

**Poster Sessions**

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**08:30–19:30, Foyer Convention Center**

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in suspension cultures as well in soft-agar colony formation assay.

**Materials and methods:** HKCSCs (Celprogen) were cultured in StemXVivo Mesenchymal Stem Cell Expansion Medium (R&D Systems). On day 3, TKIs were added to achieve final concentration of 1.5  $\mu$ M. HKCSCs were also cultured untreated in normoxia and hypoxia. Each condition was performed in triplicate. Total protein was extracted on day 6 using RIPA buffer and PIC (Phosphatase Inhibitor Cocktail, Sigma). Total mRNA was extracted using Nucleospin RNA Isolation Kit (Machery-Nagel). Label-free analysis using mass spectrometry has been performed. Subsequently, Western Blot analysis and quantitative Real-time PCR were performed using chosen antibodies and primers on the basis of MS results.

**Results and discussion:** Alterations of several proteins' expression between HKCSCs cultured in normoxia and hypoxia as well as between resistant and untreated cells have been shown.

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### LB-030

#### Production and characterization of antibodies to mycobacterial lipid antigens in rabbit

M. Shibata<sup>1</sup>, T. Naka<sup>2</sup>, S. Maeda<sup>3</sup>, N. Fujiwara<sup>4</sup>

<sup>1</sup>Department of Health and Nutrition, Faculty of Health Science, Kyo University, Koryo-cho, Kitakatsuragi-gun, Japan, <sup>2</sup>MBR Co. Ltd., Toyonaka City, Japan, <sup>3</sup>School of Pharmacy, Hokkaido Pharmaceutical University, Sapporo City, Japan, <sup>4</sup>Department of Food and Nutrition, Faculty of Contemporary Human Life Science, Tezukayama University, Nara City, Japan

The mycobacterial cell wall is rich in lipids, and the major component is mycolic acid. Cord factor is trehalose-6,6'-dimycolate, and is correlated with the formation of serpentine cords, that is the characteristic morphology of mycobacteria. We reported that anti-cord factor antibody was significantly increased in tuberculous patients, and the antigenic epitope was mycolic acid. On the other hand, the cell-wall skeleton of *Mycobacterium bovis* BCG (BCG-CWS) is composed of peptidoglycan-arabinogalactan-mycolic acid complex, and is a candidate for therapeutic agent of bladder cancer. To clarify the features of the anti-lipid antibodies in tuberculosis, it was performed to produce anti-lipid antibodies in rabbit immunized with mycolic acid-containing glycolipids (cord factor, BCG-CWS) and BCG whole bacteria as antigen. The single-immunization with BCG-CWS and cord factor in rabbit was not enough to the production of lipid-specific antibodies, and the production of the antibodies increased by the booster after 4 weeks. As a result, anti-lipid antibodies in rabbit were produced by the immunization with mycolic acid-containing glycolipids (BCG-CWS and cord factor). Anti-BCG-CWS antibody reacted to cord factor and arabinogalactan (AG), implying that both mycolic acid and AG were epitopes of this antibody. Anti-BCG-CWS antibody was produced in not only the sera of tuberculous patients but also those of healthy donors. The immunization of BCG whole bacteria is more advantageous for anti-lipid antibodies production, compared to those of BCG-CWS and cord factor alone.

### LB-031

#### Are primary and secondary metabolism in *Artemisia alba* moderated by the endogenous cytokinins levels *in vitro*?

S. Krumova<sup>1</sup>, T. Andreeva<sup>1</sup>, T. Oreshkova<sup>2</sup>, V. Motyka<sup>3</sup>, P. Dobrev<sup>3</sup>, M. Todorova<sup>4</sup>, A. Trendafilova<sup>4</sup>, N. Petrova<sup>1</sup>, S. Taneva<sup>1</sup>, K. Danova<sup>4</sup>

<sup>1</sup>Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria, <sup>2</sup>Institute of Biology and Immunology of Reproduction 'Acad. Kiril Bratanov', Bulgarian Academy of Sciences, Sofia, Bulgaria, <sup>3</sup>Institute of Experimental Botany AS CR, Prague, Czech Republic, <sup>4</sup>Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

*Artemisia alba* is a medicinal plant distributed in Southern Europe. The addition of plant growth regulators (PGR) to the growth medium *in vitro* was shown to be a tool for affecting plant morphogenesis, physiological status, as well as for targeted production of terpenoids with plausible medicinal applications (1).

In this work we study how the applied PGR influence both the primary (photosynthesis) and secondary (terpenoids) metabolism in *Artemisia alba* cultures. For this purpose we correlated the changes in the level of the endogenous cytokinins (detected by LC/MS chromatography) with: (i) the changes occurring in the photosynthetic apparatus with an accent on the macroorganization of the pigment-protein complexes involved in the light reactions of photosynthesis (characterized by circular dichroism, flow cytometry and atomic force microscopy) and the functionality of photosystem II (judged by the rate and yield of the oxygen evolution) and (ii) the plants terpenoid profile (1).

We demonstrate that plants with altered mono- and sesquiterpenoids levels also exhibit modified thylakoid membrane morphology and functionality and identify a fraction of "swollen" thylakoids which is hypothesized to indicate an early stage of senescence-like response. Our findings indicate that primary and secondary metabolism interrelations might be mediated by endogenous phytohormone levels (bioactive cytokinins in particular).

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#### References

Danova et al. (2012) Nat Prod COM 7: 1075-1076.

### LB-032

#### Correlation between the enzymatic and non-enzymatic antioxidant protection systems in *Artemisia alba* cultures

Y. Raynova<sup>1</sup>, S. Krumova<sup>2</sup>, T. Andreeva<sup>2</sup>, K. Idakieva<sup>1</sup>, Y. Markovska<sup>3</sup>, E. Wolfram-Schilling<sup>4</sup>, K. Danova<sup>1</sup>

<sup>1</sup>Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, <sup>2</sup>Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria, <sup>3</sup>Faculty of Biology, Sofia University "St Kliment Ohridski", Sofia, Bulgaria, <sup>4</sup>Zürcher Hochschule für angewandte Wissenschaften (ZHAW), Life Sciences und Facility Management – Institut für Biotechnologie, Wädenswil, Switzerland

*Artemisia alba* Turra is a fragrant shrub distributed in the southern Europe. The aerials of the plant are used in traditional medicine as a tonic and for treating intestinal disorders. In this work we study the impact of exogenously applied plant growth regulators (PGR) on the total protein profile, the architecture of thylakoid membranes and the enzyme activities in *A. alba* shoot cultures.

We have found that the aerials are rich in proteins and exhibit well developed photosynthetic (thylakoid) membrane system. The application of indole-3-butyric (IBA) acid alone led to formation of small granas and emergence of new SDS-PAGE bands in the aerials, which were absent upon application of IBA and benzyl adenine (BA). BA was found to increase the protein content in the aerial parts but to lower it in the roots and callus. The latter samples displayed marked differences in their electrophoretic profile as compared to the aerials. The native PAGE enzyme activity staining, revealed presence of antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, polyphenoloxidase, peroxidase). Data are in accordance with the activities of antioxidant enzymes obtained spectrophotometrically.

In conclusion, the application of PGR in *A. alba* *in vitro* leads to changes in the electrophoretic profile of samples derived from the plants aerials and roots and in the thylakoid membrane morphology. The changes in the polyphenol content correlated with molecular markers of lipid peroxidation and oxidative stress, suggesting a link between the enzymatic and non-enzymatic antioxidant protection of the plant *in vitro*.

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### LB-033

#### Identification of neutrophil elastase IL-36 $\gamma$ processing small molecules inhibitors

P. Davidovich<sup>1</sup>, E. Belotcerkovskaya<sup>1</sup>, S. Sura-Trueba<sup>1</sup>, S. J. Martin<sup>1,2</sup>

<sup>1</sup>Saint-Petersburg Institute of Technology, Laboratory of Cell Biotechnology, Saint-Petersburg, Russian Federation, <sup>2</sup>Trinity College, Dublin, Ireland

IL-36 $\alpha$ , IL-36 $\beta$ , IL-36 $\gamma$  are members of the extended IL-1 family that play a key role in inflammatory responses. Similar to most members of the IL-1 family, IL-36 cytokines require proteolytic processing at their N-termini for acquisition of biological activity. Upon activation, IL-36 cytokines signal through the IL-36R/IL-1RAcP complex, initiating the synthesis of a battery of pro-inflammatory cytokines and chemokines. Because IL-36 cytokines have been strongly implicated in psoriasis, strategies aimed at inhibiting their activation may have therapeutic utility in this condition. Recently, it has been found that neutrophil-derived elastase processes human IL-36 $\gamma$ , which increases the activity of this cytokine over 100-fold. Thus, small molecule inhibitors of neutrophil elastase may represent a promising approach for the treatment of psoriasis. To this end, we screened a library of small molecules to seek compounds capable of inhibiting the proteolytic activity of the latter protease. Using *in silico* docking analysis, we selected 149 potential elastase inhibitors. Using a synthetic substrate of elastase (Suc-AAPV-MCA), we identified a new family of inhibitors of this protease. These compounds were subsequently tested for their ability to inhibit elastase-mediated IL-36 $\gamma$  processing using a HeLa<sup>IL-36R</sup> reporter system, which responds to active forms of IL-36 by secreting pro-inflammatory

### LB-034

#### Syntenin silencing in cancer cells induces G<sub>0</sub>/G<sub>1</sub> cell cycle arrest and downregulates the expression of CDK4, cyclin D2 and Retinoblastoma protein

R. Kashyap<sup>1,2,3</sup>, R. Ghossub<sup>2,3</sup>, F. Lembo<sup>2,3</sup>, B. Roucourt<sup>1</sup>, P. Zimmermann<sup>1,2,3</sup>

<sup>1</sup>Laboratory for Signal Integration in Cell Fate Decision, Department of Human Genetics, Leuven, Belgium, <sup>2</sup>Centre de Recherche en Cancérologie de Marseille (CRCM), Aix-Marseille Université, Marseille, France, <sup>3</sup>Inserm, U1068; Institut Paoli-Calmettes; CNRS, UMR7258, Marseille, France

Syntenin functions as a rate limiting factor to allow the escape from degradation of syndecan heparan sulfate proteoglycans and can thereby support sustained signaling of a plethora of growth factors and adhesion molecules. Syntenin controls early developmental movements in vertebrates. In adulthood, syntenin reactivation or gain of function has been associated with the metastatic potential of melanoma and a growing number of cancers. More recently, syntenin has been reported to support breast cancer tumor growth. Here we aimed to clarify the impact of syntenin loss of function on cancer cell proliferation using cells from various origin and syntenin shRNA and siRNA silencing approaches. We found that in the mouse melanoma cell line B16F10, the human colon cancer cell line HT-29 and the human breast cancer cell line MCF-7, syntenin not solely controls migration but also proliferation and the ability to form colonies and to grow in soft agar. Using the MCF-7 cell line, we further document that syntenin controls G<sub>0</sub>/G<sub>1</sub> progression and the expression of CDK4, cyclin D2 and Retinoblastoma protein. These data highlight that syntenin supports tumor cell proliferation independently of their origin and reinforce its attractiveness as potential therapeutic target.

### LB-035

#### LRR8 heteromers form an essential component of the volume-regulated anion channel VRAC

T. Stauber<sup>1,2,3</sup>, F. K. Voss<sup>2,3</sup>, F. Ullrich<sup>2,3</sup>, J. Münch<sup>2,3</sup>, K. Lazarow<sup>2</sup>, N. Mah<sup>3</sup>, D. Lutter<sup>2,3</sup>, M. Andrade<sup>3</sup>, J. P. v.Kries<sup>2</sup>, T. J. Jentsch<sup>2,3</sup>

<sup>1</sup>Freie Universität Berlin, Institute for Chemistry and Biochemistry, Berlin, Germany, <sup>2</sup>Leibniz Institut für Molekulare Pharmakologie (FMP), Berlin, Germany, <sup>3</sup>Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany

Regulation of cell volume is pivotal for many cellular and organismal functions, such as during osmotic changes and cell growth, division and migration. A key player in this process, the volume-regulated anion channel (VRAC), opens upon cell swelling and conducts chloride and arguably organic osmolytes. Although VRAC has been vastly described and characterized by electrophysiological means, its molecular identity has remained