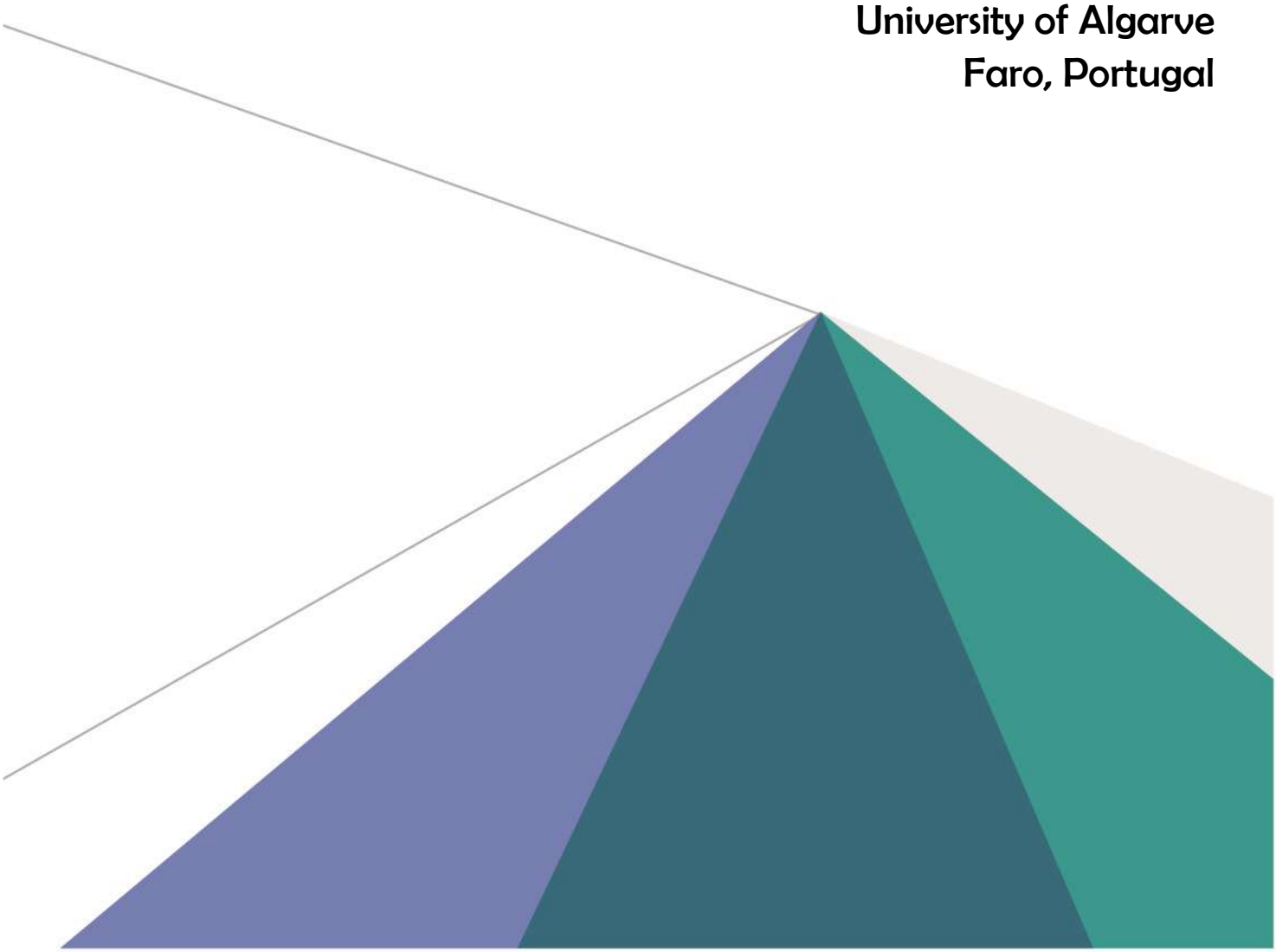
COST CM1406 Epigenetic Chemical Biology

Scientific Workshop
18 – 20 February 2019

University of Algarve
Faro, Portugal



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The Centre of Marine Sciences (CCMAR) and the Faculty of Sciences and Technology of the University of Algarve host the Epigenetic Chemical Biology COST meeting. The gathering will take place at the University of Algarve (Campus de Gambelas, Building 8), from the 18th to the 20th February 2019.

Epigenetics refers to dynamic changes that occur at the DNA, RNA and protein level in eukaryotes. Epigenetics is at the heart of gene regulation and determines which genes are activated or silenced. It is of great importance fundamentally and has many exciting translational aspects including therapeutics, diagnostics, stem cell research, microbial pathway engineering and agriculture.

The key objective of the Epigenetic Chemical Biology COST Action is to establish the first European chemical biology network focused on epigenetics.

This will provide common ground for researchers from academia, research institutes, SMEs and multinational organizations. The fruitful interactions between these sectors will lead to the creation of new chemical tools as well as leads for translational applications that impact upon human society. The Action's second objective is to increase awareness of epigenetics within the European scientific community and it will provide training for ESRs and emphasize involvement of COST inclusiveness targeted countries.

Scientific Coordinator:
A. Ganesan
School of Pharmacy, University of East Anglia

Local organizing committee:

Maria de Lurdes Cristiano
Faculty of Sciences and Technology, University of Algarve and Centre of Marine Sciences

Jorge Graça
Centre of Marine Sciences

Lília Cabral
Faculty of Sciences and Technology, University of Algarve

Alina Secrieru
Faculty of Sciences and Technology, University of Algarve

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PROGRAMME

Monday, 18 February 2019

0900-0930 Registration

0930-1000 Welcome address by Local Organizer Maria de Lurdes Cristiano and Action Chair A. Ganesan

Session chair: Marianne Rots

1000-1030 The effects of dıfferent types of calorıe restrıctıon on braın tıssue miRNA profıles of breast cancer mouse model
Bilge Tuna, School of Medicine, Yeditepe University, Istanbul, Turkey

1030-1100 The effects of different types of calorie restriction on miRNA profiles in breast cancer mouse model; comparing blood vs local breast tissue results
Soner Dogan, School of Medicine, Yeditepe University, Istanbul, Turkey

1100-1130 Coffee

1130-1200 Gene expression changes in early life neurodevelopment and sexual dimorphic brain differentiation in zebra finch telencephalon require different levels of epigenetic plasticity
Wim Vanden Berghe, Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

1200-1230 Mapping genome-wide DNA methylation patterns in gliomas in context of IDH gene mutation status and REST transcription factor binding
Bartosz Wojtas, Nencki Institute of Experimental Biology, Warsaw, Poland

1230-1400 Lunch

Session chair: Cristina Pereira

1400-1430 Epigenetic activation of microbial natural product biosynthesis
Mohammed Aldholmi, School of Pharmacy, University of East Anglia, Norwich, United Kingdom

1430-1500 Bioactivity of dioxins, genotoxic and epigenetic changes in mammary epithelial cells of lactating mothers
Bayram Yilmaz, Department of Physiology, Yeditepe University, Istanbul, Turkey

1530-1530 Action dissemination activities
Marianne Rots, Action Vice Chair

1530-1600 Coffee

1600-1630 WG1, WG2 and WG3 meetings chaired by WG leaders

1630-1830 Action Management Committee meeting

Session chair: Carmen Jeronimo

0900-0930 TERT regulation in cancer: a potential therapeutic target
Pedro Castelo-Branco, Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal

0930-1000 Using a multiple myeloma 3D co-culture model to screen for HDAC6 inhibitors
Micaela Freitas, School of pharmaceutical sciences, University of Geneva, Geneva, Switzerland

1000-1030 Mitochondrial dysfunction and non-canonical cell death induced by a selective sirtuin 1/2 inhibitor as a novel anticancer approach
Michael Schnekenburger, Laboratory of Cancer Molecular and Cell Biology, Kirchberg Hospital, Luxembourg

1030-1100 KDM5 inhibition is a novel therapeutic strategy for the treatment of KMT2D mutant lymphomas
Graham Packham, Southampton General Hospital, Southampton, United Kingdom

1100-1130 Coffee

1130-1200 The challenge and lessons of targeting DNA
Paola Arimondo, Institut Pasteur, Paris, France

1200-1230 Dual inhibition of two erasers: A COST success story
A. Ganesan, School of Pharmacy, University of East Anglia, Norwich, United Kingdom

1230-1400 Lunch

Session chair: Paola Arimondo

1400-1430 www.3d-qsar.com: a portal that allow to build 3-D QSAR models by means of any electronic device
Rino Ragno, Department of Pharmaceutical Chemistry and Technology, Sapienza Università di Roma, Roma, Italy

1430-1500 Total synthesis and bioactivity of (–)-parthenolide and its stereoisomers
Robert Freund, Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University, Jena, Germany

1500-1530 14-3-3 inhibitors for the treatment of chronic myeloid leukaemia resistance
Leire Iralde, Department of Biotechnology, chemistry and pharmacy, University of Siena, Italy

1530-1600 Coffee

1600-1800 Sightseeing in Faro

Session chair: Maria de Lurdes Cristiano

0900-0930 Epigenetic and non---epigenetic functions of dRYBP, a ubiquitin binding protein, in development and disease
Ana Busturia, Centre for Molecular Biology, Autonomous University of Madrid, Madrid, Spain

0930-1000 Genome-wide DNA methylation profiling of atherosclerotic patients in whole blood identifies inflammaging-associated biomarkers
Ken Declerck, Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

1000-1030 Molecular characterisation of Paraoxonase 1 SNP specific epigenetic modulation of obesity phenotypes (NAFLD) in response to pesticides or medical intervention strategies
Sara Diels, Department of Biomedical Sciences, Antwerp University, Antwerp, Belgium

1030-1100 Genome-wide methylome reprogramming in cancer cells by expression of an heterologous DNA demethylase
María Victoria García-Ortiz, Maimónides Biomedical Research Institute, Cordoba, Spain

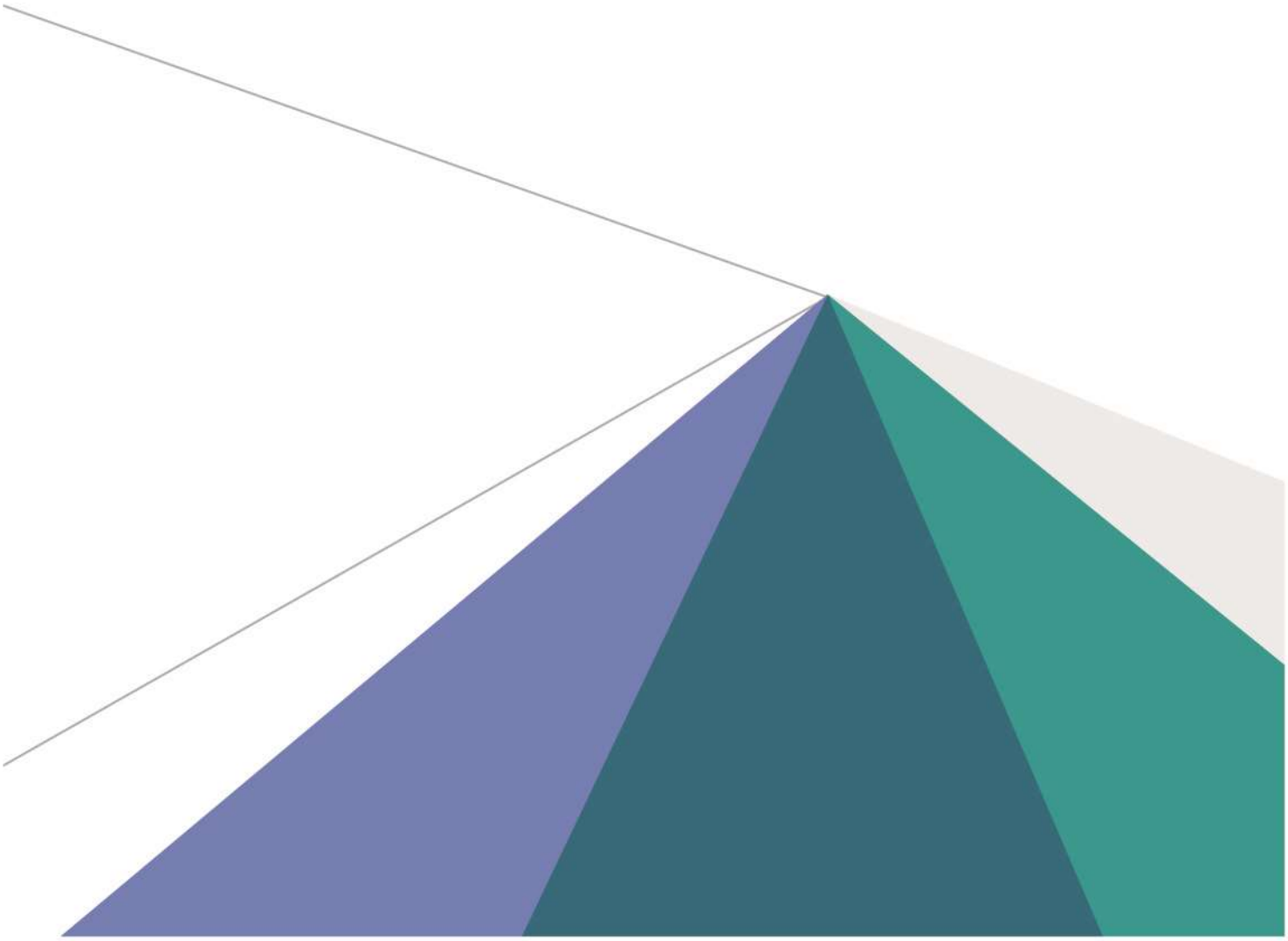
1100-1130 Coffee

1130-1200 Biological control of the ash dieback pathogen *Hymenoscyphus fraxineus* by endophytes
Frank Surup, Helmholtz Centre for Infection Research, Braunschweig, Germany

1200-1230 Closing remarks

1230-1400 Lunch

Abstracts



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Epigenetic activation of microbial natural product biosynthesis

Mohammed Aldholmi[1,2] and A. Ganesan[1]

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[2] Natural Products and Alternative Medicine, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University (University of Dammam), Dammam 31441, Saudi Arabia

Most genes responsible for the production of secondary metabolites are cryptic (silent) under laboratory conditions. The epigenetic processes including the covalent modifications of DNA and amino acids on histones have been revealed to be extensively used by organisms to regulate the expression of genes involved in natural product biosynthesis (1,2). Hence, it is possible to influence these processes during microbial fermentation by the addition of chemical epigenetic modulators (2–5).

Five *Aspergillus* strains were cultivated in the presence of HDAC inhibitors (e.g. vorinostat) or DNMT inhibitors (e.g. 5-Azacytidine). Morphological changes were monitored for seven days before extraction with Ethyl acetate or Methanol. The extracts were analysed by HPLC and LC-MS, and the metabolic profiles were compared using chromatogram overlay and Volcano plots. (6).

The overlayered chromatograms and volcano plots presented significant differences between the cultures treated with epigenetic modifiers and control cultures, in addition to differences in morphology and colour. The extract from one of the fungal cultures contained two secondary metabolites significantly induced with 5-Azacytidine and slightly induced with vorinostat. Interestingly, the induction of these metabolites was doubled when the culture was treated with both 5-Azacytidine and vorinostat suggesting a synergistic effect of these modifiers on secondary metabolism. MS analysis of the first metabolite provided molecular ions in both positive and negative mode corresponding to M+H (331.1950) and M-H (329.1840), respectively. The second metabolite was only ionizable in the negative mode providing a molecular ion with a mass of 681.3420. Databases were searched using the accurate mass and UV λ max, but no natural products were found corresponding to both accurate mass and UV profile of the induced compounds. Therefore, large-scale fermentation is in progress to isolate the compounds and elucidate their structure. Subsequently, the pure compounds will be tested for antifungal, antibacterial and cytotoxic activity.

I would like to thank Imam Abdulrahman Bin Faisal University for financial support to do my PhD in the UK.

Keywords: Natural products, epigenetic, secondary metabolites, fungi

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The challenge of targeting DNA methylation and its lessons

Paola B. Arimondo[a], Ludovic Halby[a] and Marie Lopez[b]

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Currently there is a large interest in the role of epigenetic changes in human diseases and great expectations from drugs bridling these changes. The epigenetic approach is a strategy of choice since it participates to gene regulation in living organisms and processes integrating the impact of the environment and contributing to cell plasticity [1]. Indeed, these modifications have an impact on genome activity and participate to modulate gene expression without altering the DNA sequence. Here we will discuss the limits and challenges of the chemical strategies that target these modifications and in particular DNA [3,4]. Then we will show examples of the design of compounds that modulate the methyltransferases of DNA[5] and their impact on the cancer phenotype[6]. We will show how the same chemical strategy can be applied to histone methyltransferase targeting[7]. Finally, we will present the use of these compounds as chemical probes to study the molecular mechanisms responsible of the aberrant methylation profiles.

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Epigenetic and non-epigenetic functions of dRYBP, a ubiquitin binding protein, in development and disease

Ana Busturia[1], Sol Fereres, Ricardo Aparicio, Carolina Simoes da Silva, Rocio Simón, Amanda Regojo, Jacobo Solorzano

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Epigenetic regulation of gene expression requires the function of protein complexes whose specific biochemical activities post-translationally modify histones. These modifications, such as histone mono-ubiquitylation, affect chromatin compaction and, consequently activation or repression of gene expression. Until recently it was thought that the epigenetically implemented “silenced” or “active” transcriptional states are fixed and permanently inherited. However, it now seems that these transcriptional states can be reversed, indicating plasticity of epigenetic regulation.

The RYBP (Ring and YY1 Binding Protein) protein, known in *Drosophila* as dRYBP, is a phylogenetically conserved epigenetic factor with ubiquitin binding activity. Studies showing that the RYBP/dRYBP protein functions in epigenetic regulation as well as studies in flies showing that it has the capacity to modulate the “silenced” or “active” transcriptional states by chromatin dependent mechanisms will be presented. Additionally, evidence supporting the roles for the RYBP/dRYBP protein in biological processes such as apoptosis mediated by chromatin- and transcription-independent mechanisms will also be presented.

Work in flies and mice support the idea of the importance of maintaining, perhaps through microRNAs regulation, homeostatic levels of RYBP/dRYBP and of other Polycomb and trithorax proteins to achieve normal development. We have began a long term project to investigate the Polycomb-microRNA regulatory circuit and its physiological role in organ communication. Preliminary work in progress will be presented.

TERT regulation in cancer: a potential therapeutic target

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Cancer is a group of diseases that present significant heterogeneity and distinct clinical outcomes. Limitless self-renewal is one of the hallmarks of cancer and is achieved by telomere maintenance, essentially through telomerase (h*TERT*) activation.

We have previously shown that a specific region in the hTERT promoter (termed THOR) is specifically hypermethylated in malignant cancers and hypomethylated in normal tissues and in low grade tumors lacking hTERT expression. These findings generated several important clinical and biological questions and led us to hypothesize that THOR is a cancer signature and may represent a diagnostic tool and a therapeutic target in cancer.
We established an international consortium aiming to analyze THOR in a wide variety of tumors and found that THOR shows frequent cancer-specific DNA methylation in tumor samples (*n*=1,342) of various cancer types including cancers that exhibit frequent TERT Promoter mutations. Also, THOR in its unmethylated state represses TERT promoter activity and its methylation counteracts this repressive function. Specifically, THOR adds significant prognostic information to current histopathological parameters in both Gleason 6 and 7 prostate cancer patients and in non-muscle invasive bladder cancers it is associated with higher *TERT* expression and higher-risk disease. Finally, preliminary results indicate that it is possible to specifically demethylate THOR using CRISPR/Cas9-based constructs with a consequent alteration in TERT expression.

Blood surrogate epigenetic biomarkers of atherosclerosis reveal common signature of inflamm-aging-disorders

Ken Declerck[1], Geoffrey Istas[1,2], Maria Pudenz[4], Katarzyna Szarc vel Szic[1,5], Veronica Lendinez-Tortajada[6], Montserrat Leon-Latre[7,8], Karen Heyninck[9], Guy Haegeman[9], Jose A. Casasnovas,[8,10,11] Maria Tellez-Plaza,[12] Clarissa Gerhauser[4], Christian Heiss[2], Ana Rodriguez-Mateos[2,3], Wim Vanden Berghe[1,9]

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DNA methylation is the most well-known epigenetic modification of the DNA. This epigenetic mark is crucial in controlling gene expression profiles, maintaining cellular identity, genomic imprinting and X-chromosome inactivation. Furthermore, DNA methylation is plastic and can adapt to environmental stimuli, acting as a cellular memory of past events. Whereas epigenetic DNA methylation profiling in cancer diagnostics is now well established, associations with other chronic age-associated diseases, including obesity, diabetes, cardiovascular and neurological diseases just start to be explored for prognostic, diagnostic and therapeutic applications.

Upon genome-wide DNA methylation profiling of whole blood samples of atherosclerotic patients, we characterized various atherosclerosis specific differentially methylated regions (DMRs). Interestingly, similar DMRs were also observed in other age- and inflammation-associated diseases, like obesity, cancer, Alzheimer's and Parkinson's disease, both in blood as well as in brain and tumor tissues. This suggests that inflammaging diseases share a common epigenetic signature of the immune system, which is different from the classic epigenetic clock signature. Furthermore, a cardio-protective flavanol-rich diet intervention can partially reverse this inflammaging disease associated epigenetic pattern. We found that this methylation profile mainly reflects shifts in immune cell type composition and infiltrating immune cell populations. Upon correcting for differences in immune cell composition in blood samples, we identified BRCA1 DNA methylation as a atherosclerosis-specific methylation biomarker irrespective of variations in immune cell biomarkers. How BRCA1 DNA methylation differentially promotes cancer, neurodegeneration or atherosclerosis pathologies needs further investigation.

In conclusion, atherosclerosis patient blood samples reveal inflammaging and atherosclerosis-specific DNA methylation biomarkers, which could potentially be used as lifestyle biomarkers to estimate disease risk of neurodegeneration, cardiometabolic disorders and cancer in aging populations.

A multi-omics approach to elucidate risk factors for obesity and associated liver pathology

Sara Diels[1], Ken Declerck[2] , An Verrijken[3], Eveline Dirinck[3], Sven Francque[4], Luc Van Gaal[3], Wim Vanden Berghe[2], Wim Van Hul[1]

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Obesity is a multifactorial disorder in which excess body fat has accumulated as a result of the chronic imbalance in energy homeostasis. Although key factors in the disease's origin are a high caloric diet and lack of physical activity, interactive influences of numerous factors are involved. An emerging hypothesis proposes that genetic factors within susceptibility genes interact with environmental factors through epigenetic regulation. A potential candidate for this is the paraoxonase 1 (PON1) gene. Research has identified a protective role of PON1 against adverse environmental exposure, obesity, and its comorbidities non-alcoholic fatty liver disease and atherosclerosis. Associations between the clinical metabolic phenotype and PON1 genetic variants, epigenetic DNA methylation variation, gene expression profile and enzyme activity are being examined in an obese patient cohort. Preliminary results associate promoter polymorphism -108C/T with liver cell injury parameters (p_{SAF} = 0.011; $p_{ballooning}$ = $4.13x10^{-4}$) and indicated a link between increased methylation and adverse lipid metabolism (p_{LDL}=0.073; p_{TG}=0.022). This pilot study highlights the importance of integrating genetic and epigenetic data to unravel the etiology of complex diseases. Expansion of our data set will enable us to further investigate the onset and development of metabolic alterations in obesity.

The effects of different types of calorie restriction on miRNA profiles in breast cancer mouse model; comparing blood vs local breast tissue results

Soner Dogan[1], Munevver Burcu Cicekdal[1], Umit Ozorhan[1], Isın D. Ekici[2], Emre C. Tuysuz[3], Aysegul Kuskucu[3], Omer F. Bayrak[3], Bayram Yilmaz, Bilge G. Tuna[4]

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Beneficial effects of calorie restriction (CR) has been shown in variety of pathophysiological conditions such as diabetes, asthma, neurological diseases, cardiovascular diseases (CVD), ageing and cancer. In general, two different types of CR have been applied in studies; chronic calorie restriction (CCR) and intermittent calorie restriction (ICR). Although CCR have been applied in most studies, the beneficial effects of ICR have been studied in less extend with conflicting results. No matter what the outcome of these studies are, the exact molecular mechanism of CR in physiological and/or pathophysiological conditions remains to be elucidated. The aim of the present study was to test and compare the miRNA profile in blood tissue and breast tissue under the application of two different types of CR methods; chronic calorie restriction (CCR) and intermittent calorie restriction (ICR) in MMTV-TGF-α transgenic mouse model which is post menapousal breast cancer mouse model starting at week 10 until week 82 of mouse age. Transgenic mice were enrolled in three different dietary groups; ad libitum (AL), CCR (15% of CR application compared to AL group) or ICR (one week 60 % CR application following three weeks AL feeding in cyclic manner) groups. Animals were observed weekly to see MT development. Blood samples and Mammary Fat
Pad (MFP) of animals were collected at weeks 10, 49/50 and 81/82 of mouse age to check MT development by pathological analysis. Afymetrix microarray method was used to analyze microRNA expression. Chi-square test was used to analyze MT incidence rates and grades. Although 21.4% (12/56) and 20.4% (18/88) of AL and ICR groups, respectively developed MT, only 8.7% (5/57) of CCR group of mice developed MT. For each sample total of 3,195 miRNA was analyzed (ebayes ANOVA, n=3). Then, Ingenuity Pathway Analysis (IPA) were used to investigate the roles of miRNAs specifically in adiponectin and/or leptin signalling pathways which were reported to play important roles in MT development. The miRNA profile in blood samples and mammary tissue samples of the same animals will be compared and presented in my talk. Changes with ageing (blood vs tissue) will also be presented.

Using a multiple myeloma 3D co-culture model to screen for HDAC6 inhibitors

Micaela Freitas, Muriel Cuendet

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Multiple myeloma is a hematological cancer that despite not having a strong incidence, displays a high rate of relapse and resistance to conventional therapies. It is therefore very important to find new therapeutic strategies to overcome both situations. In order to study and explore new treatments, a model that closely represents the disease should be used. Therefore, a 3D co-culture model, which includes multiple myeloma cancer stem cells, malignant plasma cells (RPMI 8226 cells) and cells from the microenvironment, mesenchymal stem cells, was established. This composition brings the model closer to the cellular and molecular complexity found *in vivo*. Currently, most multiple myeloma treatments involve the use of proteasome inhibitors. Resistance to those is mostly mediated by HDAC6 through the aggresome pathway. Panobinostat, a pan-HDAC inhibitor, is very effective overcoming proteasome resistance, however, it exhibits several unpleasant side effects. The lack of specificity of this inhibitor may be the reason for the toxicity and side effects. HDAC6 selective inhibitors could be the solution for such situation. By screening HDAC6 inhibitors in this 3D model, not only the individual response of the malignant cells is considered, but also how the spheroid, as an entity, behaves when exposed to treatment of single or combination therapies. Ultimately, this model can be combined with several techniques, providing information such as viability and protein expression of the different cell populations. This model is a very useful tool to identify compounds with potential to treat resistant multiple myeloma and quickly move from pre-clinical experiments to clinical trials.

Total synthesis and bioactivity of (–)-parthenolide and its stereoisomers

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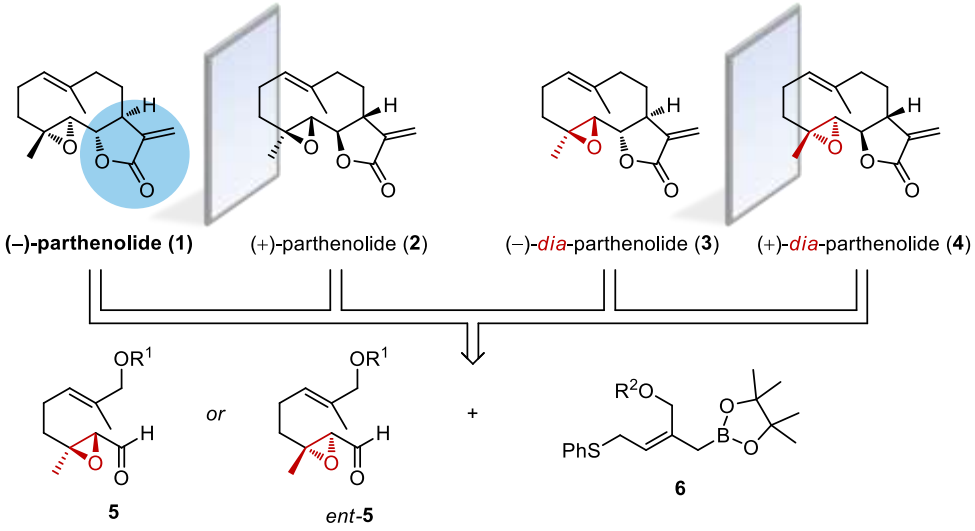

Natural products and their manipulation by synthesis are highly important for target identification and validation. In this regard, the germacrane sesquiterpene parthenolide is associated with broad anti-inflammatory and anticancer activities, *e.g.* by inhibition of NF-κB activation,[1] alteration of DNA methylation (DNMT1 inhibition),[2] and induction of oxidative stress.[3] Recently, it has been found to be a direct inhibitor of tubulin carboxypeptidase[3,4] and was reported as a highly potent (nM) stimulator for neuroregeneration *in vivo*.[5] However, only (–)-parthenolide is available from nature and synthetic access has been limited.[6–8] In this contribution, we disclose the first stereoselective total synthesis of parthenolide that enables access to all its stereoisomers by a unified work-flow.[9] It employs an allylboration of aldehydes **5**/*ent*-**5** by the novel trisubstituted allylboronate reagent **6**, with excellent stereocontrol. Furthermore, an efficient ring closure of the strained 10-membered ring scaffold was realized (80% yield). These results enabled the investigation of both the anti-inflammatory activity as well as tubulin carboxypeptidase inhibition of parthenolide stereoisomers **1**–**4**.

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Dual inhibition of two erasers: A COST success story

Adam Lee, A. Ganesan

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Eraser enzymes that return post-translationally modified lysine residues back to lysine have important physiological roles and their dysregulation is implicated in many disease conditions. The zinc-dependent histone deacetylases (HDACs) are an important example, currently with five approved drugs for haematological cancers. Another are the lysine-specific demethylases (LSDs) against which there are multiple compounds in various stages of clinical development.
In the presentation, we will describe the design and synthesis of a dual HDAC-LSD inhibitor with nanomolar potency against both targets, and in cell-based assays. Furthermore, the compound is a selective inhibitor of HDAC6 and LSD1 over other HDAC or LSD isoforms. The biological profiling was carried out through collaborations within the Action, with M. Berdasco (ES), S. Gul (DE), A. Kawamura (UK) and K. Nikolic (RS).

Genome-wide methylome reprogramming in cancer cells by expression of an heterologous DNA demethylase

María Victoria García-Ortiz[1,2,3], Teresa Morales-Ruiz[1,2,3], Iván Devesa-Guerra[1,2,3], Laura Raya-Ruiz[1,2,3], Juan R. Tejedor[4,] Gustavo F. Bayón[4,] Marta I. Sierra[4,] Mario F. Fraga[4,5,] Rafael R. Ariza[1,2,3,] and Teresa Roldán-Arjona[1,2,3,]

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Patterns of DNA methylation are disrupted in cancer cells. Understanding the functional significance of aberrant methylation in tumors remains challenging, due in part to the lack of suitable tools to actively modify methylation patterns. In animal cells, DNA demethylation involves iterative 5mC oxidation by TET enzymes followed by replication-dependent dilution and/or replication-independent DNA repair of its oxidized derivatives. In contrast, plants use a specific family of DNA glycosylases that directly excise 5mC from DNA and initiate active DNA demethylation through a base excision repair (BER) pathway. Here we show that expression of *Arabidopsis* 5-meC DNA glycosylase DME in colon cancer cells (DLD-1) initiates an active DNA demethylation process that involves the BER pathway. Interestingly, DME expression causes genome-wide changes that include both processes DNA methylation lost and gain, not randomly distributed over the genome, and linked through developmentally regulated silencing programs. DME expression partially restores the methylation pattern observed in normal tissue. Furthermore, such methylome reprogramming is accompanied by altered cell cycle responses and increased sensibility to anti-tumor drugs. Our study shows that it is possible to reprogram a human cancer DNA methylome by expression of a plant DNA demethylase.

14-3-3 inhibitors for the treatment of chronic myeloid leukaemia

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14-3-3 proteins are a highly conserved and ubiquitous family of proteins with a relative mass of 30 kDa. To date, there have been identified seven closely related isoforms conserved across mammalian species (β, ε, η, γ, τ, ζ and σ) organised mainly as homo- or heterodimers.[1] They play a key role in multiple regulatory processes such as signal transduction, cell cycle regulator, protein trafficking, metabolism, or control of apoptosis among others[2] by interaction with hundreds of proteins partners preferentially in a phosphorylation-dependent binding mode that recognizes the motifs RSXpS/TXP.[3]

c-Abl is a tyrosine kinase implicated in the regulation of proliferation, adhesion, motility and cell survival. After phosphorylation on the Thr735 residue, it binds to 14-3-3 proteins (isoforms σ and ζ) that sequester c-Abl into the cytoplasm where it induce proliferation and survival.[4] Whereas, DNA damage or oxidative stress lead to disruption of the c-Abl/14-3-3 complex, promoting nuclear translocation of the c-Abl protein that results in inhibition of grow signals, cell cycle arrest and activation of apoptosis.[5] The oncogenic form of c-Abl, Bcr-Abl, is the causative of the development and progression of chronic myeloid leukemia (CML) and is localize exclusively in the cytoplasm inducing proliferation and inhibiting apoptotic cell death.[6]

The aim of this project is to find small molecules that by disruption of the 14-3-3/c-Abl interaction could counterbalance the action of the Bcr-Abl kinase and restore the c-Abl regulatory function, promoting nuclear translocation of c-Abl and thus inducing apoptosis in CML cells. Inside the Botta's research group a series of small molecular weight compounds were designed, synthesized and subsequently validated by biophysical techniques as soft inhibitors of the 14-3-3/c-Abl interaction. Furthermore, the new molecules have demonstrated to possess antiproliferative activity against K562 cells and enhance the nuclear translocation of c-Abl at low micromolar concentrations.

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KDM5 inhibition is a novel therapeutic strategy for the treatment of KMT2D mutant lymphomas

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Loss-of-function mutations in KMT2D are a striking feature of the germinal centre lymphomas, resulting in decreased H3K4 methylation and altered gene expression. We hypothesised that inhibition of the KDM5 family, which normally opposes KMT2D through demethylating H3K4me3/2, would re-establish H3K4 methylation and restore the expression of genes repressed upon loss of KMT2D. KDM5 inhibition (KDM5i) increased H3K4me3 levels and caused an anti-proliferative response in vitro that was markedly greater in both endogenous and CRISPR-edited KMT2D mutant DLBCL cell lines, whilst tumour growth was inhibited in KMT2D mutant xenografts in vivo. Mechanistically, KDM5i appeared to drive a reactivation of KMT2D target genes, resulting in diminished B-cell receptor signalling and altered expression of BCL2 family members, including BCL2 itself. KDM5i may offer an effective therapeutic strategy for ameliorating KMT2D loss-of-function mutations in malignancies such as germinal centre lymphomas.

www.3d-qsar.com: A portal that allows to build 3-D QSAR models by means of any electronic device

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The underlying idea of any field based 3-D QSAR is that differences in a target propriety, e.g. biological activity, are often closely related to equivalent changes in shapes and intensityies of non-covalent calculated interaction surrounding the molecules (also called molecular interaction fields, MIFs). This concept was introduced in 1988 by Cramer et al with the well known Comparative Molecular Field Analysis (CoMFA). The procedure to build a MIFs based 3-D QSAR model involves the following steps: training-set selection, alignment of molecules' conformations, MIF calculation, statistical model definition, model validation and graphical interpretation. All of the above listed 3-D QSAR model building steps have been deeply investigated and several protocols have been reported and can be performed using different software so that using a single data set different 3-D QSAR models can be built obtaining similar and overlapping results. Although, to perform all the steps any user is asked to install specialized software either costly or even open source that require the user to have informatics skills. Here, it is presented very first 3-D QSAR series of web applications, accessible to all non profit organizations for free, and by which 3-D QSAR models can be easily build and graphically analyzed. Web applications are included to perform all the needed building steps and all accessible through the www.3d-qsar.com electronic address. Py-MolEdit enables the compilation of the data set through either uploading a list of molecules in any babel recognized format or by direct drawing through a java script molecular editor. Py-ConfSerch contains different conformational analysis engines to generate conformational ensembles for each dataset molecules. Py-Align through automatic molecular alignment software leads to molecular alignment on up to 16 pre-defined different templates conformations or user selected ones. Finally, Py-CoMFA web application allow the building and validation of the 3-D QSAR model in the same fashion of the original CoMFA software. Different tools are available to inspect the models' results either numerically or graphically all through a standard web browser and without the need to install any additional program. The portal can be used by any electronic device that can surf the internet as it has been designed to be fully responsive. The server is continuously under development for further applications also in the structure-based area or pharmacophoric approaches. Details and examples of all the web applications will be reported.

Mitochondrial dysfunction and non-canonical cell death induced by a selective sirtuin 1/2 inhibitor as a novel anticancer approach

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Sirtuins (SIRT) 1-7 are class III histone deacetylases (HDAC) that require nicotinamide adenine dinucleotide (NAD^+) as a cofactor to deacetylate lysines of histone and non-histone substrates. Sirtuin activities are linked to many cellular processes including regulation of gene repression, mitochondrial metabolism, cell proliferation and survival. Since SIRT1 and 2 are frequently overexpressed in cancer and the modulation of their activity appears to have beneficial effects on human diseases, there is a growing interest in the discovery of small molecule sirtuin inhibitors [1]. We recently identified a benzopyran, compound 18 (also named 711), displaying potent and selective *in vitro* inhibitory activity against SIRT1 and 2 ($IC_{50}\cong5$ µM) [2] and inducing histone and α-tubulin hyperacetylation starting in a low micromolar range in various cancer models. Furthermore, treatments with 711 strongly inhibited cancer cell proliferation ($GI_{50}\cong2$ µM after 72h of treatment) accompanied by G1 phase cell cycle arrest and reduced expression of cancer-relevant proteins (*e.g.* Mcl-1, PCNA, survivin, c-myc). Furthermore, we observed compound 711-induced ATP depletion and declined mitochondrial respiration associated with an accumulation of amorphous substances with high-electron-density in mitochondria, cristae disruption and swelling observed by transmission electron microscopy. These mitochondrial dysfunctions were accompanied by an important intra- and extracellular acidification. Chronic exposure to this compound led to reduced cancer cell viability through induction of controlled necrosis. Finally, we demonstrated that 711 has a minimal effect on healthy cell models such as hematopoietic $CD34^+$ stem cells. Taken together, this SIRTi represents a promising compound for future anti-cancer drug development by targeting mitochondrial metabolism and triggering non-canonical types of cell death, especially in cancer resistant to apoptosis and/or with metabolic addiction.

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Biological control of the ash dieback pathogen *Hymenoscyphus fraxineus* by endophytes

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Ash dieback, which has been spreading across the European continent, is threatening the very existence of *Fraxinus excelsior* L., the European ash, in continental Europe and Great Britain. *Hymenoscyphus fraxineus* (Hf) is the causal agent of ash dieback, and originates from eastern Asia, where it colonizes *Fraxinus mandshurica* asymptomatically. Biocontrol of Hf by fungal endophytes is a potential method for combating ash dieback. Our prerequisites for an antagonist are: a) synthesis of metabolites that inhibit growth of Hf, b) asymptomatic growth within *F. excelsior*, c) in planta prevention of ash dieback.
Fungal endophytes were isolated from leaves, stems and roots of *F. excelsior* and identified by multi-DNA-locus molecular phylogeny and microscopic methods. In dual culture with the pathogen, most inhibited growth of Hf. Surprisingly, Hf inhibited growth of most endophytes, suggesting that in planta there is metabolic competition between pathogen and endophyte. Of particular interest are endophytes that inhibit Hf, but themselves are barely or not at all inhibited by the pathogen, e.g. *Hypoxylon rubiginosum*. Structures from endophytes that fulfill our criteria have been elucidated and will be presented.
Our results suggest that in planta the microbial inhabitants of ash combat each other. Interactions between endophytes and pathogen are not the only ones in which toxic metabolites are involved. Hf also produces metabolites toxic to its host: viridiol (Andersson et al. 2010), volatile lactones (Citron et al. 2014), lytic enzymes (Junker et al. 2017) and the antibiotics hymenosetin and hyfraxins (Halecker et al. 2014; Surup et al 2018). Before Hf invaded Western Europe, “chemical warfare” between the microbial inhabitants and with their host was in equilibrium. Since Hf has upset this balance, we aim to restore this balance by inoculating ash trees with an endophyte that produces metabolites toxic to *H. fraxineus*.

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The effects of dıfferent types of calorıe restrıctıon on braın tıssue miRNA profıles of breast cancer mouse model

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The beneficial effects of calorie restriction on brain ageing and breast cancer development is well accepted. The hypothalamus is a brain region responsible from nutrient-related signals and energy balance. There has been shown a link between hypothalamic brain-derived neurotrophic factor (BDNF) and tumor inhibition via down regulation of leptin expression in adipocytes. Micro RNAs (miRNAs) are small non-coding RNA molecules with ranging in the size of 17-22 nucleotides which regulate about 30% of the human mRNA. The aim of this study was to understand the changes in miRNA profile in brain tissue of ageing mice usually developed mammary tumors in later stage of their life.

For this purpose, micro RNA profile was measured by using Affymetrix GeneChip™ miRNA 4.1 Array Strip in whole brain tissue samples collected from MMTV-TGFα mice into 3 different feeding protocols at weeks 10 and 82. Ad-Libitum (AL), Chronic Calorie Restriction (CCR, 15 % CR application) and Intermittent Calorie Restriction (ICR) group which were fed AL for three weeks, and following one week 60% restriction was applied from week 10 until week 82 of mouse age. Mice were sacrificed and tissues were collected at 10 and 82 weeks of mice. Total of 3163 miRNAs (pre- and mature) were screened and 53 of which were differentially expressed in diet and ageing (at least twofold change, $p < 0.05$). Compared to the week AL, 5 miRNAs were differentially expressed (4 up, 2 down regulated) in CCR, 4 miRNAs were differentially expressed (2 up, 2 down regulated) in ICR-R, 12 miRNAs were differentially expressed (3 up, 9 down regulated) in ICR-RF. In addition, 29 miRNAs were differentially expressed at week 81 compared to week 10 including miR-184-3p and miR-17-3p.

Epigenetic sensitisation of drug resistant cancers by the electrophilic steroid Withaferin A

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Withaferin A (WA) isolated from *Withania somn*ifera (Indian ginseng or Ashwagandha in Ayurvedic medicine) has recently become an attractive steroidal phytochemical under investigation in various preclinical studies for cancer treatment. Pharmacological levels of WA trigger clinically relevant anticancer effects specific to triple negative breast cancer cells, glucocorticoid therapy resistant multiple myeloma and therapy resistant neuroblastoma.
Genomewide DNA methylation, gene expression and pathway enrichment analysis revealed epigenetic suppression of multiple cancer hallmarks associated with cell cycle regulation, cell death, cancer cell metabolism, cell motility and metastasis. By means of peptide array based tyrosine phosphopeptide fingerprinting and kinome activity profiling, we found that WA inhibits various redox sensitive tyrosine kinases which are hyperactivated in hormone therapy resistant cancer cells, including TEC, BTK and HCK kinases. Furthermore quantitative chemoproteomics approaches identified covalent WA binding to epigenetic enzymes and revealed that the superior cancer therapy efficacy by WA is a consequence of simultaneous targeting of various cellular stress response pathways like proteasome degradation, lipid peroxidation, autophagy and unfolded protein stress responses which altogether exceed the cellular redox homeostasis capacity and promote noncanonical ferroptosis. Nano-targeting or prodrug formulations of WA further allow systemic application and suppressed tumor growth due to an enhanced accumulation at the tumor site. Collectively, our data propose a novel therapeutic strategy to efficiently kill heterogenous multidrug resistant cancers cells by a natural plant derived steroid WA.

Further reading:

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Mapping genome-wide DNA methylation patterns in gliomas in context of *IDH* gene mutation status and REST transcription factor binding

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Methylation of DNA regulatory regions influence gene expression. Alterations of methylome play an important role in the glioma pathogenesis. Here we have identified differentially methylated sites in gliomas of different histopathological WHO grades (I,II,III and IV) or *IDH* gene mutation status (n = 21). We used bisulphite conversion and SeqCap Epi CpGiant Methylation panel with Illumina NGS sequencing. Additionally ChIPseq analysis for REST transcription factor was performed on chromatin isolated from freshly resected glioma tumors as well as from IDH mutated and paired isogenic WT cell line. For the same brain tumor samples RNA-seq was performed. We have detected differential pathways affected in IDH mutant and WT samples. We have also noted differential REST transcription factor binding to differentially methylated promoters. We believe that REST may be a mediator of IDH-related phenotype in human gliomas.

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Bioactivity of dioxins, genotoxic and epigenetic changes in mammary epithelial cells of lactating mothers

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Persistent organic pollutants (POPs) threaten the environment and human health. These chemicals are transferred to humans through food chain and bio-accumulate in fat tissue because of their lipophilic features. Methylation of DNA promoter region is considered to be an important biomarker for breast cancer risk. DNA methylation may be related to biologically accumulated chemicals through dietary, environmental and occupational exposure. Our project was designed to investigate epigenetic changes in exfoliated human mammary epithelial cells depending on dioxin exposure, age and number of pregnancy and nursed children. Breast milk (10 ml) was collected from 200 healthy lactating mothers in Istanbul. Volunteers were asked to fill a questionnaire form with demographic, medical and nutritional information. The study protocol was approved by the Yeditepe University clinical ethics committee.

In exfoliated epithelial cells, promoter methylation of four tumor suppressor genes (RASSFF1, GSTP1, CDH1, and SFRP1) was determined by pyrosequencing of bisulfide-modified DNA. DNA damage was assessed by using comet assay (alkaline single-cell gel electrophoresis). Dioxin and estrogenic bioactivity in the milk fat was evaluated by reporter gene assay. Our results have shown that the breast milk samples may contain POPs which are both dioxin-like and genotoxic. A significant correlation was observed between Body Mass Index and genotoxicity profiles ($p<0.01$). Nursing and rate of pregnancy may have a critical effect on mehthylation of tumor suppressor genes. In this presentation, genotoxicity, methylation, xenoestrogenic and dioxin-like bioactivity findings will be discussed in relation to demographic, nutritional and medical information of the lactating mothers.

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