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Instituto de Investigaciones Biológicas Clemente Estable (IIBCE)

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Epigenetics and DNA repair

Is a chromatin remodeling process involved in the higher UV sensitivity of nucleotide excision repair defective cells?

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1) Scientific background of main research activities

We have recently shown that transcription coupled repair (TCR) failure seems not to be responsible for the increased frequency of chromosomal aberrations observed in Cockayne's Syndrome (CS) simile cells exposed to UVC, since chromosome breakpoints were distributed more random in CS cells than in normal ones instead of being concentrated on the transcribed chromosome regions as expected (Martínez-López et al., 2010) (Paper is included in deliverables).

Since DNA accessibility for DNA repair proteins is limited in nucleosomes (Thoma 1999, 2005), it can be speculated that different chromatin organization after UVC exposure in CS cells could influence the distribution of CPDs in eu- and heterochromatic regions as well as their removal by TCR subpathway of nucleotide excision repair (NER) system, leading to increased frequencies of chromosomal aberrations in these cells.

In this respect, we have observed that changes in the histone acetylation level by treating normal cells with an histone deacetylase inhibitor like trichostatin A (TSA) produce a similar sensitivity to UVC than in CS cells, increasing chromosomal aberration frequency (Figure 1).

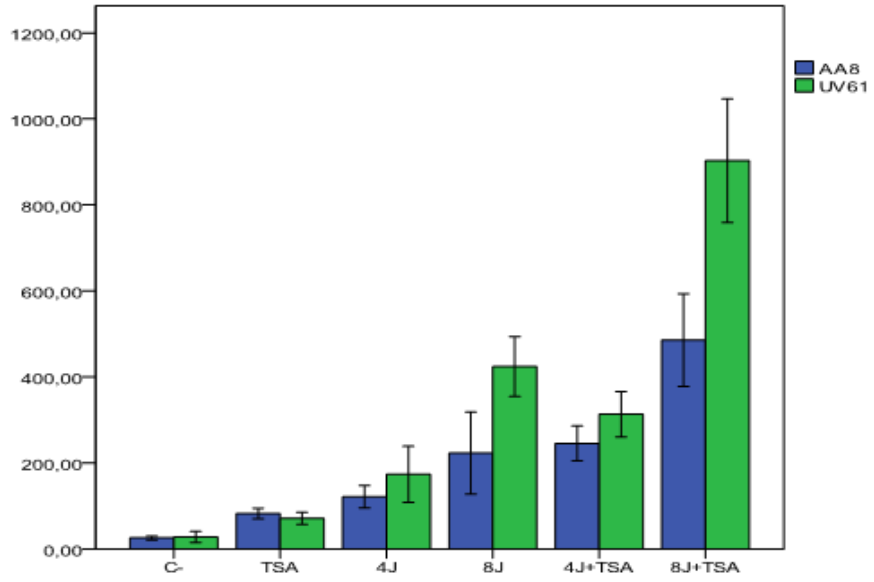


Figure 1.- A significant increase in the frequency of UVC-induced chromosomal aberrations was found in the presence of TSA (50 ng/ml) either in proficient or deficient CHO cells in TCR. C= Control; TSA= trichostatin A; 4J= 4 Joules; 8J= 8 Joules. Bars represent 2 x SE (standard error) from two independent experiments.

Besides, we have analyzed the kine tics of CPDs removal in normal and CS cells in the presence of TSA by using the com et assay plus the T4 endonuclease V which recognizes specifi cally CPDs introducing single strand breaks which increa se DNA m igration (Figures 2 and 3) as well as imm unolabeling techniques employing antibodies against CPDs and re vealed with a secondary antibody conjugated with FITC (Figure 4). An impair of CPDs removal in the presence of TSA was observed with both techniques. A more detailed study on the distribution of CPDs along euchromatic and hete rochromatic chromosome regions from normal and CS cells are being carried out at pr esent. (See abstract below: Méndez-Acuña et al., 2011). On the other hand, new experim ents em ploying hum an cells derived from CS patients are being developed to confirm our results obtained in Chinese ham ster cells (SV40 transform ed cell lines from CS patients has been recently acquired from the DNA Repair Laboratory from the University of San Pablo - USP, Brazil).

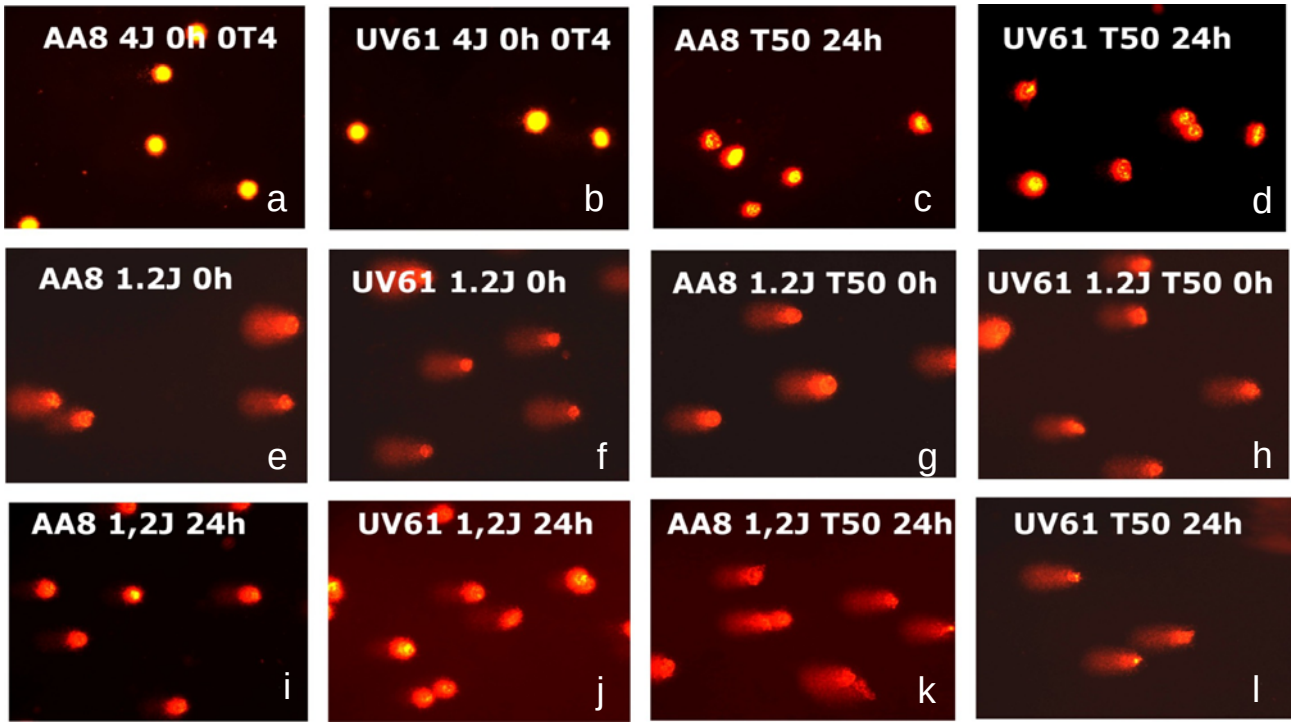


Figure 2.- Comet assay em ploying T4 endonuclease V in AA8 and UV61 cells exposed to 1,2 J/m 2 of UVC in the presence or absence of TSA (50 ng/m l), and recovered at 0h a nd 24h after UVC-irradiation. (a-b) UVC-irradiated cells (4 J/m 2) but not exposed to T4 endonucleas e V. (c-l) All nucleoids were exposed to 1 ml of T4 endonuclease V diluted in 50 ml of NET buffer. Note that after 24 h recovery tim e after UVC-irradiation, primary lesions are still not removed in AA8 as well as in UV61 cells.

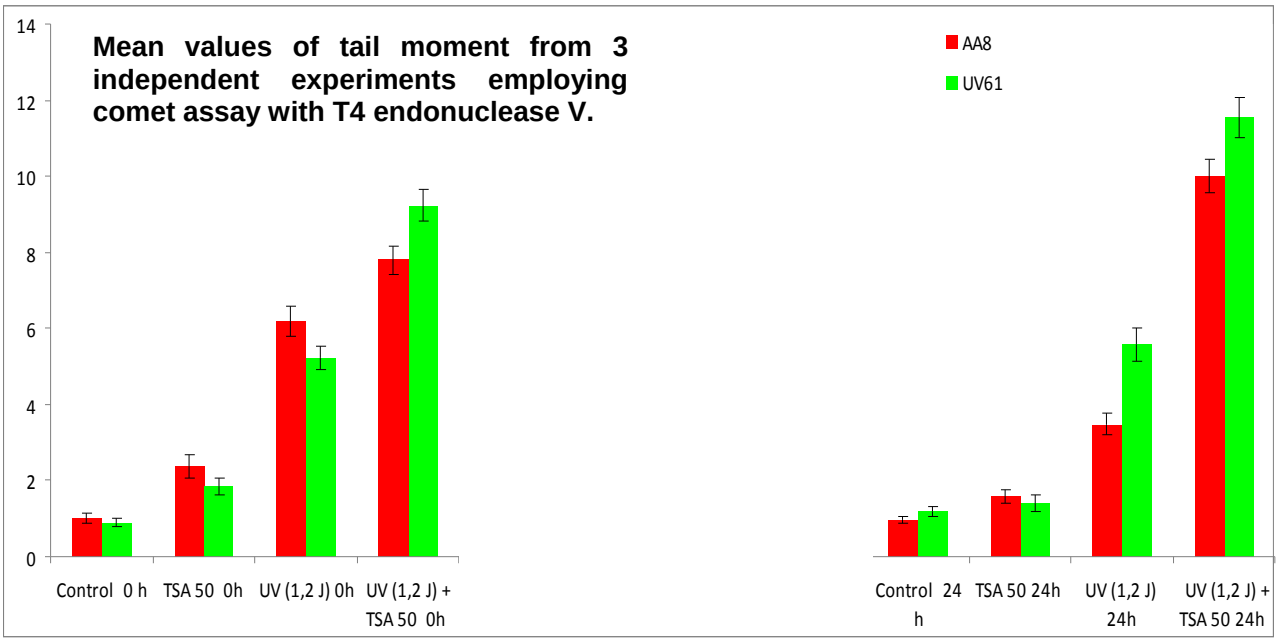


Figure 3.- Graphic representation of the effect of TSA in combination with UVC related to an impair produced by the TSA on the removal of UVC-induced primary lesions. Bars represent the standard deviations.

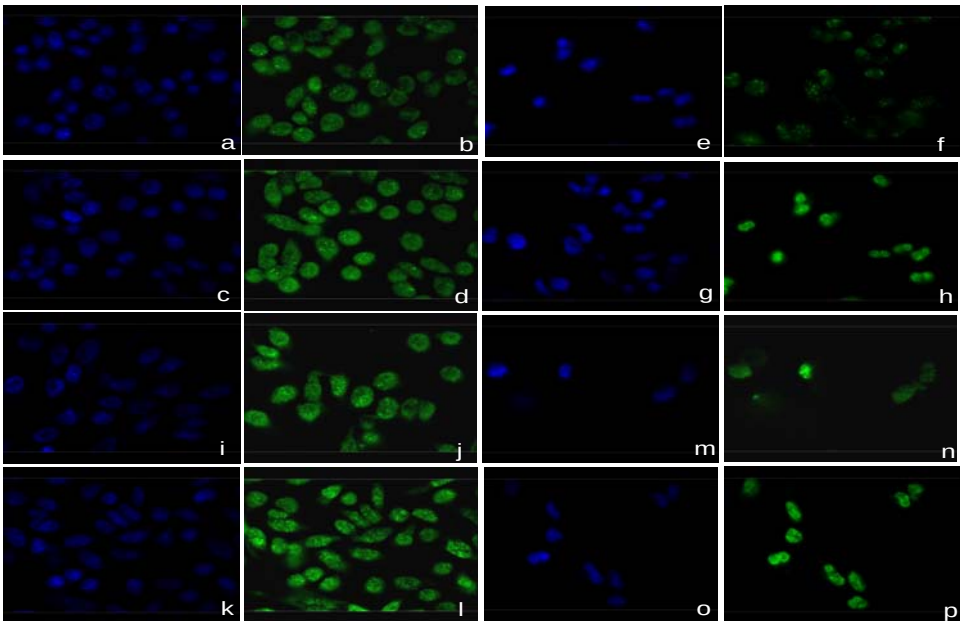


Figure 4.- Immunolabeling of cyclobutane pyrimidine dimmers (CPDs) employing anti-CPDs mouse antibodies revealed with an anti-mouse-FITC (green) and counterstained with DAPI (blue) on AA8 (a-h) or UV61 (i-p) nucleous. (a- b, i-j) Nucleous fixed with paraformaldehyde at 0 h after UVC irradiation (4 J/m2), (c-d, k-l) Nucleous fixed with paraformaldehyde at 0 h after UVC irradiation (4 J/m2) from cells pre-treated (4 h) with TSA, (e-f, m -n) Nucleous fixed with paraform aldehyde at 20 h after UVC irradiation (4 J/m2), (g-h, o-p) Nucleous fixed with paraformaldehyde at 20 h after UVC irradiation (4 J/m2) from cells pre-treated (4 h) with TSA, which produce an impair on CPDs removal by nucleotide excision repair (NER).

Although we have shown that TSA (an histone deacetylase inhibitor) is able to produce an impair in the removal of UVC-induced lesions in normal cells as it happens in CS cells, we could not find an increase in histone acetylation pattern in CS cells. On the contrary, we have observed in CS simile cells (UV61, the Chinese hamster homologue of the Cockayne's Syndrome B or CSB) a less decondensed chromatin triggered by UVC irradiation evidenced by *in situ* nick translation technique (Figures 5 and 6).

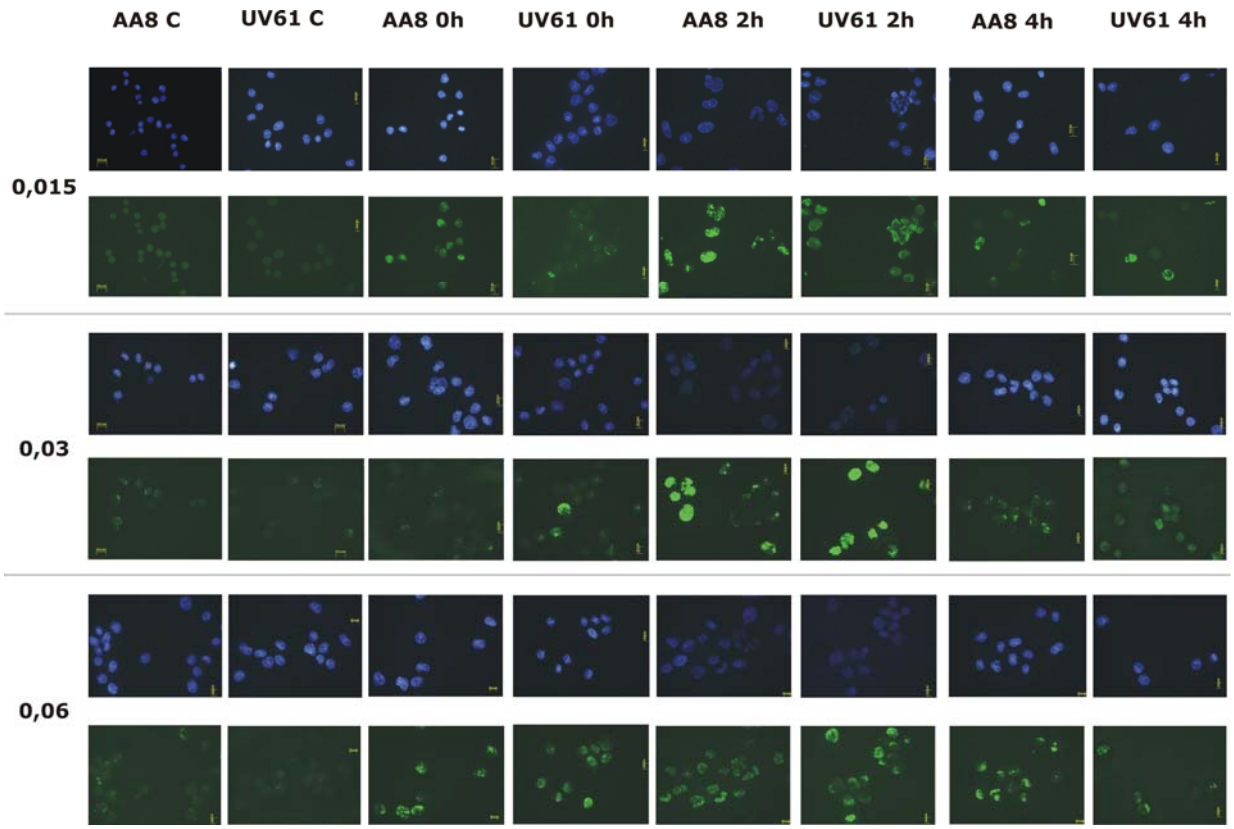


Figure 5.- *In situ* nick translation panel of fixed G1 cells from AA8 and UV61 irradiated with 4 J/m2 of UV-C and recovered at 0h, 2h and 4h post-irradiation, exposed to mixtures containing different doses of DNase I (0.015, 0.03 and 0.06 units/ml). Green fluorescence correspond to the incorporation of labeled nucleotides by polymerase I revealed with an antibody coupled with FITC. Blue fluorescence represent DAPI counterstaining.

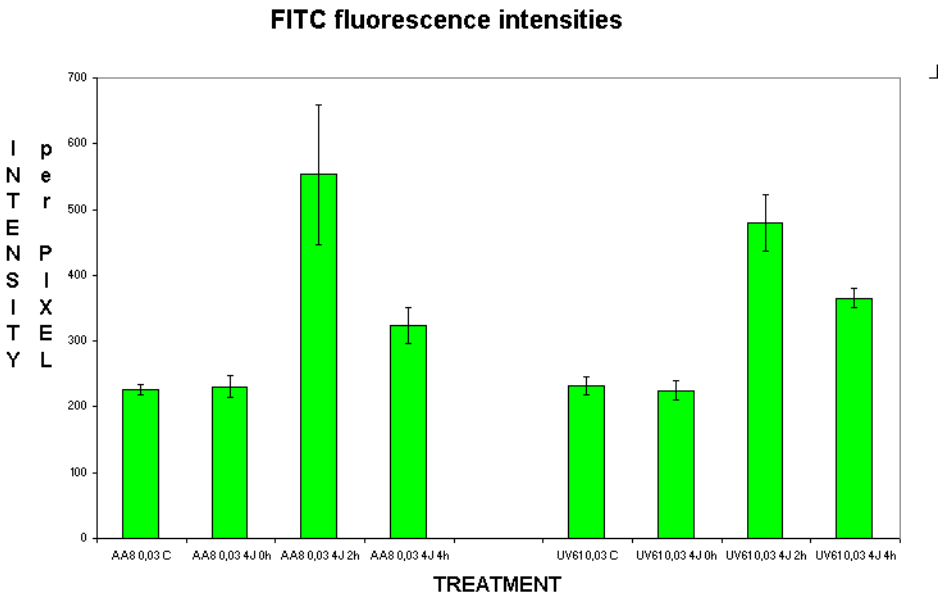
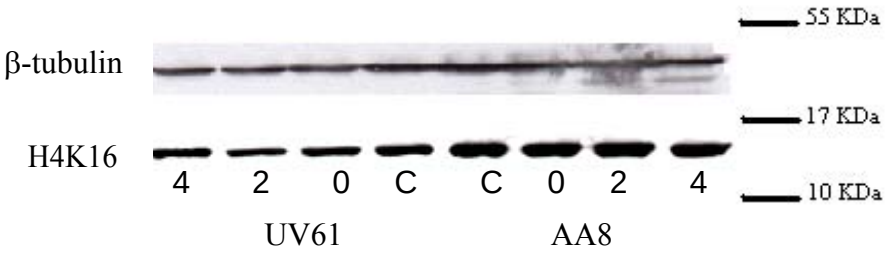


Figure 6.- Graphic representation of FITC fluorescence intensity per pixel from 50 fixed nuclei exposed to 0.03 units/ml of DNase I. Bars represent 2 x SE (standard error) from two independent experiments.

As it has been demonstrated that UVC irradiation triggers a genome-wide histone hyperacetylation at both histones H3 and H4 (Yu et al., 2005), we have investigated the histone H4 acetylation level after UVC exposure of AA8 and UV61 cells by means of western blot using antibodies against specific histone H4 acetylation sites. Since di, tri and tetra-acetylation on lysines 12, 8 and 5, respectively, from the amino terminal tail of histone H4 are considered as hyperacetylation, we have analyzed the di, tri and tetra-acetylations patterns of AA8 and UV61 after UVC irradiation. Mono-acetylation is unchanged after UVC-irradiation either in AA8 or UV61 and it is shown as loading control (Figure 7). The di-acetylation increase after 2 and 4 h after UVC irradiation in AA8 but only at 4 h in UV61 (Figure 8). However, tri-acetylation and tetra-acetylation increase also at 2 and 4 h after UVC irradiation in AA8 but almost no increase is observed in UV61 (Figures 9 and 10), indicating that CS cells could not sufficiently increase the histone acetylation level triggered by UVC irradiation as it occur in normal cells (See abstract below Martínez-López et al., 2011). Although Proietti De Santis et al. (2006) have shown previously that gene promoters from CSB controlled genes are underacetylated after UVC irradiation, we have shown a difference, for the first time, in the global histone acetylation level between normal and CS cells.



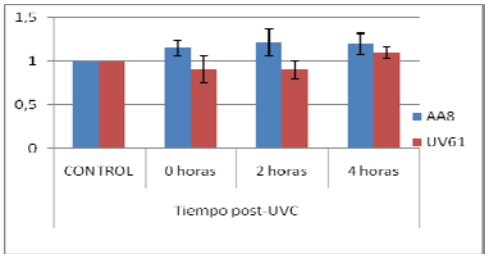
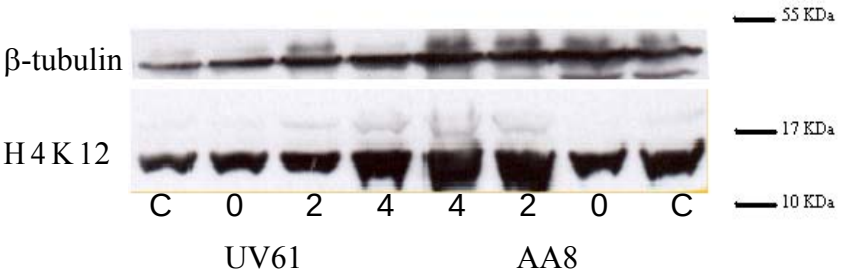


Figure 7.- Western blo t showing the histone H4 acet ylation pattern of H4K16 (mono-acetylated histone) after 0, 2 or 4 h post-U VC irradiation. Note that ther e is no differences in the mono-acetylation pattern among AA8 or UV61 samples.



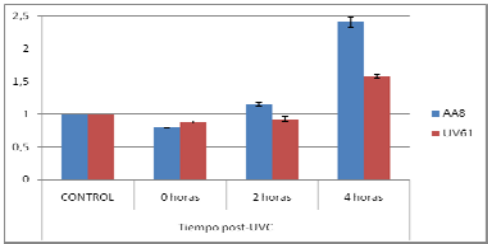
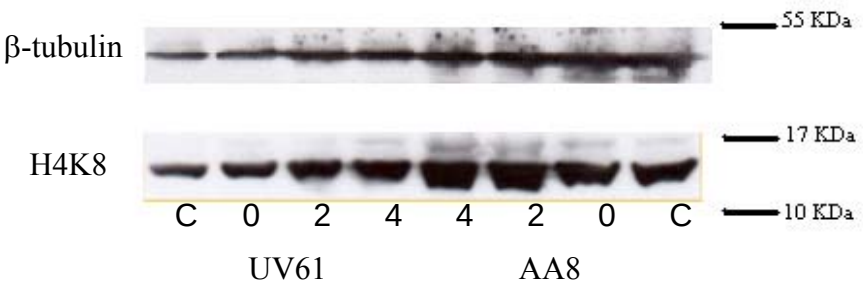


Figure 8.- Western blot showing the histone H4 acetylation pattern of H4K12 (di-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an increase in di-acetylation at 2 and 4 h after UVC while just at 4 h in UV61 cells.



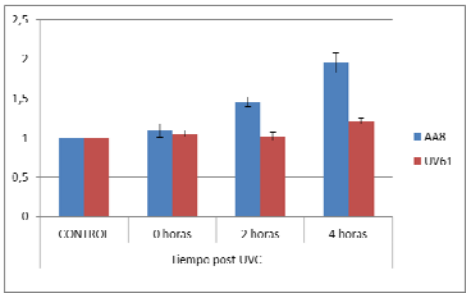
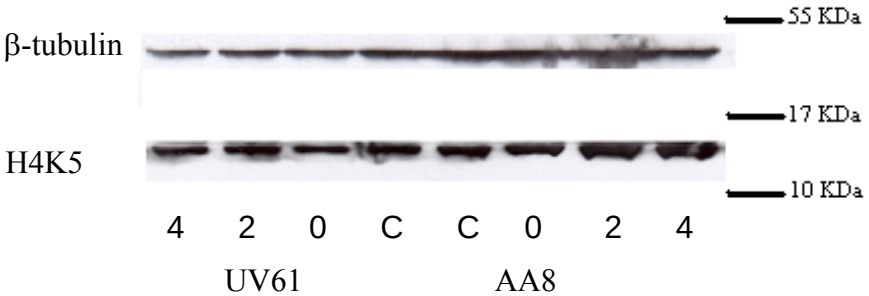


Figure 9.- Western blot of histone H4 acetylation pattern of H4K8 (tri-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an incr ease in tri-acetylation at 2 and 4 h af ter UVC while there is almost no tri-acetylation increase in UV61 cells.



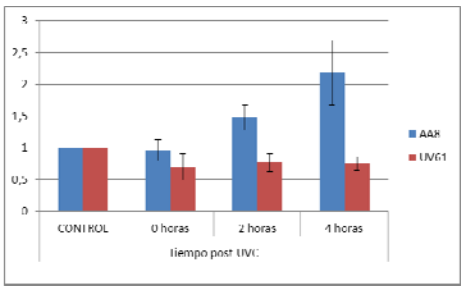


Figure 10.- Western blot of histone H4 acetylation pattern of H4K5 (tet ra-acetylated histone) after 0, 2 or 4 h post-UVC irradiation. Note that in AA8 cells there is an increase in tetra-acetylation at 2 and 4 h after UVC while there is almost no tetra-acetylation increase in UV61 cells.

The CSB protein (Cockayne's Syndrome B protein) has a key role as coupling factor to attract the histone acetyltransferase p300 and nucleotide excision repair proteins. Therefore, the 5 fold increase in chromosomal aberrations after UVC exposure (Proietti De Santis et al., 2001) could be caused by an acetylation imbalance since nucleotide excision repair proteins could not properly interact with DNA lesions in a less decondensed chromatin environment.

Impact or results obtained and future research activities

We have shown that an imbalance on histone acetylation patterns produced by histone deacetylation inhibition can produce an impair in the removal of UVC induced lesions by nucleotide excision repair. This fact could be used to sensitize tumor cells to chemoterapy or radiotherapy. Besides, we have shown some experimental evidences to support that neurodegeneration processes on CS cells could be related to changes in histone acetylation patterns.

In this respect, we have recently obtained a grant in collaboration with the Laboratory of Organic Chemistry and Laboratory of Theoretical Chemistry, both from the Faculty of Sciences of the University of the Republic of Uruguay, to "Develop of anti-cancer therapies to sensitize hypoxic tumor cells" by creating more specific histone deacetylase inhibitors (HDACi) compounds with the aim to specifically inhibits HDAC7, whose over-expression is related to the regulation of genes involved in the tumor cell survival in hypoxic conditions, in tumor cell lines.

Moreover, it is foreseen to prepare a MARIE CURIE INDUSTRY-ACADEMIA PARTNERSHIPS AND PATHWAYS (IAPP) with the aim to test some specific inhibitors of chromatin remodelling processes in animal models.

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