XXII Congreso de la SEMA
13-15 Junio 2016
Barcelona

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13-15 Junio 2016
Barcelona

Sede del congreso - Conference venue

Auditorio Fèlix Serratosa

Parc Científic de Barcelona

Edificio Claster I

C/ Baldiri i Reixac 4-12


Parc Científic de Barcelona
c/ Baldiri Reixac, 4-12 y 15
tel. +34 934 029 060
info@pcb.ub.cat
Accesos al PCB
Recepciones
Edificio Clúster I
Entrada por Baldiri Reixac, 10
Edificio Clúster II
Entrada por Baldiri Reixac, 4-8
o Av. Doctor Marañon, 8
Torres R+D+I
Entrada por Baldiri Reixac, 4-8
o Av. Doctor Marañon, 8
Edifici Hèlix
Entrada por Baldiri Reixac, 15
Restaurante Fifteen
Acceso libre
Parking
Acceso libre
Zona exclusiva para peatones
AVINGUDA DIAGONAL
BALDIRI REIXAC
AV. DOCTOR MARAÑÓN
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PASCUAL I VILA
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Facultad de Matemáticas y Estadística (UPC)
Facultad de Bellas Artes (UB)
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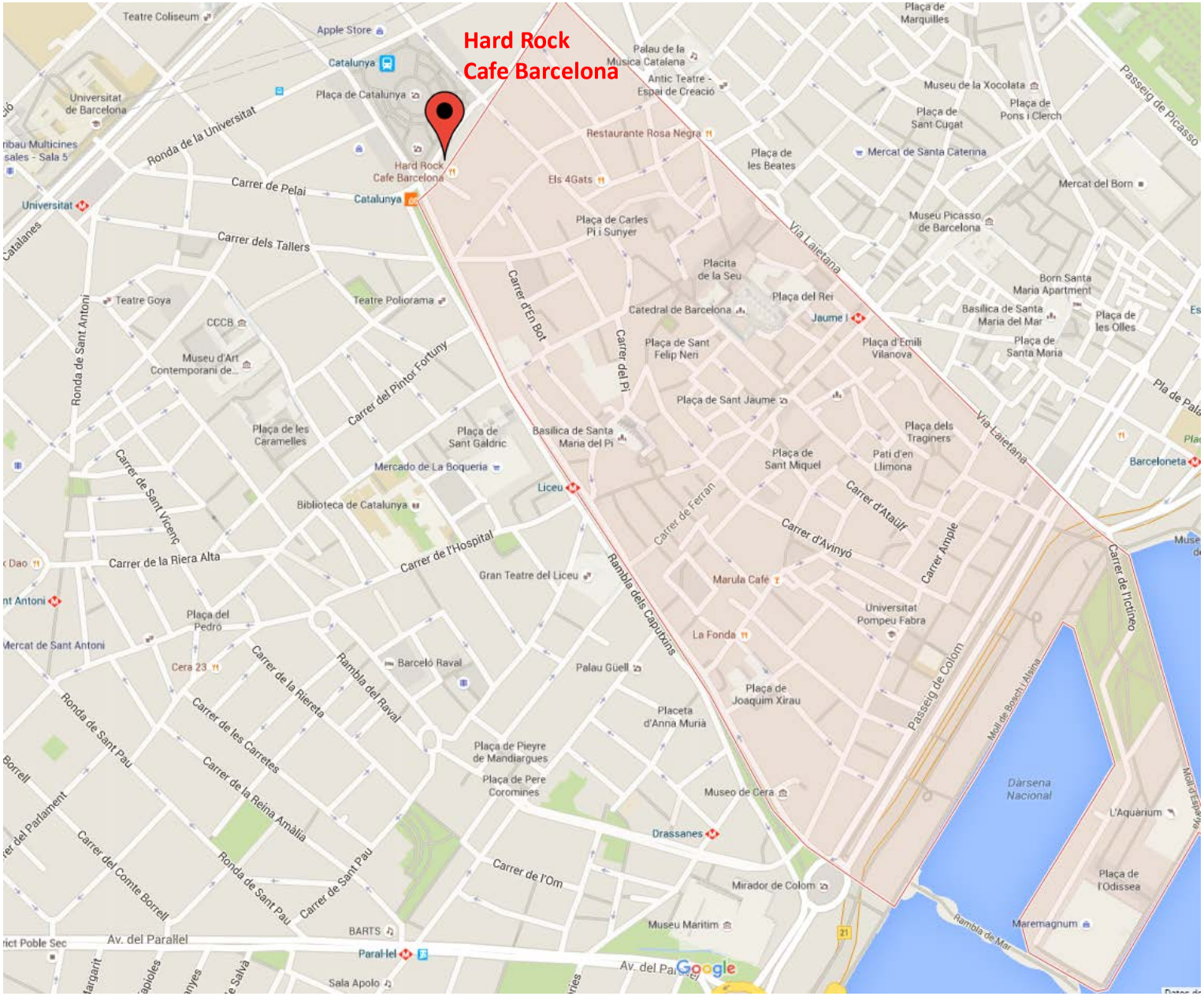
Edificio Clúster II
Edifici Clúster I
Torres R+D+I
CAMP NOU
L97
T1-T2-T3 Palau Reial
L3 Palau Reial
7-33-67-75-113-L14-L79-H6
L79-L97-H6
60-67-L79-L97
7-67-75-L14
10 km
L3 Zona Universitària
L97-H6-H69
7-54-67-75-113
L97-L79-L14
T1-T2-T3 Zona Universitària
54
54-113
T1-T2-T3 Av. Xile
113-L12
Parada de metro
L3 Palau Reial
L3 Zona Universitària
Parada de Tram
T1-T2-T3 Palau Reial -
Zona Universitària - Av. Xile
Parada de Autobús
7, 33, 54, 60, 67, 75, 113,
L12, L14, L79, L97, H6, H69
Parada de Bicing
310 Josep Samitier con Joan XXIII
El aeropuerto del Prat está a 10 km del PCB.
Para desplazarse en tren, se puede enlazar con
la línea 1 de rodalies (RENFE) que llega a la Estación de Sants,
desde donde podéis enlazar con el metro (Línia 3).
Es necesario cogerlo en dirección "Zona Universitària"
y bajar en la parada "Palau Reial".
En taxi, el trayecto aproximado es de 20 minutos.

LUGAR DE INICIO DEL TOUR

HARD ROCK CAFE BARCELONA

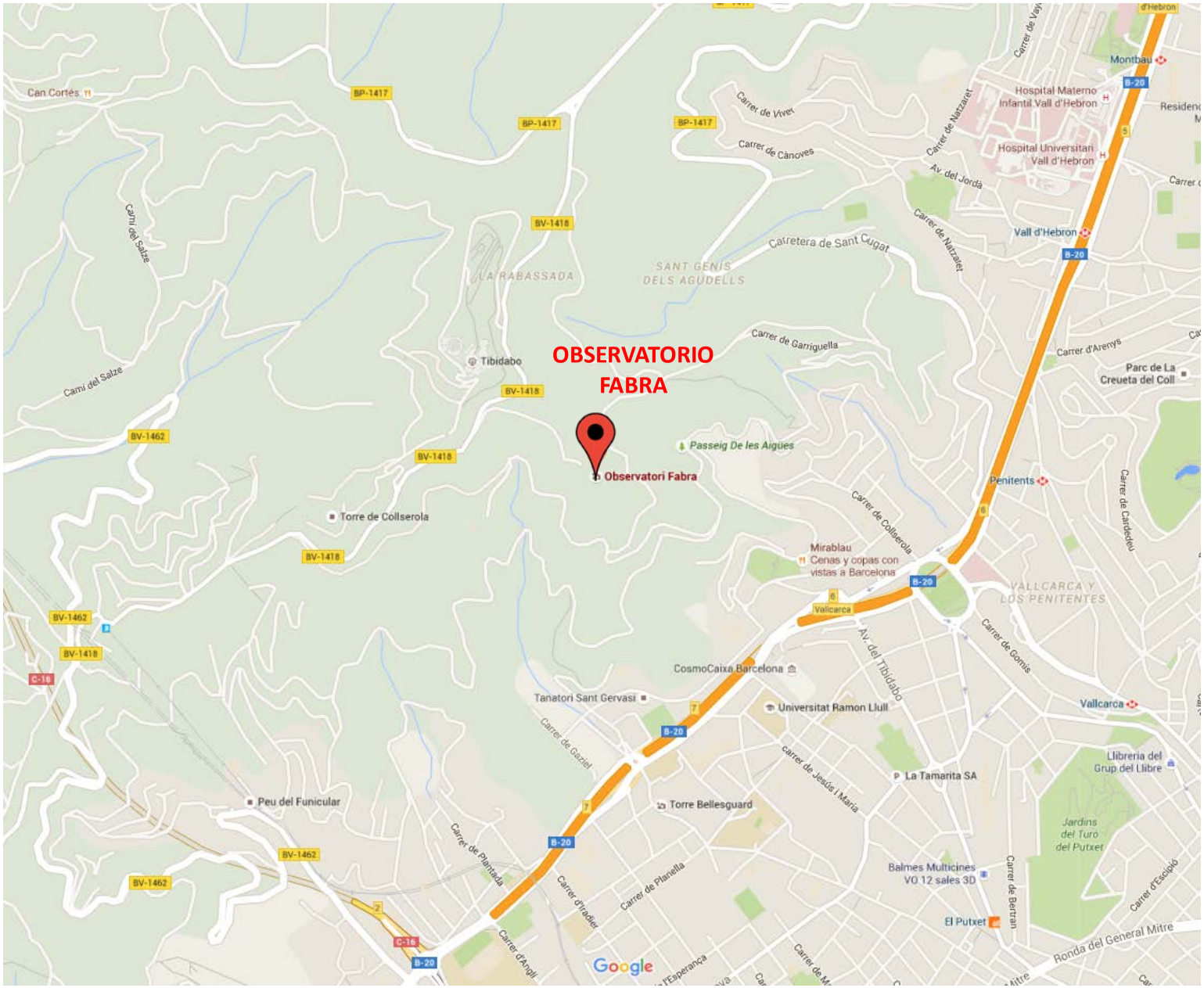
Plaça Catalunya, 21,
08002, Barcelona



CENA DEL CONGRESO

OBSERVATORIO FABRA
Camí de l'Observatori, s/n,
08001 Barcelona

Si el tiempo lo permite, la cena se celebrará en una terraza exterior. Se recomienda llevar algo de ropa de abrigo.



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PROGRAMA CIENTÍFICO – SCIENTIFIC PROGRAMME

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15:00 - 15:30 Registro y entrega de documentación - Registration

15:30 - 16:00 Acto de Inauguración Oficial de la Reunión - Opening Ceremony

Antonio Guzmán Cano, Presidente del Comité Organizador

16:00 - 18:30 Sesión I: Tecnologías aplicadas en genotoxicología: epigenética y tecnologías omicas - Session I: Genotoxicology applied technologies: epigenetics and omics technologies

Moderadores - Chairs

Amadeu Creus Capdevila (Universitat Autònoma de Barcelona)

Joaquín de Lapuente (Grupo AC Marca)

16:00 *Reactivation of epigenetically-silenced genes by the CRISPR Technology*

Iván Devesa Guerra (Universidad de Córdoba)

16:20 *Drug screening to reduce genome instability in Fanconi Anemia*

Helena Montanuy (Universitat Autònoma de Barcelona)

16:40 *Modelling the Fanconi anemia/BRCA pathway and functional analysis of genetic variants by TALEN and CRISPR-Cas9*

Nuria Muñoz (Universitat Autònoma de Barcelona)

17:00 - 17:30 Café - Coffee

17:30 *Sex- and time-dependent gene expression profile in kidneys of F344 rats after repeated OTA oral administration*

Ariane Vettorazzi (Universidad de Navarra)

17:50 *Evaluation of sex-dependent kidney oxidative stress response to ochratoxin A in F344 rats using the comet assay in combination with FPG*

José Manuel Enciso (Universidad de Navarra)

18:10 *Oxidative and genotoxic damage drop after arsenic-induced malignant transformation. Involvement of As3mt and Mth1*

Jana Peremartí (Universitat Autònoma de Barcelona)

18:30 *Iberoamerican Network of Toxicology and Chemical Safety*

Eduardo de la Peña (CSIC)

19:00 - 22:00 Visita guiada a pie por el Barrio Gótico de Barcelona – Guided Walking City tour through Barcelona´s Gothic Quater

MARTES 14 DE JUNIO – TUESDAY JUNE 14

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Moderadores - Chairs

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Luisa María Sierra Zapico (Universidad de Oviedo)

9:30 *Cisplatin resistance analysis: Insights on the usefulness of accurate DNA adducts quantitation*

Marta Espina (Universidad de Oviedo)

9:50 *Are halonitromethanes in water disinfection by-products potential human tumoral agents?*

Alicia Marsà (Universitat Autònoma de Barcelona)

10:10 *Assessment of genotoxic and carcinogenic potential of long-term exposure to monohaloacetic acids*

Constanza Cortés (Universitat Autònoma de Barcelona)

10:30 *The role of Fra-1 in arsenic-induced cell malignant transformation*

Irene Barguilla (Universitat Autònoma de Barcelona)

10:50 *Repair of DNA damage induced by alkylating agents in Arabidopsis thaliana*

Casimiro Barbado (Universidad de Córdoba)

11:10 - 11:40 **Café - Coffee**

11:40 *Tumor suppressor C53 interacts with BRCA2, regulates DSB repair and drives breast and ovarian cancer prognosis*

Jordi Minguillón (Universitat Autònoma de Barcelona)

12:00 *Effect of CeONP co-treatment in tobacco exposed lung stroma cells*

Carlos Camilleri (Universitat Autònoma de Barcelona)

12:20 *TBX15 as an antiapoptotic factor in cancer cells*

Jessica Arribas (Universitat Autònoma de Barcelona)

12:40 *Loci associated with genomic damage levels in Spanish chronic kidney disease patients*

Zuray Corredor (Universitat Autònoma de Barcelona)

13:00 *The non-canonical SOS-system of DNA-repair and mutagenesis in Acinetobacter baumannii*

Jesús Aranda (Universitat Autònoma de Barcelona)

13:30 - 15:30 Almuerzo - Lunch

16:00 - 18:25 Sesión III: Evaluación de genotoxicidad, la perspectiva de la industria - Session III: Genotoxicity assessment, an industry perspective

Moderadores – Chairs

Antonio Guzmán Cano (Esteve)

Ricard Marcos Dauder (Universitat Autònoma de Barcelona)

16:00 *Application of a Multiplexed Flow Cytometric Assay and Machine Learning to Provide Genotoxic Mode of Action Information*

Jeffrey Bemis (Litron Laboratories)

16:25 *Regulatory genotoxicity: from the bench to the Dossier*

David López (Innoqua)

16:50 *Mutagenicity prediction using in silico Methods: Gigabyte-size Petri dishes*

Antonio Sánchez (Lhasa Limited)

17:15 - 17:45 Café – Coffee

17:45 *Is there space for an industry focused research in academia? I. What can academia offer.*

Adela López de Cerain (Universidad de Navarra)

18:05 *Is there space for an industry focused research in academia? II. An industry point of view*

Antonio Rodríguez (Esteve)

18:30 - 19:30 Asamblea SEMA – SEMA Assembly

20:30 Cena – Dinner

Observatorio Fabra, Barcelona

MIERCOLES 15 DE JUNIO – WEDNESDAY JUNE 15

9:30 - 12:45 Sesión IV: Nanogenotoxicología - Session IV: Nanogenotoxicology

Moderadores – Chairs

Eduardo de la Peña de Torres (CSIC)
Antonio Rodríguez Fernández de Henestrosa (Esteve)

9:30 *Genotoxicity assessment of silica-coated iron oxide nanoparticles in human astrocytes*

Natalia Fernández-Bertólez (Dicomosa & Universidade da Coruña)

9:50 *In vitro evaluation of the genotoxicity of polymeric nanoparticles as carriers for oral drug administration*

Tamara Iglesias (Universidad de Navarra)

10:10 *Comet assay as a method for genotoxic assessment of engineered nanomaterials*

Alba García (Universitat Autònoma de Barcelona)

10:30 - 11:00 Café - Coffee

11:00 *Evaluation of flow cytometry to detect micronucleus induction by different nanoparticles in BEAS 2B cells*

Liliya Kazantseva (Universitat Autònoma de Barcelona)

11:20 *Caco-2 cells as an in vitro model to determine detrimental effects on the intestinal barrier. Studies with Ag-NPs at sub-toxic concentrations*

Laura Vila (Universitat Autònoma de Barcelona)

11:40 *Role of copper oxide nanoparticles as antigenotoxic agents. Studies in Drosophila*

Mohamed Alaraby (Universitat Autònoma de Barcelona)

12:00 *Evaluation of the genotoxic potential of silver nanoparticles in Drosophila melanogaster*

Sara Romero (Universitat Autònoma de Barcelona)

12:30 Clausura – Closing Ceremony

SESIÓN I: TECNOLOGIAS APLICADAS EN GENOTOXICOLOGIA: EPIGENÉTICA Y TECNOLOGIAS ÖMICAS – SESSION I: GENOTOXICOLOGY APPLIED TECHNOLOGIES: EPIGENETICS AND OMICS TECHNOLOGIES

Reactivation of epigenetically-silenced genes by the CRISPR Technology

Iván Devesa Guerra, Jara Teresa Parrilla Doblas, Teresa Morales Ruiz, Mª Victoria García Ortiz, Rafael Rodríguez Ariza and Teresa Roldán Arjona.

Departamento de Genética, Universidad de Córdoba. Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC). Hospital Universitario Reina Sofía, 14071, Córdoba, Spain.

The CRISPR technology has revolutionized the world of genetic editing, allowing the manipulation of the genomes of diverse organisms quickly and easily. The CRISPR (clustered regularly interspaced short palindromic repeats) methodology derives from a bacterial adaptive immune system that uses an RNA-guided nuclease (Cas9) to target and destroy invading DNA. The use of a catalytically inactive nuclease (dCas9) co-expressed with a short guide RNA (sgRNA) allows using CRISPR/dCas9 as a general platform for RNA-guided targeting of different effector proteins to specific genomic regions. Fusion of dCas9 to effector domains with epigenetic functions can be used for targeted transcriptional regulation in human cells.

To confirm that the CRISPR system allows targeted reactivation of epigenetically silenced genes, we fused dCas9 either to the transcriptional activation domain VP160, or to the catalytic core of the human p300 acetyltransferase. The targeted activity of both fusion proteins, co-expressed with different sgRNAs in human HEK293 cells, was tested on a luciferase reporter gene previously silenced by *in vitro* methylation.

Luciferase reporter assays and expression analysis by qRT-PCR showed that specific combinations of sgRNAs efficiently targeted dCas9-VP160 and dCas9-p300 for reactivation of the silenced luciferase gene. Bisulfite pyrosequencing revealed that such reactivation does not correlate with a decrease in methylation levels, suggesting that other epigenetic mechanisms are involved in the process.

Our results establish that the CRISPR system can be used as a modular and flexible DNA-binding platform for the recruitment of epigenetic effector proteins to a target DNA sequence, revealing the potential of CRISPR as a general tool for the precise regulation of gene expression in eukaryotic cells.

Notas - Notes

Drug screening to reduce genome instability in Fanconi Anemia

H Montanuy, MJ Ramírez, J Minguillón, JA Casado, B Díez, J Bueren, J Surrallés.

Genome Instability and DNA Repair Group, Universitat Autònoma de Barcelona and CIBER on Rare Diseases (CIBERER), Spain.

The only treatment of bone marrow failure (BMF) in Fanconi Anemia (FA) patients is hematopoietic stem cell (HSC) transplantation, which has a high survival rate but also important limitations including lack of available donor in many families and further increase in cancer risk in post-transplanted patients. In addition, while curative for BMF, HSC transplantation will not prevent solid tumours. In order to find new candidate drugs for preventing bone marrow failure and cancer in FA patients, we have adapted and scaled down the flow-cytometry micronucleus (MN) assay to 96 multiwell plates in order to screen large number of drugs in a short period of time. MN are biomarkers of chromosome fragility in FA as they derive from acentric chromosome fragments that are left behind in anaphase and appear in the cytoplasm of daughter cells as small nuclei. In addition, flow cytometry data allowed to check cell cycle progression which is also altered in FA cells. We initially selected 10 antioxidants and p38 inhibitors previously described in the literature as potentially beneficial for FA cells, including our previous studies in FA IPS cells (Liu et al., Nature Com 2014). We tested them for their ability to suppress spontaneous and DEB-induced chromosomal instability in FANCA-deficient cells. Our results show that antioxidants NAC, resveratrol and quercetin and anti-inflammatory drugs danazol and dasatinib reduced chromosome fragility to basal levels while also reducing G2/M arrest. Quercetin and danazol were the most effective. On the other hand, antioxidants tempol and α-lipoic acid did not exert a positive effect on chromosome fragility, nor did aldehyde chelant cysteamine, adh2 inductor alda-1 or anti-inflammatory drug doramapimod. We also tested 84 antioxidant compounds from a redox library and observed a beneficial effect of 22 additional drugs for their ability to reduce genomic instability in DEB, formaldehyde and/or acetaldehyde-treated FANCA-deficient cells. Drugs that reduced the damage caused by at least two chromosome instability inducers were tested for their ability to improve the spontaneous chromosome fragility and we are now assaying the selected drugs *in vivo* in FANCA KO mice, using the *in vivo* flow cytometry MN assay and checking for beneficial effects in terms of hematopoietic stem cell performance.

Notas - Notes

Modelling the Fanconi anemia/BRCA pathway and functional analysis of genetic variants by TALEN and CRISPR-Cas9

Muñoz Subirana N, Bogliolo M, Aza-Carmona M, Marín M,; Mina L, Ramírez M.J., Surrallés J.

Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain.

Fanconi anemia (FA) is a rare genome instability disorder clinically characterized by developmental abnormalities, high predisposition to cancer and bone marrow failure. Many of the available cell lines from FA anemia patients are difficult to grow, difficult to transduce and genetically manipulate and are not isogenic. Due to current limitations of FA cell models, we decided to knock-out three FA genes (*FANCA, FANCD1, FANCQ*) and one associated gene (*FAN1*), all of them implicated in the FA/BRCA pathway, in the genetically amenable and fast growing human HEK293T cell line. We used transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats with CRISPR associated 9 nuclease (CRISPR-Cas9) for gene KO. Specific gene disruption was proven by genetic complementation with the corresponding wildtype gene using lentiviral vectors or transient transfection. Variants of uncertain significance (VUS) were introduced in the corresponding cDNA and functionally studied by lentivirus mediated genetic complementation. We successfully generated *FANCA, FAN1, FANCD1* and *FANCQ* KO HEK293T cell lines by the use of engineered nucleases. All these cell lines are sensitive to DNA crosslinking agents, thus mimicking cells from patients with defects in the FA/BRCA pathway. Moreover, all FA genes KO cells get blocked in the G2/M phase of the cell cycle upon treatment with DNA crosslinkers, a typical FA cellular phenotype. Finally, these FA cell lines reproduce gene-specific defects: *FANCA-/-* cells have impaired FANCD2 monoubiquitination, *FANCD1-/-* cells are unable to form Rad51 foci after irradiation and are sensitive to PARP inhibitors, and *FANCQ -/-* cells are sensitive to UV-light radiation and were amenable for functional analysis of VUS.

Notas - Notes

Sex- and time-dependent gene expression profile in kidneys of F344 rats after repeated OTA oral administration

Pastor Laura, Enciso Jose Manuel, López de Cerain Adela, Vettorazzi Ariane.

Department of Pharmacology and Toxicology, Faculty of Pharmacy and Nutrition, University of Navarra, C/ Irunlarrea 1, 31008, Pamplona, Spain.

Ochratoxin A (OTA) is a natural mycotoxin produced by *Aspergillus* and *Penicillium* species with important implications for health. It is one of the most potent renal carcinogens studied in rodents, producing a higher incidence of tumors in male than in female rats. The aim of this study was to determine kidney gene expression profiles, to identify the pathways that may lead to these sex differences. For that purpose, male and female F344 rats were treated with a daily oral dose of 0.50 mg OTA/kg bw for 7 or 21 days. Gene expression was studied at basal level (male vs female controls) and after OTA treatment (treated vs control, per sex and timepoint).

In control groups, similar number of differentially expressed genes (DEG) showed sex-biased expression and 50% genes were common. 12 ToxLists were significantly altered and they were related with xenobiotic metabolism signaling, fatty acid metabolism, renal damage, nuclear factors signaling and cytochrome P450 panels.

OTA treatment increased the number of DEG over time in both sexes. In females, 528 and 2648 were DEG after 7 and 21 days respectively, and in general, a slightly tendency to downregulation was observed. In addition, 86.2% of total genes modified after 7 days were also altered after 21 days and showed the same pattern. In contrast, a higher response was observed in males than in females after 7 days, which was also different compared to 21 days. After 7 and 21 days 1088 and 2404 were DEG, but only 277 genes were common. Moreover, 82.3% of genes were upregulated after 7 days and a few of these common genes tended to downregulation after 21 days.

Less than 5% of altered genes of all groups, presented -1.5<logFC<1.5. Interestingly, the most sex-biased genes in control animals tended to be strongly downregulated in their predominant sex after OTA treatment: CYP2C9 and CYP27B1 in males and Akr1b7 and Ly6a in females.

Finally, concerning the ToxLists, females showed a similar response at both timepoints (21 common lists of 26 or 32): OTA produced changes in renal damage and toxicity, xenobiotic metabolism signaling and anti-oxidative response lists. Regarding males, 7 days group showed only 11 altered lists, being nuclear receptor signaling and glutathione depletion strongly modified. However, after 21 days, 37 lists were altered and renal damage, proliferation, fatty acid metabolism and AhR signaling were the most modified lists.

Notas - Notes

Evaluation of sex-dependent kidney oxidative stress response to ochratoxin A in F344 rats using the comet assay in combination with FPG

Enciso JM, Pastor L, Vettorazzi A, López de Cerain A, Azqueta A.

Department of Pharmacology and Toxicology, Faculty of Pharmacy and Nutrition, University of Navarra, Irunlarrea 1, 31008, Pamplona, Spain.

Ochratoxin A (OTA) is a secondary fungal metabolite that contaminates a great variety of foodstuffs. It is nephrotoxic in all animal species tested, considered a potent renal carcinogen in rodents and proposed as a possible etiological agent of the Balkan Endemic Nephropathy (BEN) in humans. Indeed, the International Agency for Research on Cancer (IARC) has classified OTA as a possible human carcinogen (group 2B). Its mechanism of action is still unknown, although oxidative stress appears to play an important role. Besides, large sex-differences have been observed in different carcinogenicity studies towards OTA-induced renal tumours.

Therefore, the objective of this study was to evaluate the sex-dependent kidney oxidative stress response to OTA in F344 rats. For that purpose, male and female F344 rats were administered by oral gavage with bicarbonate or 0.5 mg OTA/kg b.w. for 7 days, or with bicarbonate, 0.21 mg OTA/kg b.w. or 0.5 mg OTA/kg b.w. for 21 days. As this genotoxicity study was embedded in a bigger general toxicology study, the kidney samples for the comet assay were flash frozen by immersion in liquid nitrogen and stored at -80 °C until analysis. Therefore, the freezing and thawing processes of the kidney samples were also set up. The standard alkaline comet assay was used in combination with formamidopyrimidine DNA glycosylase (FPG), which detects oxidised bases.

The preliminary results of kidney samples of animals treated for 7 days will be presented. Taking into account these results, we are not able to conclude that ochratoxin A induces either single or double DNA strand breaks, or an increase of DNA oxidative damage following a sex-dependent pattern.

Notas - Notes

Oxidative and genotoxic damage drop after arsenic-induced malignant transformation. Involvement of *As3mt* and *Mth1*

Jana Peremartí[1], Jordi Bach[1], Ricard Marcos[1,2], Alba Hernández[1,2]

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[2]CIBER Epidemiología y Salud Pública, ISCIII, Spain.

Oxidative DNA damage (ODD) plays a crucial role in the carcinogenesis of the widespread and well-known human carcinogen arsenic. The key arsenic biotransformation enzyme As3mt is known to participate in the generation of ROS after a given arsenic exposure. Contrarily, Mth1 sanitizes oxidized dNTP pools to prevent incorporation of damaged bases into DNA.

In the present work, we sought to assess the role of *As3mt* and *Mth1* in the oxidative and genotoxic DNA damage generated throughout the arsenic-related transformation process. Thus, MEF cells previously transformed by 30 weeks of chronic arsenite exposure were monitored for ODD and DNA damage by the comet assay and the micronucleus assay, respectively, at different time-of-exposure intervals for 50 weeks. Expression changes of *As3mt* and *Mth1* were evaluated by real time RT-PCR at equivalent time-points. Epigenetic consequences were also analyzed.

Our results demonstrate that the oxidative and genotoxic damage of chronically exposed MEF cells increased time-dependently up to the point of transformation but dropped drastically afterwards. *Mth1* was responsible for the DNA damage decrease, as mRNA levels increased from basal to 13-33 -fold at the relapse time-point. On the other hand, *As3mt* expression followed a pattern similar to that of DNA damage. Global DNA hypomethylation was observed during the complete duration of the exposure.

As conclusion, we have demonstrated that *As3mt* and *Mth1* have differential roles in the accumulation of DNA damage linked to the transformation process. While *As3mt* acts as a sensor of damage contributing to the genotoxic effects before transformation, *Mth1* prevents the DNA damage fixation after the acquisition of the transformed phenotype. Interestingly, *Mth1* is proposed here as a new biomarker of arsenic carcinogenesis.

Notas - Notes

Iberoamerican Network of Toxicology and Chemical Safety

Oscar Herrero, Fernanda Cavieres, Rita C. Gutierrez, Araceli Píllco, Eduardo de la Peña

Mutagenesis Ambiental CSIC. Serrano 115 dpdo 28006 Madrid, Spain.

The Iberoamerican network of toxicology and chemical safety (RITSQ) was established in March 2008 and has had 79,268 visits to the website of the same of 53,759 users. Over 1,700 people from 81 countries have registered on the RITSQ and to date there have been 80 posters in presentation in meetings, and Conferences. Since its establishment we have consistently maintained the following objectives: 1. To coordinate the participation of various existing groups at universities and research organizations in Iberoamerica, involved in studies related with Toxicology, 2. To strengthen collaboration and academic exchange between PhD and Master programs in different Iberoamerica countries which have as their object the study and research in toxicology or related areas, 3. To encourage conducting joint research projects between teachers and researchers from Iberoamerica, student internships and academic events; 4. To research further the short and long term test methods used in the evaluation of carcinogenicity, mutagenicity and reproductive toxicity of substances and mixtures of chemicals, 5. To develop and standardize analytical methods for identification and identification of biomarkers of exposure, effect and susceptibility for substances and chemicals products on humans and the environment; 6. To apply methods of assessing the risk to human health and the environment of chemicals substances and products, 7. To promote scientific exchange of professionals interested food; and 8. To promote the use of alternative methods to animal experimentation (www.remanet.net).

The RITSQ continues to develop independently and their information is a link between the scientific and educational community in Iberoamerica, Portugal and Spain. Total visits to RITSQ over the years have increased from 9,609 in 2009 to 79,668 in 2016 from January to May, all these visits contribute to the dissemination of the event, collaboration, academic exchange and participation in teaching and research of existing groups at universities and research organizations in Iberoamerica, all involved in issues related to the development of Toxicology and promote the principle of the three Rs [3Rs] (refinement, replacement and reduction) and participate in the development of the application of alternative methods to the use of experimental animals.

Notas - Notes

SESIÓN II: DAÑO GENOTÓXICO, PROTECCIÓN Y REPARACIÓN – SESSION II: DNA DAMAGE, REPAIR AND PROTECTION

Cisplatin resistance analysis: Insights on the usefulness of accurate DNA adducts quantitation

Espina M.[1], M. Corte-Rodríguez[2], L. Aguado[1], M. Montes-Bayón[2], M. Sierra[3], P. Martínez-Camblor[4,5], E. Blanco-González[2], L.M. Sierra[1].

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[2]Dpt. of Physical and Analytical Chemistry, Faculty of Chemistry, C/Julián Clavería 8, 33006 University of Oviedo, Spain.
[3]Epigenetics Unit, Oncology University Institute (IUOPA), University of Oviedo, Spain.
[4]Hospital Universitario Central de Asturias (HUCA), Avenida de Roma, s/n, 33011 Oviedo, Spain.
[5]Universidad Autónoma de Chile, Carlos Antúnez 1920 Providencia, Santiago, Chile.

Although cisplatin (cis-diamminedichloroplatinum (II) or cDDP) is one of the most extensively, and rather successfully, chemotherapy drugs used in the treatment of several types of tumors, it presents the important drawbacks of toxicity and especially patient resistance. This resistance, acquired or innate, might be the result of several different processes that are ultimately related either with preventing DNA adduct formation, or with their fast removal from the exposed cell DNA. Thus, the accurate detection and quantitation of adducts might be a valuable tool in the early prediction of cisplatin resistance. Using four human cell lines of different origins and cisplatin sensitivities (A549, GM04312, A2780 and A2780cis), and low cisplatin doses (5, 10 and 20 μM for 3 hours), the relevance of DNA adduct levels as potential predictor of viability and apoptosis (used as resistance indicators) was studied alone and in combination with the intracellular Pt content, the induced genomic instability (measured with the comet assay), and the induced cell cycle alterations. Uni- and multi-variate linear regression analyses were used in this study. Cell viability, apoptosis and cell cycle changes were estimated 24 h after the end of treatment. Intracellular Pt content, adducts levels and genomic instability were determined immediately after treatment and also one hour later. Results show that cisplatin-induced G-G intra-strand adducts were detected at all concentrations and that they were the best predictor for viability and apoptosis in all the studied cell lines. The prediction improved when comet results were included in the analysis, especially in the ovarian A2780 and A2780cis cell lines.

Notas - Notes

Are halonitromethanes in water disinfection by-products potential human tumoral agents?

Alicia Marsà[1], Constanza Cortés[1], Elisabet Teixidó[1], Alba Hernández[1,2], Ricard Marcos[1,2]

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Disinfection by-products (DBPs) are formed during the process of water disinfection and represent a wide variety of chemical groups. Over the years DBPs exposure has been linked to a number of health effects, including an increased risk of bladder and colon cancer, reproductive failure and respiratory symptoms. Some DBPs species have been regulated in many countries; however, several chemical species with mutagenic capacity which potentially can affect human health remain unregulated.

Halonitromethanes (HNMs) represent one of the most abundant non-regulated groups. In contrast with previous studies conducted with these chemicals, which analyzed their genotoxic potential in short exposure periods, we determined the carcinogenic potential of two HNMs, bromonitromethane (BNM) and trichloronitromethane (TCNM), using a battery of *in vitro* genotoxicity assays in cells chronically exposed to these compounds.

Inhalation has been proven to be one of the main DBPs exposure routes. Thus, BEAS-2B pulmonary cells were exposed for 8 weeks to subtoxic concentrations of the two HNMs, to resemble a more realistic exposure. Different cell transformation markers were assessed throughout the exposure period, such as cell proliferation and morphological changes, anchorage-independent cell growth, and secretion of matrix metallo-proteinases (MMPs).

Long-term exposure to low concentrations of BNM and TCNM showed no cell-transforming ability in BEAS-2B cells, as indicated by the absence of morphological changes, no effects on cell proliferation, no increased levels of MMPs secretion, nor increased anchorage-independent cell growth capacity. Further, we assessed the capacity of long-term exposed cells to enhance tumor growth directly and indirectly by the stimulation of cells from the lung stroma. In both cases there were no changes in the growth of tumor cells.

These results suggest that BNM and TCNM are not responsible for the increased cancer risk associated to DBPs exposure, and therefore further experiments analyzing other DBPs species are necessary to determine the groups behind the observed health effects.

Notas - Notes

Assessment of genotoxic and carcinogenic potential of long-term exposure to monohaloacetic acids

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Water disinfection has been one of the biggest sanitary achievements of the past century, dramatically improving our public health status. However, a large and increasing number of chemical species, globally named water disinfection by-products (DBPs), are formed during the sanitation process. Consistent evidence has proven that exposure to DBPs increases the risk of bladder and colon cancer. This risk has been associated to some DBPs, which concentrations have been regulated by different public health agencies. However, compounds that belong to non-regulated classes still present a possible hazard, as their harmful potential still has to be tested.

Haloacetic acids are one of the most abundant classes of DBPs. Among them, monohaloacetic acids (chloroacetic acid (CAA), bromoacetic acid (BAA), and iodoacetic acid (IAA)), have been shown to present the highest cytotoxic and genotoxic effect when compared to their polyhaloacetic counterparts in short-term *in vitro* studies. Nevertheless, acute, high concentration treatments do not present a realistic outline of carcinogenicity induction. The aim of our study was to analyze the effect of these chemicals in an *in vitro* system that better emulates a real exposure scenario. Using this experimental approach, a cell line (UROtsa) derived from the main target of DBPs carcinogenicity -the bladder- was exposed to a long-term (8-10 weeks) treatment of low concentrations of all three compounds. Preliminary results confirm that all three compounds presented citotoxicity at 24 hours, showing an IC50 of 776±63 μM for CAA, 11,4±1 μM for BAA and 2,7±0,5 μM for IAA. Based on these results, two non-cytotoxic concentrations were chosen for each chemical: 10 μM and 100 μM for CAA, 0.005 μM and 0.05 μM for BAA and 0.1 μM and 0.01 μM for IAA, respectively. Preliminary data showed that 24 hours of treatment had no effect over cell proliferation or DNA genotoxic and oxidative damage at these concentrations. The acquisition of different *in vitro* cancer-like features, such as alterations in cellular morphology and proliferation, will be monitored throughout the whole exposure time. Other carcinogenic biomarkers analyzed will be genomic and oxidative DNA damage, assessed by Comet assay; chromosomal damage measured by the micronucleus test; matrix metalloproteinases activity, measured by zymography; and colony formation and promotion, tested by soft-agar assay.

Notas - Notes

The role of Fra-1 in arsenic-induced cell malignant transformation

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Arsenic is a widespread and well-known human carcinogen associated with skin, lung, bladder, liver, kidney, and prostate cancers. However, the mechanism explaining the exact relationship of chronic arsenic exposure and tumor development is unclear.

The transcription factor FOS Related Antigen 1 (Fra-1) is frequently overexpressed in epithelial cancers and has been found to be involved in tumor invasion and metastasis. The present study aims to assess whether Fra-1 plays a role in arsenic-induced malignant transformation.

Thus, MEF cells previously transformed by 30 weeks of chronic arsenite exposure were monitored for Fra-1 expression at different time-of-exposure intervals for 40 weeks. Our results demonstrate that Fra-1 is dose-dependently overexpressed 10 weeks after the acquisition of the cancer-like phenotype at the mRNA and protein level, and that the RAS-ERK1/2 signaling pathway and not MAPK or pP38 are responsible for the maintenance of the elevated Fra-1. Arsenic transformed cells with overexpressed Fra-1 showed morphological, molecular and functional characteristics of myofibroblast trans-differentiation with increased aSma, TGFb and the master EMT regulator Snail, and increased MMP2+9 secretion and invasive potential.

Therefore, this work is the first to show that Fra-1 is involved in the modulation of the malignant phenotype induced by chronic arsenic exposure.

Notas - Notes

Repair of DNA damage induced by alkylating agents in *Arabidopsis thaliana*

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Methyl methanesulfonate (MMS) is an alkylating agent that reacts with DNA causing extensive methylation of the N7 position of guanine residues, among other alkylation damages. Base excision repair (BER) is a critical pathway in cellular defence against DNA damage, but its relevance in N7-methylguanine (N7-meG) repair remains unknown. BER is initiated by DNA glycosylases that recognize and excise damaged bases, generating abasic (apurinic/apyrimidinic, AP) sites. AP sites may be processed by either AP endonucleases or AP lyases, but the factors that influence the participation of one or other type of enzyme are still unknown. Our research group has previously demonstrated that the DNA 3'-phosphatase ZDP of *Arabidopsis thaliana* is involved in the BER pathway, and ZDP deficient plants are hypersensitive to MMS. This suggests that the repair of DNA damage induced by MMS may be ZDP dependent in *Arabidopsis*.

In this work we developed an experimental system allowing *in vitro* mimicking of repair of damage induced by alkylating agents in the genome of *A. thaliana*. As a DNA substrate we used a double stranded oligonucleotide with a N7-meG residue in a defined position. Our results show that N7-meG spontaneously hydrolyzes generating an AP site. The processing of these AP sites in *zdp* mutants leads to an accumulation of 3'-P repair intermediates. In contrast, such intermediates are not observed in *fpg* mutants. These results suggest that FPG processes the AP sites arisen by spontaneous depurination of N7-meG, generating 3'-P intermediates that are hydrolyzed to 3'-OH by ZDP. We also studied why ARP, the main AP endonuclease in *Arabidopsis*, has a secondary role in this process. We found that FPG shows a marked preference for AP sites paired with C, whereas ARP shows preference for AP sites paired with G. Altogether, our results suggest that the base opposite the lesion is an important factor determining whether BER proceeds by an AP endonuclease or an AP lyase-dependent sub-pathway, thus establishing the involvement of specific proteins in subsequent steps of the repair process.

Notas - Notes

Tumor suppressor C53 interacts with BRCA2, regulates DSB repair and drives breast and ovarian cancer prognosis

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BRCA2 is an essential protein for DNA repair by homologous recombination and its deficiency leads to genome instability and tumor progression. By means of a yeast two-hybrid screen, we identified NF-κB-dependent tumor suppressor C53 as a new BRCA2 helical domain interacting protein. C53 inhibition leads to resistance to a variety of DNA damage agents and increased spontaneous and ionizing radiation-induced chromosome fragility. Resembling BRCA2, C53 is required for homologous recombination and its absence markedly upregulates single strand annealing. Finally, we show that low C53 expression strongly correlates with poor patient survival rates in over 3.500 breast and 300 ovarian cancer datasets. Our results therefore uncover a new player in DNA repair by homologous recombination and single strand annealing that modulates the prognosis of breast and ovarian cancer patients.

Notas - Notes

Effect of CeONP co-treatment in tobacco exposed lung stroma cells

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Cerium oxide nanoparticles (nanoceria; CeO_2NP) have been proposed as a new promising agent in the treatment of oxidant induced diseases, including cancer, due to its known and well demonstrated antioxidant properties. However, our previous data indicate that CeO_2NP were unable to protect cells from the carcinogenic effects of tobacco smoke condensate after long-term exposure in lung BEAS-2B cells. In fact, results rather support a synergistic role of CeO_2NP in the tobacco-induced tumor secretome.

Since lung stroma cells would better reflect the tumor secretome than epithelial cells, in this work we aim to assess the effects of tobacco smoke condensate and CeO_2NP co-treatment in lung MRC5 fibroblasts. Thus, MRC5 cells long-term exposed to 5 µM of tobacco smoke condensate alone or in combination with 5 and 7.5 µM CeO_2NP for 5 weeks were monitored for changes in the fibroblast differentiation program and signals of fibroblasts trans-differentiation. Also, we analyzed the influence of MRC5 secretome from the different treatments over the anchorage independent cell growth capacity of previously exposed BEAS-2B lung epithelial cell line.

Results evidence no protective role of CeO_2NP in MRC5 cells exposed to tobacco smoke condensates. The crosstalk between lung MRC5 stroma cells and BEAS-2B epithelial cells via secretome demonstrated that CeO_2NP co-exposure exacerbate the effects of tobacco exposure.

Notas - Notes

TBX15 as an antiapoptotic factor in cancer cells

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T-box genes regulate development processes, and some of these genes also have a role in cell proliferation and survival. TBX15, as a T-box transcription factor, is required in skeletal development; in fact, the human Cousin syndrome which is characterized by many skeleton malformations is promoted by alterations in the *TBX15* gene. Also, TBX15 has been associated with the differentiation of adipocyte cells. Recently, the methylation of the *TBX15* promoter has been suggested as a marker in prostate cancer, but its function in carcinogenesis is unknown.

Here we investigated the role of TBX15 in carcinogenesis using thyroid cancer cell lines. First, by western blot analysis, we showed that the expression of TBX15 was altered in thyroid cancer cell lines compared to normal thyroid cells. Then, we performed different functional studies including proliferation, cell viability, migration, colony formation and apoptosis assays. These studies were carried out in conditions with ectopical overexpression TBX15 through the transfection of an expression-plasmid in thyroid cancer cells, in the presence or absence of camptothecin as a cytotoxic agent.

Our results proved that TBX15 did not affect the colony formation, cell migration or cell viability; but the TBX15 transcription factor increased cell proliferation after 48 h of transfection ($P < 0.01$). Consistently, apoptosis was reduced in TBX15 transfected cells ($P < 0.01$), both in the presence or absence of camptothecin. A decrease in the proapoptotic Bax regulator and an increase in the antiapoptotic Bcl2 and Bcl-XL regulators was also observed, which indicated the implication of TBX15 in the intrinsic pathway of the cell apoptosis. Additionally, we performed a siRNA shutdown of constitutive TBX15 in the studied cell lines. Compatible with the overexpression experiments, after knocking down TBX15 an increase of apoptotic cells was observed.

Taken together, these results indicate for the first time an antiapoptotic role of TBX15 in cancer cells, suggesting a contribution of TBX15 in carcinogenesis and the potential therapeutic target of TBX15.

Notas - Notes

Loci associated with genomic damage levels in Spanish chronic kidney disease patients

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Chronic kidney disease (CKD) is a multifactorial disorder with an important genetic component, and several studies have demonstrated potential associations with allelic variants. Using different techniques such as the comet and micronucleus (MN) assay, in peripheral blood lymphocytes we have demonstrated that CKD patients present elevated levels of genomic damage. In addition, these patients also showed genomic instability, since the genomic damage induced by *in vitro* irradiation of patient's cells was significantly higher than observed in controls. Furthermore, CKD patients show deficiencies in repair oxidatively damaged DNA. In this scenario, looking for genetic variants explaining the genomic instability of CKD patients seems urgent.

Until now no studies have stablished relationships between DNA damage or genomic instability present in CKD patients and SNPs. To fill in this gap, the potential role of polymorphisms in genes involved in base excision repair (*OGG1*, rs1052133; *MUTYH*, rs3219489; *XRCC1*, rs25487), nucleotide excision repair (*ERCC2/XPD*, rs1799793, rs171140, rs13181; *ERCC4*, rs3136166); phase II metabolism (*GSTP1*, rs749174; *GSTO1*, rs2164624; *GSTO2*, rs156697), and antioxidant enzymes (*SOD1*, rs17880135, rs1041740, rs202446; *SOD2*, rs4880; *CAT*, rs1001179; *GPX1*, rs17080528; *GPX3*, rs870406: *GPX4*, rs713041) were genotyped. In addition, some genes involved in CKD (*AGT*, rs5050; *GLO1*, rs386572987; *SHROOM3*, rs17319721) were also evaluated. Our results showed significant associations with *XRCC1* (rs25487) and *ERCC2/XPD* (rs13181), as genes directly involved in DNA repair pathways. Interestingly the three genes associated to CKD (*AGT*, *GLO1*, and *SHROOM3*) showed positive associations with high levels of DNA damage, oxidatively damaged DNA, and genomic instability.

These results support our hypothesis that genomic instability can be considered a biomarker of CKD status.

Notas - Notes

The non-canonical SOS-system of DNA-repair and mutagenesis in *Acinetobacter baumannii*

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Acinetobacter baumannii is a Gram-negative bacterium which produces infections mainly in immunosuppressed patients and is characterized by its ability to rapidly develop antimicrobial resistance. In addition, one of the most striking features in comparison to most bacteria is the lack of LexA, the canonical transcriptional repressor of the SOS system. In order to study the atypical SOS system of this nosocomial pathogen, the transcriptional response of *A. baumannii* to the DNA-damaging agent mitomycin C (MMC) was studied using DNA microarray technology. Most of the 39 genes induced by MMC were related to either prophages or encoded proteins involved in DNA repair, mainly genes encoding predicted homologs to components of the error-prone DNA polymerase V (UmuDC). Electrophoretic mobility shift assays demonstrated that the product of the *A. baumannii* MMC-inducible *umuD* gene (*umuDAb*) specifically binds to a palindromic sequence present in its promoter region. Mutations in this palindromic region abolished UmuDAb protein binding. A comparison of the promoter regions of all MMC-induced genes identified four additional transcriptional units with similar palindromic sequences recognized and specifically bound by UmuDAb. Therefore, the UmuDAb regulon consists of at least eight genes, most of them encoding predicted error-prone DNA polymerase V components. Furthermore, inactivation of the *umuDAb* gene resulted in the deregulation of all DNA-damage-induced genes containing the described palindromic DNA motif, indicating that UmuDAb is the LexA analog in this bacterial species. Finally, to elucidate the role of the described UmuDC homologs in antibiotic resistance acquired through UV-induced mutagenesis, the three umuD homologs found were inactivated through the construction of *A. baumannii* knock-outs. Interestingly, all the mutants, and especially the *umuDAb* mutant, were less able to acquire resistance to rifampicin through the activities of their error-prone DNA polymerases. All these data suggest that non-canonical SOS system of *A. baumannii* provides an adaptation mechanism for this nosocomial pathogen.

Notas - Notes

SESIÓN III: EVALUACION DE GENOTOXICIDAD, LA PERSPECTIVA DE LA INDUSTRIA – GENOTOXICITY ASSESSMENT, AN INDUSTRY PERSPECTIVE

Application of a Multiplexed Flow Cytometric Assay and Machine Learning to Provide Genotoxic Mode of Action Information

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In an effort to more easily and efficiently generate genotoxic mode of action data, several biomarkers associated with cellular responses to DNA damage or overt cytotoxicity were multiplexed into a homogenous flow cytometric assay. Reagents included a detergent to liberate nuclei, a nucleic acid dye, fluorescent antibodies against γH2AX, phospho-histone H3, and p53, and fluorescent particles to serve as counting beads. The assay was applied to TK6 cells and 67 diverse reference chemicals that served as a training set. Exposure was for 24 continuous hrs in 96-well plates, and unless precipitation or foreknowledge about cytotoxicity suggested otherwise, the highest concentration was 1 mM. At 4- and 24-hrs aliquots were removed and added to microtiter plates containing the reagent mix, and robotic sampling facilitated walk-away data acquisition. Univariate analyses identified biomarkers and time points that were valuable for classifying agents into one of three groups: clastogenic, aneugenic, or non-genotoxic. A particularly high performing multinomial logistic regression model was comprised of four factors: 4 hr γH2AX and phospho-histone H3 values, and 24 hr p53 and polyploidy values. For the training set chemicals, the four-factor model resulted in 91% concordance with our a priori classifications. A test set of 17 chemicals that were not used to construct the model were evaluated, some of which utilized a short-term treatment in the presence of a metabolic activation system, and in 16 cases mode of action was correctly predicted. These initial results are encouraging as they suggest a machine learning strategy can be used to rapidly and reliably predict new chemicals' genotoxic mode of action based on data from an efficient and highly scalable multiplexed assay.

Notas - Notes

Regulatory genotoxicity: from the bench to the Dossier

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Genotoxicity assessment is a key point in the development of new substances in pharmaceutical, chemical, agrochemical and cosmetic industries. There are currently international regulations which establish how and when this assessment has to be done. Almost in all cases, positive results in genotoxicity assays mean a red flag and normally the compound does not continue in development.

As example, in the particular case of pharmaceutical industry, there are two main areas of genotoxic evaluation, 1) related to the active ingredient. This area is mainly regulated by ICH S2(R1) *Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use* and ICH M3(R2) *Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals*. These guidelines regulate the timing and the kind of assays to be performed. In a standard approach 2 *in vitro* studies (Ames + one with mammalian cells) prior to the first Phase I clinical trial and an *in vivo* study (usually a micronucleus test in rodent) prior entering in Phase II is the standard battery of assays. 2) related to the genotoxic assessment of impurities, this area is mainly regulated by ICH M7 *Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk.* Impurities present in a pharmaceutical product may have pharmacological, toxicological or/and genotoxic potential even at very low doses as the majority of the impurities are generated in chemical synthesis processes, then are highly reactive. Not only the known impurities but also the putative ones should be evaluated in a step-by-step approach.

These guidelines also define follow up studies in case of contradictory results and allow, in the case of impurities, a cross assessment of the genotoxicity potential based on QSAR approaches. Similar guidelines regulate other industry sectors.

In summary, genotoxicity is an inherent property of a compound which has to be assessed. Overall, understanding the mechanisms behind the genotoxicity potential does not mean to lower the red flag from a regulatory point of view.

Notas - Notes

Mutagenicity prediction using *in silico* Methods: Gigabyte-size Petri dishes

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The use of predictive toxicology methodologies presents the advantages of avoiding the use of animal tests and providing results in a faster way. Among the techniques employed, *in silico* toxicology has experimented a notable surge in interest, mainly because the increase in computational power has allowed the use of datasets and algorithms capable of yielding predictions as valid as a traditional *in vitro* assay. In this lecture, an overview on the predictive toxicology methods will be given, comparing *in vitro* and *in silico* approaches and showing how they fit within ICH M7 regulatory guidelines,[1] with special emphasis on *in silico* methods and how Lhasa solutions[2,3] can be used to predict mutagenicity within this framework.

Notas - Notes

Is there space for an industry focused research in academia? I. What can academia offer.

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The main objective of the University is to educate future professionals and society leaders, to investigate and to generate new knowledge that fosters the progression of science. In the field of health sciences, the University is involved in the education of future doctors, pharmacists, chemists, biochemists and biotechnologists, some of which will integrate into multidisciplinary research teams of Universities, research centres and hospitals. In recent years, clinical research within the National Health System (SNS) has been potentiated through Health Research Institutes. Since the early beginning of the 21-st century and up to the year 2008, all markers of R&D in the biomedical field have increased in Spain, both in the public and private sectors.

The objective of the pharmaceutical industry is to investigate, develop, produce and commercialize drugs that can alleviate health conditions and cure diseases. It is a business sector that invests in R&D, invigorates biomedical research and contributes to the continuous education of health professionals. The relationship between University and Industry is pivotal for the achievement of their corresponding objectives as their activities are complementary to each other. In terms of biomedical research, scientific progression requires a super-specialization achievable in a few number of research groups. New therapeutic targets, patient segmentation based on genetic properties in order to test increasingly personalized pharmacological treatments, efficacious biotechnological medicinal products with new challenges for safety assessment are some representative examples. In terms of education, the University has to adapt their degree and post-degree educative programs to the needs of the pharmaceutical sector, with a demand for highly skilled professionals, without abandoning aspects of integral student education for them to become free and non-manipulated persons with criteria and ethical sense.

What can the University offer to the pharmaceutical industry a) Future professionals: the University is a quarry and should be close to Industry. b) New ideas, treatments and approaches: biomedical research should be focused on solving the problems of diseased persons and preserving human health without conditions. However, once the idea is mature and the clinical application seems feasible then the pharmaceutical industry should take the lead for its development. c) Possible start-up of companies and support during the initial phases, thereby contributing to the economic growth and development of the business sector.

Notas - Notes

Is there space for an industry focused research in academia? II. An industry point of view

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Academia and Industry are more and more looking for a space where both sectors can converge, thus creating important synergies that could let them to achieve their goals in a more efficient manner. In this direction, a key point for setting these collaborations up is to identify which are the needs of each part in order to bring their collaboration to a successful interaction. In this session we would highlight which are the key topics that industry is seeking in academia groups to advance in their research lines, in the field of genotoxicity assessment. New techniques, the improvement of the already existing ones or new projects seems to be potential points of collaboration. The large experience of academia in basic research and the know-how generated as a consequence of such experience is clearly an added value that the private sector should take profit of. Examples of how academia has contributed in this direction to the genotoxicity area in the last years will be presented and potential topics where industry could take profit of collaboration with academia will be suggested.

Notas - Notes

SESIÓN IV: NANOGENOTOXICOLOGÍA – SESSION IV: NANOGENOTOXICOLOGY

Genotoxicity assessment of silica-coated iron oxide nanoparticles in human astrocytes

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Iron oxide nanoparticles (ION) have great potential for various biomedical and neurobiological applications such as magnetic resonance neuroimaging agents, heating mediators in hyperthermia-based cancer therapy, and molecular cargo in targeted drug/gene delivery across blood-brain barrier. For all these applications, ION must be introduced in the human body and be in contact with cells and tissues, so it is imperative to know the potential risks associated to this exposure, especially in the nervous system. ION surface may be modified by coating with a number of materials to enhance their desirable properties, biocompatibility and biodegradability. Nevertheless, surface covering can alter cellular internalization and other toxicity endpoints. Even though ION seem to be biocompatible and present low toxicity, current data on their effects on the human nervous system are scarce. Thus, the main objective of this work was to examine possible genotoxic effects of ION (silica-coated magnetite) on human glioblastoma (A172) cell line. To this aim, two treatment times (3 and 24 h), a range of ION concentrations (5-100 µg/ml), and different outcomes were tested: the standard alkaline comet assay to analyze primary DNA damage, H2AX histone phosphorylation to assess the induction of DNA double-strand breaks, and micronucleus (MN) test to determine chromosome alterations. Flow cytometry uptake analysis indicated that ION were not effectively internalized by the cells, but induced a dose- and time-dependent increase in primary DNA damage which, according to the results of H2AX assay, were not related to double strand breaks excepting for the highest concentrations and longest exposure time. Negative results in MN test indicate (i) no aneugenic effects and (ii) that the previously mentioned DNA strand breaks were not fixed upon cell division. Further research is necessary to determine the cause of the genotoxicity events detected in absence of nanoparticle uptake.

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Notas - Notes

In vitro evaluation of the genotoxicity of polymeric nanoparticles as carriers for oral drug administration

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In the last years, there has been an increased interest in the development of biocompatible and biodegradable compounds that can be used as drug delivery nanosystems for oral administration. To this aim, nanoparticles (NPs) synthesized with compounds which are commonly recognized to be safe, are used.

The aim of this study was to evaluate the genotoxicity of two polymeric (anhydride) NPs, Gantrez^{TM} (NPA) and Gantrez^{TM}–covered with mannosamine (NPA-M). The comet assay in combination with the enzyme formamidopypiridine DNA-glycosylase (FPG) and the mouse lymphoma assay (MLA) were used for this purpose. In addition, cell viability after NPs treatment was evaluated by a proliferation assay.

To study their genotoxicity potential, the comet assay was performed in L5178 TK$^{+/-}$ cells treated with the two NPs at five different concentrations (0.074-0.6 mg/mL) for 24h. Negative and positive controls were included in each experiment and three independent experiments were carried out. Furthermore, a cell proliferation assay was performed in parallel with the comet assay where cells were counted at 48 h after their incubation with the NPs at 37°C.

The MLA was conducted in L5178 TK$^{+/-}$ cells using the microwell version, according to the procedure described by the Organization for Economic Co-operation and Development guideline 490. A negative control, a positive control (MMS, 100 µM) and 10 concentrations of each NP were included in each experiment. The highest concentration tested in this test was 0.6 mg/mL; the rest of the concentrations were calculated by a decreasing factor of 3. The treatment was carried out with gentle shaking at 37°C during 24 h in a humidified CO_2 incubator. Two independent experiments were performed.

As a result, both NPs did not show any increase in the frequency of strand breaks, alkali-labile or FPG-sensitive sites in L5178 TK$^{+/-}$ cells treated at tested concentrations. Furthermore, treated cells did not indicate changes in the proliferation rate. However, NPA and NPA-M-SD did induce a statistically significant increase in the mutation frequency in this cell line at all tested concentrations after 24 h of treatment.

Notas - Notes

Comet assay as a method for genotoxic assessment of engineered nanomaterials

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Nanomaterials (NMs) display many unique and useful physico-chemical properties and, for this reason Nanothechnology has become a new vigorous discipline. Engineered NM (ENMs) are being commonly used in a wide range of applications and, consequently, humans are environmentally exposed to them. As some of the ENMs have shown strong biological reactivity there is an urgent need of information on their potential harmful effects.

The mechanism of toxicity usually involves DNA damage, for this reason genotoxicity has become an important endpoint to be evaluated in risk assessment approaches. In this context, the comet assay, or single cell gel electrophoresis (SCGE) assay, has demonstrated to be a sensitive method for detecting strand damage in the DNA as well as induction of oxidatively damaged DNA.

In the current study, we have been working with eight ENMs by using two different cells lines. i) BEAS-2B, a transformed normal human bronchial epithelium, and ii) A549, a human lung carcinoma. Previous studies of characterization by transmission electron microscopy (TEM) and dynamic light scattering (DLS) were carried out. Additionally, toxicity experiments were also carried out to select the range of concentrations to be evaluated.

Obtained results indicate that two of the tested ENMs, namely TiO_2- and SiO_2-NM, were able to induce both genomic and oxidative damage in BEAS-2B cells. With regard to the use of A549 cells they were able to detect genotoxic effects of a wider range of ENMs including TiO_2-, ZnO-, CeO_2-, SiO_2-NM and MWCNT. These results indicated that A549 cells are much more sensitive to the genotoxic effects of ENMs than BEAS-2B cells.

These results support the view that the potential genotoxicity of ENMs is modulated by different factors, including the selected cell line. According to that, more than one cell line should be included in the genotoxicity testing of ENMs.

Notas - Notes

Evaluation of flow cytometry to detect micronucleus induction by different nanoparticles in BEAS 2B cells

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Nowadays the use of nanoparticles can be seen in many goods of daily use. As the use of these compounds is increasing, the human exposure does the same. Currently, there is no clear indication of the adverse effects produced by nanoparticles, as there is high heterogeneity between studies. To evaluate the genotoxic potential of these compounds an *in vitro* micronucleus (MN) assay by using flow cytometry was performed. This is an automated approach replacing the classical method of MN scoring by microscopy. The advantages of using flow cytometry are: i) high-throughput technology that enables to analyze more than 5000 cells in several minutes; ii) there is no need to use cytochalasin B that can interfere in the analysis; iii) it is a sensitive method that has multiparametric measurements; iv) is able to determine relative cell survival, percentage of apoptotic/necrotic cells and provide information on cell cycle; and finally v) it was observed to be useful in different cell lines, such as CHO, V79, L5178Y and CHL.

Thus, the aim of this study was to evaluate the applicability of flow cytometry to determine the genotoxicity of different nanoparticles since there are few reports using this method. For this, BEAS-2B cells were treated for 48 hours with 6 different nanomaterials: titanium dioxide (NM100 and NM101), zinc oxide (NM110), multi-walled carbon nanotubes (NM401), cerium oxide (NM212) and silver (NM300K), in the frame of an EU project (NanoReg). The nanomaterial's morphology and size was obtained by using transmission electron microscopy (TEM) and dynamic light scattering (DLS). The range of concentrations to be tested was selected based on previous cytotoxicity assays. To be able to perform flow cytometry a sequential staining was applied: ethidium monoazide bromide (EMA) added before cell lysis to show apoptotic/necrotic cells and SYTOX Green added in the lysis solution for staining of MN and nuclei. Each concentration was tested in duplicate.

Results indicate that both titanium dioxide and cerium oxide were not able to induce MN formation, while zinc oxide, multi-walled carbon nanotubes and silver induced significant increases in the frequency of MN. Our conclusion is that scoring MN induction by flow cytometry is a useful tool for rapid screening of potential genotoxicants that should be combined with other methods from a battery of assays.

Notas - Notes

Caco-2 cells as an *in vitro* model to determine detrimental effects on the intestinal barrier. Studies with Ag-NPs at sub-toxic concentrations

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Engineered nanoparticles (NPs) are used in many commercial products due to their desirable characteristics for many industrial applications. In addition, some of them are being used as additives in food and packaging, increasing human exposure. This increased use of NPs and the lack of complete knowledge on their potential hazard in the gastrointestinal tract, as a main protection barrier, requires further investigations. Ag-NPs are already used in many fields, including food packaging products due to their antimicrobial and antifungical properties.

The human Caco-2 cell line is derived from colonic epithelial adenocarcinoma cells. This cell line has the capability to differentiate into small intestine enterocytes after reaching confluence, when it is grown under normal cell culture conditions. After 21 days of differentiation the cells become polarized and acquire tight junctions, microvilli and membrane transporters. This is considered a very useful model to observe uptake of nutrients and pharmaceuticals and, for these reasons, differentiated Caco-2 cells have become a model for *in vitro* studies related with the uptake and transport through intestinal barrier.

Intestinal cell toxicity of Ag-NPs was evaluated in differentiated Caco-2 cells. Moreover, intestinal integrity and paracellular permeability were also evaluated after 24 hours of NPs incubation at sub-toxic concentrations in order to mimic a realistic environmental exposure. Uptake and crossing of Ag-NPs were evaluated by confocal microscopy, TEM-EDX and ICP-MS. Furthermore, genetic damage on Caco-2 monolayer was analyzed using the COMET assay and real-time PCR. In addition, the nanomaterial was characterized for morphology and size by using TEM and DLS/LDV.

The analysis of cytotoxicity shows significant reduction of Caco-2 cells viability after 24 hours of Ag-NPs exposure. The use of sub-toxic concentrations of NPs demonstrates that the integrity and permeability remain properly after 24 hours of exposure. Ag-NPs are able to internalize in Caco-2 monolayer but only few of them are able to cross the barrier. Nevertheless, no genotoxic neither global nor oxidative damage was observed after exposure. Hence, Ag-NPs at sub-toxic doses were not able to damage our system of differentiated Caco-2 model.

Notas - Notes

Role of copper oxide nanoparticles as antigenotoxic agents. Studies in *Drosophila*

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The biological reactivity of metallic nanomaterials is attributed to their redox characteristics, which would explain their pro- or anti-cancer properties depending on exposure circumstances. In this sense, CuO nanoparticles (CuONP) have been proposed as a potential antitumoral agent.

The aim of this study is to assess if CuONP can exert antigenotoxic effects using *Drosophila melanogaster* as an *in vivo* model. Genotoxicity was induced by two well-known genotoxic compounds, potassium dichromate (PD) and ethyl methanesulfonate (EMS). The wing-spot assay and the comet assay were used as biomarkers of genotoxic effects. In addition, changes in the expression of *Ogg1* and *SOD* genes were determined. The effects of CuONP cotreatment were compared with those induced by copper sulphate (CS), an agent releasing copper ions.

A slight but significant toxic effect was observed, according to the size of the emerging adults, CS being more toxic than CuONP. Neither CuONP nor CS were genotoxic in the wing-spot assay. Although CuONP was not able to reduce the genotoxic effects of EMS exposure, it had the ability to decrease the effects induced by PD. This effect was associated to a significant reduction in the frequency of mutant twin spots resulting from mitotic recombination. Similar results were obtained with CS. In addition, CuONP were able to reduce the primary DNA damage induced by PD, as measured by using the comet assay.

Since PD effects are associated with the induction of oxidative stress, changes in the expression of *Cu,ZnSOD* and *Ogg1* genes were determined after cotreatment conditions to explain the interferences observed in the cotreatment experiments. According to the results obtained, the antigenotoxic effects of environmental relevant and non-toxic doses of CuONP can be explained by its ability to restore the expression levels of the repair gene *Ogg1* and the antioxidant gene Cu,ZnSOD, inhibited by PD treatment.

Notas - Notes

Evaluation of the genotoxic potential of silver nanoparticles in *Drosophila melanogaster*

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Drosophila has shown to be a suitable *in vivo* model to determine the toxic/genotoxic properties of nanomaterials. Among nanomaterials, silver nanoparticles are one of the most commercially available nanomaterials, mainly due to its strong antibacterial properties. These properties are being increasingly exploited in consumer products as deodorants, clothing materials, bandages and in cleaning solutions. This is the reason why silver-based nanoparticles are the most common material found among consumer nanotechnology-based products.

To increase our knowledge on the risk associated to silver nanoparticles exposure we planned to assess a wide range of biological endpoints using *Drosophila melanogaster* as a target organism. To determine if the observed effects were caused by the nanoparticulated nature or to the ions released in dissolution, all the experiments were carried out using both silver nanoparticles and silver nitrate.

Experiments to describe the main characteristics of silver nanoparticles were carried out using transmission electronic microscopy (TEM) (dry size), dynamic light scattering (DLS) (hydrodynamic size), and laser Doppler velocimetry (LDV) (zeta potential) methodologies. Viability egg-to-adult was determined to stablish the range of concentrations to be used in genotoxicity experiments. Uptake of silver nanoparticles by intestinal cells was determined by TEM. Genotoxicity was assessed by means of the comet assay using hemocytes and midgut cells as potential targets to determine the level of DNA damage.

The results obtained with both silver nanoparticles and silver nitrate will be presented and discussed.

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