

# 5G AND HEALTH

## GAPS IN THE KNOWLEDGE

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Blog BRHP-Between a Rock and a Hard Place

# Scope of the presentation

- This presentation is not a comprehensive review of the available science as a whole
- This presentation highlights some of the existing gaps in the knowledge, considered by the author as crucial in evaluation of human health risk
- This is an updated presentation of the lectures presented in Jerusalem, Israel, 2017 and Seoul, South Korea, 2018

# 5G "confusion"

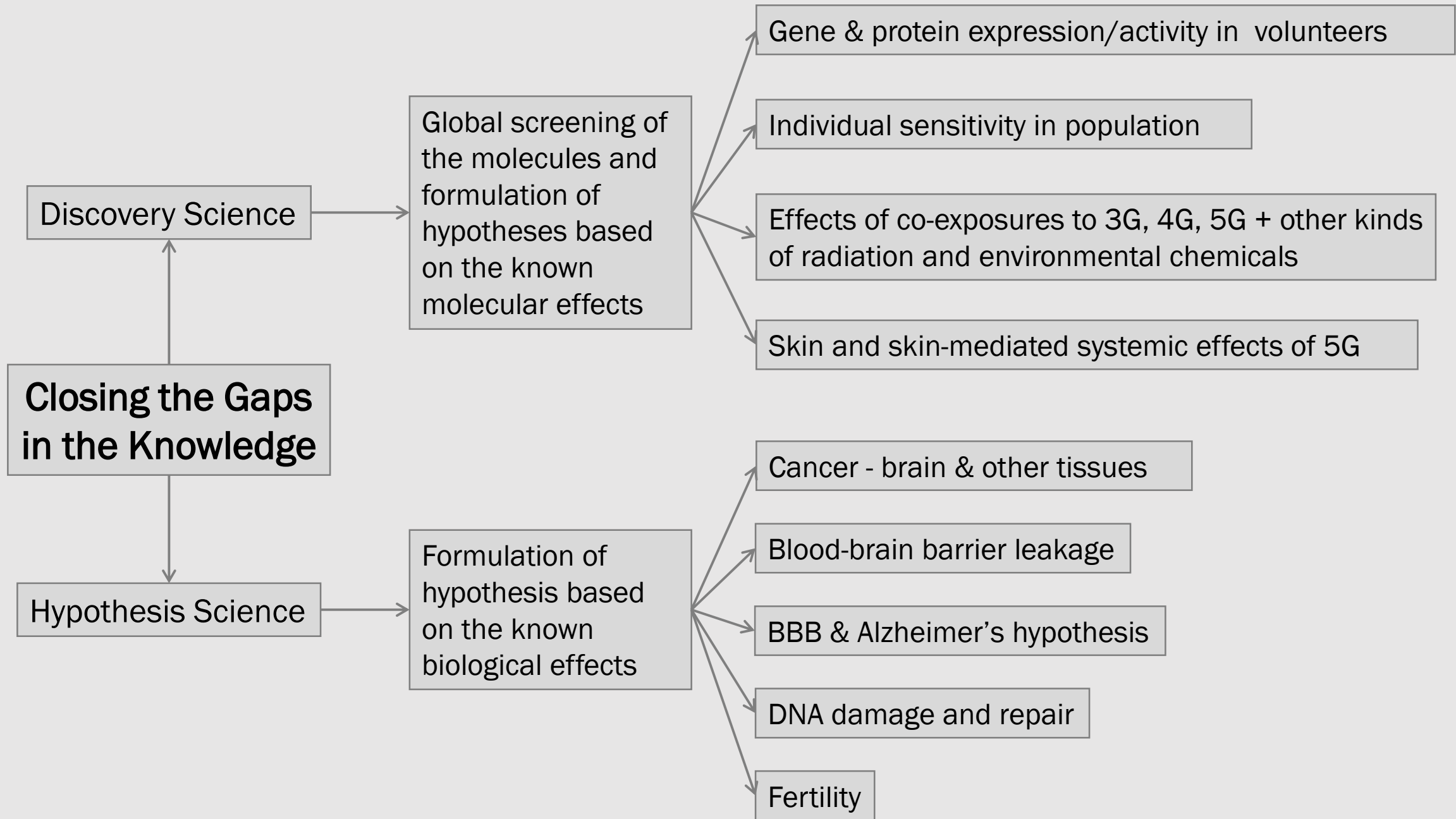
- Confusion about 5G frequencies caused by the fact that 5G is being developed as well as deployed at the same time. Even the technical standards dealing with the 5G are not all ready yet.
- 5G will combine old and new technologies: 3G, 4G, 4G LTE, 5G
- 5G will use, in due time, millimeter wave frequencies 30 GHz – 300 GHz
- In Europe governments are urged to provide frequencies of 26 GHz, 40 GHz and 66 GHz, for the use by 5G communications systems
- In Finland government reserved for 5G frequencies of 700 MHz, 3.5 GHz and 26 GHz
- In Australia, spectrum 600 MHz - 6 GHz (initially 3.5 GHz and 3.6 GHz), later on also spectrum 24 - 86 GHz (initially 26 - 28 GHz)

# Research approach

- Confirming, in human volunteers, of the existence of the effects observed in in vitro and in animal studies, is of paramount importance for the development of the reliable public health policies
- Common mistake – over-interpretation of in vitro and animal data to suggest, without human volunteer data, that effects observed in vitro and in animals “prove” health problems for humans
- Crucial questions to answer:
  - *Are there physiological RF effects occurring in humans*
  - *Are RF effects strong enough to alter human physiology in a way that could lead to health hazard*

# Thermal, non-thermal or just ‘effects’

- Terms ‘thermal’ and ‘non-thermal’ cause confusion
- Better term: ‘effects at low level exposures’ = effects induced by exposures at radiation levels permitted, or below, the current safety limits
- Epidemiology and sleep EEG studies provide compelling evidence, in humans, for the existence of non-thermal effects (=low level exposure effects)
  - *Epidemiology studies show increased brain cancer risk from the use of regular cell phones that are in compliance with the current safety limits*
  - *EEG studies show effect, even if it is not harmful it is effect at low level exposure*



# Selected research of Leszczynski group

confirming in vitro results in volunteer experiments

1.

Is stress response  
affected in vitro?

**Yes**

2.

Is protein expression  
and activity  
(phosphorylation)  
affected in vitro?

**Yes**

3.

Is protein expression  
affected in human  
volunteers?

**Yes**

# Stress response & proteomics

Nature Genetics 2001, 27, vol. 4 supplement

Leszczynski, Dariusz

[37]

## Proteomics: new way to determine possible biological effects of mobile phone radiation

Dariusz Leszczynski & Sakari Joenväära

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Despite years of research, the question of whether exposure to radiofrequency-modulated electromagnetic fields (RF-EMF) generated by mobile phones affects human health remains unsolved. We obtained a comprehensive overview of the possible extent of cellular response to RF-EMF irradiation by determining the total cellular changes in protein expression and in protein phosphorylation that occur in response to RF-EMF exposure under athermal conditions. As a model we used human endothelial cell line EA.hy926. Cells were exposed for one hour to a 900-MHz GSM signal. Immediately following exposure we harvested cells and extracted and separated proteins using two-dimensional electrophoresis. To determine changes in protein phosphorylation,  $^{32}\text{P}$  was present in the cultures during the exposure period. Using Bio-Rad's PDQUEST 6.1.0 software we identified over 1,200 proteins in two-dimensional gels ( $10 \times 20$  cm). A large number of protein spots changed expression following irradiation. In control cells we detected over 180 phosphoproteins. RF-EMF exposure has generated a large number of newly phosphorylated proteins that were not present in controls. Among the proteins with altered phosphorylation levels were shock proteins, such as hsp27. Thus the expression and phosphorylation of a large number of proteins isolated from EA.hy926 cells seems to be altered by short RF-EMF exposure, suggesting that cells mount a vigorous response to RF-EMF stress. However, whether the observed stress can cause long-lasting physiological effects remains to be determined.

## Proteomics: new way to determine possible biological effects of mobile phone radiation

“...A large number of protein spots changed expression following irradiation. In control cells we detected over 180 phosphoproteins. RF-EMF exposure has generated a large number of newly phosphorylated proteins that were not present in controls. Among the proteins with altered phosphorylation levels were shock proteins, such as hsp27. Thus the expression and phosphorylation of a large number of proteins isolated from EA.hy926 cells seems to be altered by short RF-EMF exposure, suggesting that cells mount a vigorous response to RF-EMF stress...”



# Selected studies of Leszczynski group

## STUDIES: Stress Response, Proteomics, Transcriptomics

- Leszczynski et al. Differentiation 70, 2002, 120-129
- Leszczynski et al. Proteomics 4, 2004, 426-431
- Nylund & Leszczynski. Proteomics, 4, 2004, 1359-1365
- Redmondini et al. Proteomics 6, 2006, 4745-4754
- Nylund & Leszczynski. Proteomics 6, 2006, 4769-4780
- **Karinen et al. BMC Genomics 9, 2008, 77-**
- Nylund et al. J. Proteomics & Bioinformatics 2, 2009, 455-462
- Nylund et al. Proteome Science 2010, 8:52

## Observed EFFECTS: endothelium, skin, & cell phone radiation (900 GSM at 2 W/kg)

- Activates Hsp27/p38MAPK pathway in human endothelial cell lines
- Proteomics - changes in expression and phosphorylation of numerous proteins
- Transcriptomics - changes in expression of numerous genes
- Changes detected by proteomics and transcriptomics are small
- Proteome and transcriptome changes appear to be cell type-dependent
- **Proteome of the skin appears to be affected in exposed human volunteers**

# Reviews of high-throughput research

Reviews of the published limited data from transcriptomics and proteomics indicate that RF-EMF exposures at levels permitted by the current safety limits alter expression and activity of selected genes and proteins in vitro, and possibly in vivo

## Proteomics reviews

- Leszczynski D. in Radiation Proteomics; Advances in Experimental Medicine and Biology, Vol. 990; Springer Science + Business Media B.V., The Netherlands; February 2013
- Leszczynski D. in “Methods of Cancer Risk Assessment: Current and Prospective Approaches with Special Reference to Ionizing and Non-ionizing Radiation”; Wiley-VCH, 2011, pp 265-273
- Leszczynski D. Radiation Proteomics: A Brief Overview, Reviews-2014 issue, Proteomics 14: 481-488
- Leszczynski et al. Five years later: The current status of the use of proteomics and transcriptomics in EMF research. Proteomics. 2012 Jun 18. doi: 10.1002/pmic.201200122.

## Transcriptomics review

- Parham et al. The Use of Signal-Transduction and Metabolic Pathways to Predict Human Disease Targets from Electric and Magnetic Fields Using in vitro Data in Human Cell Lines. Front Public Health. 2016; 4: 193

# Mechanism hypothesis: stress response

(so called “heat shock”) (lack of human studies)

Leszczynski et al. 2002

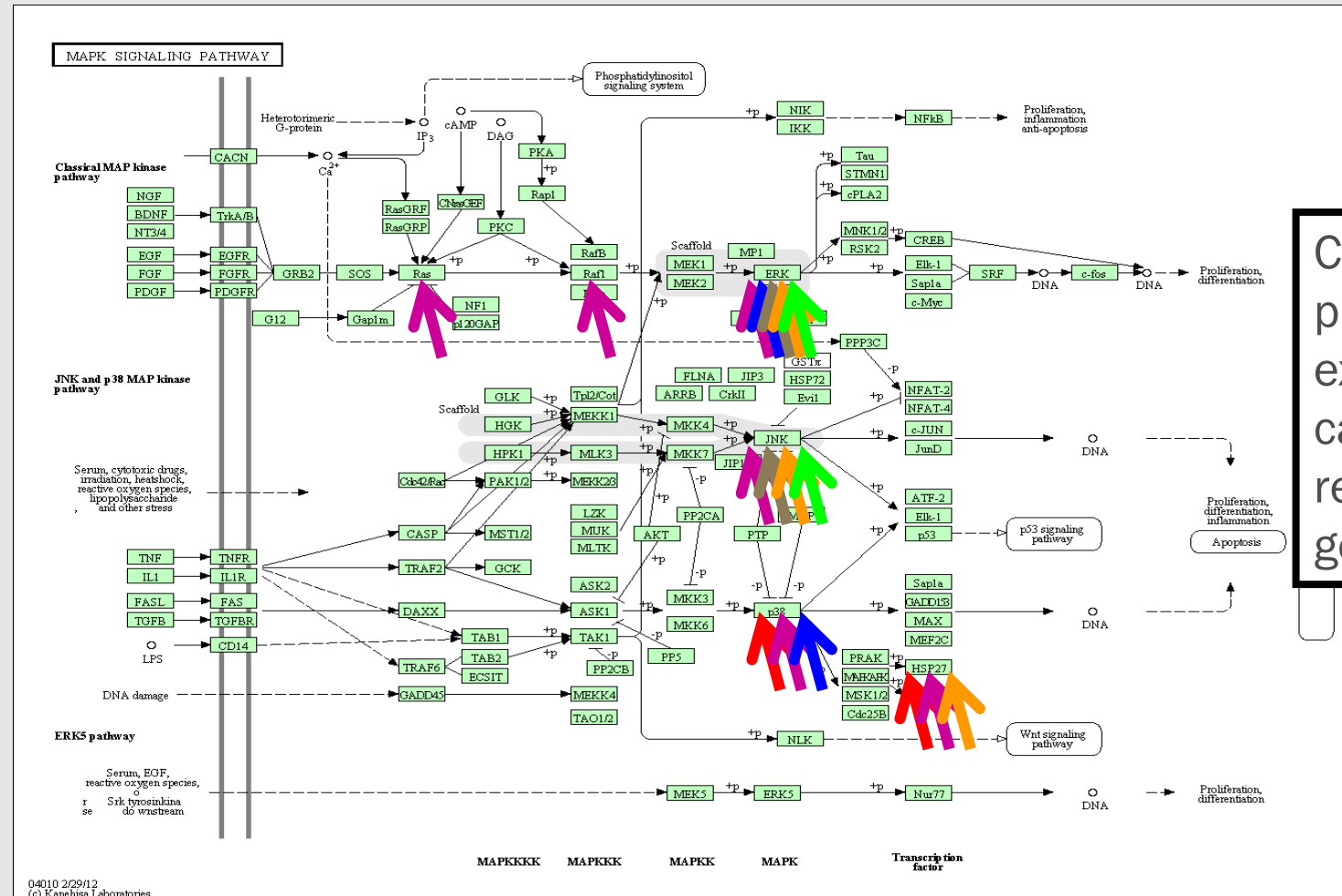
Caraglia et al. 2005

Friedman et al. 2007

Buttiglione et al. 2007

Yu et al. 2008

Lee et al. 2008



Cell proliferation & expression of cancer regulatory genes

# Mechanism hypothesis: oxidative stress

(limited human studies)

- Total of some 200 studies on oxidative stress and RF
- Only 6 studies using human blood cells and saliva
- Insufficient to determine physiological significance, if any
  - 2015, Kazemi et al. *Effect of 900 MHz Electromagnetic Radiation on the Induction of ROS in Human Peripheral Blood Mononuclear Cells. J Biomed Phys Eng* 5 (3): 105-114
  - 2015, Shivashankara et al. *Effect of cell phone use on salivary total protein, enzymes and oxidative stress markers in young adults: a pilot study. J Clin Diagn Res* 9 (2): 19-22
  - 2014, Arbabi-Kalati et al. *Effect of mobile phone usage time on total antioxidant capacity of saliva and salivary immunoglobulin a. Iran J Public Health* 43 (4): 480-484
  - 2014, Balakrishnan et al. *Hsp70 is an independent stress marker among frequent users of mobile phones. J Environ Pathol Toxicol Oncol* 33 (4): 339-347
  - 2014, Khalil et al. *Assessment of oxidant/antioxidant status in saliva of cell phone users. Electromagn Biol Med* 33 (2): 92-97
  - 2001, Moustafa et al. *Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J Pharm Biomed Anal* 26 (4): 605-608

# Mechanism hypothesis: calcium channels

(lack of human studies)

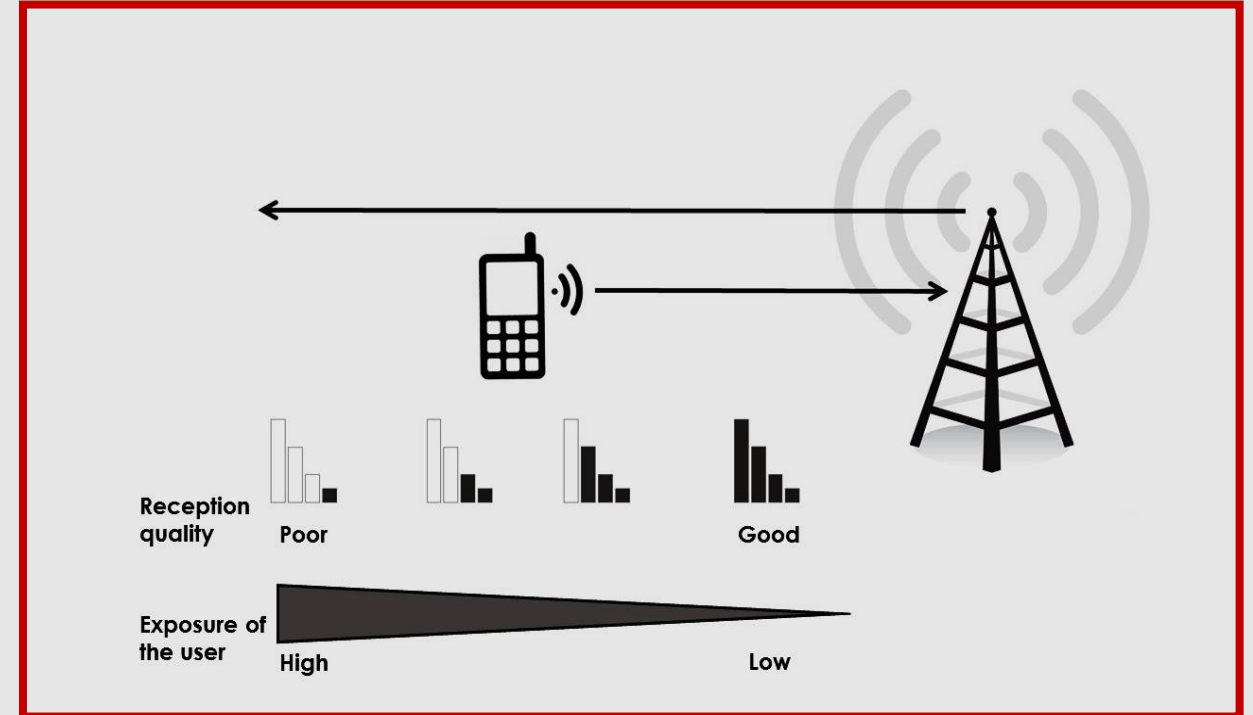
- Total of some 26 studies on voltage-gated calcium channels (VGCC) and EMF frequencies of ELF-EMF and RF-EMF
- Some important unanswered questions
  - *How alternating EMF affects VGCC by depolarization of cell membrane in vivo, when an animal (human) moves in the field*
  - *How oscillating EMF affects calcium channels. The time period of change when exposed to an AC-field in the environment is probably too fast for the channels to detect, for ELF field and particularly for RF field*
- Unconvincing VGCC blockers – any and every function of cell is possible to shut down with calcium blockers
  - *Disappearance of EMF-effect in the presence of calcium blockers does not automatically mean that EMF effect is mediated by calcium (parallel pathways)*
- Evidence still insufficient to determine physiological significance

# Epidemiology case-control studies support brain cancer risk

- International Agency for Research on Cancer 2011 classification was based on the results of Interphone and Hardell studies
- Currently, the four case-control epidemiological studies suggest the cell phone radiation increases risk of developing brain cancer in avid users
  - *Regular user (!) – no problem at all but... definition: 1 call/week for 6 months*
  - *Avid user = ca. 30 minutes/day for 10+ years*
  - *Interphone 40%; Hardell 170%, CERENAT 100%, Canadian Interphone 100% increase in glioma risk*
- Interphone 2016 analysis of full data confirms location of cancer in the most exposed parts of brain
- NOTICE: all case-control studies underestimate risk of brain cancer because of poor radiation dosimetry

# PROBLEM: Epidemiology case-control studies have no radiation exposure data

- Surrogate for radiation exposure – minutes of using cell phone
- Such surrogate leads to underestimation of the effect
- Two persons talking for the same length of time may have entirely different radiation exposure because of the different proximity to cell tower
- Persons with dramatically different radiation exposure are analyzed as if having the same exposure



# Huge number of cell phone users but no dramatic increase in brain cancer in whole population

- Brain cancer – rare disease
- Cancer latency is tens of years
- Length of use of cell phone and how avidly used
- Cancer statistics are too general – do not differentiate between types of brain cancer or age groups having it – might be misleading
- Cancer cause – cell phone radiation as co-factor for something else (?)
- My Hypothesis: Likely, only persons with higher individual sensitivity will develop cancer (results of NTP study support this hypothesis)



# Blood-Brain Barrier permeability

## Research studies from Salford & Persson research team

- *Nittby et al. Pathophysiology. 2009, 16:103-112*
- *Nittby et al. Bioelectromagnetics. 2008, 29:219-232*
- *Belyaev et al. Bioelectromagnetics. 2006, 27:295-306*
- *Salford et al. Microsc Res Tech. 1994, 27:535-542*

## Research study form US Air Force lab

- *McQuade et al. Radiation Res. 2009, 171:615-621*

## Research from China

- *Tang et al. Brain Res. 2015, 1601:92-101*

## Research from Turkey

- *Sirava & Seyhan. J. Chem. Neuroanatomy 2016, 75(Pt B):123-127*

## Research from Finland

- *Leszczynski et al. Differentiation 70, 2002, 120-129*

# Mechanism for BBB permeability:

## Stress response in endothelium

Leszczynski et al. Differentiation 70, 2002, 120-129

- Phosphorylation of Hsp27
- Phosphorylation of p38MAPK
- Activation of the p38MAPK/Hsp27 pathway
  - Weakening of cell-cell contact
  - Cell shrinking
  - Non-apoptotic cell 'blebbing'
  - Generation of growth factors

Tang et al. Brain Res. 2015, 1601:92-101

- Increased expression of mkp-1,
- De-phosphorylation of ERK
- Activation of mkp1/ERK pathway

# DNA damage & genotoxicity

- NTP study fueled debate on genotoxicity of mobile phone radiation
- Scientifically unfounded “rush to conclusions” on genotoxicity and cancer
- DNA “damage” does not automatically mean that the RF radiation is genotoxic
- DNA damage occurs also spontaneously and is repaired
- No studies to show what is the fate of the RF-induced “DNA damage”
  
- Is DNA damaged by RF or is RF impairing repair of spontaneous DNA damage?
- Is DNA damage repaired or does it persist in further generations of cells?
- Is DNA damage occurring in humans?
  
- Considering the efficiency of DNA repair mechanisms in cells, claims that mobile phone radiation is genotoxic, are not proven yet
- We do not know if mobile phone radiation exposure associated DNA damage leads to genotoxicity and mutagenicity or whether it is repaired

# Fertility: sperm and egg

- Cell phone radiation affects activity of sperm and affects binding sperm to egg
  - Falzone N, Huyser C, Becker P, Leszczynski D, Franken DR. *The effect of pulsed 900-MHz GSM mobile phone radiation on the acrosome reaction, head morphometry and zona binding of human spermatozoa. Int J Androl. 2011 Feb;34(1):20-26;*
  - Falzone N, Huyser C, Franken DR, Leszczynski D. *Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiation Research 2010 Aug;174(2):169-76*
  - Falzone N, Huyser C, Fourie F, Toivo T, Leszczynski D, Franken D. *In vitro effect of pulsed 900 MHz GSM radiation on mitochondrial membrane potential and motility of human spermatozoa. Bioelectromagnetics 29, 2008, 268-276*
- Need for studies showing that biochemistry of sperm and egg is affected
- Studies showing decline in male fertility are prone to errors because of interference of other factors cannot be excluded – e.g. chemicals, clothing

# PROBLEMS of the EHS provocation studies

by Dariusz Leszczynski, February 11, 2018

**Problem #1**  
**Research Execution**

**Methods used in provocation studies are insufficient to prove or to disprove EHS-EMF causality link**

- Scientists do not know if volunteers participating in the study have correct-self diagnosis of EHS
- Scientist introduce bias by excluding volunteers with pre-existing health problems
- Bias introduced by volunteers withdrawing from studies because of the fear of experimental radiation exposure
- Bias introduced by volunteer withdrawing from studies because of distrust in objectivity of scientists
- Scientists have no proof that methods they use are capable to detect EHS – lack of positive controls
- Bias introduced by the nocebo and placebo effects

**Problem #2**  
**Research Outcome**

False and evidence-unsupported conclusions of the studies performed on volunteers with uncertain EHS self-diagnosis and with unproven methods

**False claims of research studies mislead decision-makers leading to falsely premature conclusions that EHS is not linked to EMF exposures**

**The Solution**

Search for persons responding in significantly different way, than the rest of population, to EMF exposures by screening of tissues and body fluids with a variety of 'omics' techniques

**Correlating molecular level responses to EMF exposures with the health status and formulation of knowledge-based research hypotheses for how the EMF might affect health in long-term exposures**

# Effects of co-exposures

- Co-carcinogen studies show effects (just few performed) – cell phone radiation might potentiate effects of carcinogenic chemicals or radiation
- 6 co-carcinogenicity studies in animals were used as supportive evidence for IARC classification of cell phone radiation as possible carcinogen
- Published replication of Tillmann et al. 2010 confirmed by Lerchl et al. 2015
- Lack of co-carcinogen studies (!)

# Game changers after 2011 IARC

## ■ Epidemiology

- *Coureau G, et al. Mobile phone use and brain tumours in the CERENAT case-control study. Occup Environ Med. 2014; 71: 514-522*
- *Grell et al. The Intracranial Distribution of Gliomas in Relation to Exposure From Mobile Phones: Analyses From the INTERPHONE Study. Am J Epi. Nov. 2016; DOI: 10.1093/aje/kww082*

## ■ Animal studies – Lerchl’s group replication of Tillman et al study

- *Lerchl A, et al. Tumor promotion by exposure to radiofrequency electromagnetic fields below exposure limits for humans. BBRC 2015; 459: 585-590*
- *Tang J, et al. Exposure to 900 MHz electromagnetic fields activates the mkp-1/ERK pathway and causes blood-brain barrier damage and cognitive impairment in rats. Brain Res. 2015; 1601: 92-101*

## ■ Dosimetry – reevaluation of in vitro dosimetry by Schmid & Kuster

- *Schmid G & Kuster N. The discrepancy between maximum in vitro exposure levels and realistic conservative exposure levels of mobile phones operating at 900/1800 MHz. Bioelectromagnetics. 2015; 36:133-148*

# 5G and Skin

- Skin is the largest organ of the human body
- Involved in regulation of the immune response
- Lack of science on human skin response to RF-EMF
- Single study on RF-EMF (GSM) and skin proteome [Karinen et al. 2008]
- 5G technology spectrum 6 GHz – 100 GHz
- Above 6 GHz energy is deposited solely in the skin
- ICNIRP plans to classify skin as ‘limbs’
- Limbs are permitted to get higher exposure than the head and trunk



# Studies on skin & skin cells physiology

- Human volunteer – ca. 11 studies
  - Human in vitro – ca. 26 studies
  - Animal in vivo (rat, mice) – ca. 56 studies
  - Animal cells (rat, mice) – ca. 10 studies
  - TOTAL of ca. 103 studies
- 
- If the exposure is for long periods and non-thermal – we do not know how skin cells will respond to the deposited energy

# Human volunteer studies on millimeter-waves

- Effect on pain sensation
- Effect on acupuncture sites
- Effects on blood flow
- Skin-dependent effect on cardiovascular function and brain physiology (single study; no effect)

# Human in vitro studies

- Keratinocytes
  - Fibroblasts
  - Buccal cells
  - Lymphocytes
  - Melanoma cells
- 
- No studies on Langerhans cells or any Langerhans-like cells!

# Some in vitro effects on human cells

- Promotion of synthesis of extracellular matrix
- Induction of apoptosis
- Promotion of proliferation and G1 to S phase transition
- Inhibition of proliferation of cancer cells
- Inhibition of NO-dependent apoptosis via p38MAPK pathway
- Changes in protein expression
- Effects on NF-KB pathway via TNF-alpha and cyclophosphamide
- Effects on c-fos expression
- Lack of effects on Hsp27 and Hsp70 (no thermal effect?)
- Number of studies shows the opposite - the lack of effects...

# Specific “electromagnetic effect”

(individual sensitivity?)

Transcriptome analysis reveals the contribution of thermal and the specific effects in cellular response to millimeter wave exposure. Habauzit et al. *PLoS One*, 2014, 9:e109435-1-e109435-10

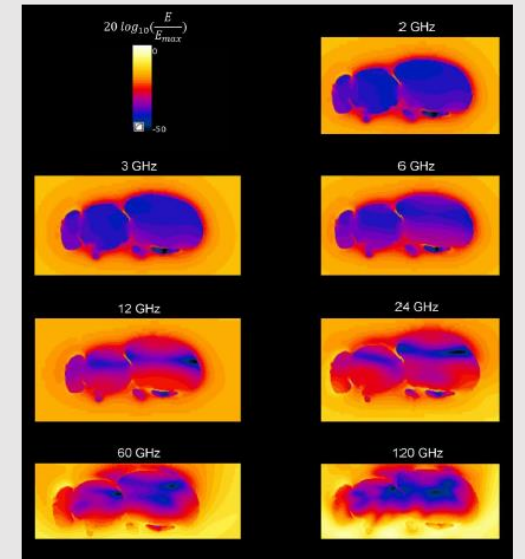
- Exposure affected gene expression
- Seven genes affected and confirmed
- Effect observed when temperature of cells increased
- When temperature was controlled effect disappeared but...
- ...just by increasing temperature it was not possible to mimic the thermal-exposure effect on genes
- Hence, proposed possibility of an “electromagnetic” component of the exposure

# Effects on microbes (skin & elsewhere)

- Inhibition of bacterial growth (53 GHz)
- Enhancement of bacterial sensitivity to antibiotics (53 GHz)
- Inhibition of growth and viability of bacteria (70 GHz)
- Effects on metabolic pathways in bacteria (53 GHz)
- Co-effects of mm-Waves and UVC (enhanced survival)
- Co effects with X-rays (repair of the damage)
- Effects on structure if bacterial genome

# Sensitivity of insects

- Exposure of Insects to Radio-Frequency Electromagnetic Fields from 2 to 120 GHz. Thielens et al. (team included Luc Martens and Wout Joseph); Scientific Reports 2018, 8:3924
  - *“...Our simulations showed that a shift of 10% of the incident power density to frequencies above 6 GHz would lead to an increase in absorbed power between 3–370%...”*
  - *“...This could lead to changes in insect behaviour, physiology, and morphology over time due to an increase in body temperatures, from dielectric heating...”*



# Serious limitations of biomedical research on millimeter-waves

- Very limited number of studies
  - *Mobile & Wireless Forum refers some 470 studies*
- Lack of studies examining human volunteers
- Lack of studies on individual sensitivity
- Complete lack of studies on chronic, long-term, exposures
- Studies from a very limited number of research groups (!)
- Lack of replication studies confirming correctness of observations (!)
- Vast majority of studies done in vitro on cells grown in laboratory



# Conclusions - Gaps in Research

- Search for sensitive sub-population using biochemistry methods of proteomics, transcriptomics and other 'omics' techniques
- Impact of co-exposures to chemicals and RF radiation
- Epidemiology with realistic RF radiation exposure data
- Finding out whether DNA damage happens in humans and, if it happens, what is the physiological relevance (e.g. cancer?)
- Examining whether human blood-brain barrier is affected and whether it affects physiology of brain
- Examining possible link between BBB leakage and Alzheimer's disease
- Skin and skin-dependent systemic responses to 5G exposures