Modulation of chemically- and oncogene-induced inhibition of gap junctional intercellular communication by chemopreventive agents

Čtveráčková L.[1], Trosko J. E. [2], Upham B. L. [2], Babica P.[1, 3]

[1] *RECETOX - Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00, Brno, Czech Republic*

[2] *Department of Pediatrics and Human Development, Michigan State University, 1129 Farm Ln, East Lansing, MI 48824, USA*

[3] *Department of Experimental Phycology and Ecotoxicology, Institute of Botany ASCR, Lidická 25/27, 602 00 Brno, Czech Republic*

E-mail:ctverackova@recetox.muni.cz

SUMMARY

Effect of investigated chemopreventive/anticancer agents to chemically-induced inhibition of GJIC:

• There were tested 6 inhibitors of GJIC – TPA (12-*O*-tetradecanoylforbol-13-acetate), Lindane, Fluoranthene, DDT, PFOA (perfluorooctanoic acid), PCP (pentachlorophenol)

•The most significant results was prevention of inhibition after exposure by Lindane (30 min) and Fluoranthene (24h)

Effect of investigated chemopreventive/anticancer agents oncogene-induced inhibition of GJIC:

• The most significant effects were induced by 6-benzylaminopurine, caffeic acid, gallic acid, metformin, naringenin and silibinin.

These *in vitro* models and endpoints represent valuable tools for future screening of chemopreventive and anticancer activity of natural products as well as for further characterization of their mechanisms of action.

Gap junctional intercellular communication (GJIC)

• Exchange of small soluble molecules (nutrients, metabolites, secondary messengers) between adjacent cells through **connexin** channels

• Critical for maintenance of **tissue homeostasis** (balance of proliferation, differentiation, apoptosis)

• Alterations of GJIC have been implicated in **carcinogenesis**, especially tumor promotion phase of cancer

• A valuable *in vitro* biomarker of **tumor promoting** and **chemopreventive effects** of chemicals

RAT LIVER EPITHELIAL CELL LINE WB-F344

•liver epithelial cells from Fischer F344 rats

•normal, non-cancerous diploid cells

• hepatic progenitor cells / stem-like cells (expression of *Oct-4*, *Sox-2*, *c-kit*, or *AFP*)

• multipotent, capable of *in vivo* and *in vivo* differentiation into hepatocytes, cardiomyocytes and biliary duct cells

• expression of connexin 43 (*Cx43*) and functional gap junctional intercellular communication (GJIC),

•contact inhibition of growth

•lack of anchorage-independent growth (AIG)

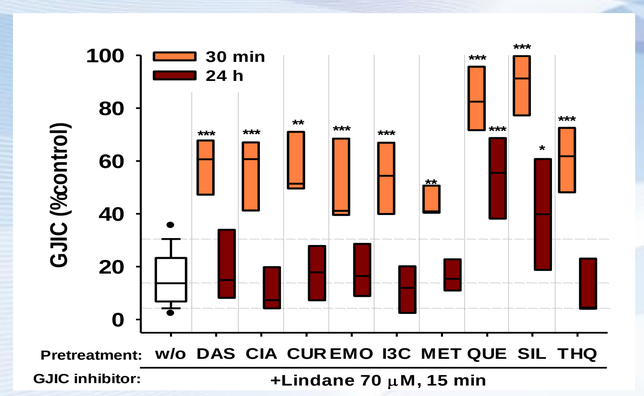
• **non-tumorigenic *in vivo***

Chemopreventive / anticancer agents used in the study: β-sitosterol, 6-benzoaminopurine, Caffeic acid, Naringenin, Silibinin, Metformin, Gallic acid, Plumbagin, Artesunate, Andrographolide, Phenethyl isothiocyanate, Curcumin, Allyl Sulfide, Emodin, Quercetin, Indole-3-carbotole, Cinnamic acid, Thymoquinone

Prevention of chemically induced inhibition of GJIC in WB-F344

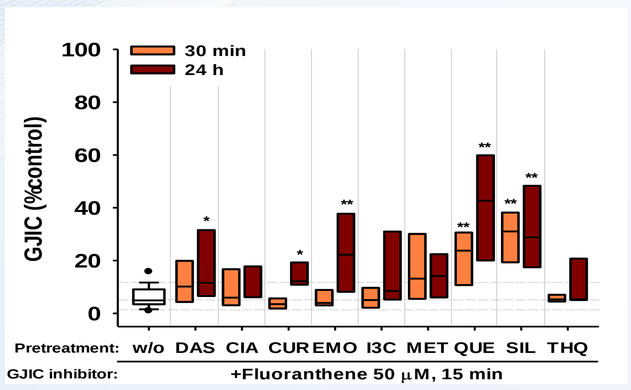
Lindane

MEK1/2-depended inhibitor GJIC



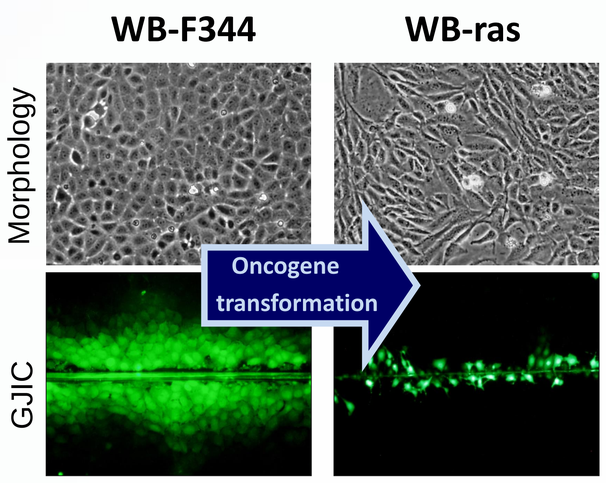
Fluoranthene

PC-PLC depended inhibitor GJIC



Investigated phytochemicals can prevent negative effects of Lindane to GJIC after 30 min of exposure and Fluoranthene after 24 hours exposure (Concentrations were 100 μM for cinnamic acid (CIA), 1 μM for curcumin (CUR), 500 μM for diallyl sulfide (DAS), 10 μM for emodin (EMO), indole-3-carbinol (I3C), 1000 μM for metformin (MET), 100 μM (30 min) or 25 μM (24 h) for quercetin (QUE), 100 μM (30 min) or 50 μM (24 h) for silibinin (SIL), 10 μM for thymoquinone (THQ).)

Acknowledgment: This research was supported by Ministry of Education, Youth and Sports of the Czech Republic: grant LH12034 (CHEMOPREV) to Babica, and project LO1214.



TRANSFORMED WB-ras CELL LINE

• prepared from WB-F344 cells by oncogene transfection or transduction H-ras

•aberrant expression, localization and phosphorylation of connexin 43

• loss of GJIC

• Hyperactivation of MAPKs

• loss of contact inhibition of growth

• capability of anchorage-independent growth (AIG)

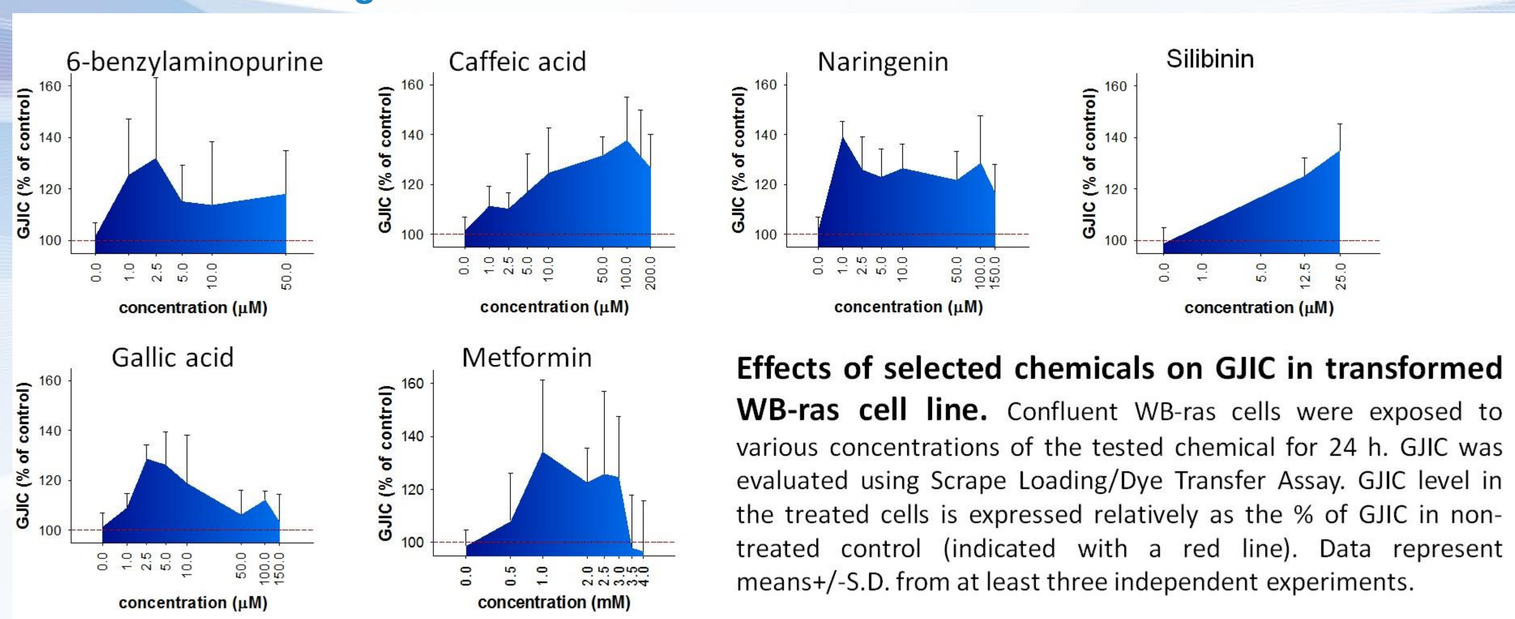
• **highly tumorigenic *in vivo***

• vector control cell line **WB-neo** has the same properties as the parental cell line WB-F344

Different chemopreventive / anticancer agents were demonstrated to suppress neoplastic phenotype of transformed WB cells. There were investigated:

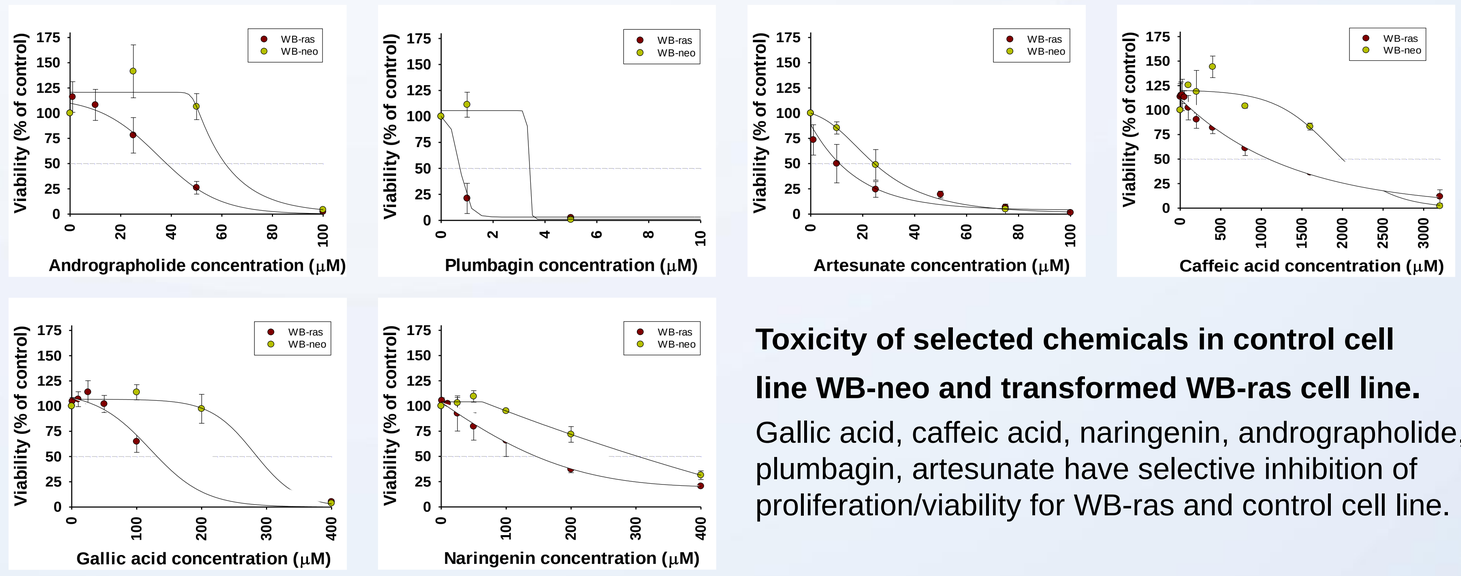
- **Restoration of GJIC**
- **Selective antiproliferative / cytotoxic effects on transformed cell lines**

Restoration of oncogen-inhibited GJIC



Effects of selected chemicals on GJIC in transformed WB-ras cell line. Confluent WB-ras cells were exposed to various concentrations of the tested chemical for 24 h. GJIC was evaluated using Scrape Loading/Dye Transfer Assay. GJIC level in the treated cells is expressed relatively as the % of GJIC in non-treated control (indicated with a red line). Data represent means+/-S.D. from at least three independent experiments.

Selective cytotoxicity



Toxicity of selected chemicals in control cell line WB-neo and transformed WB-ras cell line.

Gallic acid, caffeic acid, naringenin, andrographolide, plumbagin, artesunate have selective inhibition of proliferation/viability for WB-ras and control cell line.