Biophysical and biochemical mechanisms of the biological effects of mobile phone radiation

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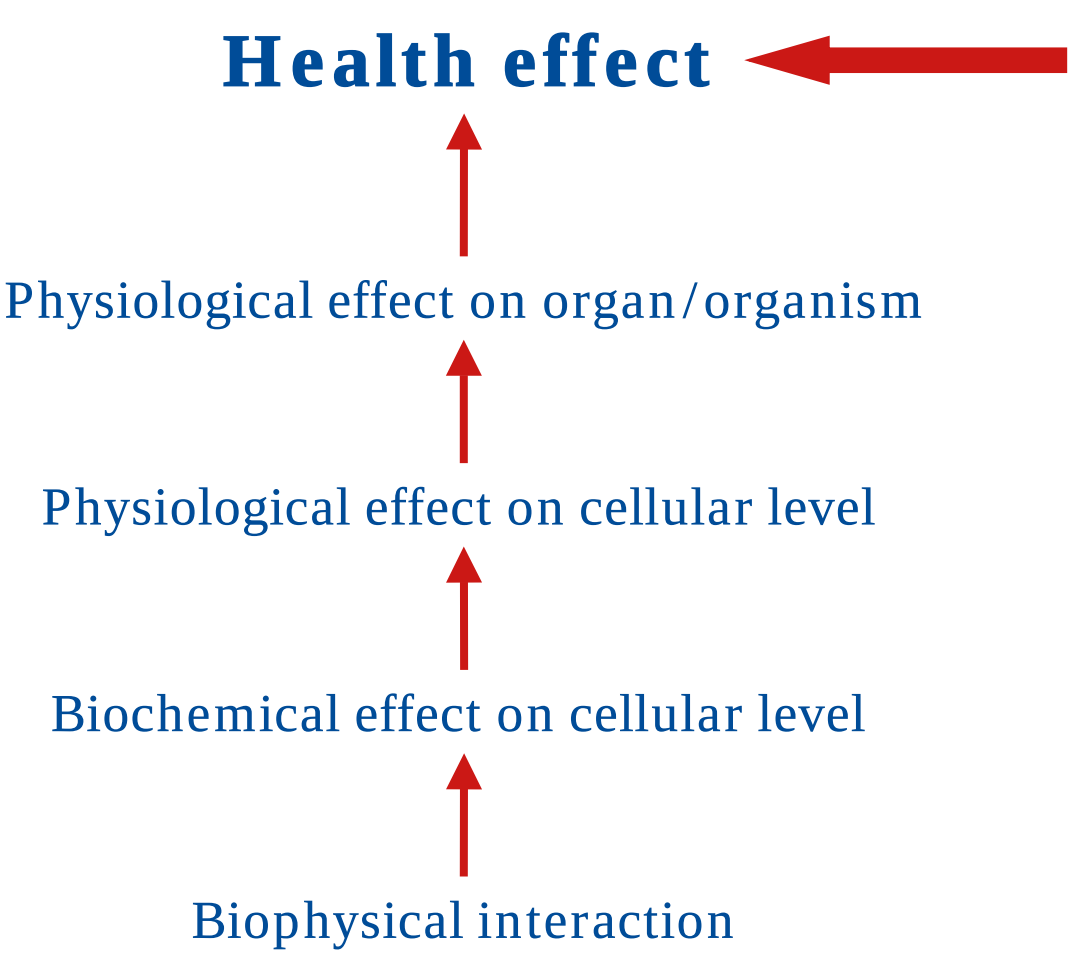


Epidemiology
Human studies
Animal studies
In vitro studies

- **Epidemiological evidence**
  - epidemiological studies lack reliable dosimetric evaluation that would be in quality comparable with the quality of cancer-related information
  - latency
  - cancer only; what about other ailments
- **Human volunteer studies**
  - lack of biomarkers of response
  - too much relying on the subjective observations
- **Animal studies**
  - useful for experimentation but how reliable data for human health risk assessment
- **In vitro studies**
  - needed for discovering the mechanisms

Lack of biophysical mechanism has at least three major consequences:

- continuing uncertainty whether the observed biological effects are real,
- it is not possible to predict the potential long term physiological effects,
- it is not possible to predict the potential impact of new frequencies and modulations

Two possible mechanisms of the RF-EMF: thermal and non-thermal.

- distinction is useful for basic research studies
- not so for health effects - for health impact are important effects themselves, independently of their thermal or non-thermal nature

Mechanism	Plausibility	Other comments
Temporal and spatial temperature gradients	Possible	Need for theoretical evaluation of temperature and energy gradients (temporal and spatial) at the microscopic (nano-scale) and macroscopic level. Determine whether such gradients can drive biochemical processes. Evaluate impact of Kapitza resistance. Consider temperature changes for times < 1millisecond and complex geometries.
Alteration of membrane potential	not plausible for brief exposures at low levels	Need to perform calculations to determine the thresholds.
Membrane rectification	Not generally plausible because small signals will result in insignificant non-linear responses.	There is experimental evidence from biological studies to support the transit time model. Pickard suggests that rectification is not possible above a few MHz.) Need to (a) define theoretical lower limits of field strength for membrane interaction (b) theoretically investigate whether multiple-cell structures create situation for rectification or whether one can get a large oscillating or rectification change in membrane potential at frequencies above 10 MHz. Examine the possibility of conducting experiments that test for harmonic responses as a means of investigating existence of non-linearity in cell system.
Polarization of structures or molecules .	Not plausible for most cases	But there is a need to theoretically investigate whether small fields could affect a process that is teetering between two nearly equal energy states. Aggregation of cells or proteins is a possible example.
RF pumping & chemical kinetics.	Not plausible due to critical damping of any modes that may or may not exist.	Need to theoretically examine: a) Isolated molecules (caged structures) to determine whether such isolation could exist and lead to realizable vibrational modes. b) The possibility and limitations of 2 -photon, 2 -phonon, or 1-photon plus 1-phonon interactions. c) The ballistic regime: Generally, a sharp resonance is not possible unless the time between collisions is long compared to binding or transit times, but ballistic transmission (as for ions in a channel) might be an exception. Therefore, need to (1) Calculate effective collision times and (2) determine whether ballistic transmission is realizable in biological systems

MMF — Mobile Manufacturers Forum

Mechanisms for Interactions of Radiofrequency Energy with Biological Systems: Principal Conclusions from a Seminar held in Washington, DC

July 23, 2001 (revised 30 Aug 01)

This report was prepared by Asher R. Sheppard, Mays L. Swicord, Sakari Lang, and Frank Gollnick and reviewed by the meeting participants to whom we are grateful for corrections and advice.

Magnetic dipole interactions	Not plausible.	Published calculations show that energy absorbed in magnetite is too small to cause significant temperature rise. No other effects in microwave range were identified as plausible. Magnetic fields effects on free radical formation should be theoretically evaluated.
Synchronization		Synchronized systems need to be theoretically examined to determine whether there may be an increased level of response in those systems that otherwise are implausible.
Electrophysiological		See "General Consideration" below.
General Consideration:		All work at the cellular level should consider multi-cell and ultra-structural analysis of inhomogeneous complex properties. 3-D structures imply limitations of in vitro studies.
Cooperative	Not plausible	No known way of coupling cooperative systems to RF energy.
Coherence	Not plausible	Critical damping would prevent appearance of any modes that might exist.

Cell Stress & Chaperones (2002) 7 (2), 200–206

Article no. csac. 2002.361

Activation of the mitogen-activated protein kinase pathways by heat shock

Sonia Dorion and Jacques Landry

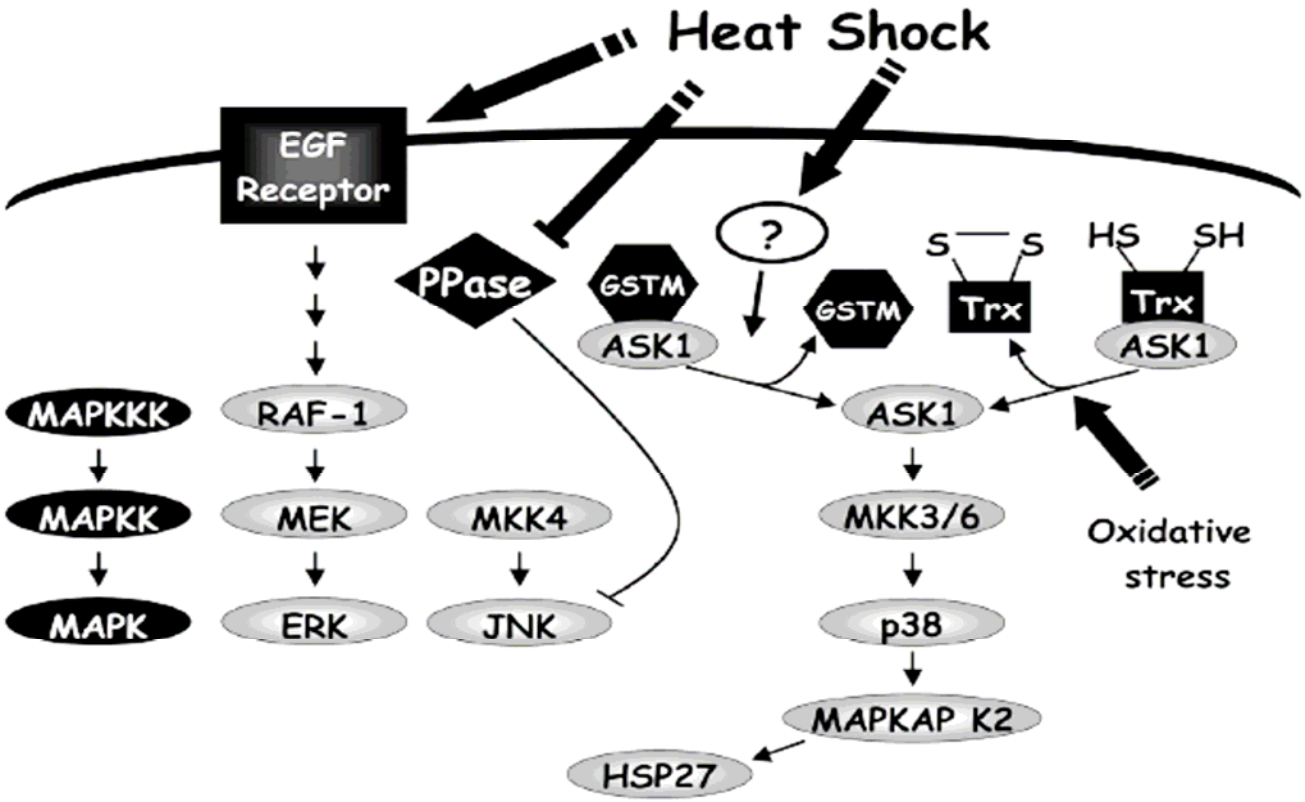
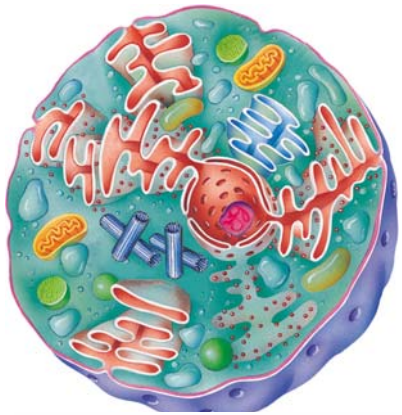


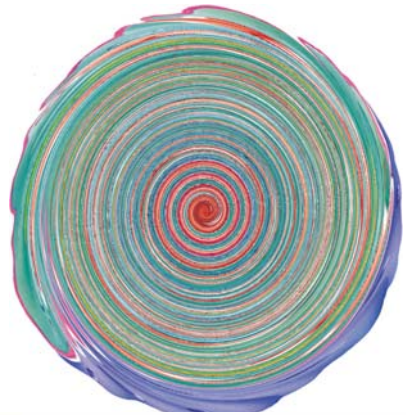
Fig. 1. Proposed mechanisms for the activation of the mitogen-activated protein kinase pathways by heat shock. Whereas the extracellular signal-regulated kinase pathway appears to be activated after the agonist-independent phosphorylation and activation of the epidermal growth factor receptor and the c-Jun N-terminal kinase (JNK) pathway, as a result of the inactivation of a protein phosphatase of JNK (PPase), the p38 pathway is activated downstream of a specific heat shock sensor or sensing pathway (indicated by the question mark). See text for details.

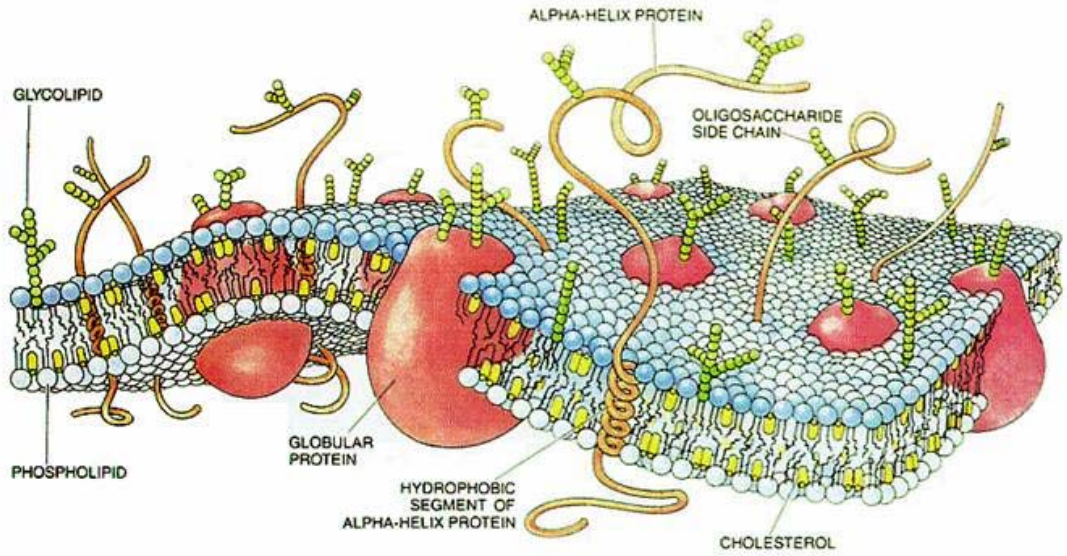
MicroDosimetry

Cell

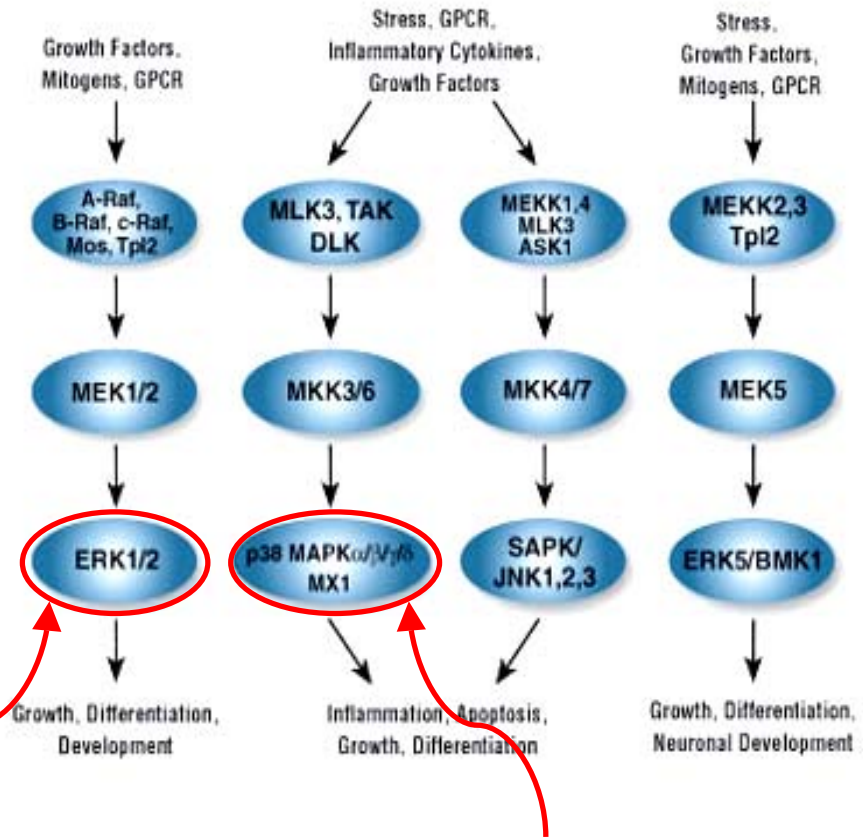


Dosimetry ”model” of cell



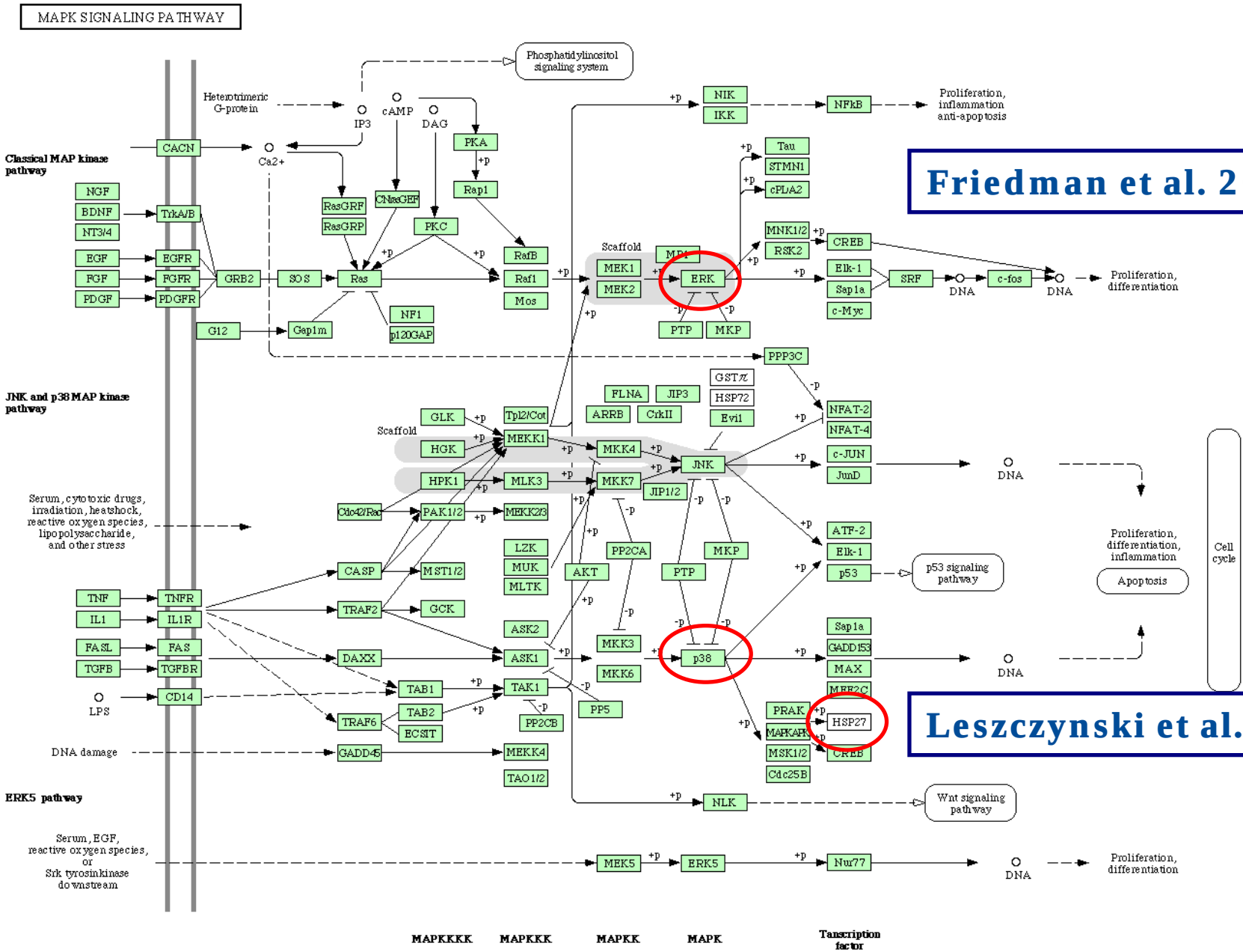


MAP KINASE SIGNALING



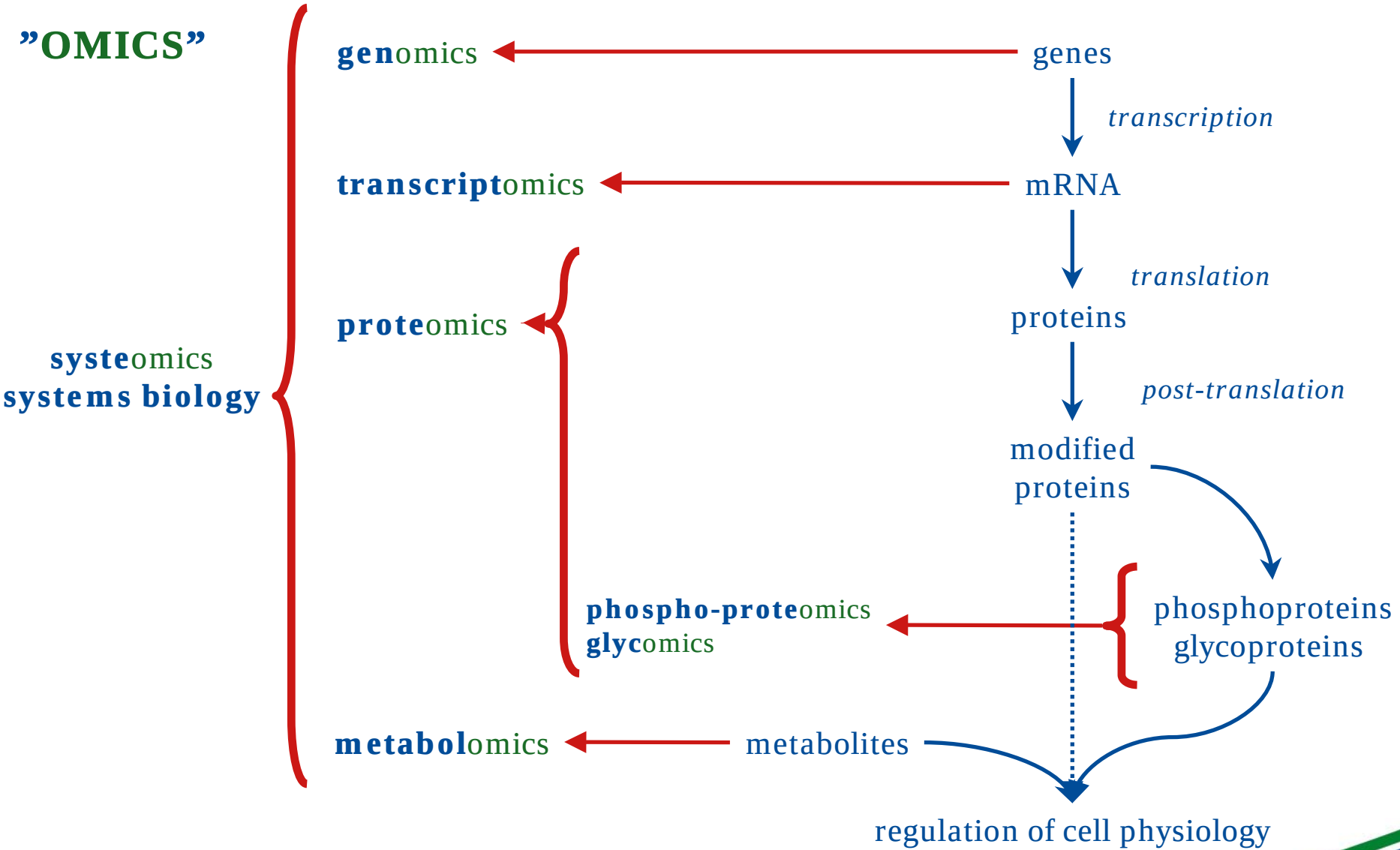
Friedman et al.
Biochem. J
2007

Leszczynski et al
Differentiation
2002



Friedman et al. 2007

Leszczynski et al. 2002


"OMICS"
genomics
genes
transcription
transcriptomics
mRNA
translation
proteomics
proteins
systeomics
systems biology
post-translation
modified proteins
phospho-proteomics
glycomics
phosphoproteins
glycoproteins
metabolomics
metabolites
regulation of cell physiology

Unlike the genome, the transcriptome and the proteome are highly dynamic and change rapidly and dramatically in response to perturbations or even during normal cellular events

strong stimulus
↓
robust response

weak stimulus
↓
response will very much depend on the transcriptome and proteome expressed by the cells at the time of exposure

Nylund R. & Leszczynski D.
Mobile phone radiation causes broad changes in gene and protein expression in human endothelial cell lines and the response appears to be genome- and proteome-dependent.
*Proteomics 2006, **6**:4769-4780*

Published studies that have examined effects of EMF using HTST approach

Cells	EMF exposure	End-point	Effect	Reference
Human HL60 cells	60 Hz magnetic field	gene expression	no	Balcer-Kubiczek et al. 2000
Human mammary epithelium & HL60	60 Hz magnetic field	gene expression	no	Loberg et al. 2000
S. cerevisiae	50 Hz magnetic field	gene & protein expression	no	Nakasono et al. 2003
E. coli	static magnetic field	gene expression	yes	Potenza et al. 2004
Human HL60 cells	60 Hz magnetic field	protein expression & phosphorylation	yes	Pipkin et al. 1999
Human fibroblasts	GSM	gene expression	yes	Pacini et al. 2002
Human EA.hy926	900 MHz GSM	protein expression & phosphorylation	yes	Leszczynski et al. 2002
Human EA.hy926	900 MHz GSM	gene & protein expression & protein phosphorylation	yes	Leszczynski et al. 2004
Human EA.hy926	900 MHz GSM	protein expression	yes	Nylund & Leszczynski 2004
Human HL60 cells	2.45 GHz	gene expression	yes	Lee et al. 2005
Rat brain	915 MHz GSM	gene expression	yes	Belyaev et al. 2006
Human U87MG glioblastoma	1900MHz	gene expression	no	Qutob et al. 2006
Human EA.hy926	900 MHz GSM	gene & protein expression	yes	Nylund & Leszczynski 2006
Human & animal cell lines	900 MHz GSM 1800 MHz GSM	gene expression	yes	Redmondini et al. 2006
C3H10T(1/2)	1900MHz	gene expression	no	Whitehead et al. 2006
TK6, HL60, Mono-Mac-6	1900MHz	gene expression	no	Chauhan et al. 2007

Ingenuity Pathways Analysis software and database

23.900 genes & 1.4 million relationships...

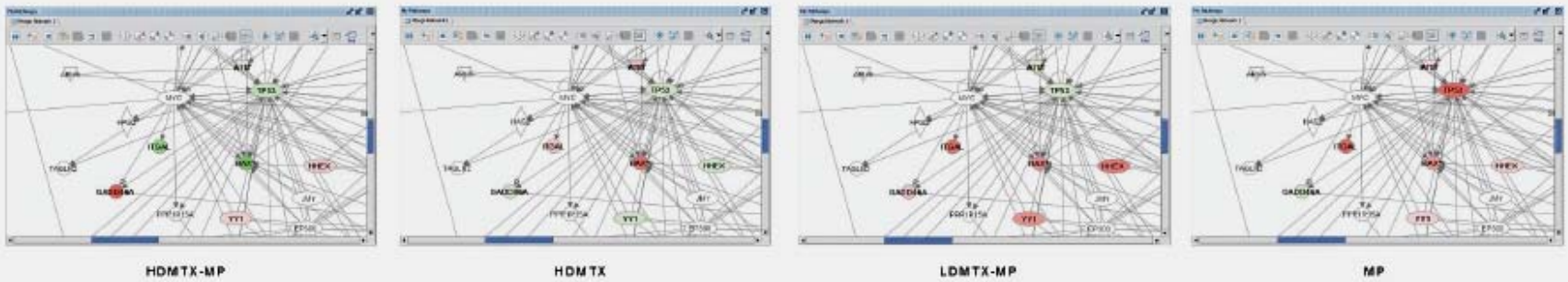


Figure 2. Biomarkers of efficacy. To determine how the expression changes in BAX and GADD45A might promote an anti-leukemic effect, we merged the networks containing these genes to create an integrated picture of biological relationships. We were able to overlay the expression values of each of the different drug treatments and easily visualize the changes in expression caused by the different dose responses. The genes BAX and ITGAL are down regulated (shown in green) in the most effective HDMTX-MP treatment while up regulated (shown in red) in the less effective treatments. The expression of GADD45A is up regulated in the combination drug treatments, HDMTX-MP and LDMTX-MP, while down regulated when treated with the individual drugs alone. We then wanted to understand how GADD45A expression was regulated and whether it was regulated in response to HDMTX-MP treatment.

Figure 3. Building pathways to understand mechanisms contributing to GADD45A expression. Using the new features of IPA 3.0, we were able to construct a pathway to identify key regulators of GADD45A expression. Starting with the GADD45A node, we were able to add nodes that are involved in expression and then overlay our experimental expression results. By doing this, we learned that IL24 increases expression of GADD45A, which leads to apoptosis and that IL24 can induce apoptosis in various cancer cells. To identify genes that function up and downstream of IL24, we grew out the pathway from IL24 using select functional relationships.

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NON-THERMAL EFFECT #1 - stress response

Problem:

Cells respond to RF-EMF levels that do not cause significant heating but the mechanism of the response is unknown.

Plausibility and Gaps in the Knowledge:

- Hsp - good model for detecting cell response
- studies published: too few and too limited (looking at 1-2 Hsp)
- there are dosimetry-reliable studies at low SAR showing changes in Hsp
- these changes were recently suggested to be possibly cell type dependent

Research Needs:

- to use stress response (panel of all stress proteins) evaluation as tool to determine the effect at low SAR
- to use stress response proteins to study the cell-type dependent response
- to use human primary cells and tissues/cells of human volunteers

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NON-THERMAL EFFECT #2 - high-throughput screening (HTST)

Problem:

Cells respond to RF-EMF levels that do not cause significant heating but the mechanism of the response is unknown.

Plausibility and Gaps in the Knowledge:

- knowing the extent of molecular level effects in combination with the known cell physiology will allow formulating more precise mechanism hypotheses for testing
- knowing responding genes and proteins will allow predicting potential long-term physiological effects and examining them
- will allow to develop, if needed ,preventive measures.

Research Needs:

- to use HTST to discover gene and protein targets of RF-EMF
- to develop new hypotheses of biophysical mechanism
- to predict possible long-term effects
- to compare existing exposures with newly developed exposures
- to use human primary cells and tissues/cells of human volunteers
- more info about use of HTST: Leszczynski & Meltz, Proteomics, 2006, 6: 4674-4677

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THERMAL EFFECT - blood-brain barrier

Problem:

Increase of temperature in brain cortex induced by RF-EMF exposure - BBB leakage?.

Plausibility and Gaps in the Knowledge:

- it is not known if the temperature is distributed uniformly or whether there are compartments within the tissue/cell that might be heated to higher temperature
- explanations using skin physiology or fever as examples are invalid without comparable scientific evidence
- dosimetry does not consider (i) compartmentalization and (ii) movement of charged molecules is not free but is strictly regulated by the surrounding molecules (active transport).

Research Needs:

- develop models and dosimetry on single cell level where lipid bilayer delineated organelle compartments will be taken into consideration
- determine what impact has the rate of brain heating on brain physiology

Summary…

- **Human volunteer studies**
  - defined sub-populations to compare
  - exposure - whole body or local
  - samples - blood, body fluids, solid tissues
  - examined endpoints
    - protein expression & protein activity (e.g. phosphorylation)
    - metabolites
    - gene expression
- We need studies that could answer whether cells from different people respond differently to the exposure using "feelings"-independent test
- In vitro cellular studies - there where volunteer studies not possible
- *Sufficient amount of data ("critical mass") to make comparisons and to find if there exist commonly affected proteins, metabolites or genes*

HERMO-SKIN

Effect of mobile phone radiation on protein expression in human skin

Anu Karinen Sirpa Heinävaara, Reetta Nylund & Dariusz Leszczynski

STUK - Radiation and Nuclear Safety Authority,
Helsinki, Finland

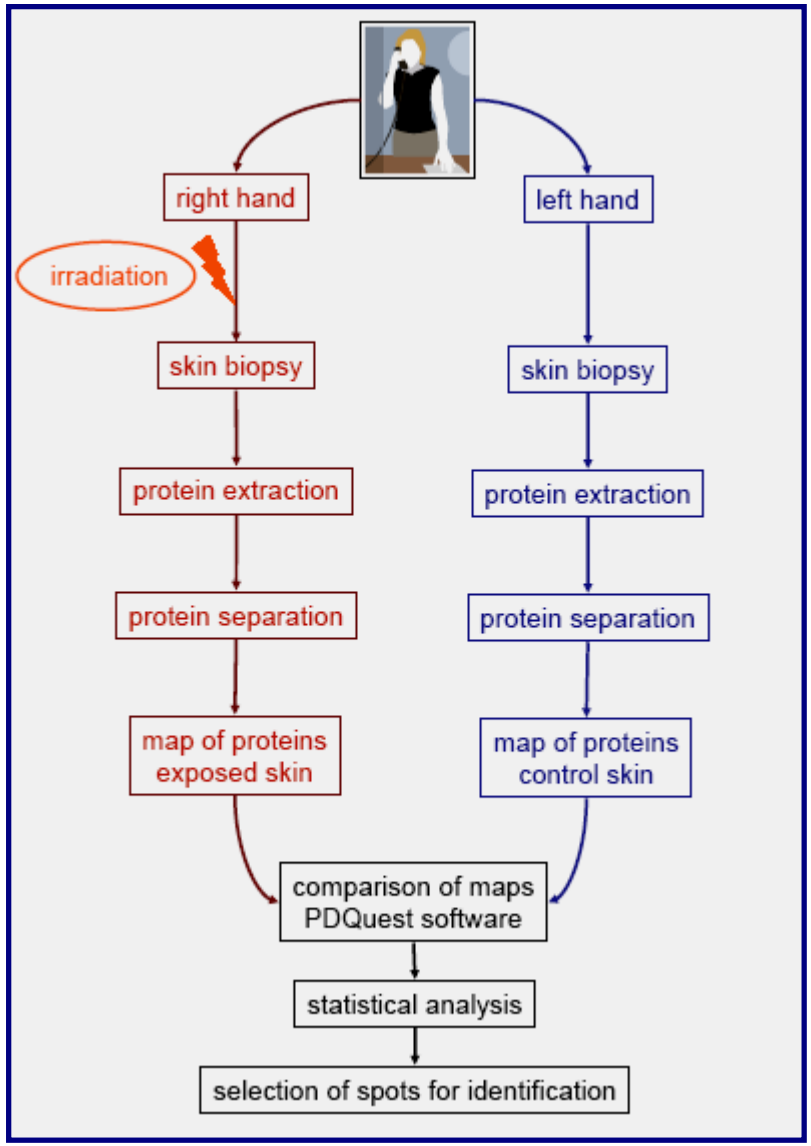
INTRODUCTION

- Our previous in vitro studies have shown that exposure of human endothelial cell lines for 1 hour to 900MHz GSM signal at SAR of 2.4 W/kg induced changes in protein expression and protein activity (phosphorylation).

  Leszczynski et al., Differentiation 2002, 70, 120-129
  Nylund & Leszczynski. Proteomics 2004, 4, 1359-1365
  Nylund & Leszczynski. Proteomics 2006, 6, 4769-4780

- However, it is not known whether similar response could occur in mobile phone users.

- In the present study we wanted to determine whether the exposure of the skin of human volunteers to 900 MHz GSM signal will induce changes in protein expression.


right hand
left hand
irradiation
skin biopsy
skin biopsy
protein extraction
protein extraction
protein separation
protein separation
map of proteins exposed skin
map of proteins control skin
comparison of maps PDQuest software
statistical analysis
selection of spots for identification

CONCLUSIONS

- there might be proteins in human skin that respond to mobile phone radiation
  - eight significantly altered proteins found in 10 volunteers
  - larger sample of volunteers needed because of variability
- this result agrees with results of our earlier in vitro studies (published in 2002, 2004, 2006)
- we have analyzed only small fragment of proteome but the numbers of affected proteins agree with our previously published data
- at this point it is not possible to determine whether these small changes might have any effect on skin physiology
- this feasibility study indicates that the proteomics approach may be successfully used to discover proteins that respond to mobile phone radiation