German Mobile Telecommunication Research Programme

International Workshop on Action Mechanisms

Title:

Influence of GSM-signals on differential gene expression in isolated human blood cells

B.Differential gene expression
- first results-

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Fraunhofer Institut
Toxikologie und
Experimentelle Medizin

Study Design

A. Isolation of human peripheral lymphocytes

B. Treatment with GSM signals

C. Gene expression analysis

1. Microarrays (Affymetrix)
2. Real-Time PCR of 20 selected genes

D. Western Blotting

1. Specification: Hsp-27, Hsp-27-P; p38MAKP, Hsp-70, p-21, C-Jun and C-Myc
2. Selection of 10 other proteins

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Study Design

Human blood lymphocytes were exposed to GSM 1800 signals with specific absorption rates (SAR) of 0 / 0.2 / 2 / 5 W/kg for 1 and 48 h.

treatment (W/kg)	0	0,2	2	5	Treatment time	age
Test person (m/f)	10/10	10/10	10/10	10/10	1 h	between 50 and 60 years
Test person (m/f)	10/10	10/10	10/10	10/10	48 h	
Test person (m/f)	10/10	10/10	10/10	10/10	1 h	between 18 and 21 years
Test person (m/f)	10/10	10/10	10/10	10/10	48 h	

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A. Isolation of human blood Lymphocytes

Blood collection (in cooperation with)

Prof. Dr. med. Rainer Blasczyk

Institute of Transfusion Medicine

Hannover Medical School (MHH)

* Agreement of the Ethics Committee (MHH)

B. Treatment with GSM signals

in cooperation with Dr. A. Bahr, Dr. C. Adami
(IMST, Kamp-Lintfort)

Installation of 4 resonators and the software.

(Resonators were coded)

Identification of pre-selected proteins by Western Blotting

positive controls

HSP-27 P-HSP-27 HSP-70 p-21	blood lymphocytes*
p38MAPK C-Jun	Balb3T3
C-Myc	mouse lung adenocarcinoma**

*** cultivated at 42ºC/2 h**

**** trangenic mouse strain expression the protooncogene c-myc in lung cells**

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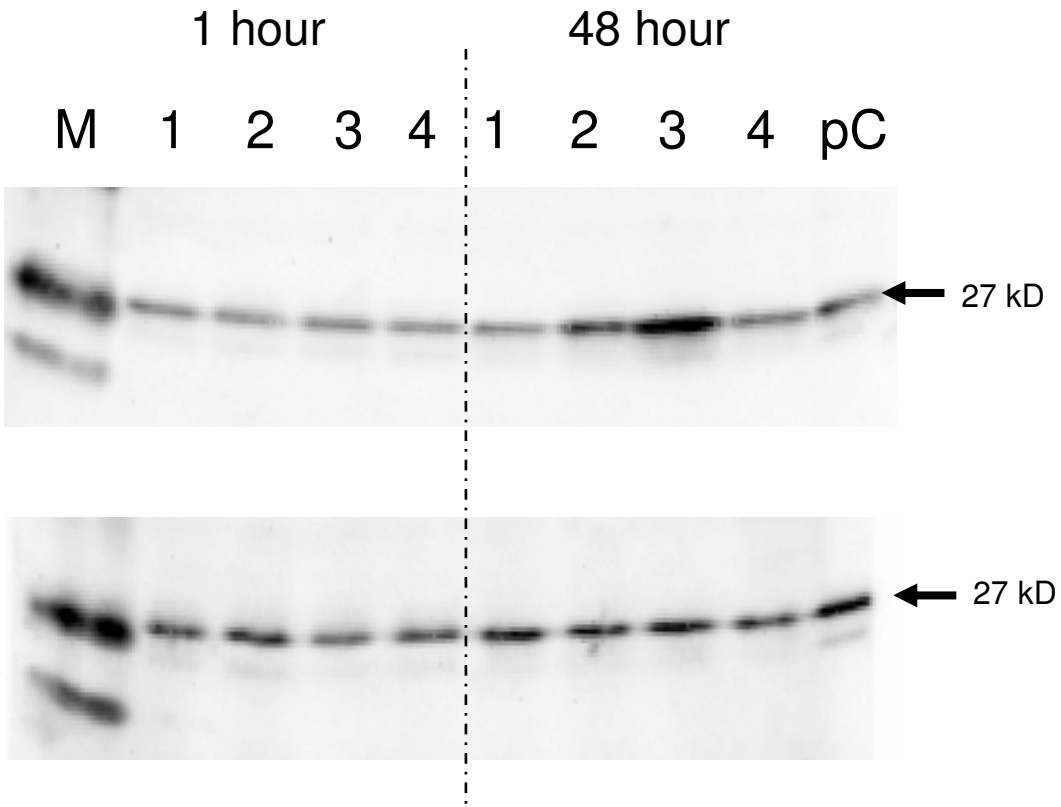
Results from Western blotting

HSP-27



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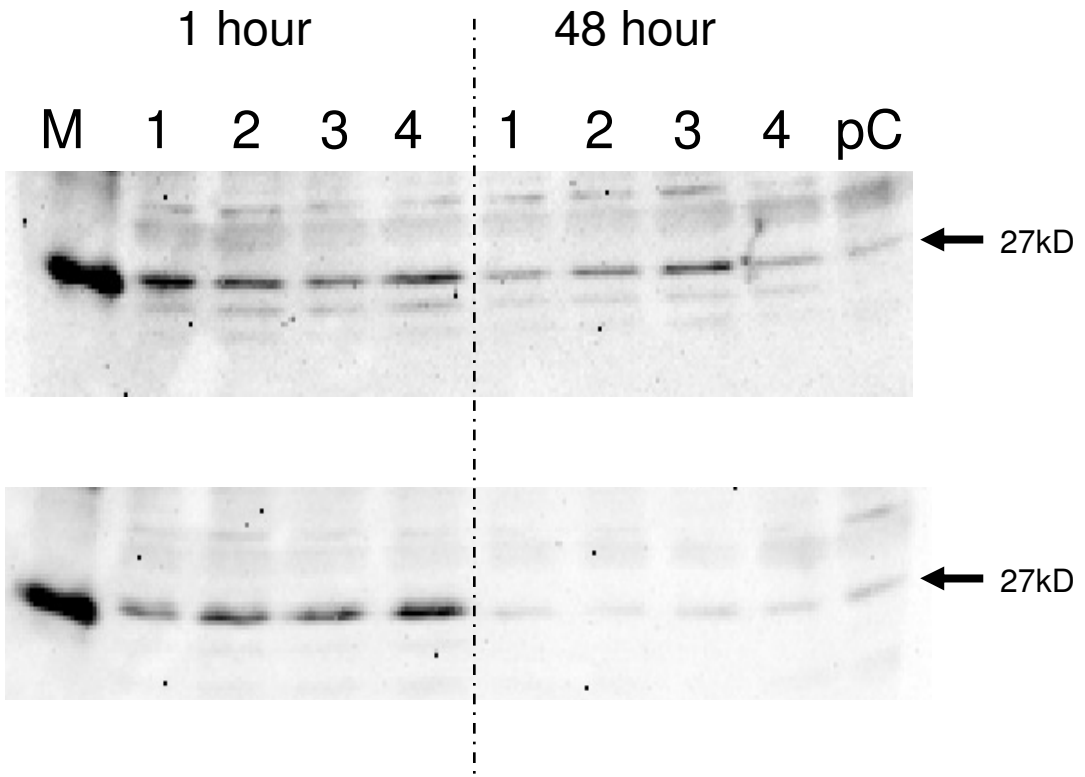
Results from Western blotting

P-HSP-27:



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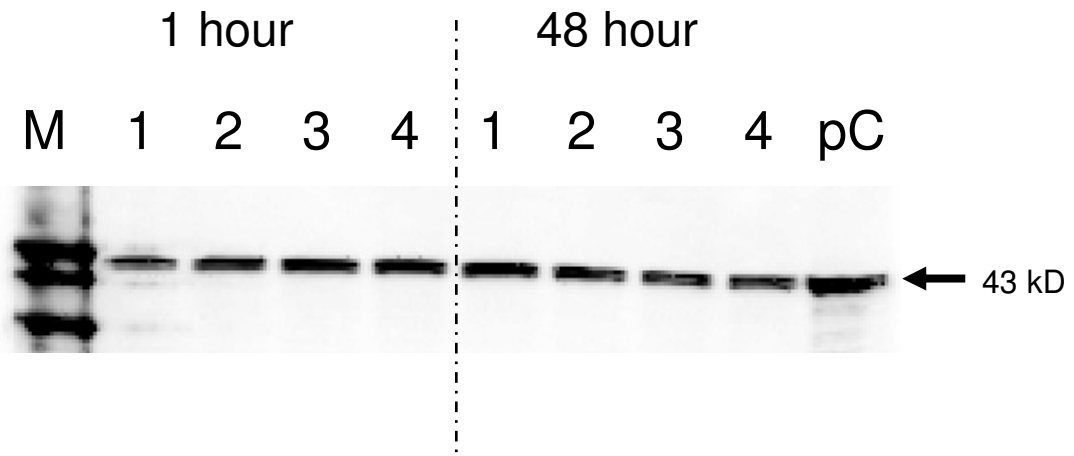
Results from Western blotting

HSP-70



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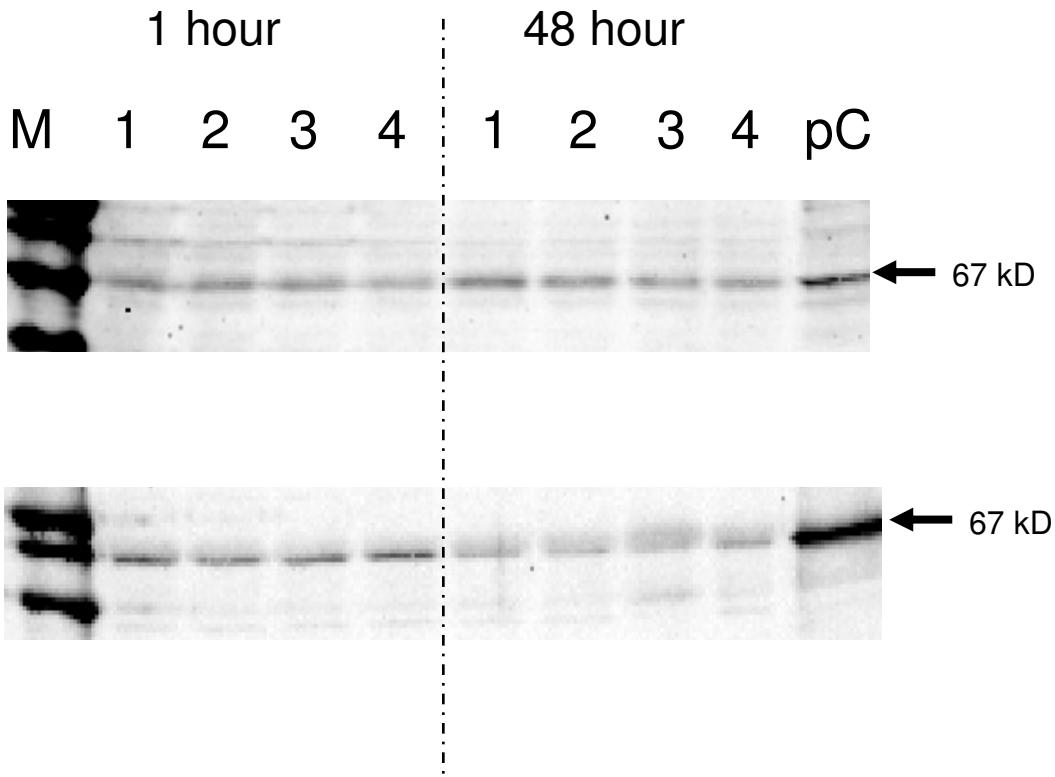
Results from Western blotting

C-Myc



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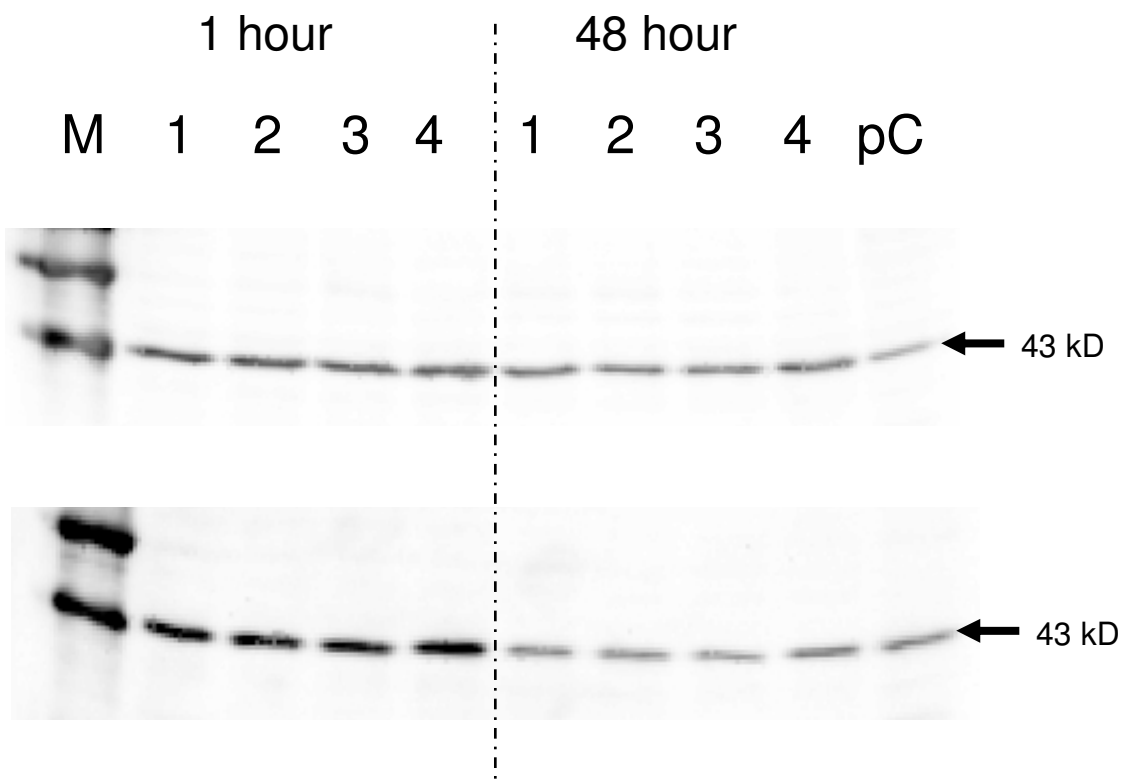
Results from Western blotting

p38-MAPK



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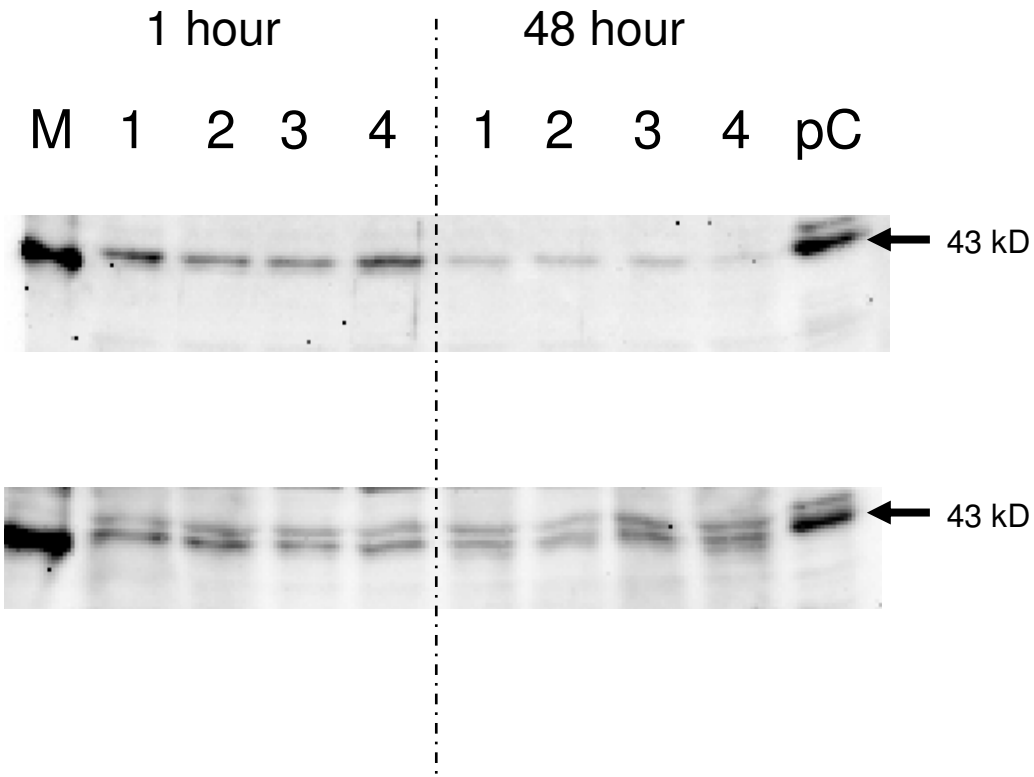
Results from Western blotting

C-Jun



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Summary (Western blotting)

Signal intensity											
	1 hour				48 hours					1 h > 48 h	1 h < 48 h
Protein	R1	R2	R3	R4	R1	R2	R3	R4			
HSP-70	no difference				no difference					no change	no change
P-HSP-27	no difference				+	++	+++	+	4/20	9/20	
					++	++	++	+	1/20		
HSP-27	no difference				+	++	+++	+	9/20		6/20
					++	++	++	+	1/20		
					+	++	++	+	1/20		
					+	++	+	+	1/20		
c-Jun	no difference				no difference				-	17/20	
c-Myc	no difference				no difference				-	2/20	
p38MAPK	no difference				no difference				-	2/20	
p-21	No signal detected										

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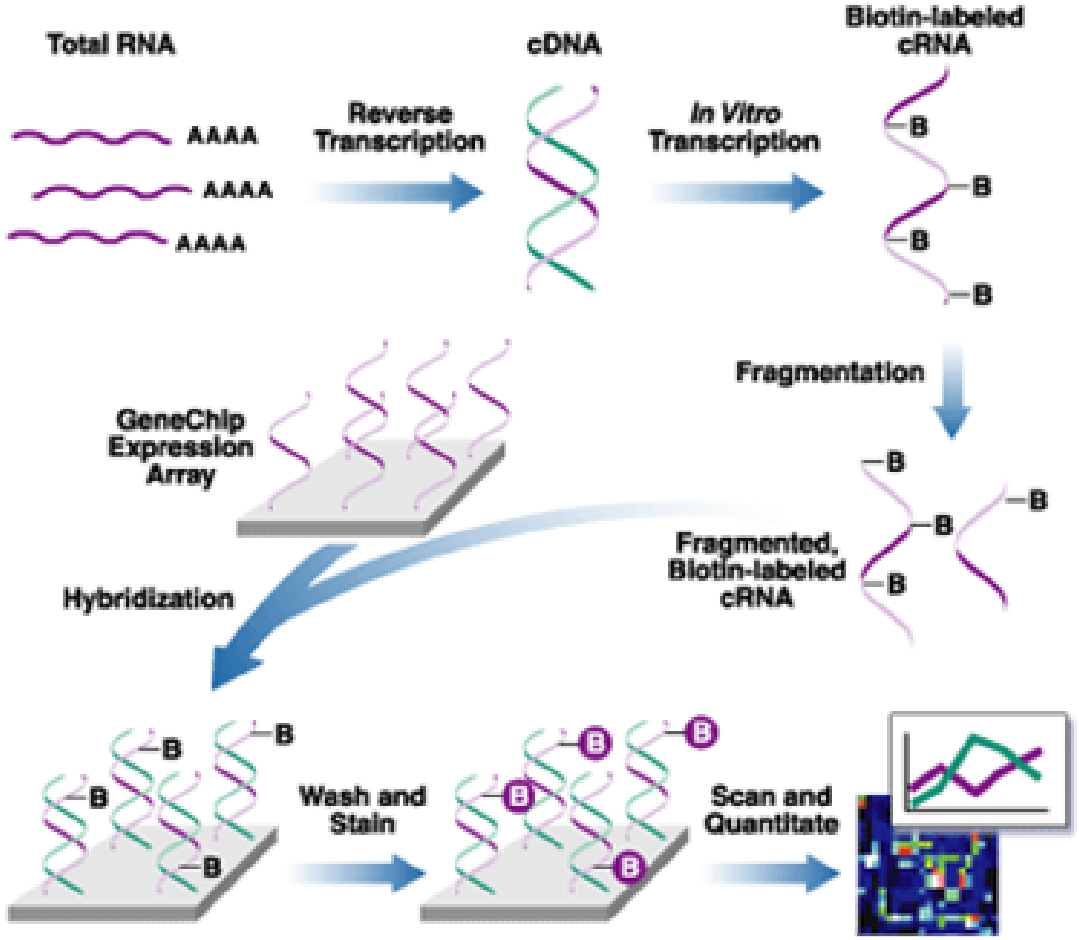
C. Gene expression analysis

1. Microarray gene expression analysis
   (Affymetrix)

   Genchip: Human Genome U133 Plus 2.0 Array

   Expression analysis of the whole human genome
   (>47.000 Transkripts)

Gene expression analysis (Affymetrix)



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Gene expression analysis (Affymetrix)

Gene expression analysis from human blood lymphocytes exposed to GSM 1800 signals of 0 (control) / 2 / 5 W/kg for 1 and 48 h of each blood donor.

Regulated genes will be identified by comparing the control groups with the treated groups.

Significantly altered regulated genes will be identified with statistical methods.

Microarray analysis of 320 genome wide gene expression analysis will be completed in June.

Biostatistics and Bioinformatics

Identification of significantly altered regulated genes.

- **Hierarchical gene cluster analysis**
- **Self-organizing map (SOM)**
- **Annotation of altered regulated genes**

Biostatistics and Bioinformatics

- **Hierarchical gene cluster analysis**

  expression values (signal log ratios) and experimental conditions (dose, age and sex) and genes will be clustered using average linkage clustering and uncentered correlation as distance metric.

- **self-organizing map (SOM)**

  SOM-algorithm will be applied to the microarray data. In this case the signal values and not the fold changes are the basis for this mathematical algorithm.

Biostatistics and Bioinformatics

Annotation of regulated genes

cellular function of regulated genes will indicate mechanisms of action of GSM-signals.

aim:

Identification of networks of regulated genes, which are relevant for a possible risk assessment.

C. Gene expression analysis

Validation of microarray data

- Real-time-qPCR of 20 genes
- Western blotting of 10 additional proteins

Selection will be dependent from results of microarray gene expression analysis.