Tissue and Cell 68 (2021) 101478

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Exposure to 2.45 GHz radiofrequency modulates calcitonin-dependent activity and HSP-90 protein in parafollicular cells of rat thyroid gland

E. López-Martín [a,b,*], F.J. Jorge-Barreiro [b], J.L. Relova-Quintero [c], A.A. Salas-Sánchez [d,e], F. J. Ares-Pena [d]

[a] CRETUS Institute, Morphological Sciences Department, Faculty of Medicine, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain
[b] Morphological Sciences Department, Faculty of Medicine, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain
[c] Physiology Department, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, 15782, Santiago de Compostela, Spain
[d] CRETUS Institute, Applied Physics Department, Faculty of Physics, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain
[e] ELEDIA@UniTN – DISI – University of Trento, 38123, Trentino-Alto Adige, Italy

ARTICLE INFO

Keywords:
Thyroid
Electromagnetic fields
SAR
Parafollicular cells
Calcitonin-positive cells

ABSTRACT

In this study we analyzed the response of parafollicular cells in rat thyroid gland after exposure to radiofrequency at 2.45 GHz using a subthermal experimental diathermy model. Forty-two Sprague Dawley rats, divided into two groups of 21 rats each, were individually exposed at 0 (control), 3 or 12 W in a Gigahertz Transverse Electro-Magnetic (GTEM) chamber for 30 min. After radiation, we used simple or fluorescence immunohistochemistry to measure calcitonin cells or cellular stress levels, indicated by the presence hyperplasia of parafollicular cells, heat shock protein (HSP) 90. Immunomarking of calcitonin-positive cells was statistically significant higher in the thyroid tissue of rats exposed to 2.45 GHz radiofrequency and cell hyperplasia appeared 90 min after radiation at the SAR levels studied. At the same time, co-localized expression of HSP-90 and calcitonin in parafollicular cells was statistically significant attenuated 90 min after radiation and remained statistically significantly low 24 h after radiation, even though parafollicular cell levels normalized. These facts indicate that subthermal radiofrequency (RF) at 2.45 GHz constitutes a negative external stress stimulus that alters the activity and homeostasis of parafollicular cells in the rat thyroid gland. However, further research is needed to determine if there is toxic action in human C cells.

1. Introduction

Currently, the incidence of thyroid cancer has increased in many countries (Qian et al., 2019; Carlberg et al., 2016) and numerous occupational and environmental agents have been proposed as thyroid carcinogens (Zhang et al., 2019; Albi et al., 2017; Baccarelli et al., 2000). There are numerous studies that associate RF radiation with changes in gland morphology (Misa-Agustiño et al., 2015; Rajkovic et al., 2006) and activity (Mortazavi et al., 2009; Eşmekaya et al., 2006), as well as increased cancer incidence (Milham and Morgan, 2008).

This has led researchers to study whether EMFs can provoke apoptosis in animal models (Eşmekaya et al., 2006) with decreased antioxidant mechanisms (Kunt et al., 2016) in thyroid tissue and to look for evidence of carcinogenic and mutagenic capacity (Wang et al., 2011) in the human thyroid gland. Some occupational epidemiological studies on humans in various countries (Lope et al., 2006; Milham, 2009; Kato et al., 2015) have indicated a causal relationship between non-ionizing radiation and thyroid cancer.

Recently, some authors have been analyzing the research outcomes published by the USA National Toxicology Program (NTP) concerning the toxicology and carcinogenicity of radiofrequency radiation (RF) in rats and mice, comparing the results with epidemiological studies in humans. They found some evidence that RF exposure increases the risk of developing thyroid cancer, and clear evidence that RF radiation is a multi-site carcinogen (Hardell and Carbelg, 2019). Other related research has described how the incidence of thyroid cancer increases with increased duration and frequency of cell phone use when there are genetic variants in some genes. (Luo et al., 2020).

C cells are mainly confined to groups of thyroid lobules located centrally in the rear upper portions of both thyroid lobes (Schmid,

* Corresponding author at: CRETUS Institute, Morphological Sciences Department, Faculty of Medicine, University of Santiago de Compostela, 15782, Santiago de Compostela, Spain.
E-mail address: melena.lopez.martin@usc.es (E. López-Martín).

https://doi.org/10.1016/j.tice.2020.101478
Received 14 August 2020; Received in revised form 25 November 2020; Accepted 25 November 2020
Available online 26 December 2020
0040-8166/© 2020 Elsevier Ltd. All rights reserved.

2015).

Hyperplasia of C cells or parafollicular cells in the thyroid gland may be caused by pathological conditions such as thyrotoxicosis (Zbucki et al., 2019) or nodular goiter (Dadan et al., 2003). These processes may also precede or be associated with medullary thyroid cancer (MTC) (De Lellis et al., 2004). Virtually all types of MTC synthesize and secrete the peptide hormone calcitonin (Schmid, 2015). Thus, calcitonin is a very useful immunohistochemical marker for the histological diagnosis of MTC (Harach et al., 1992). Exposing the thyroid gland of animal models to electromagnetic fields in vivo may constitute a physical stimulus that induces hyperplasia in parafollicular cells (Rajkovic et al., 2006).

Cell damage in pathological conditions provokes the expression and release of inducible types of heat shock proteins (HSPs) (Taha et al., 2019). HSP-90 is associated with cell growth, proliferation, and differentiation. (Li and Buchner, 2013). Normal cells only secrete HSP-90 under stress, whereas tumor cells secrete it continually (Li et al., 2012). The HSP-90α subtype is heavily expressed by cancer cells and secreted to extracellular space as free HSP-90 α (Eguchi et al., 2018), while HSP-90β is found in oncosomes (Ono et al., 2018).

Earlier, we described in our laboratory the qualitative distribution of the HSP-90 α/β chaperones in the cytoarchitecture of the thyroid gland and in the parafollicular cells. The distribution suggests that this stress protein constitutes an important component of glandular architecture and is probably dedicated to maintaining glandular structure and morphology in the parafollicular cells of a rat model exposed to 2.45 GHz radiation (Misa-Agustiño et al., 2015). In a related study, we also found a decrease in HSP-90 α/β levels in rat thyroid gland after interaction with radiofrequency at 2.45 GHz. (Misa-Agustiño et al., 2012). Other research indicates that expression of HSP is altered in thyroid pathologies (Wallin et al., 1992), especially in malignant hyperplasia processes such as MTC (Soudry et al., 2017). However, no research has been done to determine if the stress levels measured by HSPs after exposure to non-ionizing radiation are indicators of an immune system response in the parafollicular cell population. Such a response would be similar to what occurs in neoplastic processes (Nolan et al., 2015; Chatterjee et al., 2007) or other rapid protective responses to environmental stimuli including heat, hypoxia, UV or gamma irradiation (Cheng et al., 2010).

Our hypothesis suggests that non-ionizing radiation may constitute a toxic environment that could alter the morphology, function, and stress response of parafollicular cells. The stress response can be measured by looking at HSP-90 α/β levels. Therefore, the objective of this study was to analyze parafollicular cell response and the expression of HSP-90 α/β in rat thyroid C cells after exposure to 2.45 GHz radiofrequency using a subthermal experimental diathermy model.

2. Methodology

2.1. Animals

Adult female Sprague-Dawley rats were used in the study. The rats weighed 170−190 g and were housed in individual cages with free access to food and water. They were kept at 22 °C under a light / dark regime of 12:12 h. Animals with a synchronized estrogen cycle (the same age and weight, under the same light/dark conditions) were used in all experiments, to eliminate any confounding factor or endocrine interference. However, there is already evidence that estrogen may act directly to modulate the proliferation and functioning of female rat thyroid cells (Santin et al., 2011; Misa-Agustiño et al., 2012).

All experiments were carried out in accordance with the Spanish (RD53/2013) and European Communities Council Directive (2010/63/EU and 86/609/CEE), ARRIVE guidelines, the UK Animals (Scientific Procedures) Act, 1986, and associated guidelines. All experimental protocols were approved by the University of Santiago de Compostela (USC) Committee on Institutional Bioethics of Animal Care and Use (15005AE/11/FUN.01). In addition, every effort was made to minimize the number of animals used in the study and their suffering.

Table 1
SAR values in thyroids and bodies of the test rats, calculated from the power (P) and electrical field (E).

	Mean SAR in Thyroid (W/kg)	Peak SAR in 1 g Thyroid	Mean SAR in body (W/kg)	Peak SAR in 1 g body
P = 3 W E = 40.28 V/m	$0.1 \pm 10{\cdot}10^{-3}$	$0.07 \pm 4{\cdot}10^{-3}$	$0.03 \pm 19{\cdot}10^{-3}$	$0.18 \pm 9{\cdot}10^{-3}$
P = 12 W E = 80.56 V/m	$0.4 \pm 12{\cdot}10^{-3}$	$0.3 \pm 10{\cdot}10^{-3}$	$0.16 \pm 4{\cdot}10^{-3}$	$0.8 \pm 2{\cdot}10^{-3}$

2.2. Experimental design

A total of 42 female Sprague-Dawley rats were distributed in the following groups:

Group A (n = 21): Animals subjected to a single exposure for 30 min and slaughtered after 90 min. The rats were divided into three subgroups (n = 7) according to the intensity of microwave radiation applied: 0 W (control), 3 W and 12 W. The rats were kept alive for 90 min and then euthanized and perfused with fixative.

Group B (n = 21): Animals were subjected to a single exposure for 30 min and slaughtered after 24 h. The rats were divided into three subgroups (n = 7) according to the intensity of microwave radiation applied: 0 W (control), 3 W and 12 W. The rats were kept alive for 24 h and then euthanized and perfused with fixative.

2.3. Experimental radiation system protocol and description of the numerical simulations by finite difference time domain (FDTD) SAR estimation

Radiofrequency energy was applied from a generator connected to an amplifier that was connected to the directional coupler that powered the GTEM chamber. The rat was placed in the region of maximum field uniformity within the chamber. The directional coupler allowed us to measure the input power and reflected power. The input power was read by a power meter that also controlled the purity of the input signal, while the reflected power value was obtained directly by means of a spectrum analyzer. All measuring instruments were certified and calibrated by Agilent Technologies. System components and operation are described in detail (Jorge-Mora et al., 2010; Misa-Agustiño et al., 2012).

The rat was immobilized in a methacrylate holder so that maximum radiation could be applied to the left front leg according to the "diathermic model" (Misa-Agustiño et al., 2012, 2015). The specific absorption rate (SAR) values were estimated with the help of SEM-CAD X 20 and a software tool based on FDTD (Schmid and Partner Engineering A.G., 2009).

The SARs were estimated by applying a correction factor to the values obtained from the numerical simulations, using a ratio that relates the weight of the model rat to the weights of the experimental rats (see Table 1).

2.4. Tissue extraction and preparation

After radiation, the rats were kept alive for 90 min or 24 h and then euthanized by injection with sodium pentobarbital. For tissue extraction, the thyroid gland of each animal was removed and immersed in a fixation solution using 10 % formaldehyde in phosphate buffer. Four hours after fixation, the tissue was again placed in the same solution of 10 % formaldehyde in phosphate buffer to remove water, then all tissues were cooled to 4 °C. After 24 h, the fixing agent was replaced with 70 % ethanol (in water) twice in a 2 h interval and stirred for 12 h. It was then replaced with 96 % ethanol twice in a 2 h interval and subsequently immersed in 100 % ethanol twice in a 2 h interval. An hour later, the alcohol was replaced with 100 % toluene and left for 12 h. All tissues

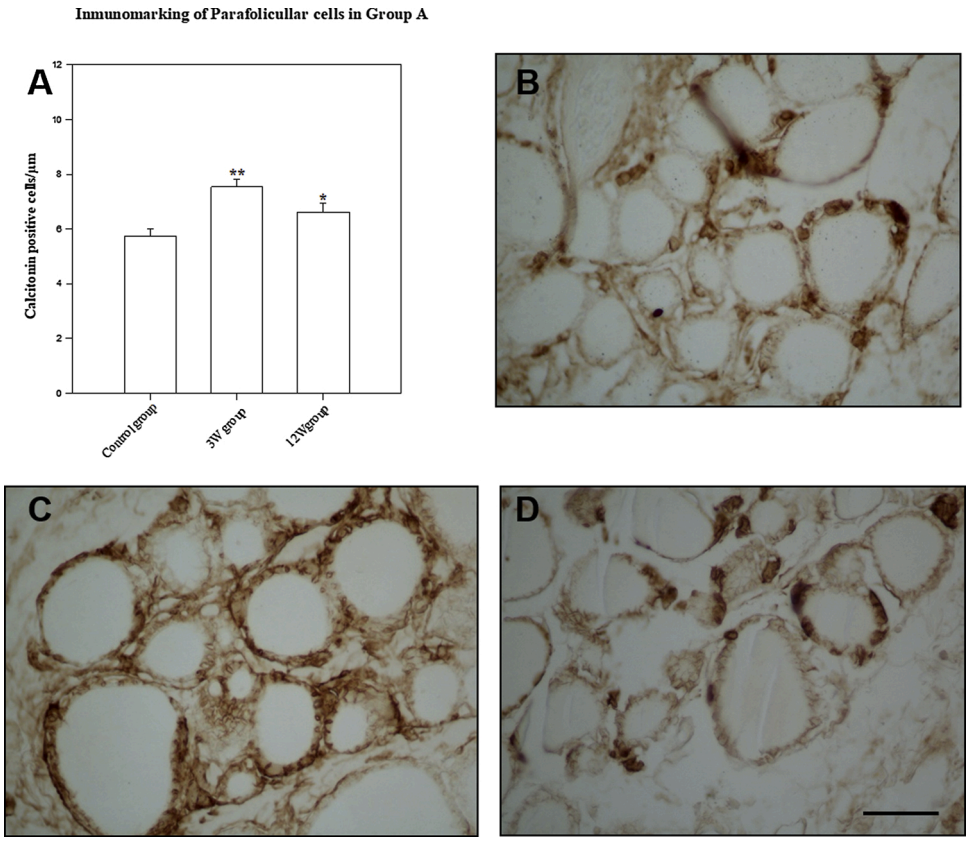


Fig. 1. Histogram and microphotographs showing immunomarking of parafollicular cells in Group A (animals subjected to a single exposure for 30 min and studied after 90 min); (A) histogram of calcitonin-positive cells in thyroid gland. *Average significant differences ($p < 0.05$) between 0 W, 3 W and 12 W subgroups. The photographs show calcitonin-positive cell immunomarkers for control or 0 W (B), 3 W (C) and 12 W (D) in the thyroid gland. Scale bar =50 µm.

were then immersed three times in liquid paraffin (Paraplast plusPlus, Leica) that had been melted in an oven at 58 °C. The pieces were submerged for 2 h each time, leaving the samples completely saturated with paraffin.

After the final immersion, the pieces were placed in a mold and allowed to cool and solidify to a consistency suitable for cutting. Once all the tissues had been embedded in paraffin blocks, a microtome was used on the blocks to make a series of 5 mm cuts which were then joined to create a tape several millimeters long. Sections were selected from each tape at 120 mm intervals (each section 24, 120/5) and mounted on microscope slides for study. The slides were placed in an oven at 37 °C for at least 24 h, after which the tissues were dewaxed by incubating them in an oven at 60 °C for 30 min and then immersing them in Xylol twice for 5 min each time.

2.5. Simple direct immunohistochemistry

The thyroid sections were pretreated for 1 h with 10 % normal rabbit serum and 0.25 % Triton X-100 in 0.02 % potassium- phosphate-buffered saline (KPBS) and then exposed overnight at room temperature to mouse anti-HSP-90 antibody (1:1000 in KPBS, Santa Cruz Biotechnology) or anti-human calcitonin polyclonal antibody (1:1 DAKO) for 24 h at room temperature. Sections were incubated for an hour and a half in Envision + Dual Link Rabbit/Mouse from DAKO and labeled with 3,3'-diaminobenzidine in imidazole-HCl-buffered H_2O_2 solution (DAKO). Subsequently, the sections were hydrated in distilled water, dehydrated with 70 %, 96 %, and 100 % alcohol, then submerged in xylol and mounted. The samples were examined under a conventional brightfield microscope to observe the follicular and parafollicular cells, connective tissue, and colloid of the thyroid gland (see Fig. 1).

2.6. Double indirect immunofluorescence staining

The thyroid sections were pretreated for 1 h with 10 % normal rabbit serum and 0.25 % Triton X-100 in 0.02 % potassium- phosphate-buffered saline (KPBS) and then exposed overnight at room temperature. The primary antibody was applied for overnight and tissues were exposed to a higher concentration of mouse anti-HSP-90 polyclonal antibody (1:100 in KPBS, Santa Cruz Biotecnology) and anti-human Calcitonin (1:1, DAKO) for 24 h at room temperature. Sections were then rinsed with KPBS, incubated for 90 m with fluorescent mouse anti-mouse Cy3 antibody (1:100 in KPBS, Sigma) or α-anti-rabbit Alexa 488 (1:200 in KPBS), hydrated in distilled water and then mounted with glycerol.

2.7. Histological parameters, quantification, and statistical analysis

2.7.1. Cell quantification corresponded to the technique applied

- A morphological count was taken for simple immunohistochemistry of the parafollicular cells: n = 32 sections of 4 µm thickness. 'The area was divided into four equal quadrants by intersecting perpendicular lines, forming horizontal (X) and vertical (Y) axes. Parafollicular cells were observed at 400x magnification and counted in each quadrant, moving counterclockwise from the first quadrant (A1) to the others (A2, A3, A4), to obtain data for each section of the two animal groups (A and B).
- Co-localization counts were done at 200x magnification in n = 20 sections for each group of double-fluorescence immunohistochemistry: anti-mouse Cy-3 for HSP-90 (red), and Alexa 488 α-rabbit for calcitonin-positive cells (green) and co-localizations (yellow).

Table 2
Percent average values ± SEM of calcitonin-positive (C+) cells in thyroid gland at 0, 3, and 12 W power 90 min (90 min) or 24 h (24 h) after exposure of animals to 2.45 GHz radiofrequency ($p < 0.05$ or $p > 0.05$). Two-way ANOVA followed by multiple comparisons versus control group (0 W) using the Holm-Sidak method.

Power applied	*N*	90 min POST-EXPOSURE NUMBER OF CALCITONIN (+) CELLS	*P value**	24 h POST-EXPOSURE NUMBER OF CALCITONIN (+) CELLS	*P value**
0 W	20	5.6 ± 0.3		6 ± 0.1	
3 W	20	7.8 ± 0.2	*p=0.0000251*	6.2 ± 0.2	*p >0.05*
12 W	20	7.4 ± 0.2	*p = 0.0298*	6.1 ± 0.2	*p >0.05*

Table 3
Results of two-way ANOVA (post-exposure times, different power, post-exposure time x power) for C cells in the thyroid gland that co-localized with heat stress protein HSP-90 at 0, 3, and 12 W power 90 min (90 min) or 24 h (24 h) after exposure of animals to 2.45 GHz radiofrequency.

Effects	*p-values*
Post exposure time	*0.057*
Different power	*<0.001*
Post-exposure time x different power	*0.352*

2.7.2. Statistical analysis

The results in the text and figures are expressed as averages and standard error of the mean (SEM) ($p < 0.05$). The following statistical tests were applied:

- One-way ANOVA using the radiation factor (irradiated or non-irradiated) to analyze the immunoreactivity results for calcitonin-positive cells, followed by multiple comparison versus control group (Holm-Sidak method).
- Two-way ANOVA using radiation power (0, 3 and 12 W) X time after radiation (90 min or 24 h) for morphological study of calcitonin-positive cells that were co-localized with HSP-90 at 20 × . This was done with double fluorescence immunohistochemistry for the different groups (Tables 2 and 3) followed by multiple comparisons versus control group (Holm-Sidak method).

All statistical analyses were performed with Sigma Plot 14.0 software (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Immunohistochemistry sample

3.1.1. Calcitonin-positive cells

Positive immunomarking for calcitonin and parafollicular cells increased in the thyroid tissue of rats exposed to 2.45 GHz radiofrequency and cellular hyperplasia appeared. After doing the parafollicular cell counts for the animal groups exposed and not exposed to radiation, statistical analyses were performed (see Figs. 1 and 2).

Ninety minutes after radiation (Group A), the animals showed differences in mean values among treatment groups. The differences were

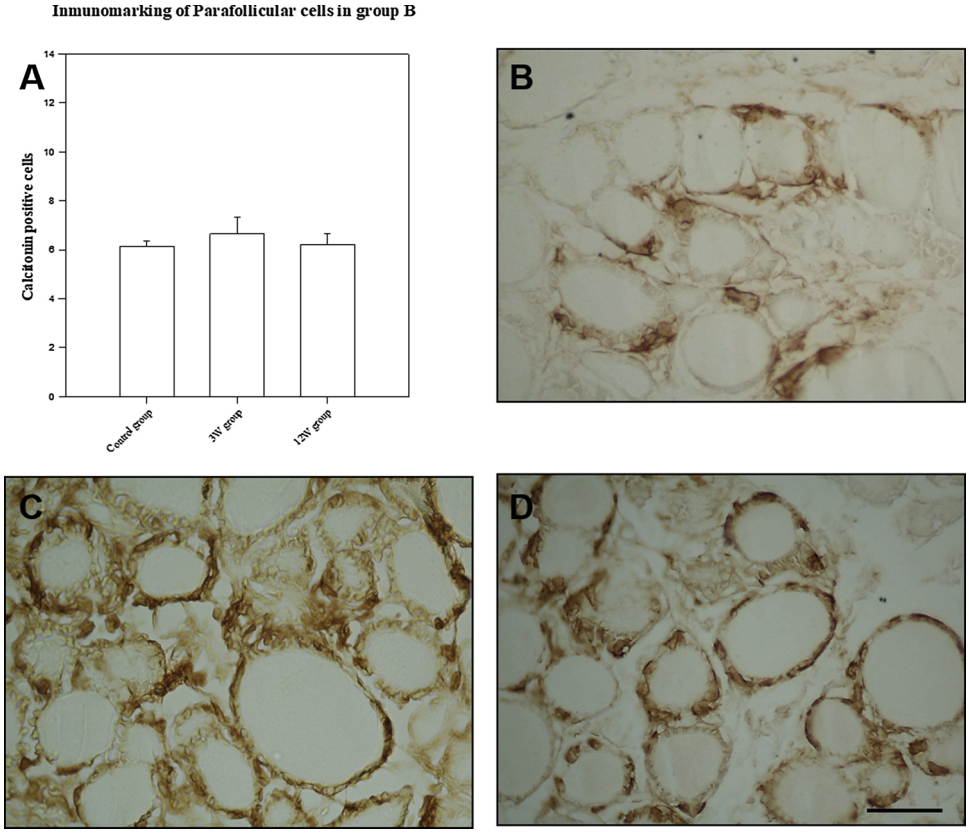


Fig. 2. Histogram and microphotographs showing immunomarking of parafollicular cells in Group B (Animals subjected to a single exposure for 30 min and studied after 24 h); (A) histogram of calcitonin-positive cells in thyroid gland. *Average significant differences ($p < 0.05$) between 0 W, 3 W and 12 W subgroups. The photographs show the calcitonin-positive cell immunomarkers for control or 0 W (B), 3 W (C) and 12 W (D) in the thyroid gland. Scale bar =50 μm.

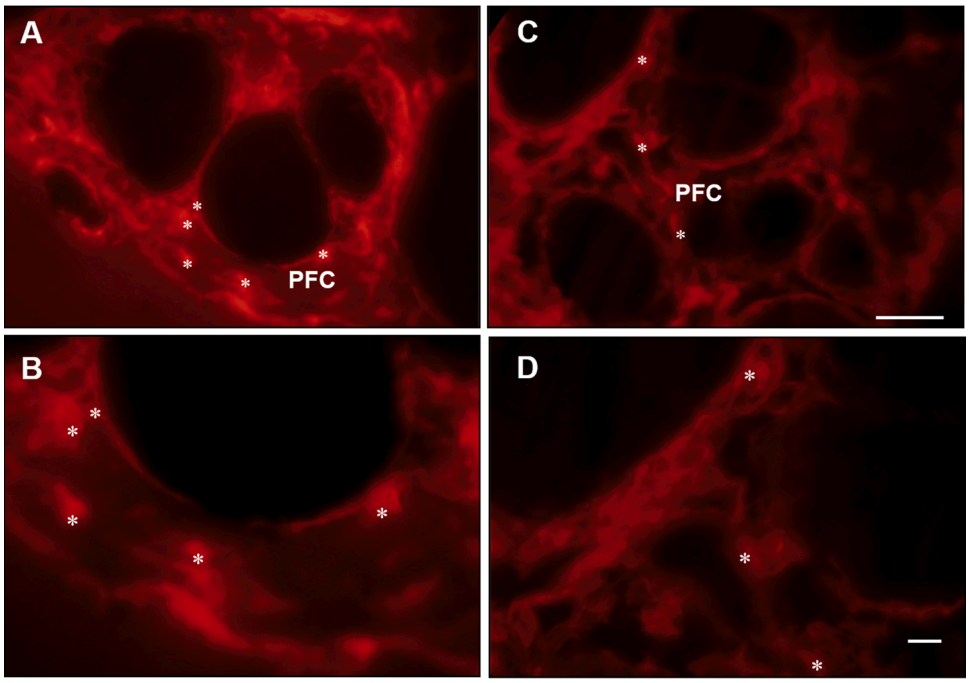


Fig. 3. Photographs of rat thyroid tissue with HSP-90 fluorescence immunomarking show differences in expression in parafollicular cells in thyroid gland on animals euthanized 90 min after exposure to 3 and 12 W at 40x magnification (photos A and C) and at 100x magnification (photos B and D). Asterisks * indicate parafollicular cells (PFC). Calibration bars (A, C) 40 μm; (B, D) 20 μm.

statistically significant ($p < 0.001$) and greater than what would be expected by chance.

For all Pairwise Multiple Comparison Procedures (Holm-Sidak method), the overall significance level was 0.05. Parafollicular cell levels in radiated animals exposed to 3 W or 12 W power showed statistically significant differences with respect to the control or non-radiated group ($p = 0.0000251$; $p = 0.0298$, respectively). However, differences in cell counts between the radiated groups were not statistically significant (see Table 2 and Fig. 1). Twenty-four hours after radiation (Group B), the differences in mean values among the animal treatment groups were not great enough to exclude the possibility that they were due to random sampling variability. Therefore, the differences were not statistically significant ($p = 0.984$) (see Table 2 and Fig. 2).

3.2. Results of fluorescence immunohistochemistry

3.2.1. HSP-90 in the thyroid gland

The presence of HSP-90 was also visualized through Cy3 red immunofluorescence, which clearly showed how this cytoplasmic protein was distributed around the colloid borders of the follicles but was not expressed within them, but was not expressed in the inter-cellular unions of the follicular (see Fig. 3). Cytoplasmic expression of HSP-90 was also observed in the parafollicular cells (see Fig. 3). This may indicate the vulnerability of this type of cell to stress or that the protein helps to maintain parafollicular cell function.

3.2.2. Double immunofluorescence staining marking for HSP- 90 and calcitonin

Compared to non-radiated animals, the co-localized expression of HSP-90 and calcitonin in the parafollicular cells decreased considerably when the animals were radiated with 3 and 12 W. Co-localization increased progressively as the time after radiation increased.

The following criteria were applied to the analysis of the results for calcitonin + HSP-90 co-localization:

The difference in mean values among the power levels, after allowing for the effects of differences in time after exposure, was greater than would be expected by chance and was considered statistically significant ($p =< 0.001$). A multiple comparison procedure was used to isolate which group(s) differed from the others.

Table 4
Percent average values ± SEM of C cells in the thyroid gland that co-localized with heat stress protein HSP-90 at 0, 3, or 12 W power ninety minutes (90 min) after exposure of animals to 2.45 GHz radiofrequency ($p < 0.001$). Two-way ANOVA followed by multiple comparisons versus control group (0 W) using the Holm-Sidak method.

Power applied	N	90 min POST-EXPOSURE % OF CELLS WITH CO-LOCALIZED CALCITONIN AND HSP-90 (+)	P value*
0 W	20	97% ± 0.01	
3 W	20	67% ± 0.04	*0.001*
12 W	20	68%± 0.06	*<0.001*

The difference in mean values among post-exposure times, after allowing for the effects of differences in power, was not great enough to exclude the possibility of it being due to random sampling variability. It was not considered statistically significant ($p = 0.057$). See Table 3.

The effect of different power levels did not depend on time after exposure. There was no statistically significant interaction between power and time after exposure ($p = 0.352$). See Table 3.

There were no statistically significant differences between co-localization values recorded 90 min and 24 h after radiation for the powers used (3 or 12 W), including the control group. Ninety minutes after radiation, there were statistically significant differences in the co-localization of calcitonin (+) and HSP-90 cells in animals exposed to 12 W and/or 3 W radiation, compared to non-radiated control animals (see Table 4 and Fig. 4). However, 24 h after radiation, no statistically significant differences appeared in the co-localization of calcitonin (+) and HSP-90 cells in animals exposed to 12 W and/or 3 W radiation, with respect to non-radiated control animals (see Table 5 and Fig. 5).

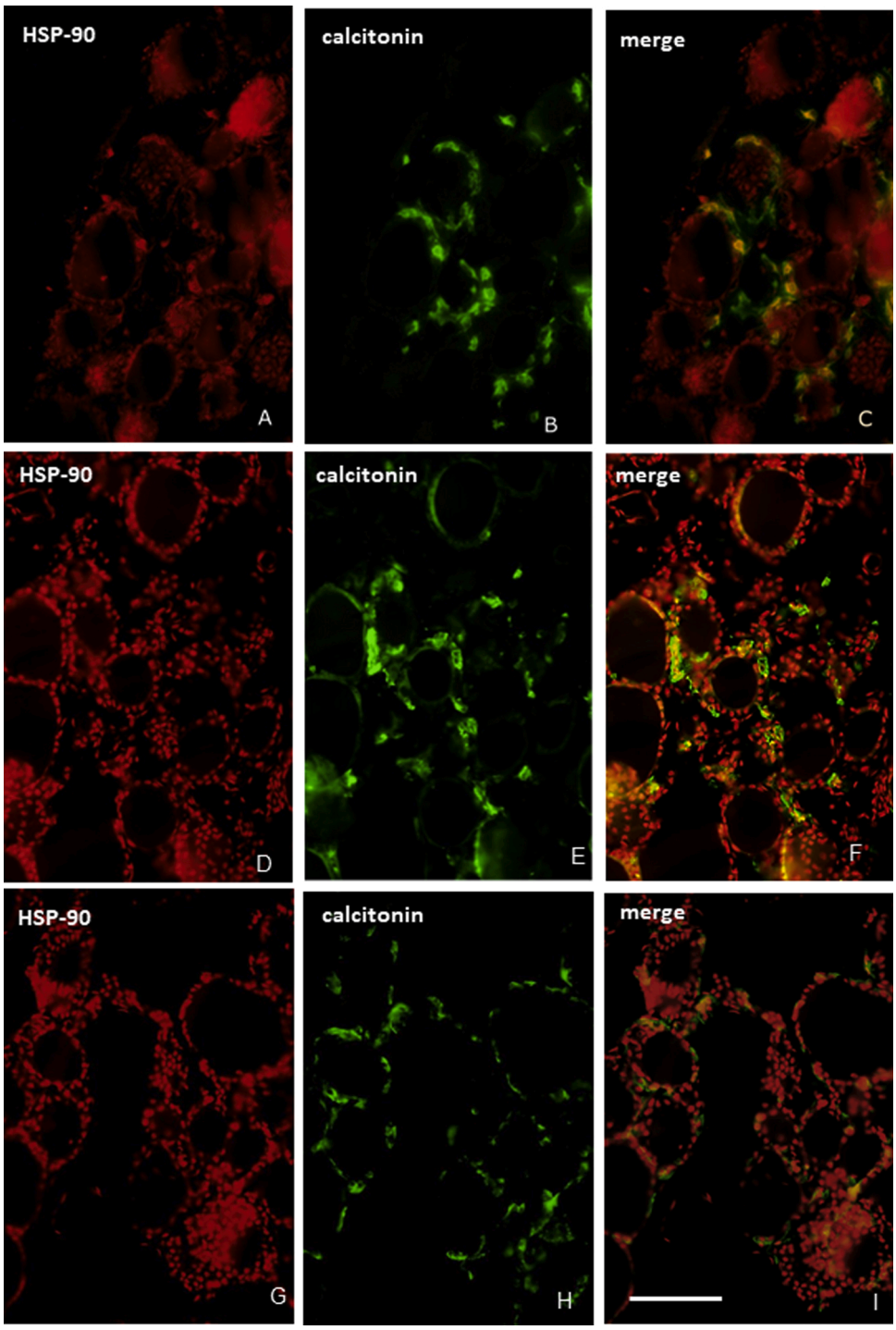


Fig. 4. Photographs showing double immunofluorescence staining of parafollicular cells in Group A (animals subjected to a single exposure for 30 min and studied after 90 min) in radiation subgroups for 0 W (A,B,C) 3 W (D,E,F) and 12 W (G,H,I) at 20x magnification. Red indicates HSP-90-positive cells (A,D,G), green indicates calcitonin-positive cells (B,E,H) and yellow indicates co-localization of both cell types (C,F,I). Scale bar =60 μm.

4. Discussion

The main findings of this experimental study are related changes in the thyroid gland, which were provoked by the acute interaction of electromagnetic fields at 2.45 GHz radiofrequency. Accordingly, we observed 1) hyperplasia of parafollicular cells in female rats in the experimental diathermy model; and 2) attenuation of cytoplasmic expression of cellular stress proteins HSP-90 α/β in thyroid gland cells that were positive for calcitonin after exposure to non-ionizing radiation. The relevance of changes in the parafollicular cells as an effect of electromagnetic fields is discussed here.

4.1. Non-ionizing radiation induces stress and modifies the activity of parafollicular cells in rats

A wide variety of stimuli can modify the activity of the parafollicular cells and be associated with cancer (Vitale et al., 2017), autoimmune diseases (Baschieri et al., 1989), goiter (Dadan et al., 2003), hyper/-hypothyroidism (Zbuckiet et al., 2007) or single-gene disorder in the hypothalamic-pituitary axis and thyroid (Gilloteaux and Pardhan,

Table 5
Percent average values ± SEM of C cells in the thyroid gland that colocalized with thermal stress protein HSP-90 at 0, 3, and 12 W power 24 h (24 h) after exposure of animals to 2.45 GHz radiofrequency ($p < 0.001$).Two-way ANOVA followed by multiple comparisons versus control group (0 W) using the Holm-Sidak method.

		24 h POST-EXPOSURE % OF CELLS WITH CO-LOCALIZED CALCITONIN (+) AND HSP-90	
Power applied	*N*		*P value**
0 W	20	97%±0.005	
3 W	20	81%±0.07	*0.052*
12 W	20	79%±0.04	*0.054*

2015). C-cell hypoplasia is induced in environments of hypo/hypergravity (Albi et al., 2012), while continuous exposure to low-frequency electromagnetic fields (50 Hz) provokes an increase in the number of parafollicular cells (Rajkovic et al., 2005). It should be noted that all physiological and/or pathological changes to the gland are associated with changes in its cytoarchitecture (Dadan et al., 2003), thus establishing a mutual relationship between changes in the follicular and parafollicular cells (Dadan et al., 2004; Zbucki et al., 2007). Recent research has described follicular-parafollicular structural changes in the thyroid gland from acute and repeated exposure to hyper-frequencies (Misa-Agustiño et al., 2015). These include increases in the follicular epithelium, parafollicular cells and interfollicular tissue from exposure to low-frequency EMFs (Rajkovic et al., 2006). Alterations have also been reported in thyroid function and in hormonal levels of T3, T4 and TSH from exposure to EMFs with radiofrequencies (Mortavazi et al., 2009; Eşmekaya et al., 2006) and low frequencies (Rajkovic et al.,

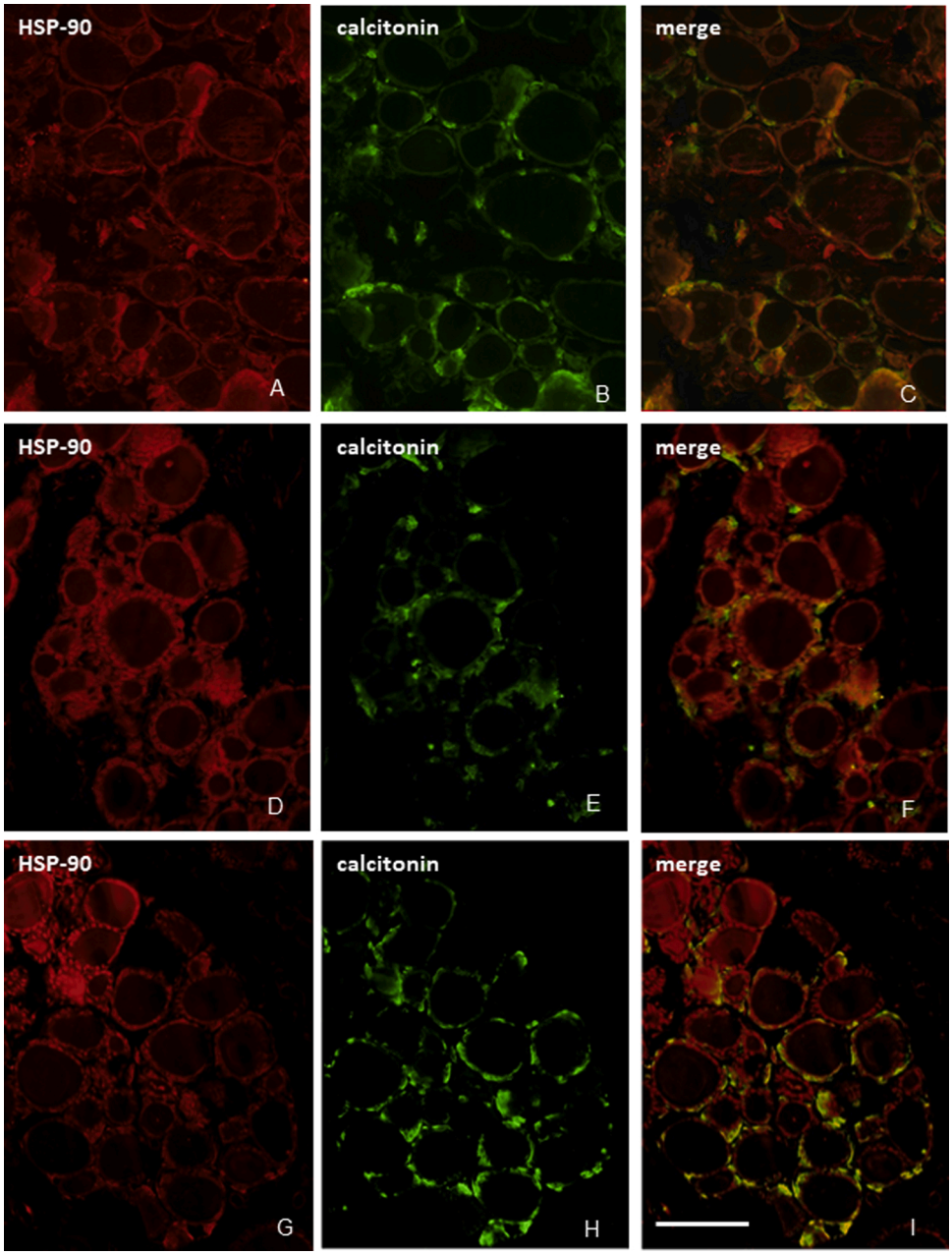


Fig. 5. Photographs showing double immunofluorescence staining of parafollicular cells in Group B (animals subjected to a single exposure for 30 min and studied after 24 h) in radiation subgroups for 0 W (A,B,C) 3 W (D,E,F) and 12 W (G,H,I) at 20x magnification. Red indicates HSP-90-positive cells (A,D,G), green indicates calcitonin-positive cells (B,E,H) and yellow indicates co-localization of both cell types (C,F,I). Scale bar =60 μm.

2005), though not from the combined interaction of two radio-frequencies (Jin et al., 2013). Though an increment in the cellular propagation of thyrocytes was reported at the experimental level (Hilly et al., 2013), the carcinogenic capacity of non-ionizing radiation in primary thyroid cells could not be demonstrated (Silva et al., 2015). Furthermore, despite the proliferation of epidemiological studies in diverse countries due to the increased incidence of thyroid tumors, a demonstrated causal relationship between exposure to electromagnetic fields and increased incidence of thyroid cancer has not been established (Luo et al., 2019; Carlberg et al., 2016; Kato et al., 2015). Nevertheless, oncology researchers have recently claimed that there is evidence of greater risk of developing thyroid cancer and clear evidence that RF radiation is a carcinogenic agent (Hardell and Carbelg, 2019). In any case, the high levels of RF with SAR that reach the thyroid gland in humans as they are emitted from numerous RF devices (Lauer et al., 2013) and the opinion of the International Agency for Research on Cancer (IARC) regarding the possible carcinogenic effect of RF-EMFs from mobile telephones and other devices that emit electromagnetic fields in the 30 kHz – 300 GHz frequency range (Baan et al., 2011; IARC., 2013) make it necessary to adopt a principle of precaution with the general population. This applies especially to children and youth, whose thyroid tissue is still developing (Sangün et al., 2015). Similarly, thyroid radiosensitivity is greater (Sangün et al., 2015; Gandhi et al., 2012) in physiological states of metabolic increase, such as pregnancy (Mollee et al., 2011), and thyroid function should be protected (Lauer et al., 2013).

Notably, we found higher numbers of C cells in radiated rats, compared to control animals ($p < 0.001$), which has been described by other authors in association with increased calcitonin (Rajkovic et al., 2005). It should also be kept in mind that elevated pro-calcitonin may constitute an indicator of thyroid medullary cancer (Karagiannis et al., 2016) when associated with malignant proliferation of parafollicular cells (Emmertsen, 1985). This difference in the relationship between C-cell hyperplasia and thyroid medullary cancer in tests for GLP-1 receptors in rodents and humans gives cause to reflect on the difficulty of extrapolating some test results from laboratory animals to humans (Rosol, 2013) and the need for long-term testing in humans (Kannan and Nasr, 2015).

All these facts allow us to confirm that the C-cell response to non-ionizing radiation and other external stress stimuli in the thyroid gland is associated with changes in the cellular cytoarchitecture (Misa-Agustiño et al., 2015) as well as with specific functional changes in species that do not always induce mutagenic action. Finally, it is advisable to explore whether interaction with EMFs causes C-cell hyperplasia and elevated levels of calcitonin in humans, since it could also have implications for calcium metabolism in the bones (Andresen et al., 2008; Faour and Gilloteaux, 2017).

4.2. Non-ionizing radiation modulates expression of heat shock protein 90 in calcitonin-positive cells

HSP-90 is a constitutional protein that forms part of the architecture of the thyroid gland at the follicular level, in the septa and membranes (Misa-Agustiño et al., 2015) as well as in the cytoplasm of the follicular and parafollicular cells (see Fig. 3), to help maintain cellular homeostasis (Taipale et al., 2010). There is a close relationship between heat shock proteins and certain thyroid pathologies (Wallin et al., 1992; Palladino et al., 2016) and elevated HSP levels can constitute a diagnostic biomarker for thyroid cancer in some cases (Mo et al., 2012; Soudry et al., 2017). This chaperone regulates crucial functions in human carcinogenesis, such as cellular proliferation, angiogenesis, metastasis and apoptosis (Fulda et al., 2010; Taipale et al., 2010). It is also related to malignant transformation and tumor progression (Miyata et al., 2013). At the cellular level, elevated expression of this protein, which activates oxidative stress mechanisms and apoptosis in the thyroid cells (Kostic et al., 2010), could constitute a biomarker for environmental stress (Sabbah et al., 1996) caused by toxic substances (Fahmi et al., 2017), ultraviolet radiation (Chen et al., 2009) or hypoxia (Jain et al., 2014).

In prior research, we found that acute exposure to 2.45 GHz provoked an overall decline of HSP-90 in rat thyroid tissue (Misa-Agustiño et al., 2012). In the present study, we identified a decrease in the co-localization of this chaperone in the parafollicular cells 90 min after exposure of the thyroid gland to non-ionizing radiation (see Fig. 4) but no change in apoptotic activity (Misa-Agustiño et al., 2012). In the parafollicular cells not exposed to non-ionizing radiation, simultaneous expression of calcitonin and HSP-90 was greater than 95 %, indicating an important protective and constitutional function of this protein in the thyroid cytoarchitecture (see Tables 2, 3 and Figs. 4, 5). A decline in this protein due to the interaction of electromagnetic fields with the thyroid gland constitutes a negative stimulus for the maintenance of cellular homeostasis. This could lead to an imbalance in the cellular cycle and, over prolonged periods, apoptosis (Trepel et al., 2010; Barrott and Haystead, 2013).

Inhibition of HSP-90 has also been found to provoke the simultaneous degradation of multiple proteins involved in the oncogenic signaling pathways, making it a therapeutic target for thyroid medullary cancer (Lin et al., 2017; Kamal et al., 2003). In the last few years, however, research has suggested that the radiosensitizing effect of EMFs could have therapeutic benefits and improve the effectiveness of radiotherapy (Artacho-Cordón et al., 2013). The combined action of both types of radiation has been explored for various breast cancer cell lines and provided satisfactory results (Salinas-Asensio et al., 2019). Some authors suggest that the thermal and non-thermal effects of EMFs have potential therapeutic applications for inducing apoptosis, developing oxygen-reactive species and increasing membrane permeability in malignant cells (Saliev et al., 2014). In the present study, we found that non-ionizing radiation provoked a decline in HSP-90 in thyroid C cells. Other authors have described how this chaperone diminishes in association with the appearance of apoptosis in human epidermoid carcinoma cells exposed to EMFs (Caraglia et al., 2005). Such findings lead us to think, as other authors suggest, that possible therapeutic actions should be explored for non-ionizing radiation (Saliev et al., 2019) as a coadjuvant for radiotherapy in neoplasias, including thyroid cancer (Vadalà et al., 2016). As with other thyroid cancer treatments, the levels of this chaperone after applying EMFs could also constitute a therapeutic prognostic biomarker (Lin et al., 2017).

5. Conclusions

These facts indicate that RF at 2.45 GHz constitutes a negative external stress stimuli that alters the activity and homeostasis of parafollicular cells in rat thyroid gland. However, long-term studies would be needed to determine whether it induces toxicity in humans or if non-ionizing radiation modulates HSP-90 in human C cells, which would imply exploring its potential for therapeutic interventions.

CRediT authorship contribution statement

E. López-Martín: Conceptualization, Methodology, Software, Visualization, Investigation. **F.J. Jorge-Barreiro:** Software, Validation. **J.L. Relova-Quintero:** Supervision. **A.A. Salas-Sánchez:** Data curation, Writing - original draft. **F.J. Ares-Pena:** Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This work was supported in part by the FEDER/Ministerio de Ciencia, Innovación y Universidades-Agencia Estatal de Investigación under

Project TEC2017-86110-R. The work of A.A.S.-S. was supported by the Xunta de Galicia under the Postdoctoral Fellowship with ref. ED481B 2018/008.

References

Albi, E., Curcio, F., Spelat, R., Lazzarini, A., Lazzarini, R., Cataldi, S., Loreti, E., Ferri, I., Ambesi-Impiombato, F.S., 2012. Loss of parafollicular cells during gravitational changes (microgravity, hypergravity) and the secret effect of pleiotrophin. PLoSOne 7 (12). https://doi.org/10.1371/journal.pone.0048518 e48518.

Albi, E., Krüger, M., Hemmersbach, R., Lazzarini, A., Cataldi, S., Codini, M., Beccari, T., Ambesi-Impiombato, F.S., Curcio, F., 2017. Impact of gravity on thyroid cells. Int. J. Mol. Sci. 18 (5), 972.

Andresen, C.J., Moalli, M., Turner, C.H., Berryman, E., Pero, R., Bagi, C.M., 2008. Bone parameters are improved with intermittent dosing of vitamin D3 and calcitonin. Calcif. Tissue Int. 83 (6), 393–403.

Artacho-Cordón, F., Salinas-Asensio, M.M., Calvente, I., Ríos-Arrabal, S., León, J., Román-Marinetto, E., Olea, N., Núñez, M.I., 2013. Could radiotherapy effectiveness be enhanced by electromagnetic field treatment? Int. J. Mol. Sci. 14 (7), 14974–14995.

Baan, R., Grosse, Y., Lauby-Secretan, B., El Ghissassi, F., Bouvard, V., BenbrahimTallaa, L., Guha, N., Islami, F., Galichet, L., Straif, K., 2011. WHO international agency for research on cancer monograph working group. Carcinogenicity of radiofrequency electromagnetic fields. Lancet Oncol. 12 (2011), 624–626.

Barrott, J.J., Haystead, T.A., 2013. HSP 90, an unlikely ally in the war on cancer. FEBS J. 280, 1381–1396.

Baschieri, L., Castagna, M., Fierabracci, A., Antonelli, A., Del Guerra, P., Squartini, F., 1989. Distribution of calcitonin- and somatostatin-containing cells in thyroid lymphoma and in Hashimoto's thyroiditis. Appl. Pathol. 7, 99–104.

Caraglia, M., Marra, M., Mancinelli, F., D'Ambrosio, G., Massa, R., Giordano, A., Budillon, A., Abbruzzese, A., Bismuto, E., 2005. Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. J. Cell. Physiol. 204 (2), 539–548.

Carlberg, M., Hedendahl, L., Ahonen, M., Koppel, T., Hardell, L., 2016. Increasing incidence of thyroid cancer in the Nordic countries with main focus on Swedish data. BMC Cancer 16, 426.

Chatterjee, M., Jain, S., Stühmer, T., Andrulis, M., Ungethüm, U.te, Kuban, R.J., Lorentz, H., Bommert, K., Topp, M., Krämer, D., Müller-Hermelink, H.K., Einsele, H., Greiner, A., Bargou, R.C., 2007. STAT3 and MAPK signaling maintain overexpression of heat shock proteins 90alpha and beta in multiple myeloma cells, which critically contribute to tumor-cell survival. Blood. 109 (2), 720–728. https://doi.org/10.1182/blood-2006-05-024372.

Chen, H., Xia, Y., Fang, D., Hawke, D., Lu, Z., 2009. Caspase-10-mediated heat shock protein 90 beta cleavage promotes UVB irradiation-induced cell apoptosis. Mol. Cell. Biol. 29 (13), 3657–3664.

Cheng, C.F., Fan, J., Zhao, Z., Woodley, D.T., Li, W., 2010. Secreted heat shock protein-90alpha: a more effective and safer target for anti-cancer drugs? Curr. Signal Transduct. Ther. 5, 121–127.

Dadan, J., Zbucki, R., Sawicki, B., Winnicka, M.M., Puchalski, Z., 2003. Activity of the thyroid parafollicular (C) cells in simple and hyperactive nodular goitre treated surgically - preliminary investigations. Folia Morphol. (Praha) 62, 443–445.

Dadan, J., Zbucki, R., Andrzejewska, A., Winnicka, M.M., Puchalski, Z., 2004. Activity of thyroid parafollicular (C) cells in rats with hyperthyroidism - preliminary ultrastructural investigation. Rocz. Akad. Med. Bialymst. 49, 132–134.

De Lellis, R.A., Lloyd, R.V., Heitz, P.U., Eng, C., 2004. WHO histological classification of thyroid and parathyroid tumours. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of Endocrine Organs. IARC Press, Lyon.

Eguchi, T., Sogawa, C., Okusha, Y., Uchibe, K., Iinuma, R., Ono, K., Nakano, K., Murakami, J., Itoh, M., Arai, K., et al., 2018. Organoids with cancer stem cell-like properties secrete exosomes and HSP90 in a 3D NanoEnvironment. PLoS One 13, e0191109.

Emmertsen, K., 1985. Medullary thyroid carcinoma and calcitonin. Dan. Med. Bull. 32 (1), 1–28.

Eşmekaya, M.A., Seyhan, N., Ömeroğlu, S., 2006. Pulse modulated 900 MHz radiation induces hypothyroidism and apoptosis in thyroid cells: a light, electron microscopy and immunohistochemical study. Int. J. Radiat. Biol. 86, 1106–1116.

Fahmi, T., Branch, D., Nima, Z.A., Jang, D.S., Savenka, A.V., Biris, A.S., Basnakian, A.G., 2017. Mechanism of graphene-induced cytotoxicity: role of endonucleases. J. Appl. Toxicol. 37 (11), 1325–1332.

Faour, O., Gilloteaux, J., 2017. Calcitonin: survey of new anatomy data to pathology and therapeutic aspects. Transl. Res. Anat. 6, 4–15.

Fulda, S., Galluzzi, L., Kroemer, G., 2010. Targeting mitochondria for cancer therapy. Nat. Rev. Drug Discov. 9, 447–464.

Gandhi, O.P., Morgan, L.L., de Salles, A.A., Han, Y.Y., Herberman, R.B., Davis, D.L., 2012. Exposure limits: the underestimation of absorbed cell phone radiation, especially in children. Electromagn. Biol. Med. 3, 34–51.

Gilloteaux, J., Pardhan, D., 2015. Crinophagy in thyroid follicular and parafollicular cells of male obese zucker rat. Ultrastruct. Pathol. 39 (4), 255–269.

Harach, H.R., Wilander, E., Grimelius, L., Bergholm, U., Westermark, P., Falkmer, S., 1992. Chromogranin A immunoreactivity compared with argyrophilia, calcitonin immunoreactivity,ç. Histopathology of C Cells and Medullary Thyroid Carcinoma 57 and amyloid as tumour markers in the histopathological diagnosis of medullary (C-cell) thyroicarcinoma. Pathol. Res. Pract. 188, 123–130.

Hardell, L., Carbelg, M., 2019. Comments on the US National Toxicology Program technical reports on toxicology and carcinogenesis study in rats exposed to whole-body radiofrequency radiation at 900 MHz and in mice exposed to whole-body radiofrequency radiation at 1,900 MHz. Int. J. Oncol. 54, 111–127.

Hilly, O., Silva, V., Mizrachi, A., Ariel, O., Raiter, A., Hauptman, Y., Hardy, B., Feinmesser, R., 2013. Effect of non-ionizing electromagnetic radiation at mobile phone frequency on human thyroid cells. In: World Congress on Thyroid Cancer 10th-14th July 2013. Book of Abstracts. Toronto, Canada.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 2013. Volume 102. Non-ionizing Radiation, Part II: Radiofrequency Electromagnetic Fields [includes Mobile Telephones]. IARC, Lyon. http://monographs.iarc.fr/ENG/Monographs/vol102/mono102.pdf.

Jain, K., Suryakumar, G., Ganju, L., Singh, S.B., 2014. Differential hypoxic tolerance is mediated by activation of heat shock response and nitric oxide pathway. Cell Stress Chaperones 19 (6), 801–812.

Jin, Y.B., Choi, H.D., Kim, B.C., Pack, J.K., Kim, N., Lee, Y.S., 2013. Effects of simultaneous combined exposure to CDMA and WCDMA electromagnetic fields on serum hormone levels in rats. J. Radiat. Res. 54 (3), 430–437.

Jorge-Mora, M.T., Alvarez-Folgueiras, M., Leiro, J., Jorge-Barreiro, F.J., Ares-Pena, F.J., Lopez-Martín, E., 2010. Exposure to 2.45 GHz microwave radiation provokes cerebral changes in induction of HSP90 α/β heat shock protein in rat. Prog. Electromagn. Res. B Pier B 100, 351–379.

Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M.F., Fritz, L.C., Burrows, F.J., 2003. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425, 407–410.

Kannan, S., Nasr, C., 2015. Should we be concerned about thyroid cancer in patients taking glucagon-like peptide 1 receptor agonists? Cleve. Clin. J. Med. 82 (3), 142–144.

Karagiannis, K.A., Girio-Fragkoulakis, C., Nakouti, T., 2016. Procalcitonin: a new biomarker for medullary thyroid cancer? A systematic review. Anticancer Res. 36 (8), 3803–3810.

Kato, I., Young, A., Liu, J., Abrams, J., Bock, C., Simon, M., 2015. Electric blanket use and risk of thyroid cancer in the women's health initiative observational cohort. Women Health 55 (7), 829–841.

Kostic, I., Toffoletto, B., Toller, M., Beltrami, C.A., Ambesi-Impiombato, F.S., Curcio, F., 2010. UVC radiation-induced effect on human primary thyroid cell proliferation and HLA-DR expression. Horm. Metab. Res. 42 (12), 846–853.

Kunt, H., Şentürk, I., Gönül, Y., Korkmaz, M., Ahsen, A., Hazman, Ö., Bal, A., Genç, A., Songur, A., 2016. Effects of electromagnetic radiation exposure on bone mineral density, thyroid, and oxidative stress index in electrical workers. OncoTargets Ther. 9, 745–754.

Lauer, O., Frei, P., Gosselin, M.C., Joseph, W.M., Röösli, J., Fröhlich, M.J., 2013. Combining near- and far-field exposure for an organ-specific and whole-body RF-EMF proxy for epidemiological research: a reference case. Bioelectromagnetics. 34, 366–374.

Li, J., Buchner, J., 2013. Structure, function and regulation of the hsp90 machinery. Biomed. J. 36 (3), 106–117.

Li, W., Sahu, D., Tsen, F., 2012. Secreted heat shock protein-90 (Hsp90) in wound healing and cancer. Biochim. Biophys. Acta 1823 (3), 730–741.

Lin, S.F., Lin, J.D., Hsueh, C., Chou, T.C., Yeh, C.N., Chen, M., Wong, R.J., 2017. Efficacy of an HSP90 inhibitor, ganetespib, in preclinical thyroid cancer models. Oncotarget 8 (25), 41294–41304.

Lope, V., Pérez-Gómez, B., Aragonés, N., López-Abente, G., Gustavsson, P., Floderus, B., Dosemeci, M., Silva, A., Pollán, M., 2006. Occupational exposure to ionizing radiation and electromagnetic fields in relation to the risk of thyroid cancer in Sweden. Scand. J. Work Environ. Health 32 (4), 276–284.

Luo, J., Deziel, N.C., Huang, H., Chen, Y., Ni, X., Ma, S., Udelsman, R., Zhang, Y., 2019. Cell phone use and risk of thyroid cancer: a population-based case-control study in Connecticut. Ann. Epidemiol. 29, 39–45.

Luo, J., Li, H., Deziel, N.C., Huang, H., Zhao, N., Ma, S., Ni, X., Udelsman, R., Zhang, Y., 2020. Genetic susceptibility may modify the association between cell phone use and thyroid cancer: a population-based case-control study in Connecticut. Environ. Res. 182, 109013. https://doi.org/10.1016/j.envres.2019.109013.

Milham, S., 2009. Most cancer in firefighters is due to radio-frequency radiation exposure not inhaled carcinogens. Med. Hypotheses 73 (5), 788–789.

Milham, S., Morgan, L.L., 2008. A new electromagnetic exposure metric: high frequency voltage transients associated with increased cancer incidence in teachers in a California school. Am. J. Ind. Med. 51 (8), 579–586.

Misa-Agustiño, M.J., Leiro, J., Jorge-Mora, M.T., Rodriguez-Gonzalez, J.A., Jorge-Barreiro, F.J., Ares-Pena, F.J., López-Martín, E., 2012. Electromagnetic fields at 2.45 GHz triggers changes in Heat Shock Proteins 90 and 70 without altering the apoptotic activity on the thyroid gland of rat. Biol. Open 1, 831–838.

Misa-Agustiño, M.J., Jorge-Mora, T., Jorge-Barreiro, F.J., Suarez-Quintanilla, J., Moreno-Piquero, E., Ares-Pena, F.J., López-Martín, E., 2015. Exposure to non-ionizing radiation provokes changes in rat thyroid morphology and expression of HSP-90. Exp. Biol. Med. (Maywood) 240 (9), 1123–1135.

Miyata, Y., Nakamoto, H., Neckers, L., 2013. The therapeutic target Hsp90 and cancer hallmarks. Curr. Pharm. Des. 19, 347–365.

Mo, J.H., Choi, I.J., Jeong, W.J., Jeon, E.H., Ahn, S.H., 2012. HIF-1α and HSP90: target molecules selected from a tumorigenic papillary thyroid carcinoma cell line. Cancer Sci. 103 (3), 464–471.

Mollee, T.S., Finken, M.J., van Weissenbruch, M.M., Rotteveel, J., 2011. Normal thyroid function in young adults who were born very preterm. J. Pediatr. Endocrinol. Metab. 24 (11–12), 887–891.

Mortavazi, S., Habib, A., Ganj-Karami, A., Samimi-Doost, R., Pour-Abedi, A., Babaie, A., 2009. Alterations in TSH and thyroid hormones following mobile phone use. Oman Med. J. 24 (4), 274–278.
Nolan, K.D., Franco, O.E., Hance, M.W., Hayward, S.W., Isaacs, J.S., 2015. Tumor-secreted Hsp90 subverts polycomb function to drive prostate tumor growth and invasion. J. Biol. Chem. 290 (13), 8271–8282.
Ono, K., Eguchi, T., Sogawa, C., Calderwood, S.K., Futagawa, J., Kasai, T., Seno, M., Okamoto, K., Sasaki, A., Kozaki, K.I., 2018. HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells. J. Cell. Biochem. 119 (9), 7350–7362.
Palladino, G., Notarangelo, T., Pannone, G., Piscazzi, A., Lamacchia, O., Sisinni Spagnoletti, L., Toti, P., Santoro, A., Storto, G., Bufo, P., Cignarelli, M., Esposito, F., Landriscina, M., 2016. TRAP1 regulates cell cycle and apoptosis in thyroid carcinoma cells. Endocr. Relat. Cancer 23 (9), 699–709.
Qian, Z.J., Jin, M.C., Meister, K.D., Megwalu, U.C., 2019. Pediatric thyroid cancer incidence and mortality trends in the United States, 1973-2013. JAMA Otolaryngol. Head Neck Surg. 145 (7), 617–623.
Rajkovic, V., Matavulj, M., Johansson, O., 2005. The effect of extremely low-frequency electromagnetic fields on skin and thyroid amine- and peptide-containing cells in rats: an immunohistochemical and morphometrical study. Environ. Res. 99 (3), 369–377.
Rajkovic, V., Matavulj, M., Johansson, O., 2006. Light and electron microscopic study of the thyroid gland in rats exposed to power-frequency electromagnetic fields. J. Exp. Biol. 209 (Pt 17), 3322–3328.
Rosol, T.J., 2013. Ontarget effects of GLP-1 receptor agonists on thyroid C-cells in rats and mice. Toxicol. Pathol. 41 (2), 303–309.
Sabbah, M., Radanyi, C., Redeuilh, G., Baulieu, E.E., 1996. The 90 kDa heat-shock protein (hsp90) modulates the binding of the oestrogen receptor to its cognate DNA. Biochem. J. 314 (Pt 1), 205–213.
Saliev, T., Tachibana, K., Bulanin, D., Mikhalovsky, S., Whitby, R.D., 2014. Bio-effects of non-ionizing electromagnetic fields in context of cancer therapy. Front Biosci. (Elite Ed) 1 (6), 175–184.
Saliev, T., Begimbetova, D., Masoud, A.R., Matkarimov, B., 2019. Biological effects of no ionizing electromagnetic fields: two sides of a coin. Prog. Biophys. Mol. Biol. 141, 25–36.
Salinas-Asensio, M.M., Ríos-Arrabal, R., Artacho-Cordón, F., Olivares-Urbano, M.A., Calvente, I., León, J., Núñez, M.I., 2019. Exploring the radiosensitizing potential of magnetotherapy: a pilot study in breast cancer cells. Int. J. Radiat. Biol. 17, 1–9.
Sangün, Ö., Dündar, B., Çömlekçi, S., Büyükgebiz, A., 2015. The effects of electromagnetic field on the endocrine system in children and adolescents. Pediatr. Endocrinol. Rev. 13 (2), 531–545.
Schmid, K.W., 2015. In: Raue, F. (Ed.), Histopathology of C Cells and Medullary Thyroid Carcinoma in Medullary Thyroid Carcinoma. Biology-Management-Treatment. Springer. Ch. 2, 41-60, ISBN-978-3-319-22541-22548.
Schmid & Partner Engineering A.G, 2009. Reference Manual for the SEMCAD Simulation Plat-form for Electromagnetic Compatibility, Antenna Designand Dosimetry. www.semcad.com.
Silva, V., Hilly, O., Strenov, Y., Tzabari, C., Hauptman, Y., Feinmesser, R., 2015. Effect of cell phone-like electromagnetic radiation on primary human thyroid cells. Int. J. Radiat. Biol. 21, 1–9.
Soudry, E., Stern, S., Shavit, B., Hardy, S., Morgenstern, T., Hadar, R., Feinmesser, R., 2017. Heat shock proteins HSP90, HSP70 and GRP78 expression in medullary thyroid carcinoma. Ann. Diagn. Pathol. 26, 52–56.
Taha, E.A., Ono, K., Eguchi, T., 2019. Roles of extracellular HSPs as biomarkers in immune surveillance and immune evasion. Int. J. Mol. Sci. 20 (September (18)), 4588. https://doi.org/10.3390/ijms20184588. Published online 2019 Sep17.
Taipale, M., Jarosz, D.F., Lindquist, S., 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat. Rev. Mol. Cell Biol. 11, 515–528.
Trepel, J., Mollapour, M., Giaccone, G., Neckers, L., 2010. Targeting the dynamic HSP90 complex in cancer. Nat. Rev. Cancer 10, 537–549.
Vadalà, M., Morales-Medina, J.C., Vallelunga, A., Palmieri, B., Laurino, C., Iannitti, T., 2016. Mechanisms and therapeutic effectiveness of pulsed electromagnetic field therapy in oncology. Cancer Med. 5 (11), 3128–3139.
Vitale, G., Gaudenzi, G., Circelli, L., Manzoni, M.F., Bassi, A., Fioritti, N., Faggiano, A., Colao, A., NIKE Group, 2017. Animal models of medullary thyroid cancer: state of the art and view to the future. Endocr. Relat. Cancer 24 (1), R1–R12.
Wallin, G., Brönnegård, M., Grimelius, L., McGuire, J., Tørring, O., 1992. Expression of the thyroid hormone receptor, the oncogenes c-myc and H-ras, and the 90 kD heat shock protein in normal, hyperplastic, and neoplastic human thyroid tissue. Thyroid 2 (4), 307–313.
Wang, D., Feng, J.F., Zeng, P., Yang, Y.H., Luo, J., Yang, Y.W., 2011. Total oxidant/antioxidant status in sera of patients with thyroid cancers. Endocr. Relat. Cancer 18 (6), 773–782.
Zbucki, R.L., Winnicka, M.M., Sawicki, B., Szynaka, B., Andrzejewska, A., Puchalski, Z., 2007. Alteration of parafollicular (C) cells activity in the experimental model of hypothyroidism in rats. Folia Histochem. Etcytobiol. 45 (2), 115–121.
Zhang, Y.L., Li, P., Liu, Z.Y., Yi, J.P., Chen, Y., Zhang, M., Lin, Q., 2019. Does relatively low iodine intake contribute to thyroid cancer? An ecological comparison of epidemiology. Medicine (Baltimore) 98 (41), e17539.