair and severe stress response. Findings that stem cells are most sensitive to microwave exposure and react to more frequencies than do differentiated cells may be important for cancer risk assessment and indicate that stem cells are the most relevant cellular model for validating safe mobile communication signals [82].

Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells. The data support the proposition that cell phones may have a potential to cause hazardous effects on the genome; however, in in vivo conditions, the duration of exposure and the capacity of DNA repair may prevent the development of cancer to an extent [83]

One of the interesting questions raised in all studies was the identity of the molecular acceptor of mobile phone irradiation. Results of Friedman *et al.* [57] indicated that one such early acceptor should be NADH oxidase, which appears to be localized in the plasma membrane and converts the irradiation into ROS.

However, the earliest target receptor of HF-EMF could be calcium ions stored on the external face of cell membrane. The HF-EMF induction pathway is resumed in figures 2 and 3. EMF stimulus may lead to Ca^{++} ion movement, directly or indirectly, and near the plasma membrane toward NAD(P)H oxydase protein, a calcium-dependent enzyme. Its activity leads to the production of superoxide ion and hydrogene peroxide. The production of ROS alternatively would also activate some calcium channels, then enhancing the calcium ions that are required for signal transduction. ROS can function as cellular second messengers that are likely to modulate many different proteins leading to a variety of responses. The transcription factor, NF-κB, is maintained in the cytoplasm through interactions with an inhibitor of NF-κB (IκB), but upon dissociation, it moves into the nucleus and promotes cancer cells proliferation, angiogenesis, and metastasis (Fig. 2). On the other hand, EMF would activate ion movements and would allow the activation of calcium channels, then triggering NAD(P)H oxidase inside the cell and inducing a signal transduction to oncogene expression (Fig. 3). In turn, a cell survival homeostasis following EMF stimuli thus can be described (Fig. 4). The EMF stimuli would trigger the expression of transcription factors that develop a permanent loop of transcription factor gene expression. Transcription factors then induce the permanent expression of oncogenes involved in cell survival that would allow the development of tumour cells (Fig. 4).

The microenvironment that exists during chronic inflammation can contribute to cancer progression [7, 84]. Macrophages are key mediators of the interaction between inflammation, immunity and cancer. The role of macrophages in cancer has received attention due to the discovery of their tumor-promoting effects [85-87]. Inhibition in macrophages during tumorigenesis at certain time points may interfere with host-suppressive effects on metastasis formation, and demonstrates that timing and cell specificity may be the key determinants of the impact of NF-κB inhibitors as a cancer therapy [88]. Tumor growth increases inflammation and recent studies have indicated that in the presence of a tumor or a malignancy in general in the organism, a high oxidative stress status can be detected [7].

Panagopoulos and Margaritis [89] showed that GSM 900MHz radiation is slightly more bioactive than DCS 1800MHz radiation, at the same exposure durations and under equal radiation intensities. Exposure to mobile telephony radiation may have adverse health effects and it should be restricted by more rigorous exposure criteria. Since the radiation intensity in their experiments was 45 and 90 times lower than the current exposure limits for 900 and 1800 MHz, respectively, it seems to suggest a significant reduction of the current exposure limits.

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Captions.

Figure 1: The mitogen activated protein kinase (MAPK) signaling cascades identified to date are: extracellular signal regulated kinase 1/2 (ERK), c-Junterminal kinase (JNK), p38MAPK and stress activated protein kinase (SAPK). Elements of the three MAPkinase pathways that have been identified as activated by EMF are shown as the shaded circles, according to Weisbrot et al. [56].

Figure 2: Cell survival after EMF stimuli. Scheme representation of the proposed mechanism that mediates the phosphorylation of transcription factors upon mobile phone irradiation. Electromagnetics field (900 MHz) would shift calcium ions that triggers the nearby enzyme NAD(P)H oxidase. The enzyme generates reactive

oxygen species (ROS) that can induce membrane proteins which are involved in formation of signal message. The signal transduction through the cell membrane activates calciproteins then MAP kinases and transcription factors that should the occasion arise oncogene expression. In turn, EMF (900MHz) could activate calcium channels that would allow the induction of the cell transduction signal cascade.

Figure 3: Cell transduction signal after EMF stimuli. Following the targeting of various agents (Activation) on cell membrane, IκB proteins are phosphorylated by a protein kinase complex (IKK) and a regulation element (NEMO), then IκB are ubiquitinated. Proteasomal degradation is proceeded allowing the translocation of the NF-κB complexes in the nucleus where they bind the gene promoters which are invoved in the cell response (Lobry and Weil 2007). EMF can activate a cell membrane calcium channel which allows triggering of membrane NADPH oxidase. The latter enzyme is part of a mechanism producing reactive oxygen species (ROS) that can activate transcription factors (TF) such as AP-1 or NF-κB. Oxidizing stimuli in the cytosol lead to post-translational transcription factor modification that facilitates translocation to the nucleus.The factor of transcription can bind DNA site of oncogene promoters that trigger cell survival and tumour development.

Figure 4: Cell survival homeostasis after EMF stimuli. EMF would trigger cell signal transduction through membrand receptor. Transcription factors which are activated, woud allow the expression of gene invoved in cell survival together with own ttranscription factor genes resulting in permanent cell survival and tumour development.

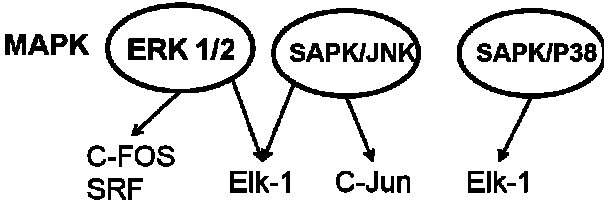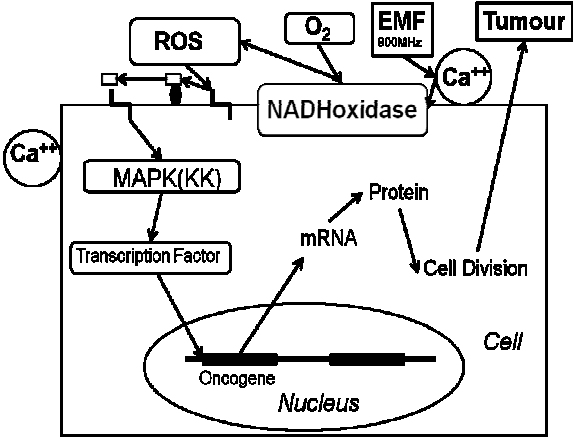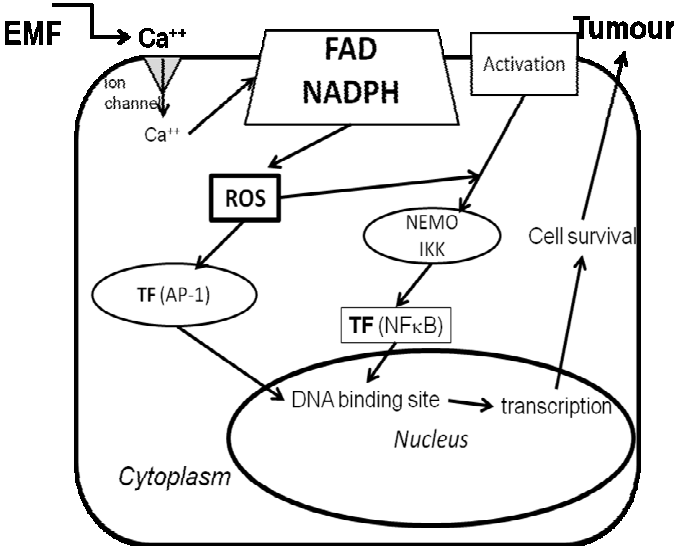**Figure 1.** Mitogen activated protein kinase (MAPK) signaling cascades.

Figure 2: Cell survival after EMF stimuli.

Figure 3: Cell transduction signal after EMF stimuli.

Figure 4: Cell survival homeostasis after EMF stimuli.