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sema2017

XXIII Reunión Científica de la Sociedad Española de Mutagénesis Ambiental

OVIEDO, 14, 15 y 16 DE JUNIO DE 2017

EDIFICIO SEVERO OCHOA, CAMPUS DEL CRISTO

Organiza: Unidad de Genotoxicidad y Reparación del DNA del IUOPA, en el grupo de Espectrometría de Masas y Análisis Biomédico de la Universidad de Oviedo

INVITACIÓN

La Unidad de Genotoxicidad y Reparación del DNA, del Instituto Universitario de Oncología del Principado de Asturias, dentro del grupo de Espectrometría de Masas y Análisis Biomédico, de la Universidad de Oviedo, y en nombre de la Sociedad Española de Mutagénesis Ambiental (SEMA), tiene el placer de invitar a la XXIII Reunión Científica de la Sociedad Española de Mutagénesis Ambiental, que se celebrará en Oviedo, del 14 al 16 de junio de 2017.

Universidad de Oviedo

SOCIEDAD ESPAÑOLA DE MUTAGENESIS AMBIENTAL

INSTITUTO UNIVERSITARIO DE ONCOLOGIA
OBRA SOCIAL CAJASTUR

SEDE DE LA REUNIÓN

Salón de Actos del Edificio Severo Ochoa
C/ Fernando Bonguera s/n
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33006 Oviedo

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La cuota de inscripción para esta Reunión Científica, hasta el 20 de mayo, es:

Becarios y estudiantes de doctorado	100 €
Socios SEMA	165 €
No Socios	185 €

Después del 30 de mayo los precios se incrementarán en 20 €.
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RESÚMENES/ABSTRACTS

Los resúmenes deberán ajustarse a las especificaciones que se detallan más abajo, e indicar en cuál de las cuatro Sesiones Científicas se incluye:

1. Respuesta al daño en el DNA: reparación/ DNA damage responses: DNA repair.
2. Genotoxicidad y Antigenotoxicidad. Ecogenotoxicología / Genotoxicity and Antigenotoxicity. Ecogenotoxicology.
3. Epigenética y tecnologías "ómicas" aplicadas a Mutagénesis y Carcinogénesis / Epigenetics and omic technologies applied to Mutagenesis and Carcinogenesis.
4. Nanogenotoxicología. Evaluación / Nanogenotoxicology. Evaluation .

Deberán enviarse, antes del 5 de junio, por e-mail a la dirección: **sema2017@uniovi.es**

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- **Interlineado**: 1,5
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Se encontrará en un link anexo una plantilla por si quiere utilizarse.

ALOJAMIENTO

Existen varios hoteles en el centro de la ciudad, a una distancia de 5 min, o menos, de una parada de autobús, de la línea (C2) que llega al Campus del Cristo donde se encuentra la sede. La frecuencia de esta línea es de aproximadamente 10 minutos.

- Hotel Campoamor
- Hotel NH Principado
- Princesa Munia Hotel y Spa
- Fruela Hoteles
- Ayre Hotel Ramiro I
- AC Hotel Oviedo Forum
- Hotel Exe Oviedo Centro
- Ayre Hotel Oviedo

En la página siguiente se encuentran localizados en el mapa del centro de Oviedo, así como las paradas de las líneas de autobús que llevan hasta la sede. También se pueden consultar en el siguiente mapa online específico para **sema**2017: https://goo.gl/Ev8HcO. Puede consultarse tanto en ordenador como en dispositivos móviles.

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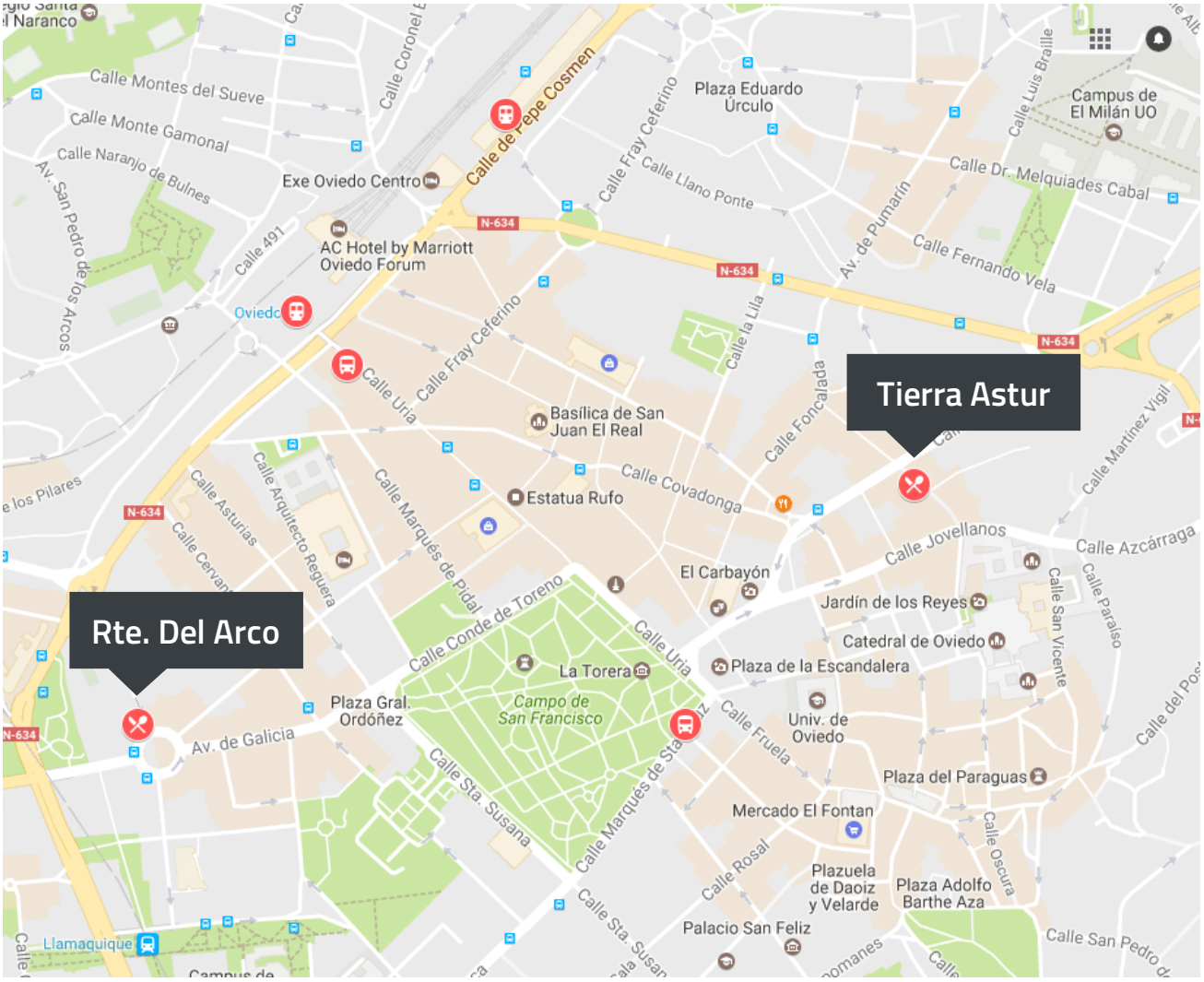

PROGRAMA CIENTÍFICO

Miércoles 14

14.30-15.30 Entrega de documentación / Registration

15.30-16.00 Ceremonia de apertura / Opening ceremony

16.00-17.15 CONFERENCIA INAUGURAL / OPENING CONFERENCE.
MODERADORA: L. María Sierra
"Regulation and dynamics of DNA repair in a chromatin context"
Dr. Haico van Attikum, Leiden University Medical Center

17.15-17.30 Café/Coffee

17.30-19.00 SESIÓN 1: Nanogenotoxicología. Evaluación
SESSION 1: Nanogenotoxicology. Evaluation
MODERADORES: Amaya Azqueta y Amadeu Creus

17.30-18.00 **"Human astrocytes DNA repair competence: influence of oxide nanoparticle surface coating"**
Fernández-Bertólez, N.; Costa, C.; Brandão, F.; Fraga, S.; Teixeira, J.P.; Pásaro, E.; Laffon, B.; Valdiglesias, V.
Universidad de A Coruña

18.00-18.30 **"Interaction of silver nanoparticles with differentiated CACO-2 cells monolayers"**
Vila L., García-Rodríguez A., Cortés C., Marcos R., Hernández A.
Universidad Autónoma de Barcelona

18.30-19.00 **"Estudio comparativo de la absorción, el almacenamiento celular y citotoxicidad de diferentes nanocompuestos de hierro usados en el tratamiento de la anemia"**
Turiel Fernández, D.; Bettmer, J.; Sierra, L.M.; Montes-Bayón, M.
Universidad de Oviedo

19.30-21.00 Visita guiada por la ciudad / Guided tour of the city

21.00 Espicha asturiana en Tierra Astur / Asturian "Espicha" at Tierra Astur

Jueves 15

9.30-11.00 SESIÓN 2: Respuesta al daño en el DNA: reparación
SESSION 2: DNA damage responses: DNA repair
MODERADORES: Ricard Marcos y Maite Roldán-Arjona

9.30-10.00 **"Arsenic trioxide enhances the antitumor activity of cisplatin in bladder tumor cell line models, via inhibition of FA/BRCA repair pathway"**
Peremartí J., Marcos R., Hernández A.
Universidad Autónoma de Barcelona

10.00-10.30 **"Implicación de la actividad AP liasa de FPG en la reparación de los sitios abasicos generados por depurinación de N7-metilguanina"**
Barbado, C.; Córdoba-Cañero, D.; Ariza, R.R.; Roldán-Arjona, T.

10.30-11.00 **"Genomic instability as an indicator of chronic kid ney disease patients"**
Pastor, S., Marcos, R.; Coll, E.
Universidad Autónoma de Barcelona

11.00-11.30 Café/Coffee

11.30-12.00 PRESENTACIÓN PROGRAMA "COMETA 5.1" / PRESENTATION PROGRAM "COMETA 5.1"
"Ensayo Cometa: mejoras para su análisis"
Corrales, J.A.; Muñiz, R.; Sierra, L.M.
Universidad de Oviedo

12.00-13.15 CONFERENCIA INVITADA/INVITED CONFERENCE.
MODERADORA: L. María Sierra
"Environmental programming of respiratory allergy: utility of a child's spit epigenome"
Dr. Sabine Langie, Flemish Institute for Technological Research

13.15-15.00 Comida / Lunch

15.00-16.15 CONFERENCIA INVITADA / INVITED CONFERENCE.
MODERADORA: L. María Sierra
"**El efecto de la exposición a nanopartículas sobre el epigenoma"**

Dr. Mario F. Fraga, Centro de Investigación en Nanomateriales y Nanotecnología-CINN (CSIC)

16.15-17.15 SESIÓN 3: Epigenética y tecnologías "ómicas" aplicadas a Mutagénesis y Carcinogénesis
SESSION 3: Epigenetics and omic technologies applied to Mutagenesis and Carcinogenesis
MODERADORES: Blanca Laffon y Antonio Guzmán

16.15-16.45 **"Development of a CRISPR-based system to reactivate epigenetically silenced genes in human cells"**
Devesa Guerra, I.; Parrilla Doblas, J.T.; Morales Ruiz, T.; García Ortiz, M.V.; Rodríguez Ariza, R.; Roldán Arjona, T.
Universidad de Córdoba

16.45-17.15 "**5-MeC Precise quantitation in genomic DNA and specific sequences: relevance in cisplatin resistance"**
Espina, M.; Iglesias-González, T.; Montes-Bayón, M.; Sierra, M.I.; Blanco-González E.; Sierra L.M.
Universidad de Oviedo

17.15-17.45 Café / Coffee

17.45-18.45 SESIÓN 3 (continuación) / SESSION 3 (continuation)

17.45-18.15 "***AS3MT*** **and** ***MTH1*** **genes as new markers in the arsenic-induced malignant transformation"**
Barguilla I., Peremartí J., Bach J., Marcos R., Hernández A.
Universidad Autónoma de Barcelona

18.15-18.45 "**Determination of gene copy number variations: usefulness of multiplex PCR in combination with gel electrophoresis-mass spectrometry (GE-ICP-MS)"**
Fernández Asensio, A.; Iglesias, T.; Blanco González,E.; Montes-Bayón, M.; Sierra, L.M.
Universidad de Oviedo

19.00-19.45 Asamblea anual de la Sociedad Española de Mutagénesis Ambiental
Annual meeting of the Spanish Environmental Mutagenesis Society

21.00 Cena del Congreso en Restaurante Del Arco / Congress Dinner at Del Arco Restaurant

Viernes 16

9.30-11.30 SESIÓN 4: Genotoxicidad y Antigenotoxicidad. Ecogenotoxicología
SESSION 4: Genotoxicity and Antigenotoxicity. Ecogenotoxicology
MODERADORES: Eduardo De La Peña y Elisa Blanco-González

9.30-10.00 **"Is micronucleus frequency in peripheral lymphocytes and buccal cells related to Frailty syndrome in older adults?"**
Sánchez-Flores, M.; Marcos-Pérez, D.; Maseda, A.; Lorenzo-López, L.; Millán-Calenti, J.C.; Bonassi, S.; Pásaro, E.; Valdiglesias, V.; Laffon, B.
Universidad de A Coruña

10.00-10.30 **"Are monohaloacetic acids genotoxic and/or carcinogenic under long-term exposure conditions?"**
Marsà, A.; Cortés, C.; Hernández, A.; Marcos, R.
Universidad Autónoma de Barcelona

10.30-11.00 "**Influence of wave length, frequency and duty cycle on the induction of DNA damage by pulsed versus continuous ultraviolet radiation"**
García-Valbuena, C.; Rodríguez, R.; Cotarelo, A.; Gutiérrez, D.; Álvarez, P.; Ferrero, F.; Campo, J.C.; Valledor, M.; Sierra, L.M.
Universidad de Oviedo

11.00-11.30 **"Usefulness of the new developed "COMETA 5.1" software in the analysis of Comet assay nucleoids: comparisons with Komet5"**
Moreno, O.; Fernández, P.; Corrales, J.A.; Muñoz, R.; Aguado, L., Toledo, R., Sierra, L.M.
Universidad de Oviedo

11.30-12.00 Café / Coffee

12.00-13.15 CONFERENCIA DE CLAUSURA / CLOSING CONFERENCE.
MODERADORA: L. María Sierra
"**Fanconi anemia: new genes, new partners and new syndromes"**
Dr. Jordi Surrallés, Hospital San Pau y Universidad Autónoma de Barcelona

13.15 Ceremonia de clausura / Closing ceremony

CONFERENCIAS INVITADAS

INVITED CONFERENCES

REGULATION AND DYNAMICS OF DNA REPAIR IN A CHROMATIN CONTEXT

Haico van Attikum

Leiden University Medical Center. Department of Human Genetics. The Netherlands

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Our cells receive tens of thousands of different DNA lesions per day. Failure to repair these lesions will lead to cell death, mutations and genome instability, which contribute to human diseases such as neurodegenerative disorders and cancer. Efficient recognition and repair of DNA damage, however, is complicated by the fact that genomic DNA is packaged, through histone and non-histone proteins, into a condensed structure called chromatin. The DNA repair machinery has to circumvent this barrier to gain access to the damaged DNA and repair the lesions. By using a cross-disciplinary approach that combines novel and cutting-edge genomics approaches with bioinformatics, genetics, biochemistry and high-resolution microscopy, we identified several chromatin-modifying enzymes and showed how these enzymes regulate DNA repair in chromatin to maintain genome stability and counteract human diseases. At the meeting, I will present some of our recent findings and indicate the current status of our work.

ENVIRONMENTAL PROGRAMMING OF RESPIRATORY ALLERGY: UTILITY OF A CHILD'S SPIT EPIGENOME

Sabine A.S. Langie[1,2], Matthieu Moisse[3], Katarzyna Szarc vel Szic[4], Ellen Van Der Plas[1], Gudrun Koppen[1], Sylvie Remy[1], Guy Van Camp[5], Diether Lambrechts[3]; Greet Schoeters[1,4], Wim Vanden Berghe[4] and Patrick De Boever[1,6]

[1]Environmental Risk and Health unit, Flemish Institute of Technological Research (VITO), Mol, Belgium; [2]Theoretical Physics, Hasselt University, Diepenbeek, Belgium; [3]VIB Vesalius Research Center, KU Leuven, Leuven, Belgium; [4]Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium; [5]Laboratory of Cancer Research and Clinical Oncology, Center for Medical Genetics, University of Antwerp, Edegem, Belgium; [6]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium

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Epigenetic DNA methylation changes can be part of the underlying molecular mechanisms leading to complex diseases. Early life exposures like parental lifestyle and exposure to chemicals can alter DNA methylation patterns, and thereby predispose the child to develop respiratory allergy (RA) later in life. Longitudinal birth cohorts are instrumental to study disease development, but DNA biomarker research is hampered because blood sampling is kept to a minimum for practical and ethical reasons. Saliva is a non-invasive and convenient source of DNA that can be used for biomarker research. In this study, we aimed at discovery and confirmation of differential methylation regions (DMR) in saliva of children with RA when comparing to controls.

Saliva samples collected in the two independent longitudinal birth cohorts (Flanders Environment and Health Surveys FLEHS1 & FLEHS2) were analysed using Illumina Methylation 450K BeadChips. A statistical analysis pipeline was developed in R to identify genome-wide differential methylation. We identified 27 DMRs in saliva from 11y old allergic children (self-reported/doctor's diagnosed RA, Phadiatop IgE $\geq$ 0.35 kU/L; N=26) vs. controls (no self-reported/diagnosed RA, Phadiatop IgE< 0.35 kU/L; N=20) in the FLEHS1 cohort. A set of 8 DMRs was selected for further validation by iPLEX MassArray analysis. First, iPLEX analysis was performed in the same 46 FLEHS1 samples that were previously analysed on the 450K methylation arrays, to allow technical validation. iPLEX results correlated significantly with the 450K methylation array data ($P<0.0001$), though iPLEX analysis confirmed 5 of the 8 identified DMRs in the FLEHS1 study.

Aiming for biological confirmation, we studied these DMRs in an independent birth cohort FLEHS2. Due to a lack of blood samples to measure IgE levels in the FLEHS2 cohort, cases and controls were identified as: 1) cases = doctor's diagnosed/self-reported RA symptoms ever (N=19); and 2) controls = no self-reported/diagnosed RA (N=20). When studying the 8 DMRs by means of iPLEX analysis in the FLEHS2 cohort, only a DMR in the *GLI2* gene showed a statistically significant difference in methylation between RA cases and controls. GLI2 has a regulating role in IL4 signalling and can modulate T-helper differentiation and allergic disease, and might thus be an interesting DNA methylation marker to study for further biomarker development.

Interestingly, the RA-related hypermethylation in *GLI2* correlated significantly with life time exposures towards air pollution markers PM_{10}, NO_2 and O_3. Using the statistical framework developed by Valeri and VanderWeele (*Psychol Methods, 2013*), *GLI2* hypermethylation was observed to partially mediate the effects of PM_{10}, NO_2 and O_3 on RA.

This project is providing novel insights in the molecular mechanisms that may predispose children to RA development. We are among the first to show the utility of saliva to identify DNA methylation marks in children that are relevant for RA.

EL EFECTO DE LA EXPOSICIÓN A NANOPARTÍCULAS SOBRE EL EPIGENOMA

Mario Fdez Fraga

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En los últimos años se ha producido un gran incremento en la utilización de nanocompuestos y nanomateriales en áreas como la industria química, cosmética o alimentaria. Partículas de tamaños menores de 100nm implican cambios en las propiedades físicas y químicas de los materiales con respecto a las que presentan partículas de mayor tamaño. Estos cambios se deben, en gran medida, al incremento de la superficie de contacto de las nano partículas con su entorno. Macroscópicamente, hay sustancias que cambian de color por debajo de un cierto tamaño de partícula. Otras, por ejemplo, pueden cambiar su conductividad o solubilidad.

Aunque los nanocompuestos también tienen importantes aplicaciones en el campo de la biomedicina, los efectos de la exposición a este tipo de materiales sobre la salud humana todavía son poco conocidos. En este sentido, en los últimos años, los mecanismos epigenéticos se han postulado como posibles intermediarios entre los factores externos o medioambientales y nuestros genes. Los mecanismos epigenéticos se refieren a procesos químicos que regulan de forma estable la función del genoma sin afectar a la secuencia de DNA. Uno de los mecanismo epigenéticos mejor conocido es la metilación del DNA genómico, una modificación química esencial para la vida de mamíferos cuya alteración está relacionada con envejecimiento y cáncer.

La metilación del DNA, al igual que otros procesos epigenéticos, puede verse afectada por factores externos como la dieta, el ejercicio, la contaminación ambiental, el alcohol y el tabaco. Además, existen evidencias de que alguna región específica del DNA genómico puede sufrir alteraciones en respuesta a exposición a ciertos tipos de nanopartículas. Recientemente, en nuestro laboratorio analizamos, a nivel de genoma completo, las alteraciones en los patrones de metilación del DNA genómico en respuesta a exposición a nanopartículas de óxido de titanio y nanotubos de carbono. En mi charla presentaré y discutiré los resultados obtenidos.

FANCONI ANEMIA: NEW GENES, NEW PARTNERS AND NEW SYNDROMES

Jordi Surrallés

Genetics Service, Hospital de Sant Pau, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, and CIBER on Rare Diseases, Barcelona, Spain.
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The biomedical relevance of genome maintenance is illustrated by the severe clinical consequences of mutations in DNA repair genes. A clear example are genes involved in the repair of DNA interstrand-cross links and double strand breaks by homologous recombination (HR), such as BRCA1, BRCA2, PALB2, BRIP1, or RAD51C. Mutations in these genes cause familial breast cancer and Fanconi anaemia (FA) in monoallelic or biallelic carriers, respectively. Furthermore, the proteins encoded by many of these genes are crucial for the modulation of the response of cancer cells to chemotherapeutics, including cisplatin and PARP inhibitors. Therefore, the identification of additional components of this DNA repair pathway is of extreme biomedical importance. By using whole exome sequencing and interactomics we have recently identified and functionally studied novel components of this pathway and uncovered their association to Fanconi anemia and breast and colon cancer susceptibility. Functional studies of variants identified in these novel genes are of critical importance to understand their role in cancer predisposition or FA-related bone marrow failure and to correlate the genetic variant with the clinical outcome. Newly acquired knowledge about FA/BRCA pathway promises to provide a cure in the near future.

SESIÓN 1: NANOGENOTOXICOLOGÍA. EVALUACIÓN.

SESSION 1: NANOGENOTOXICOLOGY. EVALUATION.

HUMAN ASTROCYTES DNA REPAIR COMPETENCE: INFLUENCE OF IRON OXIDE NANOPARTICLE SURFACE COATING

Fernández-Bertólez, N.[a,b]; Costa, C.[c,d]; Brandão, F.[c,d]; Fraga, S.[c,d]; Teixeira, J.P.[c,d]; Pásaro, E.[a]; Laffon, B.[a]; Valdiglesias, V.[a,d]

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[b]Universidade da Coruña, Department of Cellular and Molecular Biology, Facultad de Cienicas, Campus A Zapateira s/n, 15071 A Coruña, Spain

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In recent years, iron oxide nanoparticles (ION) have gained importance in diagnosis and treatment of human nervous system pathologies, and many of them are able to cross the blood-brain barrier. However, ION safety is not entirely clear yet. Studies describing possible genotoxic damage or repair alterations induced by ION exposure are still very scarce, especially in nervous cells. Moreover, it is known that ION surface coatings may modify their advantageous features as well as their potential toxicity. The aim of this study was to assess the possible effects of ION on DNA repair ability, and the influence of nanoparticle surface coating. DNA repair competence assay, based on alkaline comet assay, was performed in human astrocytes (A172) treated with silica-coated (S-ION) and oleic acid-coated (O-ION) ION in the presence and absence of serum, using H_2O_2 as challenging agent. DNA damage detected immediately after challenging the cells with H_2O_2 (before repair) was compared with residual damage after a 30 min incubation-period (after repair). Incubations with ION were conducted before inducing DNA damage (3 or 24h), during damage induction, or during the repair period. Data obtained showed a significant decrease in DNA damage assessed after the repair period for both ION tested, regardless if exposure was performed prior H_2O_2 treatment, in co-exposure, or during the subsequent DNA repair period. Results obtained for the two different surface coatings tested were very similar, indicating absence of influence of this feature. Presence of serum did not affect the results at any condition either.

Acknowledgements: This work was supported by Xunta de Galicia (ED431B 2016/013), hCOMET COST Action CA15132, and the project NanoToxClass (ERA

ERA-SIINN/001/2013). N. Fernández-Bertólez was supported by an INDITEX-UDC fellowship. F. Brandão was supported by the grant SFRH/BD/101060/2014, funded by the Portuguese Foundation for Science and Technology (subsidized by national fund of MCTES).

INTERACTION OF SILVER NANOPARTICLES WITH DIFFERENTIATED CACO-2 CELLS MONOLAYERS

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In the past few years, nanoparticles (NPs) have revolutionized consumable goods, becoming an important asset of their production, as well as a component of the supplies themselves. In particular, AgNPs have gained popularity in pharmaceutical and food industry supplies, given its antimicrobial properties. However, the internalization mechanism and the actual risk posed by these NPs have not been fully elucidated yet. Ingestion is one of the main entry routes of xenobiotics. As such, the use of an *in vitro* model mimicking the enteric epithelium as target model could assess the hazard of AgNPs. To this end, the human colon adenocarcinoma Caco-2 cell line has been used due to its capability to differentiate and form a well-structured cell monolayer.

In this study, we have used the aforementioned model to evaluate different parameters that could be altered by AgNPs exposure, such as cell viability, the monolayer integrity, and permeability, as well as cellular uptake and translocation of the NPs. Induction of DNA damage, both genotoxic and oxidative, as well as expression of different genes coding for specific markers of differentiated enterocytes were also evaluated.

The obtained results show that no significant effects were observed on the integrity and permeability of the monolayer after AgNPs exposure, although cellular uptake was demonstrated by confocal microscopy. Despite this, no translocation to the basolateral chamber was observed with any of the different experimental approaches used. The genotoxic effects evaluated using the comet assay indicate that AgNPs exposure induces a significant increase in the levels of oxidative DNA damage, although it was not able to induce direct DNA breaks, at the tested conditions.

ESTUDIO COMPARATIVO DE LA ABSORCIÓN, EL ALMACENAMIENTO CELULAR Y CITOTOXICIDAD DE DIFERENTES NANOCOMPUESTOS DE HIERRO USADOS EN EL TRATAMIENTO DE LA ANEMIA

Turiel Fernández, D.[1]; Bettmer, J.[1]; Sierra Zapico, L.M.[2]; Montes-Bayón, M.[1]

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La anemia por deficiencia de hierro es debida a un desajuste en los niveles de hierro. Dentro de los suplementos para tratar esta enfermedad, las sales de Fe (II) son las más utilizadas, aunque se ha encontrado que pueden producir efectos secundarios indeseados. Como alternativa, algunas formas de Fe (III) han sido propuestas por ser potencialmente mejor toleradas en el intestino. Desafortunadamente, la absorción de las mismas es bastante pobre. Para mejorar esto, se han ido desarrollando distintas alternativas teniendo especial relevancia el Fe nanoparticulado. Este trabajo se ha centrado en un estudio comparativo entre la absorción, el almacenamiento y la citotoxicidad de diferentes nanocompuestos de hierro usados como candidatos para la anemia en deficiencia en hierro: nanopartículas de hierro recubiertas de sacarosa y otras presentes de forma natural en la ferritina, comparadas también con una fuente de Fe (II) iónico. Los estudios se llevaron a cabo en dos líneas celulares de cáncer de colon, HT29 y Caco2.

Los resultados obtenidos mostraron una mayor absorción en el caso de las nanopartículas recubiertas de sacarosa, 1.7 veces mayor que en el caso de la ferritina y 3 mayor que en el Fe (II). La especiación del hierro citosólico reveló la presencia de dos especies mayoritarias en todos los tratamientos: una perteneciente a la ferritina y otra, desconocida y de mayor peso molecular que resultó ser producto de una interacción inespecífica de las NPs con componentes celulares. En cuanto a la toxicidad celular y el daño en el ADN, las nanopartículas recubiertas de sacarosa son las que mayores descensos de viabilidad generan, pero siempre con valores para la viabilidad superiores al 80%. La genotoxicidad ensayada con el ensayo del Cometa reveló daños significativos en el caso de las NPs de Fe y en ninguno de los otros tratamientos.

SESIÓN 2: RESPUESTA AL DAÑO EN EL DNA: REPARACIÓN

SESSION 2: DNA DAMAGE RESPONSE: DNA REPAIR

ARSENIC TRIOXIDE ENHANCES THE ANTITUMOR ACTIVITY OF CISPLATIN IN BLADDER TUMOR CELL LINE MODELS, VIA INHIBITION OF FA/BRCA REPAIR PATHWAY.

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Arsenic trioxide (ATO) is an inorganic arsenic derivative administered to treat certain types of cancers, although its adverse side-effects and unknown mechanism(s) of action may confine its therapeutic effect in clinical practice.

This work aims to determine whether ATO is able to potentiate the antitumor effects of the ICL-inducer cisplatin (CDDP) in bladder cancer cells via FA/BRCA disruption. Thus, SW800, 5637 and T24 cell lines were co-treated with different doses of ATO and CDDP, alone or in combination. The integrity of the FA/BRCA repair pathway was analyzed after the treatments, together with cell viability and several characteristic bladder cancer features.

Our results show that ATO co-treatments reduce the cellular capacity to trigger the CDDP-dependent FA/BRCA function in all cases, as indicated by a significant decrease in the FANCD2 ubiquitination and foci formation. Consequently, combinations of ATO and CDDP synergistically induce toxicity in bladder cancer cells, along with a significant decrease in SW800, 5637 and T24 anchorage-independent cell growth, migration capacity, and secretion of matrix metalloproteinases.

This is the first study demonstrating the potential beneficial effects of ATO and CDDP combined therapy via FA/BRCA disruption.

IMPLICACIÓN DE LA ACTIVIDAD AP LIASA DE FPG EN LA REPARACIÓN DE LOS SITIOS ABASICOS GENERADOS POR DEPURINACIÓN DE N7-METILGUANINA

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La ruta de reparación por escisión de bases (BER) repara sitios abásicos (sitios AP) generados por rotura espontánea del enlace N-glicosídico o por la acción enzimática de ADN glicosilasas que escinden bases dañadas. Los sitios AP son sustrato de AP endonucleasas y de AP liasas, pero se desconocen los factores implicados en la participación de uno u otro tipo de enzimas en la ruta BER. Nuestro grupo ha descrito previamente que plantas de *Arabidopsis thaliana* deficientes en la ADN 3'-fosfatasa ZDP muestran hipersensibilidad a MMS, un agente alquilante que genera mayoritariamente N7-metilguanina (N7-meG). En el presente estudio, demostramos que la N7-meG se hidroliza de forma espontánea, dando lugar a sitios AP que son procesados por la AP liasa FPG de Arabidopsis. El intermediario de reparación generado por FPG presenta un extremo 3´-P, que es convertido a su vez por ZDP en un extremo 3´-OH que permite finalizar la reparación. Las proteínas FPG y ZDP interaccionan *in vitro*, y la inactivación de FPG restaura la resistencia a MMS en las plantas deficientes en ZDP, lo que indica que su hipersensibilidad al agente alquilante se debe a la acumulación de intermediarios 3´-P no procesados. Mientras que los sitios AP surgidos por hidrólisis espontánea y enfrentados a C son un sustrato óptimo para la AP liasa FPG, los generados enzimáticamente por ADN glicosilasas y enfrentados a G son procesados preferentemente por la AP endonucleasa ARP. Nuestros resultados sugieren que, al menos en plantas, el origen enzimático o no de un sitio AP y la base a la que se encuentre enfrentado determinan si será procesado por AP liasas o AP endonucleasas.

GENOMIC INSTABILITY AS AN INDICATOR OF CHRONIC KIDNEY DISEASE PATIENTS

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Chronic kidney disease (CKD) is a multifactorial disease characterized by a decrease of glomerular filtration rate. Cardiovascular disorders in these patients are the main cause of mortality, followed by cancer and infections.

It is known that genomic damage is related to the development of cancer and cardiovascular diseases. In previous studies, and using different techniques such as the comet and micronucleus (MN) assays in peripheral blood lymphocytes, we demonstrated that CKD patients present elevated levels of genomic damage, since the first stages of the disease. We demonstrated that these patients showed intrinsic genomic instability, since genomic damage induced by *in vitro* irradiation of patient's cells was significantly higher than that observed in controls. Furthermore, CKD patients showed deficiencies in repair oxidized damaged DNA. In addition, we identified a positive correlation between the levels of DNA damage and the risk of mortality. Thus, the levels of DNA damage could be used as a biomarker of bad prognosis in patients with CKD. The increase of the genomic damage on CKD patients could be attributed to the increase of the oxidative stress, uremic toxins, deficiency of antioxidants, or to the decrease in the DNA repair capacity. In that sense, our group have demonstrated that the administration of antioxidants to CKD patients submitted to hemodialysis reduce the oxidative DNA damage.

We have also look for genetic variants of genes involved in base excision repair (*OGG1, MUTYH, XRCC1*), nucleotide excision repair (*ERCC2, ERCC4*), phase II metabolism (*GSTP1, GSTO1, GSTO2*) and antioxidant enzymes (*SOD1, SOD2, CAT, GPX1, GPX3, GPX4*) trying to explain the genomic instability of CKD patients. Our results showed significant associations with *XRCC1* (rs25487) and *ERCC2* (rs13181), as genes directly involved in DNA repair pathways. These results support our hypothesis that genomic instability can be considered a biomarker of CKD status.

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PRESENTACIÓN PROGRAMA "COMETA 5.1"

PRESENTATION PROGRAM "COMETA 5.1"

ENSAYO COMETA: MEJORAS PARA SU ANÁLISIS

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El ensayo cometa es sobradamente conocido en el estudio del daño del DNA. En este trabajo fruto de la colaboración entre los departamentos de Informática (área de Lenguajes y Sistemas Informáticos) y de Biología Funcional (área de Genética) se presenta una nueva herramienta que introduce ciertas mejoras respecto a las existentes en la actualidad.

El objetivo que se persigue es doble, por una parte aumentar la velocidad con la que el operador efectúa los análisis y por otra mejorar la precisión de las medidas, en casos por ejemplo donde el daño es muy pequeño, es decir la cola del cometa se acorta mucho o donde la cabeza tiene una estructura irregular.

La primera de las innovaciones consiste en la determinación automática del área de interés, esto es del rectángulo dentro del cual está contenida la cabeza y cola del cometa. Pare ello se emplea la técnica de proceso digital de imágenes de detección automática de BLOBs (Binary Large Objects). Esta técnica permite localizar todos los núcleos de cierta relevancia presentes en una imagen y determinar sus áreas de análisis asociadas.

La segunda innovación consiste en un grupo de mejoras para que el operador pueda marcar mediante inspección visual la zona donde se considera que se encuentra el centro de la cabeza. Esto es útil especialmente en los casos donde la destrucción del núcleo es asimétrica y los histogramas obtenidos se alejan del modelo de daño estándar provocando una separación incorrecta de las zonas de cabeza y cola y generando de esta manera resultados poco o nada precisos.

SESIÓN 3: EPIGENÉTICA Y TECNOLOGÍAS "ÓMICAS" APLICADAS A MUTAGÉNESIS Y CARCINOGÉNESIS

SESSION3: EPIGENETICS AND OMIC TECHNOLOGIES APPLIED TO MUTAGENESIS AND CARCINOGENESIS

DEVELOPMENT OF A CRISPR-BASED SYSTEM TO REACTIVATE EPIGENETICALLY SILENCED GENES IN HUMAN CELLS

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DNA methylation is an epigenetic mark associated to gene silencing, and its targeted removal is a major goal of epigenetic editing. In human cells, DNA demethylation involves iterative 5-methylcytosine (5-mC) oxidation by TET enzymes followed by replication-dependent dilution and/or replication-independent DNA repair of its oxidized derivatives. In contrast, plants use specific DNA glycosylases that directly excise 5-mC and initiate its substitution for unmethylated C in a base excision repair process.

The CRISPR 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 epigenetics effector domains can be used for targeted transcriptional regulation in human cells.

In this work, we have fused dCas9 to the catalytic domain of Arabidopsis ROS1 5mC DNA glycosylase (ROS1_CD), and we have explored the possibility of directing ROS1_CD glycosylase activity to a specific target sequence in human cells. As control, TET1 human protein has been also fused to dCas9. 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 single or combined sgRNAs efficiently targeted dCas9-ROS1_CD for reactivation of the silenced luciferase gene. In contrast, no reactivation was detected when those same gRNAs were used to target dCas9-TET1. Bisulfite pyrosequencing revealed that reactivation induced by dCas9-ROS1_CD correlates with certain decrease in DNA methylation levels.

These findings suggest the use of plant 5mC DNA glycosylases for targeted active DNA demethylation and gene reactivation in human cells.

5-MeC PRECISE QUANTITATION IN GENOMIC DNA AND SPECIFIC SEQUENCES: RELEVANCE IN CISPLATIN RESISTANCE

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Cisplatin is a widely used chemotherapeutic drug, whose major drawback is patient resistance due to, among others, changes in genome methylation profiles. Cytosine methylation at CpG sites is one of the most important epigenetic marks in vertebrates, and seems to be related to cisplatin resistance when studied at promoter regions of specific genes. However, the relationship between global DNA methylation and cisplatin resistance is not clear.

We have developed a novel technique to precisely quantitate 5-methyl-2′-deoxycytidine (5-MeC), based on high performance liquid chromatography (HPLC) followed by ultraviolet absorbance detection, and used it to study methylation both in genomic DNA and in specific sequences, which were isolated with streptavidin coated magnetic beads and 5'-biotinated oligonucleotides. Cisplatin sensitive (A2780) and resistant (A2780cis) human cell lines, treated for 3h, with 40 μM, were used for this analysis. Fragments of 700-1000 bp size, from the promoter regions of *BAX*, *CASP3*, *BCL2*, *TP53*, *ERCC1*, *ERCC4*, and *POLQ* genes, were selected and their methylation levels were analyzed both in treated and untreated cells. Bisulfite pyrosequencing was used for comparisons. Gene expression was determined, with quantitative RT-PCR using Taqman probes, to evaluate possible relationships with promoter methylation.

Results revealed that: (i) cisplatin treatment increased global genome methylation in both cell lines; (ii) methylation levels between 5 and 29% were detected for all the analyzed promoters, except for that of *ERCC4* gene; (iii) methylation differences between cell lines were detected for *BAX* and *ERCC1* promoters; and (iv) treatment effects on methylation profile were found for *BAX* in A2780cis cells. Moreover, the new method seems to be

more sensitive than bisulfite pyrosequencing. Finally, the detected methylation profiles of *BAX* promoter were apparently related to changes in gene expression.

AS3MT AND *MTH1* GENES AS NEW MARKERS IN THE ARSENIC-INDUCED MALIGNANT TRANSFORMATION

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Arsenic is a widespread and well-known human carcinogen associated with different kinds of cancer. Although the mechanisms of action by which chronic arsenic exposure leads to tumor development are not fully unveiled, it has been found that oxidative DNA damage plays an important role in the process. AS3MT is a key enzyme in arsenic biotransformation known to participate in the generation of ROS after a given arsenic exposure, while MTH1 has a role sanitizing oxidized dNTP pools and preventing the incorporation of damaged bases into the DNA.

In this work, MEF cells were chronically exposed to arsenite and monitored for oxidative and genotoxic DNA damage at different time-of-exposure intervals for 50 weeks, in order to assess the role of *As3mt* and *Mth1* in the arsenic-related transformation process. Thus, expression changes of these genes were evaluated by real-time RT-PCR at equivalent time-points.

Our results demonstrate that the oxidative and genotoxic damage increased time-dependently up to the cell's transformation point -reached after 30 weeks of exposure- but dropped drastically afterwards. Ongoing knock-down experiments could elucidate *As3mt* and *Mth1*'s role on the transformation process, validating the correlation found between their mRNA levels and the DNA damage pattern observed during arsenite exposure.

In conclusion, the study of the differential role of *As3mt* and *Mth1* in the accumulation of DNA damage linked to the transformation process has shown that *As3mt* acts as a damage sensor, contributing to the genotoxicity observed before transformation, while *Mth1* prevents the DNA damage fixation after the acquisition of the transformed phenotype and it is proposed as a new biomarker of arsenic carcinogenesis.

DETERMINATION OF GENE COPY NUMBER VARIATIONS: USEFULNESS OF MULTIPLEX PCR IN COMBINATION WITH GEL ELECTROPHORESIS-MASS SPECTROMETRY (GE-ICP-MS).

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DNA copy number variations (CNVs), defined as genomic structural variations caused by gain (duplications) or loss (deletions) of specific genome regions, are known to be involved in cancer as well as in chemotherapy response like cisplatin resistance. Their determination is performed mainly by real-time PCR and digital PCR, in single or in multiplex reactions. Both qPCR and digital PCR require fluorescent labels that limit their uses. Therefore, new methodologies enabling CNVs determination without labeling are needed. In this work we have studied the potential of the combination between end-point multiplex PCR and gel electrophoretic separations (GE) coupled with inductively coupled plasma detection (ICP-MS), monitoring the P present in the DNA backbone. The quantitative dimension can be obtained by using inorganic phosphate standards with known concentration as well as the calibration of the GE system in terms of the separation as function of the base pair number of the fragments. We have applied this methodology to study CNV in human ovary cancer cell lines. We have chosen three genes that are thought to be related to cisplatin chemotherapy response such as *CCNE1*, *ERBB2* and *GSTM1*, using *ACTB* as reference gene. We have found that the OVCAR-3 cell line presented around three more copies of *CCNE1* than the reference gene. We have also observed that both OVCAR-3 and A2780 cell lines may be heterozygous for the null *GSTM1* mutant allele.

SESIÓN 4: GENOTOXICIDAD Y ANTIGENOTOXICIDAD. ECOGENOTOXICOLOGÍA

SESSION 4: GENOTOXICITY AND ANTIGENOTOXICITY. ECOGENOTOXICOLOGY

IS MICRONUCLEUS FREQUENCY IN PERIPHERAL LYMPHOCYTES AND BUCCAL CELLS RELATED TO FRAILTY SYNDROME IN OLDER ADULTS?

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Average age of populations around the world is rapidly increasing. This aging situation is leading to an inversion of the population pyramids and to relevant increases in healthcare expenditure. In this new context, frailty has emerged as a more reliable way to estimate biological age. Frailty is a condition of vulnerability involving an increased risk of poor outcomes in older adults, including disability and mortality. Genomic instability has been proposed to be a primary cause of the aging phenotype since most age-related diseases and aging signs are associated with it. Therefore, implementation of clinical data with genomic instability biomarkers would have the potential of anticipating recognition of frail individuals and improving frailty outcomes. The objective of the present work was to assess the possible relationship between micronucleus (MN) frequency – a biomarker of genomic instability – evaluated both in peripheral blood lymphocytes and in exfoliated buccal cells, and frailty status in a population of older adults aged 65 and over. Results obtained showed that frail individuals had significantly higher frequencies of MN in lymphocytes and of binucleated buccal cells, as well as lower frequencies of pyknotic and condensed chromatin buccal cells, than pre-frail and non-frail subjects. When nutritional status and cognitive status of the individuals were considered, similar results were obtained, revealing increases in the rates of MN in lymphocytes and of binucleated buccal cells, together with decreases in the frequencies of pyknotic buccal cells, in individuals at risk of malnutrition/malnourished or with cognitive impairment compared to those with normal nutrition or without cognitive

impairment. Additional research is necessary to further understand the connection between genomic instability and frailty syndrome in the elderly.

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ARE MONOHALOACETIC ACIDS GENOTOXIC AND/OR CARCINOGENIC UNDER LONG-TERM EXPOSURE CONDITIONS?

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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. 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. 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 has not been tested yet.

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 for 8 weeks to sub-toxic concentrations of all three compounds. 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 metalloproteinases (MMPs).

Long-term exposure to low concentrations of the HAAs showed no cell-transforming ability in UROtsa cells, however, cell toxicity experiments suggest an increased resistance to oxidative damage in long-term exposed cells. Further experiments are necessary to determine non-carcinogenic effects of a long-term exposure to this class of DBPs.

INFLUENCE OF WAVE LENGTH, FREQUENCY AND DUTY CYCLE ON THE INDUCTION OF DNA DAMAGE BY PULSED VERSUS CONTINUOUS ULTRAVIOLET RADIATION

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The development of light-emitting diodes (LEDs) in a broad wavelength range, that includes ultraviolet (UV) radiation (UV-LED), has considerably improved the advantages of these sources of UV radiation, compared to the traditional low-pressure mercury lamps. UV-LEDs present long-life, are compact, robust and efficient, low-energy demanding, do not need pre-heating and do not produced mercury as waste. Moreover, they can be easily prepared for different wavelengths, and the emitted radiation can be modulated, varying both pulse frequency and width, or duty cycle.

However, little is known about the effect of this modulation on DNA damage induction. We have designed a versatile and easy to use lighting system that enables pulsed emission at different duty cycles and pulse frequencies, with UV-LEDs of 260 and 285nm. We have used it to check the effect of these variations on DNA damage induction, using the *Salmonella* reverse mutation assay, which detects mutations that revert the histidine-dependent genotype of *Salmonella typhimurium* strains. Among the strains available, TA102 was designed to specifically study the mutagenicity of some agents, including UV radiation.

Results showed, first of all, that pulse radiation could be more mutagenic than the continuous one, depending of the combined studied parameters. Pulse frequency analysis of 100, 500 and 5000Hz, revealed a clear effect, independent of the wavelength, since the lower the frequency, the higher the induced mutation frequency.

Duty cycle results, analysing 25 and 50%, were however controversial, because it apparently depended on the used UV-LED. The wavelength analysis confirmed higher mutagenic activity at 260 than at 285nm.

USEFULNESS OF THE NEW DEVELOPED "COMETA 5.1" SOFTWARE IN THE ANALYSIS OF COMET ASSAY NUCLEOIDS: COMPARISONS WITH KOMET5

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There are, currently, numerous software tools available to analyze DNA damage in nucleoids of the comet assay. Although some of them are free-to-use, those that offer the best features and reliability are protected under commercial license and must be purchased at rather high prices. In addition, even the commercial ones present problems in the recognition of some nucleoid heads and, moreover, results depend on the program user.

We are developing new software for the analysis of comet results, "Cometa 5.1". The key features of this new software are essentially three: an automatization in the process of selecting the areas containing nucleoids, a better determination of the head areas and a new and user-friendly interface. With them, the user goes through the assay photographs in a fast and reliable way, reducing time and especially subjectivity. Additionally, when the automatic determination fails, the user can manually select the area of the comet and reposition the head center.

The performance of this software was checked against the commercial Komet 5 (Kinetic Limited, UK). Although results demonstrated that there were not differences on the decision about treatment genotoxicity, in terms of operation time the developed software was the most efficient one, due to its comet recognition system; in the manual, or interactive, use it was more objective and reliable; finally, it allowed the analysis of rather damaged cells.

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