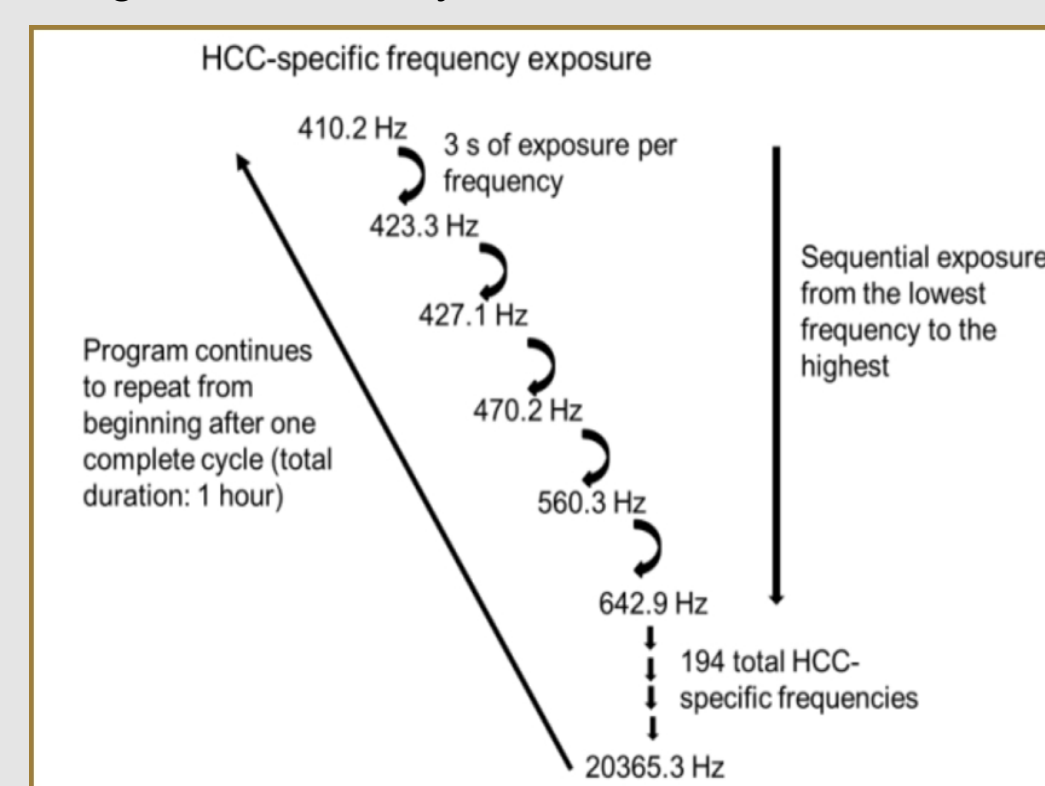


# The anti-proliferative effects of RF EMF amplitude-modulated at tumor-specific frequencies are mediated by calcium

## Introduction

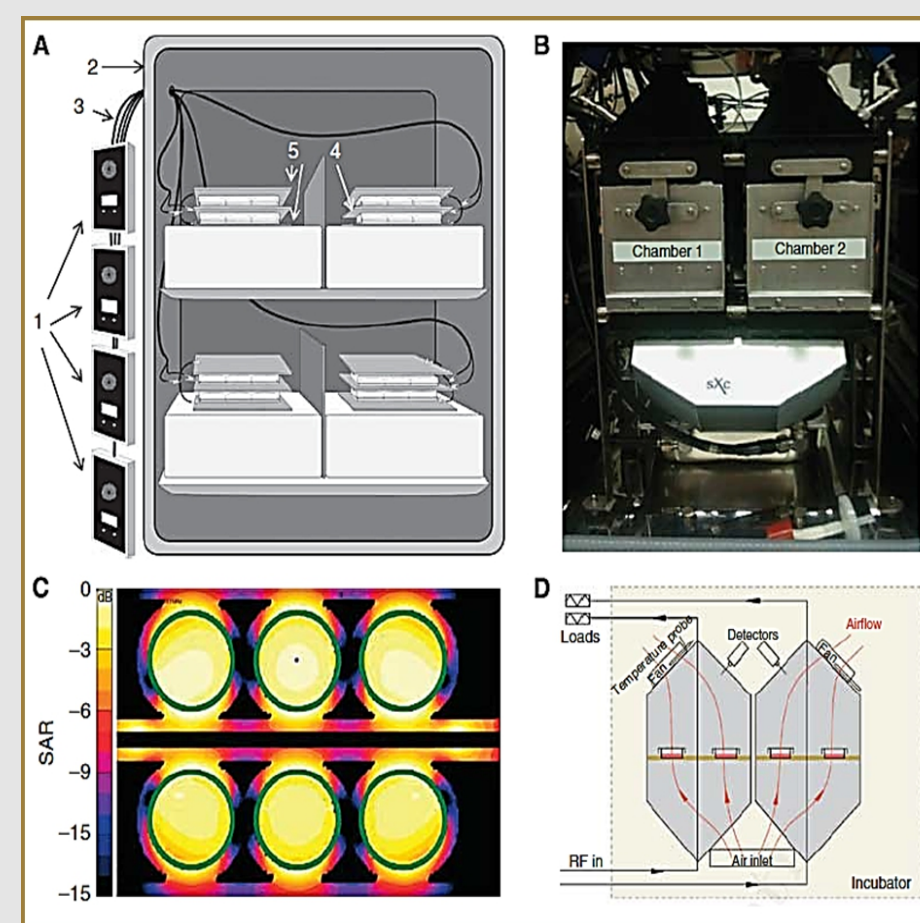
Whole body administration of low-level radiofrequency electromagnetic fields, amplitude-modulated (AM RF EMF) at specific frequencies ranging from 400 Hz to 21 kHz has efficacy in advanced hepatocellular carcinoma (HCC) (Costa et al. 2011) and may be used for cancer diagnosis (Abstract # 15657). *In vitro* studies demonstrate that AM RF EMF treatment inhibits cancer cell growth in a tumor-specific manner (Zimmerman et al. 2012). RNA-seq and microRNA analysis of HCC cells treated with AM RF EMF reveal altered expression of several mRNAs belonging to the IP3/DAG signaling pathway. Given the central modulatory effect of calcium in this pathway, we hypothesized that calcium modulates the growth inhibitory effect of RF EMF.



## Methods

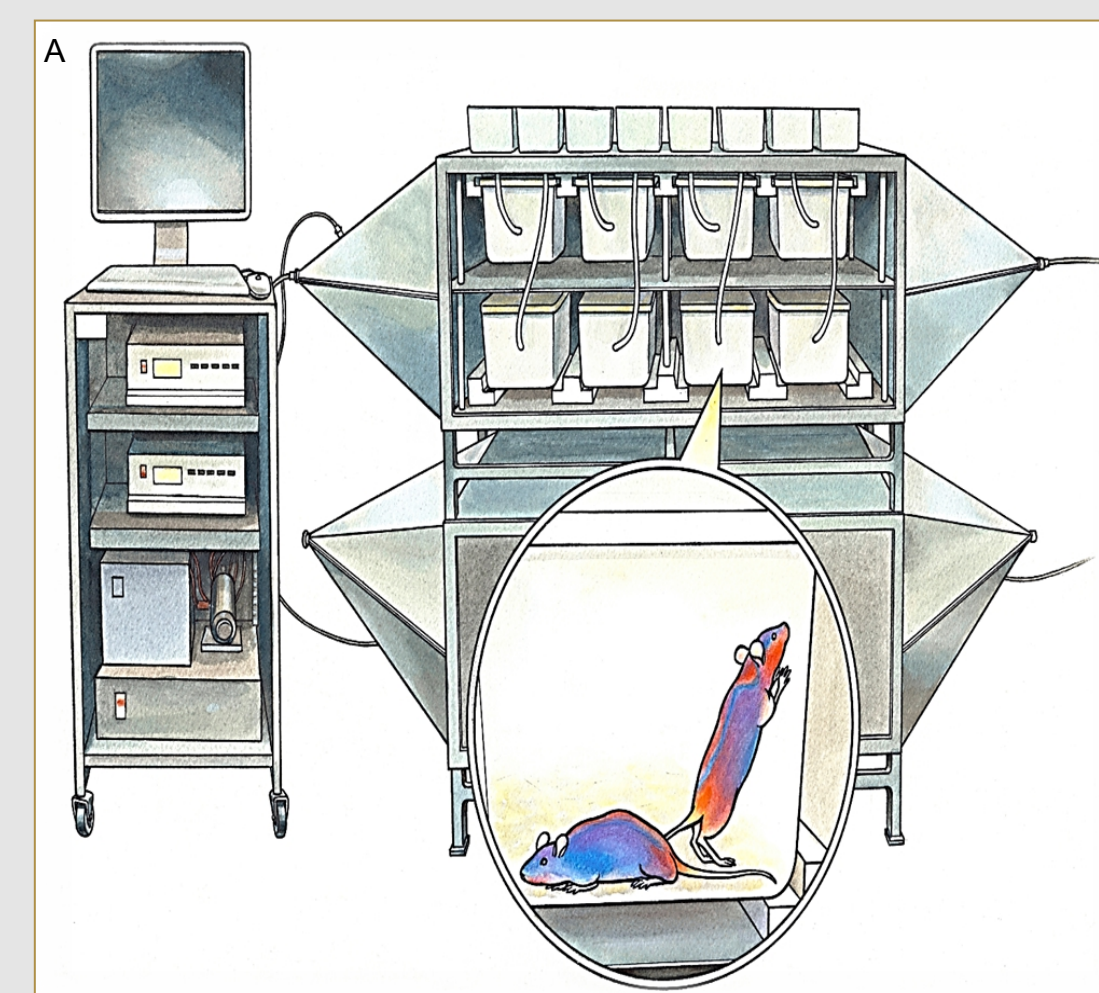
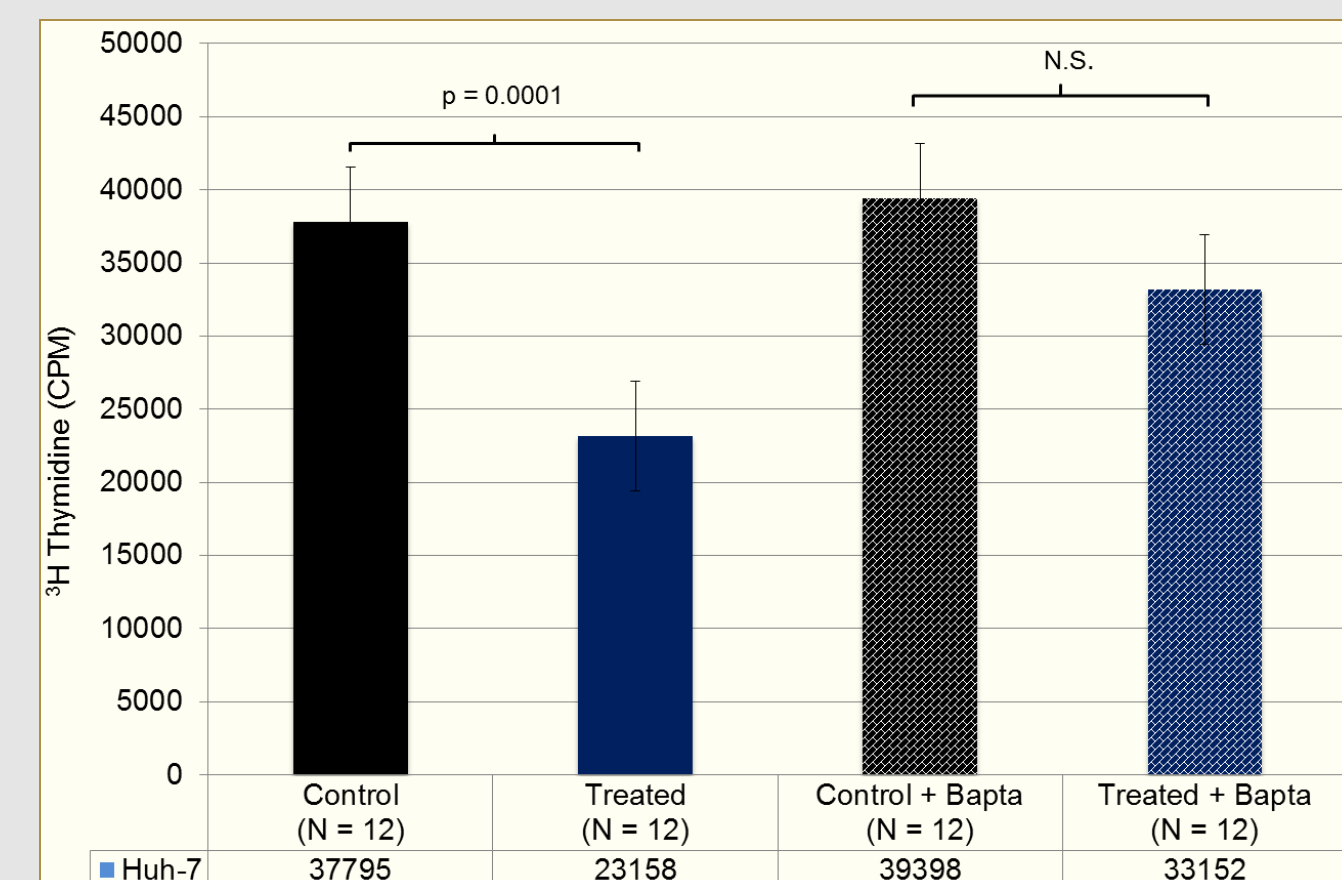
**AM RF EMF exposure *in vitro*:** Cell lines were exposed to 27.12 MHz radiofrequency electromagnetic fields using exposure systems designed to replicate *in vivo* exposure levels. Experiments were conducted at an SAR of 0.03 and 0.4 W/kg. Cells were exposed for three hours daily, seven days in a row. Cells were exposed to tumor-specific modulation frequencies previously identified in patients with a diagnosis of Hepatocellular Carcinoma or modulation frequencies never identified in patients with a diagnosis of cancer (Zimmerman et al. 2012). Huh-7 cells were cultured in the presence or absence of BAPTA, a Ca<sup>2+</sup> chelator, for three consecutive hours daily, seven days in a row. Proliferation was measured by tritiated thymidine (<sup>3</sup>H) assay.

**AM RF EMF exposure *in vivo*:** NOD SCID mice (Jackson Lab) were subcutaneously injected with 7.0 x 10<sup>6</sup> Huh-7 cells. Using a custom-designed exposure system in which RF EMF setup consists of two TEM exposure cells configured as half wave resonators (sXv-27, IT'IS, Zurich, Switzerland) we exposed mice to 27.12 MHz electromagnetic fields modulated at HCC-specific or randomly chosen frequencies. A separate control group consisted of mice not exposed to any AM RF EMF. Upon establishment of a palpable tumor, measuring at a minimum of 0.5 cm in any dimension, exposure with HCC-specific RF EMF or randomly chosen frequencies was initiated. An additional group of mice were not exposed to any RF EMF. Mice were exposed to AM RF EMF for three hours daily, for the duration of their lives. Mice receiving treatment were exposed to HCC-specific or randomly chosen AM RF EMF at a SAR of 0.4 W/kg and euthanized following excessive tumor burden. Tissues were collected/fixed for immunohistochemistry.



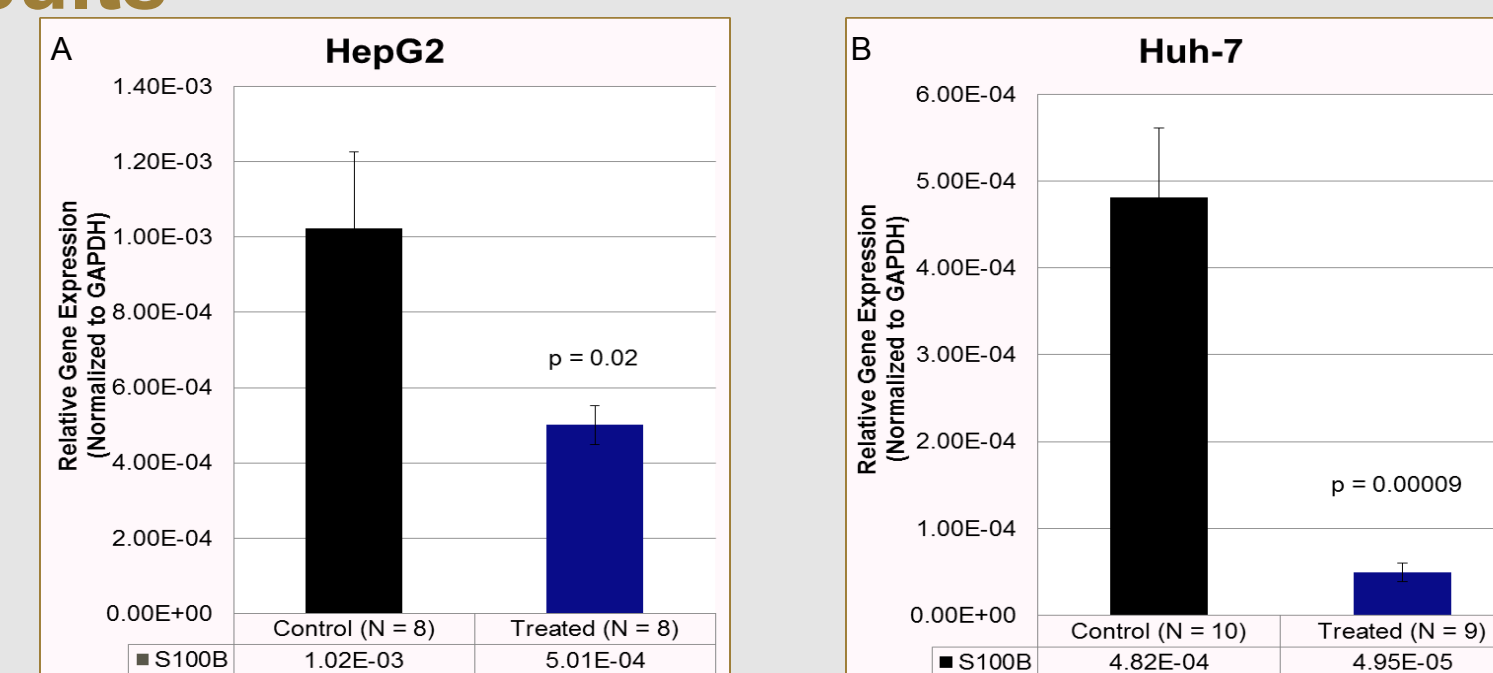
IP3 / DAG Pathway (HepG2)	
Gene	Fold Change
ANXA1	-2.47
ARHGDI1B	-2.14
MBOAT1	+9.85
<b>S100B</b>	<b>-10.14</b>

**Table 1. IP3/DAG Pathway.** Pathway analysis of RNA-seq data from the HepG2 (HCC) cell line has identified the IP3/DAG pathway as being modulated by HCC-specific RF AM EMF. Fold changes of key genes in the IP3/DAG pathway are shown.



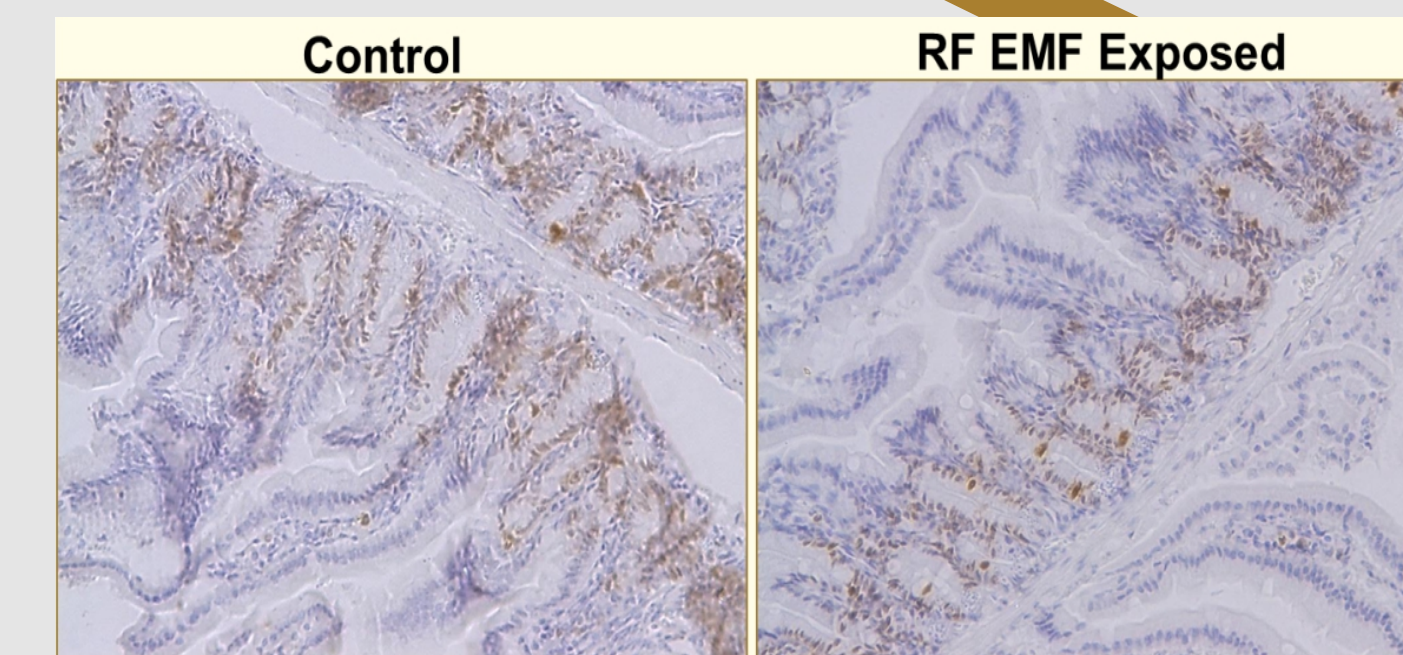
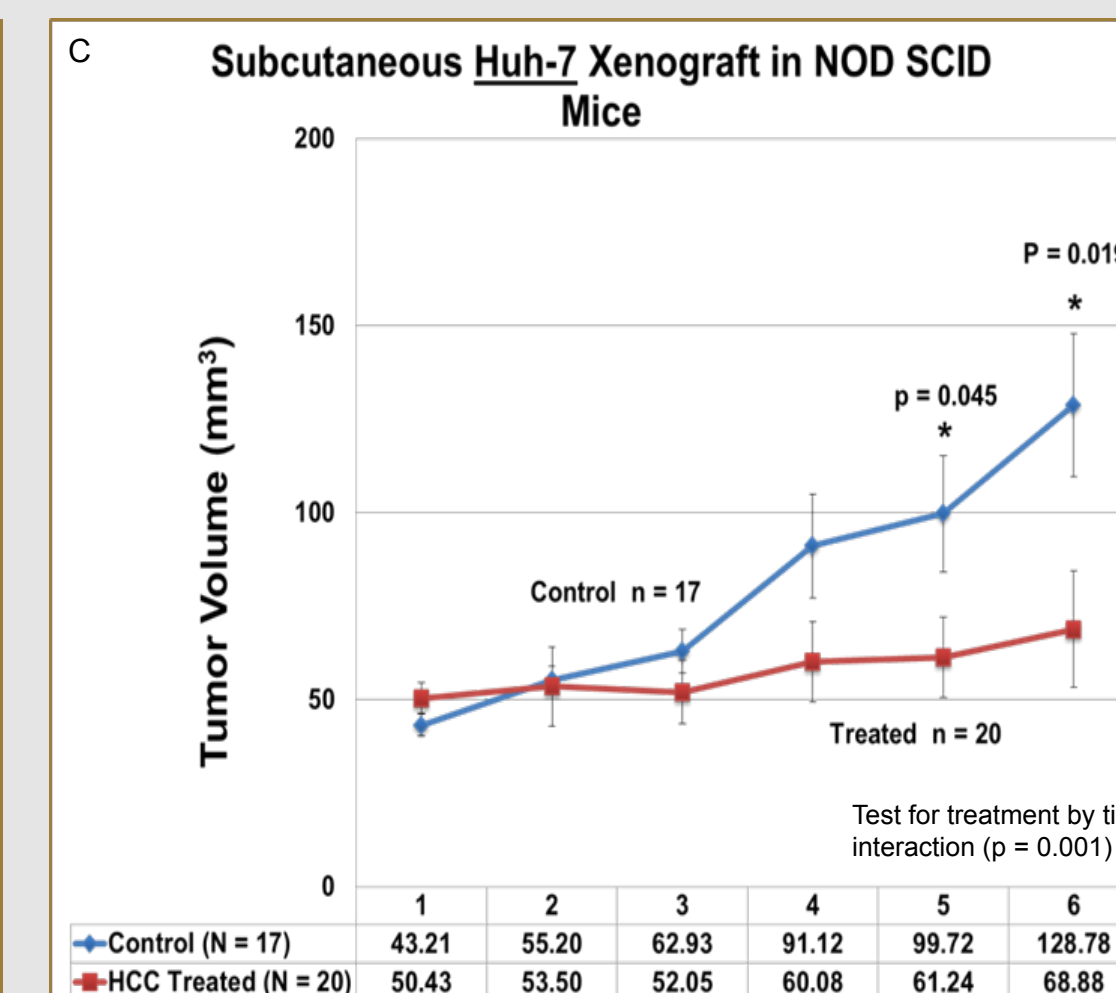
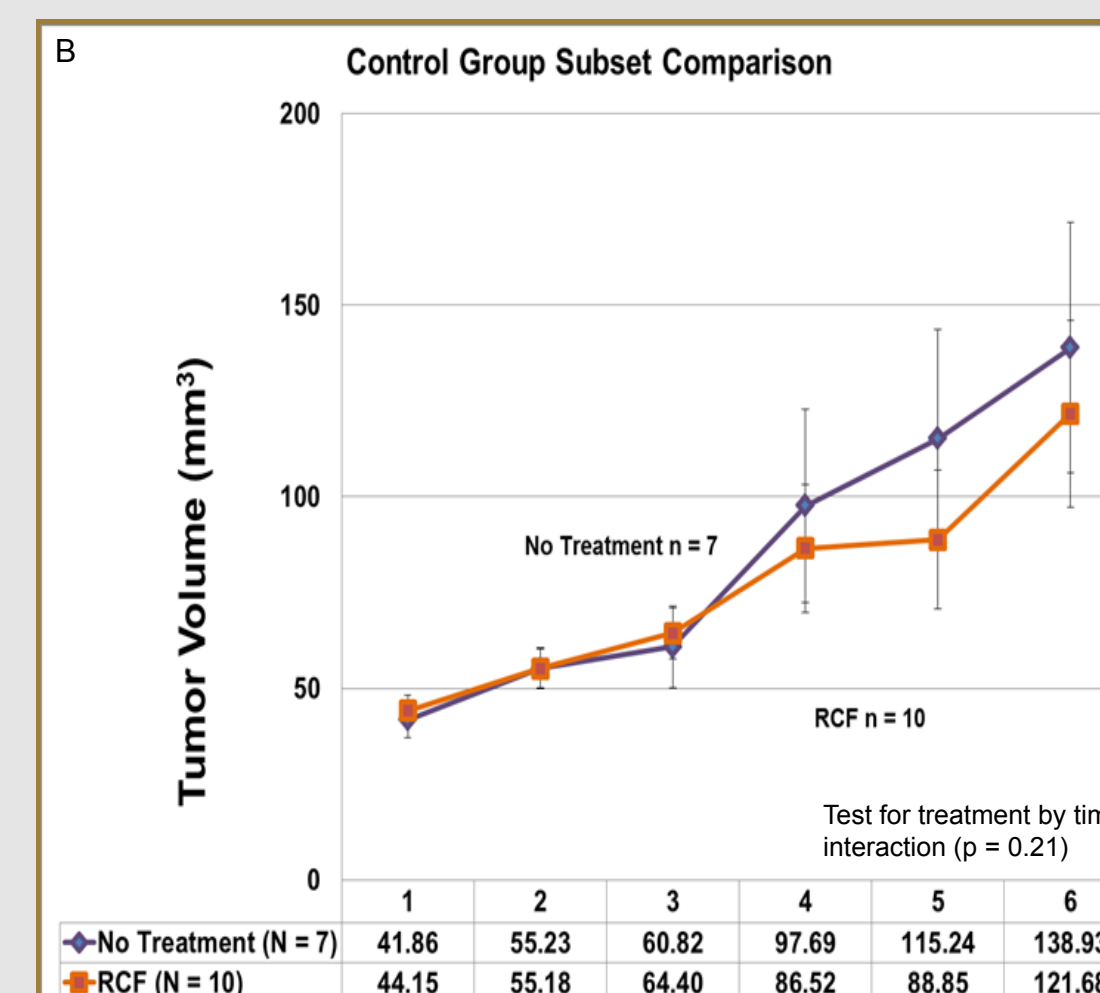
**Figure 3. *In vivo* AM RF EMF exposure.** A. Mice bearing human tumor xenografts (Huh-7) are exposed 3 hours per day to either tumor-specific AM RF EMF or control exposure. B. Control mice received either No Treatment or Random chosen AM RF EMF exposure 3 hours per day. Test for treatment by time interaction (p = 0.21). C. The growth of xenografts is significantly slower in mice exposed to HCC-specific frequencies: Test for treatment by time interaction (p = 0.001). Between group comparison is also significant at Week 5 (p = 0.045) and Week 6 (p = 0.019). P-value was calculated via student's t-test.

## Results



**Figure 1. S100 calcium binding protein B (S100B) gene expression is down regulated:** qRT-PCR gene expression of S100B in two HCC cell lines: HepG2 and Huh-7. A: HepG2, fold change of -2.04, Student's T-Test, p = 0.02. B: Huh-7: fold change of -9.74, Student's T-Test, p = 0.00009.

**Figure 2. Anti-proliferative effects of HCC-specific AM RF EMF in the presence or absence of BAPTA.** Huh-7 cells were treated with randomly-chosen or HCC-specific frequencies in the presence or absence of BAPTA. Randomly-chosen frequencies compared to HCC-specific group in the absence of BAPTA is statistically significant (p = 0.0001). HCC-specific compared to HCC-specific + BAPTA is statistically significant (p = 0.0034). Randomly-chosen frequencies compared to randomly-chosen frequencies + BAPTA was not significantly different (p = 0.0592). Quantification of tritiated thymidine incorporation assay shows the average of two different experiments. Statistical analysis was performed 2-way ANOVA; groups were compared using post-hoc pairwise comparison.



**Figure 4. Ki-67 staining of mouse small intestine:** No noticeable difference in staining of mouse small intestine was identified between groups. Magnification 200x

## Conclusions

- IP3/DAG signaling pathway is modulated by tumor-specific AM RF EMF in HCC.
- S100B mRNA is significantly decreased in HCC cell lines exposed to HCC-specific AM RF EMF.
- Ca<sup>2+</sup> chelation abrogates the AM RF EMF induced proliferative inhibition of HCC cells.
- Ca<sup>2+</sup> signaling is involved in mediating the effect of AM RF EMF induced proliferative inhibition in Hepatocellular Carcinoma cell lines.
- The growth of Huh-7 tumor xenografts is significantly inhibited by HCC-specific AM RF EMF.
- There is no difference in the growth of Huh-7 xenografts exposed to randomly chosen frequencies or not exposed to any AM RF EMF.
- Ki-67 stained small intestine shows no difference when comparing the control or treated groups indicating that AM RF EMF is a novel precision oncology therapeutic approach.

## References

- Costa, F. P., et al. (2011). "Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields." *British Journal of Cancer* 105(5): 640-648.
- Zimmerman, J. W., et al. (2012). "Cancer cell proliferation is inhibited by specific modulation frequencies." *British Journal of Cancer* 106(2): 307-313..

## Contact Information

E-mail: [hjimenex@wakehealth.edu](mailto:hjimenez@wakehealth.edu)