

## The Induction of Epstein-Barr Virus Early Antigen Expression in Raji Cells by GSM Mobile Phone Radiation\*

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Mobile phones are widely used nowadays and there have been many reports suspecting mobile phone radiation-induced cancer during the past few years<sup>[1-5]</sup>. But the results to date have given no consistent or convincing evidence of a causal relation between radiofrequency field exposure and cancer risks<sup>[6-7]</sup>. On May 31, 2011, International Agency for Research on Cancer (IARC), which is affiliated with World Health Organization (WHO), published a 10-year research report aimed at identifying whether the use of mobile phone would increase the incidence of brain tumors, but failed to conclude due to contradictory results, because of the different research methods and study biases<sup>[8]</sup>. However, IARC now lists radiofrequency electromagnetic fields, including radio frequency electromagnetic fields from wireless phones, into Group 2B classification (possibly carcinogenic to humans), as lead, acetaldehyde and fight AIDS drugs<sup>[9]</sup>.

Raji Cell line carries the latent Epstein-Barr Virus (EBV) genome and expresses the Epstein-Barr virus nuclear antigen (EBNA). Raji is sometimes referred to as an EBV "non-producer" since the integrated EBV genome carries deletions attributed to preventing the formation of virus particles. A variety of antigens, such as the Epstein-Barr virus early antigen (EBV-EA), can be expressed when Raji cells are stimulated by chemical or physical carcinogens<sup>[10]</sup>. Clinical studies

have shown that EBV-EA is linked to malignancies including Burkitt's lymphoma, T-cell lymphoma, gastric cancer, nasopharyngeal cancer and some breast cancer<sup>[11]</sup>. EBV-EA gene has been identified as a cancer-related gene<sup>[12]</sup>, so we observe the effect of mobile phone radiation on the expression of EBV-EA gene to investigate the relationship between mobile phone radiation and cancer genesis.

### Raji Cells

Raji Cell line came from Chinese Academy of Sciences Cell Bank. Cells were placed at an appropriate seeding density per 25 cm<sup>2</sup> flask, cultured in RPMI-1640 supplemented with 10% FBS, incubated at 37 °C in a 5% CO<sub>2</sub> and 95% air-humidified incubator, and kept a 3-day passage cycle.

### Radiation Experiment

Raji cells were divided into four groups, 3 flasks per group. Group A and Group B were not exposed to any radiation. Group C and Group D were exposed for 4 h to microwaves emitted from a GSM 900 MHz mobile phone every day. In the experimental process, the geometric center of the cell culture flask bottom was put over the mobile phone antenna and 1 mm from the surface of the mobile phone. Theoretically speaking, averaged Specific Absorption Rate (SAR) values in 1 g were 0.020 mW/g, and they were performed with the SPEAG DASY5 Systems. The

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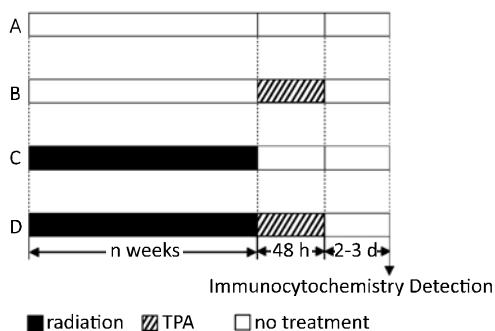
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radiation experiments were carried out in 4 consecutive weeks. Group B and Group D were cultured in the medium with 1 ng/mL 12-O-tetradecanoylphorbol-13-acetate (TPA) used as tumor promoter for 48 h before each test<sup>[13]</sup>. Cells were sampled to be tested by immunocytochemistry with EBV-EA IgG antibodies every week. Figure 1 shows the experimental flowchart of  $n$  ( $n=1, 2, 3, 4$ ) weeks' radiation experiment.



**Figure 1.** Experimental flowchart of  $n$  ( $n=1, 2, 3, 4$ ) weeks' radiation. A: Radiation (-)TPA(-); B: Radiation (-)TPA(+); C: Radiation (+)TPA(-); D: Radiation (+)TPA(+).

### Immunocytochemical Study

Cells of each group were centrifuged and resuspended in an appropriate volume of PBS. Put 100  $\mu$ L cell suspension ( $10^4$ - $10^5$ ) on a glass slide; placed the slide in the humidified chamber; and allowed the cells to adhere electrostatically to a monolayer at room temperature. After 60 min, added 50  $\mu$ L of ice-cold pyroacetic spirit to fix the cells, and rinsed 3 times with PBS (5 min each time)

to remove the fixative. Briefly, slides were incubated with monoclonal mouse EBV-EA IgG antibody (1:100) overnight at 4 °C. PBS was used instead of primary antibodies as blank control. Glass slides were washed with PBS, and incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Zhongshan Golden Bridge Biotechnology Company Limited) for 30 min at 37 °C in an incubation chamber. Substrate was developed with 3, 3'-diaminobenzidine tetrahydrochloride (DAB, Zhongshan Golden Bridge Biotechnology Company Limited), and incubated for 5 min. The reaction was terminated by rinsing the slides in ddH<sub>2</sub>O. Photographed three horizons of each cell smear (at least 500 cells) where the cells were uniformly distributed, and counted the number of positive cells and total cells.

### The Comparison of EBV-EA Positive Rate

Table 1 shows the positive rate of immunocytochemistry after radiation experiment for each week. No EBV-EA positive cells emerged in Group C in the first two weeks. After 3 weeks' radiation, an average of 6 positive cells per 501 cells emerged. Cooperating with tumor promoter (TPA), positive cells appeared earlier in Group B and D. It is obvious that the positive rate in Group B was higher than that in Group C. Furthermore, the positive rate in Group D was higher than that of Group B or Group C at the same time point. Therefore, the increase rate of the positive rate in Group D was the highest.

The positive rate in each group was analyzed by using repeated measures and the multivariate analysis

**Table 1.** The Positive Rate in Immunocytochemistry after Radiation Experiment for Each Week (%)

Radiation Time (weeks)	A			B			C			D		
	Number of Positive Cells	Number of Total Cells	Positive Rate (%)	Number of Positive Cells	Number of Total Cells	Positive Rate (%)	Number of Positive Cells	Number of Total Cells	Positive Rate (%)	Number of Positive Cells	Number of Total Cells	Positive Rate (%)
1	0.0±0.0	500.0±0.0	0	0.0±0.0	500.0±0.0	0	0.0±0.0	500.0±0.0	0	0.0±0.0	500.0±0.0	0
2	0.0±0.0	500.0±0.0	0	0.7±0.5	706.0±54.2	0.09	0.0±0.0	500.0±0.0	0	1.0±0.0	666.7±38.7	0.15
3	0.0±0.0	500.0±0.0	0	14.0±1.4	569.7±13.9	2.46	6.0±0.8	501.3±0.9	1.2	24.0±1.6	510.0±6.2	4.7
4	0.0±0.0	500.0±0.0	0	20.0±2.9	551.7±36.7	3.61	10.0±2.2	600.0±75.2	1.65	44.7±5.9	579.0±54.5	7.69

**Note.** (1)A: Radiation (-)TPA(-), B: Radiation (-)TPA(+), C: Radiation (+)TPA(-), D: Radiation (+)TPA(+); (2) After 2 weeks, the positive rate in Group C is significantly different from that in Group B ( $P<0.05$ ); the positive rate in Group D is significantly different from that in Group C ( $P<0.01$ ). After 3 and 4 weeks, the positive rate in Group C is significantly different from that in Group A and B ( $P<0.001$ ); the positive rate in Group D is significantly different from that in Group A, B, and C ( $P<0.001$ ). The experiment was run in triplicate.

of variance. The results showing the P-value next to "time" and "time\*group" were all low enough ( $P < 0.05$ ). The test between groups indicates that the variable group was significant. The test within subjects reveals that time effects were significant; in other words, the groups did change over time. And the positive rates in Group B, C and D got higher over time. Moreover, the interaction within a group with the passage of time is significant, which means that the groups not only changed over time but also changed in different ways. It can be concluded that "radiation" and "TPA" are two different factors that function independently. And the coupling effect of "radiation" and "TPA" in EBV-EA gene expression is dissimilar with the sum effect of them.

The results indicate that mobile phone radiation could induce the expression of EBV-EA and the induction is even more evident with the presence of tumor promoter such as TPA.

#### REFERENCES

1. Repacholi MH, Basten A, Gebiski V, et al. Lymphomas in E mu-Pim1 Transgenic Mice Exposed to Pulsed 900MHz Electromagnetic Fields. *Radiat Res*, 1997; 147(5), 631-40.
2. Richter E, Berman T, Ben-Michael E, et al. Cancer in Radar Technicians Exposed to Radiofrequency/Microwave Radiation: Sentinel Episodes. *Int J Occup Environ Health*, 2000; 6(3), 187-93.
3. Stang A, Anastassiou G, Ahrens W, et al. The Possible Role of Radiofrequency Radiation in the Development of Uveal melanoma. *Epidemiology*, 2001; 12(1), 7-12.
4. Hardell L, Mild KH, Carlberg M. Case-control Study on the Use of Cellular and Cordless Phones and the Risk for Malignant Brain Tumours. *Int J Radiat Biol*, 2002; 78(10), 931-6.
5. Hardell L, Carlberg M, Mild KH. Case-control Study on Cellular and Cordless Telephones and the Risk for Acoustic Neuroma or Meningioma in Patients Diagnosed 2000-2003. *Neuroepidemiology*, 2005; 25(3), 120-8.
6. Lin, JC. Cancers in Normal Mice Exposed to Cell Phone Radiation. *IEEE Microwave Magazine*, 2009; 10(5), 120-2.
7. de Vocht F, Burstyn I, Cherrie JW. Time trends (1998-2007) in brain cancer incidence rates in relation to mobile phone use in England. *Bioelectromagnetics*, 2011; 32(5), 334-9.
8. <http://curecancer.com/352/whether-mobile-phones-cause-brain-cancer-research-for-10-years-ended-in-failure>. February 4, 2011.
9. Lin JC. The Curious Case of the IARC Working Group on Radio Frequency Electromagnetic Fields and Cell Phones. *IEEE Microwave Magazine*, 2011; 12(6), 32-6.
10. Feederle R, Kost M, Baumann M, et al. The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators. *EMBO J*, 2000; 19(12), 3080-9.
11. Middeldorp JM, Brink AA, van den Brule AJ, et al. Pathogenic roles for Epstein-Barr virus (EBV) gene products in EBV-associated proliferative disorders. *Crit Rev Oncol Hematol*, 2003; 45(1), 1-36.
12. Li HP, Leu YW, Chang YS. Epigenetic changes in virus-associated human cancers. *Cell Research*, 2005; 15(4), 262-71.
13. Kanamori M, Tajima M, Satoh Y, et al. Differential Effect of TPA on Cell Growth and Epstein-Barr Virus Reactivation in Epithelial Cell Lines Derived from Gastric Tissues and B Cell Line Raji. *Virus Genes*, 2000; 20(2), 117-25.