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Evidence of bystander effect induced by radiofrequency radiation in a human neuroblastoma cell line

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ABSTRACT

In previous studies we demonstrated that radiofrequency (RF) electromagnetic fields (EMF) is able to reduce DNA damage induced by a subsequent treatment with genotoxic agents, resembling the adaptive response, a phenomenon well known in radiobiology. In this study we report on the capability of the culture medium from SH-SY5Y neuroblastoma cells exposed to 1950 MHz to elicit, in recipient non-exposed cells, a reduction of menadione-induced DNA damage ($P < 0.05$; comet assay), indicating the capability of non-ionizing radiation to elicit a bystander effect. A comparable reduction was also detected in cultures directly exposed to the same EMF conditions ($P < 0.05$), confirming the adaptive response. In the same exposure conditions, we also evidenced an increase of heat shock protein 70 (hsp70) in culture medium of cells exposed to RF with respect to sham exposed ones ($P < 0.05$; western blot analysis), while no differences were detected in the intracellular content of hsp70. On the whole, our results evidence a protective effect of RF against menadione-induced DNA damage in directly and non-directly exposed cells, and suggest hsp70 pathway to be investigated as one of the potential candidate underpinning the interaction between RF exposure and biological systems.

1. Introduction

Bystander effect (BE) and Adaptive Response (AR) are phenomena typically triggered by very low doses and/or dose-rate of ionizing radiation (IR). While BE is defined as the propagation of the effects from cells directly irradiated (targeted cells) to non-irradiated cells (bystander cells), the AR is manifested by an increased resistance to the damaging effects of higher doses administered to the same cells (Preston 2005).

BE was described in *in vitro* experiments for the first time by Nagasawa and Little in chinese hamster ovary cells irradiated with extremely low doses of α-particles. The authors detected the induction of sister chromatid exchanges both in irradiated cells and in nuclei of many cells that were not traversed by an α-particle (Nagasawa and Little 1992).

Since then, evidence has been accumulated in *in vivo* and *in vitro* studies on the induction of BE triggered by several agents on different experimental models and by measuring a variety of biological endpoints. These observations have been reviewed by several authors (Chai and Hei 2008; Rzeszowska-Wolny et al., 2009; Hei et al., 2011; Verma and Tiku 2017; Widel 2016), but the mechanisms determining the onset of such phenomenon are still a matter of investigation (Bright and Kadhim 2018). BE in most cases refers to the propagation of adverse effects but beneficial/protective effects have also been reported (Coates et al., 2004; Spray et al., 2013), providing both the so called “kiss of life” and “kiss of death” (Waldren 2004).

Evidence suggests that it can be mediated by direct cell–cell communications, via gap junctions, and by extracellular soluble factors within cell microenvironment (Kadhim and Hill 2015), including reactive oxygen species (Lyng et al., 2011), nitrogen oxide species (Matsumoto et al., 2001), cytokines (Facoetti et al., 2009; Desai et al., 2013) and calcium fluxes (Shao et al., 2006).

Moreover, it has been reported that exosomes released from IR-

Abbreviations: AR, adaptive response; BE, bystander effect; CM, conditioned medium; DMEM, Dulbecco’s modified Eagle’s medium; DMSO, Dimethyl sulfoxide; EDTA, Ethylene diamine tetracetic acid; EMF, electromagnetic fields; FBS, foetal bovine serum; HRP, horseradish peroxidase; hsp70, heat shock protein 70; IR, ionizing radiation; MD, menadione; NIR, non-ionizing radiation; RF, radiofrequency; SE, standard error; Sh, sham; SAR, specific absorption rate; UMTS, universal mobile communication system.

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exposed cells may be responsible for BE (Albanese and Dainiak 2003; Al-Mayah et al., 2012; Al-Mayah et al., 2015). Exosomes are small membrane microvescicles present in all biological fluids (EL Andaloussi et al., 2013) whose content consists of lipids, nucleic acids, and proteins including heat shock proteins (hsps) (Hunter-Lavin et al., 2004; Lancaster and Febbraio 2005; De Maio and Vazquez 2013), an important group of cell response proteins, highly conserved and ubiquitous, whose function varies depending on the specific hsp and on the physiological context.

In response to a variety of physiological and environmental factors, including heat, transient expression of hsps occurs that is normally thought of as a protective mechanism in a range of both *in vitro* and *in vivo* models (Hightower 1991; Ohtsuka and Suzuki 2000). A number of studies suggest that also non-ionizing radiation (NIR) exposure both at extremely low frequency (Zeni et al., 2017) and at radiofrequency (RF) can activate the cellular stress response and cause increased hsps expression, both on the mRNA and protein levels, and hsp70 is one of the most investigated (McNamee and Chauhan 2009).

In the last years, our research group collected evidence that exposure to NIR, in the range of RF and with modulation schemes employed for wireless technologies, is able to protect cell cultures from the damage induced by a subsequent treatment with chemical or physical agents. Such phenomenon has been observed under different experimental conditions in terms of electromagnetic characteristics, cell models and biological endpoints with features resembling the IR-induced AR (Sannino et al., 2009, 2011, 2014, 2017, 2019; Zeni et al., 2012; Falone et al., 2018; Romeo et al., 2019). Experimental results published by independent research groups confirmed our findings and extended such observation to *in vivo* studies. Literature published until 2014 was reviewed, and gaps in knowledge were identified (Vijayalaxmi et al., 2014). More recently, other *in vitro* (Ji et al., 2016; He et al., 2017) and *in vivo* (Zong et al., 2015; Mortazavi et al., 2017) investigations extended the variety of experimental models and electromagnetic conditions able to elicit similar response in different assays.

In the present paper, we investigated whether RF exposure is capable to trigger a bystander phenomenon in a human neuroblastoma cell model, SH-SY5Y, and whether a correlation exists between AR and BE. To this purpose, we tested the experimental conditions that in a previous investigation reduced the DNA damage (AR) in SH-SY5Y cells due to a subsequent treatment with menadione (MD; 2-methyl-1,4-naphtoquinone), a DNA-damaging inducer acting via oxidative imbalance in mammalian cells (Falone et al., 2018). The comet assay was applied to test DNA damage both in directly RF-exposed cells and in cells treated with culture medium deriving from RF-exposed cells. Moreover, the intracellular and extracellular levels of hsp70 were analyzed in the western blot in RF- and sham-exposed cells as a first step to explore the possible mechanism mediating RF-induced protective effects.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum (FBS) were from Domenique Dutscher (Brumath, France), L-glutamine, trypsin-EDTA and penicillin/streptomycin were from Biowhittaker (Verviers, Belgium). Triton X-100, N-lauryl sarcosine, menadione (MD), RIPA buffer solution, protease inhibitor cocktails, skim milk powder and Tween were from Sigma (St. Louis, MO). Dimethyl sulfoxide (DMSO), NaOH and Na_2EDTA were from Baker (Deventer, The Netherlands). Tris and trypan blue were from BDH (Poole, England); NaCl was from Carlo Erba (Milan, Italy). Normal-melting-point agarose, low-melting-point agarose, ethidium bromide and protein assay kit were from Bio-Rad Laboratories (GmbH, Munich, Germany). Total Exosome Isolation kit, PVDF membrane and precast 4–12% SDS-PAGE were from Thermo Fisher Scientific (Milano, Italy). Vinculin was from Abcam (Milano, Italy). hsp70 antibody and ECL were from Santa Cruz Biotechnology Inc (Milano, Italy). HRP-linked anti-mouse antibody was from ImmunoReagent Inc (Raleigh, USA).

2.2. RF exposure and dosimetry

A well-established RF signal generation and exposure system was used to expose samples to 1950 MHz, UMTS (Universal Mobile Communication System) signal, under strictly controlled environmental and electromagnetic conditions, and with high efficiency (>70%) and low disuniformity degree (about 30%) of the SAR distribution in the biological samples. Details on design, realization and characterization can be found in our previous papers (Romeo et al., 2013; Falone et al., 2018). Briefly, the signal generation, conditioning, and monitoring chain was composed by a RF signal generator (Agilent, E4432B), a microwave amplifier (Microwave Amplifier, AM38A-0925-40-43), a −6 dB power splitter (HP, 11667A) and two bidirectional power sensors (Rohde and Schwarz, NRT-Z43). The exposure chambers consisted of two, twin, short-circuited, rectangular waveguides (WR430, 350 mm long), connected to the power sensors by a coaxial adapter (Maury Microwave R213A2, VSWR: 1.05) at the feeding end. A third, identical waveguide was used for sham-exposed cultures (same procedure of the corresponding exposed cultures but without field application). The three waveguides were hosted in the same cell culture incubator (Forma Scientific, Model 311) at 37 ± 0.5 °C, 95% air and 5% CO_2 atmosphere. In each waveguide, two 35 mm Petri-dishes (Corning, catalogue n. 430165), containing cell cultures were placed in the distal positions of a four-layer Plexiglas stand and exposed simultaneously to 0.3 W/kg SAR. The central positions hosted dummy cultures.

For AR and BE experiments, one of the two powered waveguides was used to expose cell cultures to be analyzed 1) after RF exposure alone and 2) after subsequent treatment with MD. The other waveguide was used to expose 1) cell cultures destined to donate conditioned medium (see section *Experimental Protocol*) to recipient cell cultures, and 2) cell-free culture medium to be transferred to recipient cell cultures. For hsp70 analysis, the two powered waveguides hosted duplicate cultures from independent batches of cells to obtain two independent experiments in a single run.

A continuous monitoring of incident and reflected power in each waveguide was carried out to keep the required SAR constant over the entire exposure period. Monitoring of temperature inside cell cultures was carried out by means of a fiber optic thermometer (FISO Technologies, FisoUMI4), and the detected variations were within the accuracy range of the instrument (37 ± 0.3 °C).

2.3. Cell model and handling

Human SH-SY5Y neuroblastoma cell line was purchased by ATCC cell culture collection (Manassas, VA USA), and cells were cultured in DMEM, supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin and routinely checked for mycoplasma infection.

Cells were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 in a standard incubator, supplied with fresh culture medium every 48 h, and kept exponentially growing by splitting them once a week by trypsin treatment (200 mg/ml trypsin-EDTA solution for 5 min).

For consistency and reproducibility, the same batch of reagents was used, and experiments were carried out on cells from passages 3–10.

2.4. Experimental protocol

For the experiments, 8×10^5 cells were seeded 48 h before treatments in 3 ml complete medium in Petri dishes, and grown for a total of 71 or 72 h, based on the applied protocol.

RF exposure at 1950 MHz, UMTS signal, was given continuously for 20 h (h 48–68) at 0.3 W/kg SAR.

In the bystander experiments, 1 h before harvest (h 71), conditioned

medium (CM) was obtained from RF/Sham exposed cultures after filtering through a 0.22 μm filter (Merck Millipore, Burlington, USA), to avoid transferring the cells and debris to recipient cell cultures. Recipient cell cultures were set up with the same cell density and grown in a separate incubator until the reception of CM and, where required, MD (1 mg/ml in DMSO, dissolved immediately before treatments) was added at 10 μM final concentration.

In the AR experiments, MD was directly added to the RF/sham-exposed cells.

Four independent experiments were carried out. Each experimental run included 12 randomly assigned cultures to provide treated/exposed and CM producer/recipient cultures as follows:

1. Untreated control (incubator)
2. Sham control (Sh)
3. RF-exposed (RF)
4. MD-treated (MD)
5. Sham-exposed and MD-treated (Sh + MD)
6. RF-exposed and MD-treated (RF + MD)
7. Sham control for medium transfer
8. Untreated control to receive sham-conditioned medium and MD (ShCM + MD)
9. RF-exposed for medium transfer
10. Untreated control to receive RF-conditioned medium and MD (RFCM + MD)
11. RF-exposed medium (without cells) for medium transfer
12. Untreated control to receive RF-conditioned medium without cells and MD (RFCM w/o cells + MD).

All cultures were processed for comet assay, except for cultures n. 7, 9 and 11 that served to produce medium to be transferred to bystander cultures. In particular, cultures 1 to 6 were used to evaluate AR while cultures 8, 10 and 12 were used to assess BE.

A schematic representation of the applied protocol is shown in Fig. 1.

In addition, six independent experiments were carried out to measure the intracellular and extracellular levels of hsp70 following 20h RF/sham-exposure at 71 h from cell seeding.

All the experiments were carried out in blind, i.e. the researchers involved in the analysis were not aware of the exposures/treatments, and data were decoded after completion of all analyses.

2.5. Assay procedures

2.5.1. Alkaline comet assay

The method developed by Singh and co-workers was employed (Singh et al., 1988), with further modifications to obtain a consistent DNA migration in the control cells and a subsequent higher sensitivity. After trypsinization, cell viability was assayed (trypan blue dye exclusion method), resulting higher than 85% in all samples. About 10×10^4 cells were suspended in 100 μl low-melting point agarose (0.5% w/v), and sandwiched between a lower layer of 1% normal-melting agarose at 37 °C, and an upper layer of low melting point agarose (0.5% w/v) on microscope slides. After 60 min treatment at 4 °C in a freshly prepared cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 25 mM NaOH, pH 10, 1% N-lauryl sarcosine, 1% Triton X-100 and 10% DMSO), slides were drained and placed in a horizontal gel electrophoresis tank with fresh alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH 13) and left in the solution for 40 min at 4 °C to allow the equilibration and DNA unwinding to express alkali labile damage. Using the same buffer, electrophoresis was carried out at 4 °C for 40 min at 30 V by using an Amersham Pharmacia Biotech power supply (Uppsala, Sweden), with a current amplitude adjusted to 340 mA by modulating the buffer level. Slides were then rinsed three times with Tris (400 mM, pH 7.5) and left in distilled water for 5 min. Slides were air-dried and stained just before analysis with 12 μg/ml ethidium bromide. For each condition, 2 slides were set up and images of 300 randomly selected nuclei (150 from each slide) were analyzed using a computerized image analysis system (Delta Sistemi, Rome, Italy) fitted with a Leica DM BL fluorescence microscope (Leica Microsystems, Mannheim, Germany) at 200X magnification. The system acquires images and evaluates the percentage of DNA migrated in the tail as a measurement of DNA integrity (Zeni and Scarfi 2010).

2.5.2. Measurement of hsp70 levels by western blot

To quantify the intracellular levels of hsp70, after trypsinization, RF-exposed and sham-exposed cells were collected by centrifugation, viability was tested (Trypan blue dye exclusion method), and resulted unaffected (data not shown). Then cells were lysed in 50 μl RIPA buffer plus protease inhibitor cocktails.

To quantify the extracellular levels, culture medium from RF/Sham exposed samples was filtered through a syringe filter 0.22 μm pore size, and 1.5 ml of filtered medium was mixed with 0.75 ml of Total Exosome Isolation kit reagent. After overnight incubation at 4 °C, samples were centrifuged at 10000 g × 1 h at 4 °C, and pellets containing exosomes were resuspended in 50 μl RIPA buffer plus protease inhibitor cocktails.

In both cases, protein concentration was measured on supernatants with a protein assay kit, following the instructions of the manufacturer. Western blot analysis of hsp70 was performed according to standard procedure. Briefly, about 20 μg of proteins deriving from both cell pellets and extracellular pellet were separated on 4–12% SDS-PAGE gel followed by transfer to a PVDF membrane. After blocking with 5% skim milk powder in Tris –buffered saline (TBS)-Tween for 1 h, the membrane was incubated with specific antibody against hsp70 diluted to 1:1000. HRP-linked anti-mouse secondary antibody was used at 1:2000 dilution. Immunocomplexes were visualized by enhanced chemiluminescence and autoradiography according to the manufacturer protocol, and quantified by densitometric analysis with Image J software (NIH, Bethesda, MA, USA), using the vinculin levels as loading control for normalization (Zanotti et al., 2018).

2.6. Statistics

Data for each condition were expressed as mean ± SE, and unpaired Student's *t*-test was applied to analyze all results. P values below 0.05 were considered as indication of statistically significant differences.

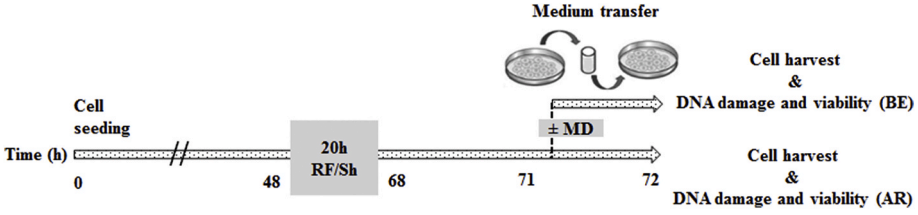


Fig. 1. Schematic representation of the radiofrequency (RF)/Sham (Sh) exposure timing and of the experimental protocols adopted in the study. MD, BE and AR stand for menadione, bystander effect and adaptive response, respectively.

3. Results

3.1. RF exposure does not induce DNA damage while RF pre-exposure reduces MD-induced damage in directly exposed and in bystander cells

Since the determination of cell survival is critical with respect to biological significance of comet results (Albertini et al., 2000), cell viability was assayed in all samples (trypan blue dye exclusion method) and it resulted higher than 85% (data not shown).

The % DNA in the tail of control (Incubator), sham (Sh), RF-exposed (RF) and MD-treated SH-SY5Y cells is presented in Fig. 2. The results showed that sham exposure did not alter the basal level of DNA damage (Sh *vs.* Incubator; $P > 0.05$), and thus sham was considered as reference control for all the RF exposed samples. RF exposure (1950 MHz, 0.3 W/kg SAR) also did not induce DNA damage (RF *vs.* Sh; $P > 0.05$). MD treatment, as expected, resulted in an increased DNA damage with respect to the other treatments (MD *vs.* Incubator, $P < 0.001$; MD *vs.* Sh and MD *vs.* RF, $P < 0.005$). These results confirm those observed in an earlier study in which SH-SY5Y cultures were assayed under the same conditions (Falone et al., 2018).

The % DNA in the tail of cells RF/sham -exposed and subsequently treated with MD, and of bystander cells is reported in Fig. 3 (a) as mean ± SE of four independent experiments. Representative pictures of DNA migration in SH-SY5Y cells treated for RF-induced AR and bystander experiments are presented in Fig. 3 (b).

We evidenced that cells directly exposed to RF were capable of reducing the effect of the subsequent treatment with MD with a 58% reduction in the % DNA in the tail (RF + MD *vs.* Sh + MD; $P < 0.05$), confirming the induction of AR in the experimental conditions adopted. This effect resulted propagated to bystander cells with a comparable reduction of 43% (RFCM + MD *vs.* ShCM + MD; $P < 0.05$). Such a reduction was not detected in cell cultures receiving the RF conditioned medium without cells (RFCM w/o cells + MD *vs*. ShCM + MD; $P > 0.05$).

3.2. RF exposure increases extracellular but not intracellular levels of hsp70

In Fig. 4 the results of western blot analysis of intracellular **(a)** and extracellular **(b)** levels of hsp70 in RF- and sham-exposed samples are presented, where the densitometric analysis is reported as mean ± SE of six independent experiments.

A slight but not statistically significant increase in the intracellular fraction and a significant increase ($P < 0.05$) in the extracellular fraction of hsp70 levels were detected in RF-exposed samples compared to sham-exposed ones.

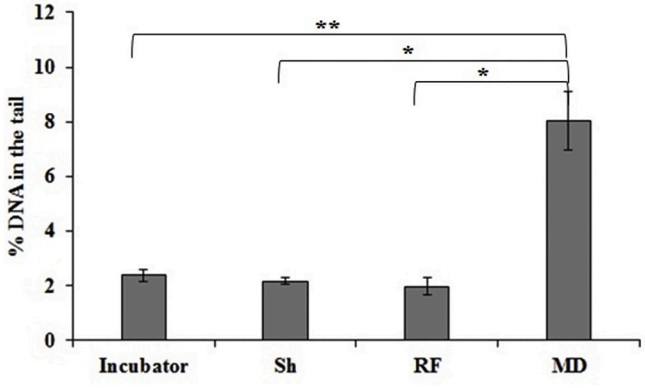


Fig. 2. % DNA in the tail in SH-SY5Y cells is reported for control (Incubator), sham (Sh), RF- exposed (RF) and MD-treated samples. Each data point represents the mean ± SE of 4 independent experiments. Two tailed unpaired Student's *t*-test: **$P < 0.001$; *$P < 0.005$.

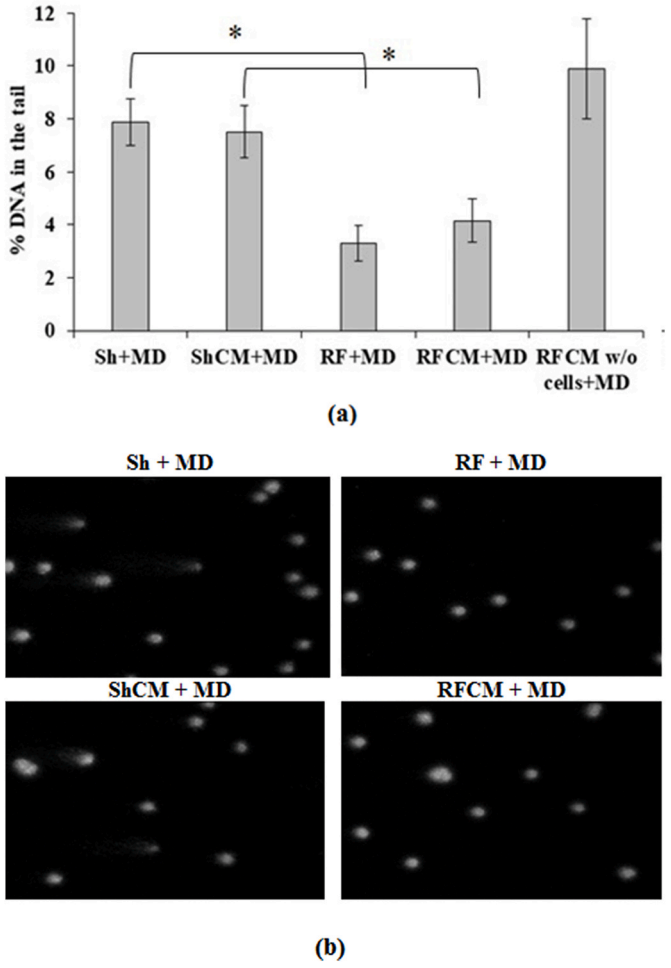


Fig. 3. % DNA in the tail in SH-SY5Y cells is reported in cultures RF/sham-exposed and MD treated (Sh + MD; RF + MD), and in bystander cells (ShCM + MD; RFCM + MD). % DNA in the tail of cells bystandered with RF-exposed medium in absence of cells is also reported (RFCM w/o cells + MD). Each data point represents the mean ± SE of 4 independent experiments. *$P < 0.05$ (two tailed unpaired Student's *t*-test) **(a)**. Representative pictures of DNA migration; 200X images have been acquired by a CCD camera mounted on a Leica DM BL fluorescent microscope **(b)**.

4. Discussion

Here we presented the experimental evidence that RF exposure is capable of eliciting a bystander response in not directly exposed SH-SY5Y cells. In particular, in the experimental conditions adopted, the culture medium transferred from RF-exposed cells to unexposed recipient cells was capable of inducing a 43% reduction of MD-induced DNA damage. The same effect was not detected in cell cultures receiving the RFCM without cells, therefore we can rule out that the effect could derive from some modification in the composition of culture medium due to RF exposure. Rather, the effect could arise from secreted factors released by exposed cells and transferred to recipient cells.

Some indications of BE induced by NIR, including RF, can be found in the literature, referring to the propagation of detrimental effects. In 2007, Mothersill and co-workers exposed human foreskin cells to static and time-varying magnetic fields generated by a 1.5 T magnetic resonance imaging device, and evidenced absence of effects on cloning efficiency in directly exposed cultures, while a significant reduction was found in bystander cells (Mothersill et al., 2007). More recently, Calatayud and co-workers used magnetic nanoparticles along with RF and observed cell death in both targeted and bystander cells (Calatayud et al., 2016). Mortazavi and co-workers found that a 2h exposure of

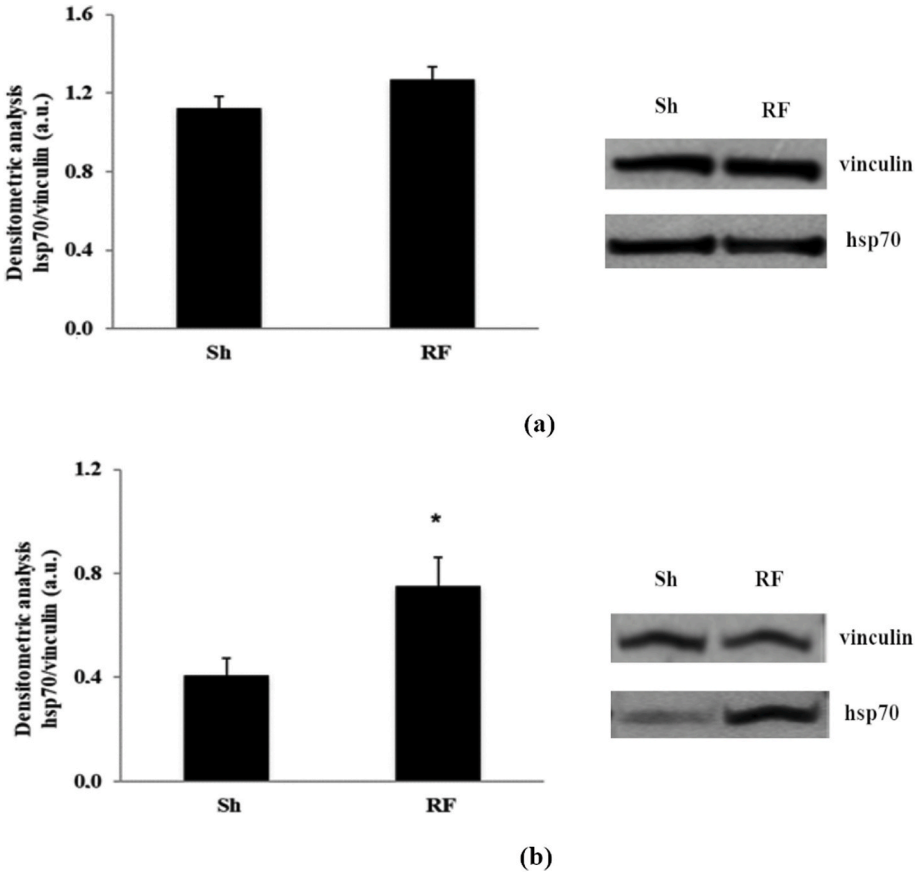


Fig. 4. Western blot analysis of hsp70 levels in SH-SY5Y cells exposed for 20 h to 1950 MHz, 0.3 W/kg SAR and to sham condition. Densitometric analysis of hsp70/vinculin and the western blot of a representative experiment are presented in panel (a) and (b), for intracellular and extracellular levels, respectively. Mean ± SE of six independent experiments. *P < 0.05 (two tailed unpaired Student's *t*-test).

Jurkat cells to 900 MHz, GSM signal, induced apoptosis. The same effect was also detected in cultures treated with medium transferred from directly RF-exposed cells (Mortazavi et al., 2015). In a further study, the same research group reported similar results in Chinese hamster ovary cells exposed for 24 h in the same conditions at SAR value of 2 W/kg: directly exposed and bystander cells showed a significant decrease in clonogenic ability as well as a significant increase in DNA damage (Jooyan et al., 2019).

Our results are consistent with the ability of RF in inducing BE, but in the experimental conditions here adopted a beneficial instead of detrimental effect is transferred via bystander. By comparing the results obtained in our experiments in AR and BE protocols, we can state that the RF-induced AR can also occur in unexposed bystander cells via transferable factors. As a matter of fact, RF pre-exposure resulted in a 58% reduction in MD-induced damage also in directly exposed cells, confirming our previous findings on the induction of AR on this cell model (Falone et al., 2018).

Such strict relation between AR and BE was also reported for low doses of IR. Matsumoto and co-workers pointed out that the acquisition of radioresistance may be also induced in a bystander manner (Matsumoto et al., 2007). Consistently, Iyer and Lehnert found a protective effect in human lung fibroblasts directly irradiated with a 1 cGy gamma rays and challenged with 2 and 4 Gy, and this effect was also detected in not directly irradiated bystander cells (Iyer and Lehnert 2002). Similar results were reported by Buonanno and co-workers in human skin fibroblasts pre-irradiated with 20 cGy from 0.05 GeV protons and challenged with 50 cGy of 1 GeV/u iron ions (Buonanno et al., 2015).

The mechanisms that underlie the IR-induced AR in bystander cells, such as the identification of secreted factors in the culture medium and cellular/molecular events that bestow a condition of protection are under investigation, while very limited data are available in the literature on such phenomenon induced by NIR. Nevertheless, in both cases it is possible to speculate about how some potential mechanisms may contribute to the effect. It could be hypothesized that inducible proteins are triggered by RF exposure, that lead to the protection of cells against DNA damage. Among them, hsps are a good candidate.

It is well established that during stress condition, hsps are not circumscribed to the intracellular environment but they are also actively secreted in extracellular media (Hunter-Lavin et al., 2004; Lancaster and Febbraio 2005; De Maio and Vazquez 2013). Because hsps and their associated factors are induced by a variety of stressors, they have been also proposed as possible biomarkers of RF-field exposure. Several research papers are available on this topic, and an exhaustive review article was published by McNamee and Chauhan in 2009, where the authors conclude that the majority of the results does not suggest a role for RF in inducing a generalized cellular stress response, although in some cases an increased hsps expression was observed, and hsp70 was one of the most investigated (McNamee and Chauhan 2009). More recent investigations confirm such a conclusion (Terro et al., 2012; Valbonesi et al., 2014; Koyama et al., 2016) although it should be pointed out that the above mentioned studies have been conducted by taking into account the intracellular hsps levels only, without considering the hsps release in culture medium.

In the present study, as a first step in investigating the mechanisms involved in the RF-induced AR and BE we measured the intracellular and extracellular levels of hsp70 in RF/sham exposed cultures. A statistically significant increase in the level of hsp70 in the culture medium of RF-exposed cells was found, in absence of cell death, and it was associated with a slight, but not significant increase in the intracellular levels. It is worth noting that, a possible thermal increase responsible for the

hsp70 release can be ruled out since the exposure system employed in this study was strictly controlled in terms of temperature and dosimetry.

The relationship between hsps induction and DNA repair mechanisms has been in the spotlight during the past decades (Sottile and Nadin 2018), and a very recent review presents an analysis of the mechanisms by which hsps can regulate DNA repair signaling pathways in mammalian cells (Dubrez et al., 2020). There is also evidence in the literature of a possible role of hsp70 expression in protecting cells against primary DNA damage induced by a variety of genotoxic agents or to protect DNA from further damage (Abe et al., 1995; Gao et al., 2004; Niu et al., 2006; Kotoglou et al., 2009).

The increase in extracellular level of hsp70 here reported could contribute to the protective effect induced by RF against the MD-induced DNA damage in SH-SY5Y cells in terms of DNA-repair mechanisms. This finding adds to our previous results where we reported on the negation of RF-induced AR when 3-aminobenzamide was used as poly (ADP-ribose) polymerase inhibitor (Sannino et al., 2019), and on the involvement of 8-oxoguanine DNA glycosylase (OGG1), a critical DNA repairing enzyme, in the induction of RF-induced AR (Falone et al., 2018).

5. Conclusions

Our results contribute to the already published evidence on RF-induced BE, and extend it highlighting that also beneficial effects can be transferred via bystander and RF-induced AR can also occur in unexposed bystander cells. The results on hsp70 are preliminary and suggest a possible mechanism underpinning the observed effect of RF exposure. Further and focused investigation is mandatory to provide direct proof that hsp70 pathway is involved in RF-driven protective effects.

Author contributions

Olga Zeni: Conceptualization, data curation, methodology, writing – original draft, writing – review & editing; Stefania Romeo: Conceptualization, data curation, investigation, methodology, writing – review & editing; Anna Sannino: Conceptualization, data curation, investigation, methodology, writing – review & editing; Rosanna Palumbo: Conceptualization, data curation, investigation, methodology, writing – review & editing; Maria Rosaria Scarfì: Conceptualization, methodology, writing – original draft, writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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