SOCIETA' ITALIANA DI MUTAGENESI AMBIENTALE

CHROMOSOME INSTABILITY: MECHANISMS AND HEALTH EFFECTS

WORKSHOP

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ABSTRACT BOOK

INVITED LECTURES
AND SHORT ORAL COMMUNICATIONS

(IN THE ORDER OF PRESENTATION)

SESSION 1

CHROMOSOME STRUCTURAL DYNAMICS

CAN CHROMOSOME INTEGRITY BE MAINTAINED ALWAYS AND EVERYWHERE?

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Genome integrity is known to be disturbed with age. This age-dependent impairment has been classically attributed to telomere length attrition. Telomeres shorten with age in proliferating cells of most human somatic tissues. Telomere dysfunction, which is known to influence both the structure and the number of chromosomes in cells, also affects DNA repair fidelity. Telomere function now emerges as a factor potentially contributing to increasing the sensitivity of aging human cells to DNA-damaging agents. Since eroded telomeres are sensed and act as DNA double strand breaks, they can interact with radiation-induced DNA breaks, thus sharply increasing the possibility of misrejoining and revealing itself as an important factor that definitely contributes to genomic instability and radiation sensitivity. In the light of more recent work carried out in our laboratory, it is clear that age-dependent radiation sensitivity and genomic instability is not only linked to telomere dysfunction but also to a progressive deterioration of DNA damage cell response. This is even more relevant when the malfunction of DNA-repair proteins has been observed in the scenario of low-dose radiation exposure. Concerned about the risks of mammography screening, our research group has analyzed the ability of human mammary epithelial cells to cope with mammogram-induced DNA damage. In a recent study, we show that the dose received by the breast surface per mammogram X-ray exploration induces increased frequencies of DNA double strand breaks to aged—but not to young—human mammary epithelial cells. This is consistent with recent IRCP published data that classifies breast tissues amongst those that are most sensitive to radiation and also with epidemiological studies that reveal increased carcinogenic risks of radiation exposures at older ages. When faced with low-dose radiation induced double strand breaks, aged mammary epithelial cells trigger a slow response, thus inducing increased amount of genetic damage. In this new scenario where telomere dysfunction and DNA repair impairment is produced by the sole act of proliferative cell aging, radiation sensitivity might acquire a temporal aspect: shortened telomeres and decreased repair efficiency in aged cells may potentially increase radiation sensitivity in elderly organisms.

Organisms are continuously exposed to DNA damaging agents, consequently, cells have developed an intricate system known as the DNA damage response to detect and repair DNA lesions. To restore genome integrity, the cell must overcome important hurdles in space and time so as to rapidly sense and initiate the correct signalling and repair programs. However, DNA damage response is not triggered with the same efficacy everywhere. Specifically, the micronuclear environment strongly hinders a proper DNA damage response. The almost total absence of DNA damage response factors recruitment to micronuclear DNA double strand breaks and helix-distorting base lesions indicate that micronuclei are almost incapable of generating an effective DNA damage response. Nuclear envelope defects have been identified as responsible for this defective micronuclear damage response. The data collected in this laboratory suggest that the DNA damage response machinery is not ready for action everywhere. Only the cell nucleus with intact envelope structure provides the adequate concentration of proteins for interacting with damaged DNA and triggering an efficient response. In the case of micronuclear DNA lesions, the chromatin encapsulated in micronuclei does not benefit from the intricate and efficient web of DDR players of the cell, and chromosome instability and radiation sensitivity would be favored under these circumstances when micronuclei is eventually incorporated into daughter nuclei. Thus micronuclei, which have mainly been considered as indicators of ongoing genomic instability, now emerge as a source of instability at the same time. Altogether, this reveals a new dimension in the significance of micronucleation within the carcinogenesis process.

THE G4 LIGAND RHPS4 INDUCES CHROMOSOMAL INSTABILITY AND INCREASED SENSITIVITY TO X-RAYS IN GLIOBLASTOMA CELLS

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It is well known that one of the phenotypic hallmarks of cancer, *i.e.*, unlimited replicative potential, is intimately related to the maintenance of telomeres, the nucleoproteic complexes located at the end of eukaryotic linear chromosomes. A number of published works revealed that uncapped/dysfunctional telomeres can be obtained by pharmacological stabilization of specific secondary structures that are physiologically present at telomeres known as G-quadruplex (G4). G4 interacting compounds, such as the RHPS4 drug, destabilize telomere architecture leading to end-to-end telomeric fusions and consequently to chromosomal instability.

In addition, in recent years telomeres have been recognized as a new player in radiation sensitivity and in particular telomere loss or dysfunction were indicated as critical parameters in the cellular response to ionizing radiation.

The aim of the present study is to shed light on the possible synergistic effect of the combined treatment with RHPS4 and ionizing radiation on radioresistant U373 human glioblastoma cells. Percentage of dysfunctional telomeres progressively increased with time of exposure whereas Q-FISH analyses revealed that telomere length did not decrease as response to RHPS4-treatment. To investigate the effect on chromosomal stability of the drug alone or in combination with IR, cytogenetic experiments were performed using direct labeled FITC-pancentromeric and Cy3-telomeric PNA probes. Dicentrics and telomere fusions were higher in the combined treatment samples if compared to X-irradiated samples. In addition, it was observed a higher percentage of unrejoined DSBs and a reduced surviving fraction in the combined treatment with respect to the only X-ray exposure.

Further experiments are in progress to support the hypothesis of a direct correlation between the number of dysfunctional telomeres induced by RHPS4 and the cellular radiosensitivity measured by the induction of unstable rearrangements such as dicentrics and telomere fusions.

DIVERGING ROLES OF THE WRN HELICASE AND THE MUS81 ENDONUCLEASE IN THE MAINTENANCE OF CHROMOSOME INTEGRITY FOLLOWING REPLICATION STRESS

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The integrity of eukaryotic chromosomes relies on a sophisticated mechanism that allows DNA lesion repair, faithful replication and chromosome segregation. An accurate duplication of chromosomes is crucial to the maintenance of genome stability, since any replication error has the potential to introduce harmful DNA lesions, such as DNA double-strand breaks or gaps, giving raise to gross chromosomal rearrangements. However, to counteract this eventuality, eukaryotic cells have evolved multiple pathways that permit completion of chromosome replication even in the presence of replication stress. Despite the redundancy in these mechanisms, some of them are preferred over other because less prone to formation of chromosome rearrangements.

Over the years, many factors participating to one or multiple replication recovery mechanisms have been identified, and most of them have been found mutated in human chromosome instability syndromes or have been associated to cancer predisposition, such as ATR, RecQ helicases, BRCA1/2 or Fanconi anemia proteins. Only in the last few years, several groups started to investigate in human cells how these multiple factors are regulated and inter-related, as well as to appreciate the cellular hierarchy in their usage at perturbed forks. Recently, these studies have became increasingly important, not only for basic science, but also for cancer biology as it is now well acknowledged that genome instability, which is a hallmark of cancer cells, is primarily acquired because of chronic replication stress.

Our results suggest that a correct response to perturbed replication relies on the Werner syndrome helicase (WRN), which is a tightly-regulated protein that interfaces with multiple checkpoint factors. Absence of WRN determines excessive replication fork collapse and chromosome instability, mostly through activation of more error-prone pathways. Moreover, we identified the predominant of these pathways, which grants survival of WS cells at cost of genome instability, and we observed that it relies on the MUS81/EME1 endonuclease.

Novel findings showing how MUS81/EME1 may replace WRN during replication fork recovery, will be reported, and how activation of back-up pathways during replication stress may correlate to chromosome instability, and may be exploited in a target therapy of cancer will be discussed.

THE EFFICIENCY OF HOMOLOGOUS RECOMBINATION AND NON-HOMOLOGOUS END JOINING SYSTEMS IN REPAIRING DOUBLE-STRAND BREAKS DURING CELL CYCLE PROGRESSION

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This study investigated the efficiency of Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) repair systems in rejoining DNA double-strand breaks (DSB) induced in CCD-34Lu cells by different γ-ray doses. The kinetics of DNA repair was assessed by analyzing the fluorescence decrease of γ-H2AX foci measured by SOID (Sum Of Integrated Density) parameter and counting foci number in the time-interval 0.5-24 hours after irradiation. Comparison of the two methods showed that the SOID parameter was useful in determining the amount and the persistence of DNA damage signal after exposure to high or low doses of ionizing radiation. The efficiency of DSB rejoining during the cell cycle was assessed by distinguishing G1, S, and G2 phase cells on the basis of nuclear fluorescence of the CENP-F protein. Six hours after irradiation, γ-H2AX foci resolution was higher in G2 compared to G1 cells in which both NHEJ and HR can cooperate. The rejoining of γ-H2AX foci in G2 phase cells was, moreover, decreased by RI-1, the chemical inhibitor of HR, demonstrating that homologous recombination is at work early after irradiation. The relevance of HR in DSB repair was assessed in DNA-PK-deficient M059J cells and in CCD-34Lu treated with the DNA-PKcs inhibitor, NU7026. In both conditions, the kinetics of γ-H2AX demonstrated that DSBs repair was markedly affected when NHEJ was absent or impaired, even in G2 phase cells in which HR should be at work. The recruitment of RAD51 at DSB sites was, moreover, delayed in M059J and in NU7026 treated-CCD-34Lu, with respect to DNA-PKcs proficient cells and continued for 24 hours despite the decrease in DNA repair. The impairment of NHEJ affected the efficiency of the HR system and significantly decreased cell survival after ionizing radiation, confirming that DSB rejoining is strictly dependent on the integrity of the NHEJ repair system.

COMPARATIVE GENOMIC HYBRIDIZATION STUDIES ON MESOTHELIOMA SHOW A PARALLEL FATE OF 1p21-1p22 AND 9p21 BANDS, AND A CHROMOSOMALLY STABLE SUB-GROUP

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Malignant Pleural Mesothelioma (MPM) is a cancer whit a poor prognosis. Unfortunately, robust conclusions on the landscape of the chromosomal rearrangements could not be made, given the limited sample sets studied. In order to improve the knowledge on the field, we collected (pooled) all the results deriving from studies on comparative genomic hybridization (CGH). This allowed a better description and novel insights on the alterations occurring in 179 MPM tissues (74 epithelioid, 48 sarcomatoid, 57 biphasic) and 8 cell lines (4, 1, and 3, respectively). Some chromosomal bands were involved in aberrations with a frequency exceeding 20% for -1p21, -9p, -14q, and -22q for the epithelioid, -4, +5p, +8q21→ter, -9p, -13, and -14q for the sarcomatoid, and -9p, -14q, and -22q for the biphasic type. We found a statistical significant association between losses at 9p21 and 1p21-1p22 (21% of the samples showed a contemporary loss of the two chromosomal bands, $P=7.74x10^{-8}$). The association held also within each histological group. These findings suggest an underlying mechanism related to MPM tumorigenesis. Finally, 15% of epithelioid, 13% of sarcomatoid, and 13% of biphasic MPM tissues did not show genomic alterations. Interestingly, previous studies suggested a less aggressive behavior of MPM in relation to a low degree of chromosomal alterations. However, according to our review of the literature, only few studies considered this aspect. More studies are warranted to understand whether chromosomal rearrangements could be included as diagnostic and prognostic biomarker in the clinical practice.

SESSION 2

CHROMOSOME SEGREGATION AND ANEUPLOIDY

EFFECTS OF ANEUPLOIDY ON CHROMOSOME SEGREGATION AND CELLULAR PHENOTYPE

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Cancer cells display distinct aneuploid karyotypes (i.e., abnormal chromosome numbers) and typically mis-segregate chromosomes at high rates, a phenotype referred to as chromosomal instability (CIN). Recent studies have shown that anaphase lagging chromosomes arising due to merotelic kinetochore attachment (attachment of a single kinetochore to two spindle poles instead of just one) are the major chromosome segregation defect responsible for whole- chromosome instability in cancer cells. Indeed, we find that whereas lagging chromosomes occur at significantly higher frequencies in CIN vs. non-CIN cells, other mitotic chromosome segregation defects (e.g., chromosome bridges or acentric fragments) occur at similar frequencies in CIN and non-CIN cancer cells. While it is readily apparent how chromosome mis- segregation in the form of anaphase lagging chromosomes can cause aneuploidy, the effect aneuploidy has on chromosome segregation is unclear. To test the effects of aneuploidy on chromosome segregation we utilized the colorectal cancer cell line DLD1 (2n=46) and variants of this line containing defined artificial trisomies for chromosomes 7 and 13 (DLD1+7 and DLD1+13, respectively). We found that DLD1+7 and DLD1+13 cells displayed higher rates of chromosome mis-segregation compared to the parental cell line. Furthermore, we found that cells with trisomy 13 display a distinctive cytokinesis failure phenotype. Interestingly, chromosome 13 encodes for *SPG20* (Spastic Paraplegia 20, or Spartin), a gene involved in cytokinesis completion. We showed that up-regulation of SPG20, brought about by trisomy 13, is both required and sufficient for the cytokinesis failure phenotype. Indeed, overexpression of Spartin in DLD1 cells reproduced the cytokinesis failure phenotype observed in DLD1+13 cells. We further determined that Spartin overexpression prevented localization of Spastin, another cytokinesis protein, at the midbody. Finally, we showed that siRNA-mediated Spartin knock down rescued both the cytokinesis failure defect and Spastin midbody localization in the DLD1+13 cell line. Overall, our study shows that aneuploidy *per se* induces chromosome mis-segregation in cancer cells. Moreover, our data indicate that different aneuploidies can yield distinct cellular phenotypes/behaviors that are driven by up-regulation of specific genes encoded on the aneuploid chromosome.

MECHANISMS TO MAINTAIN CENTROMERE STABILITY IN HUMAN CELLS

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Human centromeres are composed of highly organized arrays of repetitive alpha satellite DNA. Extensive sequence homology of the repeats within each centromere indicates that homologous recombination (HR) plays an active role in maintaining the repeat structure, but also implies that this process must be tightly regulated to avoid loss of centromeric DNA, which could result in kinetochore malfunctions and cause genome instability. We hypothesize that the mechanism(s) that suppresses mitotic recombination at centromeres may become compromised during ageing and tumorigenesis, leading to aneuploidy. To test this, we have utilized the CO-FISH technique to monitor centromere recombination in proliferating human cells. We observed an increase in sister chromatid exchanges and rearrangements at centromeres of a variety of cancer cells. To investigate the underlying molecular mechanisms that maintain centromere integrity, we down-regulated the centromere- specific histone H3 variant CENP-A and observed an increase in centromeric re- arrangements in both cancer and primary cells. Additionally, as the level of CENP-A is altered during cellular senescence, our preliminary data indicate that cellular senescence also leads to an increase in centromeres rearrangements. While CENP-A is an epigenetic determinant for kinetochore formation, which is critical for microtubule attachment and chromosome segregation, induction of aberrant attachments during mitosis did not enhance centromere rearrangements. Thus, we suggest that CENP-A provides a mechanism to suppress centromere recombination independently of its role in regulating microtubule attachments. We are currently dissecting the mechanism by which CENP-A represses centromere re-arrangements and the consequences of the alpha-satellite repeats recombination. The potential mechanisms by which the centromere integrity is compromised during ageing and tumorigenesis will also be discussed.

INHIBITORS OF TOPOISOMERASE 1 INDUCE ARREST OF COLON CANCER HCT116 CELLS AT THE END OF MITOSIS

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Topoisomerase I (Top1) inhibitors, used in cancer therapy for the treatment of a variety of solid tumours, are semi-synthetic drugs derived from the alkaloid camptothecin obtained from the Chinese tree Campotheca acuminate. Their main molecular mechanism of action relies on stabilization of the cleavable complex formed by Top1 and DNA with generation of single strand breaks. Irreversible DNA damage occurs when the stabilized drug-DNA-Top1 complex collides with replication machineries, leading to replication fork stalling and eventually double strand breaks. Tumour cell treatment with Top1 poison may cause not only cell cycle arrest or delay and cell death, but also chromosome damage. Even though DNA damage and consequently chromosome aberrations mainly occur during the S phase of cell cycle at sites where the replication fork is arrested, DNA can be damaged also during the G2 phase. However, the mechanism underlying this effect has not been clarified yet. Two hypotheses have been proposed: a) latest part of DNA duplication might take place also in the G2 phase, b) since the inhibition of Top1 interferes also with the stability of genes during their transcription, camptothecins may induce breaks at the level of genes expressed in G2. Moreover, these drugs frequently induce karyotype alterations, for example polyploidy, through the total inhibition of sister chromatid separation, or aneuploidy, when the phenomenon concerns only some chromosomes.

Different clones of the colon cancer HCT116 cell line, proficient or deficient for the mismatch repair component MLH1, poly(ADP-ribose)polymerase 1 (PARP-1) and the tumour suppressor p53 were studied . Tumour cells were exposed to graded concentrations of SN38, the active metabolite of irinotecan, which is a camptothecin derivative commonly used for colon cancer chemotherapy. Initially, we determined the SN38 concentrations that caused significant chromosomes damage (treating cells in G2 for 3 hours), but that allowed cells to reach partly mitosis without causing a complete G2 arrest. Besides chromosome damage (as control of the efficacy of the drug) we analysed mitosis progression in control cultures and in those treated with SN38. The progression through mitosis was evaluated counting the percentage of cells in the different phases of mitosis (prophase, metaphase, anaphase, telophase and cytodieresis). Cells were stained with DAPI and using antibodies recognizing tubulin, to better characterize the different phases of mitosis, or centromere protein F (CENPF) that labels cells in G2 and mitosis, and phosphorylated H2AX as a marker of DNA damage.

SN38 treatment induced an increase in the percentage of cells accumulated at the second part of mitosis after metaphase/anaphase transition checkpoint. This effect was not observed in cells exposed to bleomycin, which is known to cause a strong G2 arrest, or aphidicolin that blocks cells mainly in early S phase. The arrest in the last phase of mitosis (cytodieresis) has been associated also to damage of telomeres, that can be targeted by camptothecins. Thus, telomere sensitivity to SN38 was analysed in HCT116 derived cell clones by FISH.

MMR deficient HCT116 cells (devoid of hMLH1 expression) were more sensitive to SN38 as compared to MMR proficient counterpart. Stable silencing of PARP1 expression resulted in higher sensitivity to SN38 clastogenicity and a higher percentage of cells arrested at the end of mitosis, compared either to control cultures and also with those expressing MLH1. PNA-FISH analysis showed that SN38 may induce a decrease in the number of telomeres positive for the PNA probes, suggesting a damage at that level.

ENDOGENOUS DNA DAMAGE AND DNA REPAIR DEFICIENCY IN DOWN SYNDROME FIBROBLASTS

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DNA damage has been suggested to significantly contribute to the etiology of several human diseases, including genetic diseases and tumors. In particular, DNA damage and deficiency in DNA repair have been associated with diseases, such as Xeroderma Pigmentosum (XP), Cockaine syndrome (CS), Trichothiodystrophy (TTD), and other genetic diseases. In the last years, defects in DNA repair have also been called into question for age-related diseases, such as Alzheimer disease (AD), Parkinson disease (PD), and Down syndrome (DS). Increased accumulation of DNA lesions, and specifically those produced by oxidative damage, possiblly in association with defects in DNA repair, have been indicated as one of the factors involved in the etiology of these syndromes. We are studying the involvement of DNA damage and its repair in some aspect of DS because these patients are prone to develop tumors, particularly some leukemias, in addition to aging early. In this work we have investigated the presence of endogenous DNA damage and the repair of oxidative lesions by the base excision repair (BER) mechanism in fetal, as well as adult dermal fibroblasts obtained from DS patients.

The results have shown that DS fibroblasts are characterized by signs of endogenous DNA damage, and activation of the DNA damage response, as evidenced by the appearance of the phosphorylated form of histone H2AX (g-H2AX), increased levels of p53, and by Thr68 Chk2 protein phosphorylation. The presence of cells showing a typical comet tail, when tested by the Comet assay, in DS cell cultures grown under normal conditions, further supported this conclusion. In addition, activation of the DNA damage response pathway was indicated by the increase in the number of cells entering a senescent state, as determined by the increase in beta-galactosidase. The cloning efficiency after oxidative DNA damage induced by potassium bromate ($KBrO_3$), indicated that both fetal and adult DS fibroblasts were more sensitive to this type of lesions, than cells from normal donors. These results suggest a deficiency in the BER process, because DS cells did not show recovery of DNA damage induced by $KBrO_3$, as assessed by the Comet test. An increase in the chromatin-bound form of XRCC1 in DS fibroblasts, observed both in the absence and in the presence of DNA damage, pointed to a possible cause of DNA repair defects. These results suggest that DS cells are prone to genome instability which may lead to cell senescence, and ultimately to carcinogenesis, not only because of an increase in DNA damage, but also because of defects in DNA repair.

ACTIVATION OF THE HIPPO TUMOR SUPPRESSOR PATHWAY LIMITS THE PROLIFERATION OF GENOMICALLY UNSTABLE TETRAPLOID CELLS

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Tetraploid cells, which are a common byproduct of cell division failures, are genetically unstable and have the capacity to facilitate tumorigenesis. Approximately 20% of all solid tumors exhibit a near-tetraploid karyotype, suggesting that tetraploidy plays significant roles in both the development and/or progression of human malignancies. Countering this oncogenic effect of tetraploidy is a p53-dependent tumor suppression mechanism that limits the proliferation of tetraploid cells by promoting a durable G_1 cell cycle arrest and cellular senescence. However, unlike other pathways that activate p53 and promote G_1 arrest in response to stress, such as the DNA damage response, the cellular defects and corresponding signaling mechanisms that trigger G_1 arrest in tetraploid cells are poorly understood. To address this fundamental unresolved aspect of cancer biology, we developed a novel genome-wide RNAi screening assay to identify proteins that are necessary to activate G_1 cell cycle arrest in tetraploid cells. As a complementary approach, we also performed *in vitro* evolution experiments to identify physiologically relevant adaptations made by rare tetraploid cells that enable their sustained proliferation. We demonstrate that cytoskeletal stress imparted by cytokinesis failure leads to activation of the Hippo tumor suppressor pathway in tetraploid cells both *in vitro* and *in vivo*. Hippo pathway induction in tetraploid cells is triggered by functionally reduced intracellular tension and is mediated by activation of Lats2 kinase. Notably, analysis of a broad spectrum of human cancers reveals that high-ploidy tumors frequently adapt to overcome Hippo signaling, suggesting that functional inactivation or bypass of this pathway may be a prerequisite for the development of such tumors. Understanding the adaptations acquired by tetraploid cells in order to sustain their proliferation may provide new therapeutic avenues to selectively kill abnormal high-ploidy cancer cells while sparing normal healthy diploids (*ploidy-specific lethality*).

CYTOKINESIS DEFECTS DUE TO HIPK2 DEFICIENCY PROMOTE CHROMOSOMAL INSTABILITY AND INCREASE TUMORIGENICITY

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Homeodomain-Interacting Protein Kinase 2 (HIPK2) is a S/T kinase involved in cell fate decision in development and in response to stress. In the DNA damage response HIPK2 modulates the activity of several proteins in a p53-dependent and independent manner. Reduction of HIPK2 expression was shown to impair apoptosis, and a few mechanisms of HIPK2 inactivation have been identified in human cancers. We have recently demonstrated a novel HIPK2 function in the control of cytokinesis. HIPK2-depleted cells fail abscission and accumulate tetraploid and multinucleated cells.

To analyze HIPK2 deficiency effects on the predisposition to chromosomal instability (CIN) and tumorigenicity, we induce transformation in *Hipk2* +/+ and -/- MEFs by expressing E1A and Ras oncogenes. We found that HIPK2 depletion facilitates transformation induced by these oncogenes. *Hipk2* -/- E1A/Ras MEFs became aneuploid more readily than *Hipk2* +/+ E1A/Ras MEFs, suggesting that oncogene induced CIN is exacerbated in the absence of HIPK2. Importantly, *Hipk2* -/- E1A/Ras MEFs formed more aggressive and aneuploid tumors in nude mice. In addition, we will present data suggesting an inverse correlation among HIPK2 protein level, aneuploidy and high grade of malignancy in pancreas tumors.

Overall, our data show that loss of HIPK2 induces aneuploidy and results in an increased ability of cells to become malignant and aggressive, indicating that HIPK2 oncosupressor activity is not only linked to its pro-apoptotic function, but also to its role in cytokinesis and ploidy maintenance.

MECHANISM OF CANCER CELL KILLING BY MPS1 INHIBITORS

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MPS1, a mitotic kinase that is overexpressed in several human cancers, contributes to the alignment of chromosomes to the metaphase plate as well as to the execution of the spindle assembly checkpoint (SAC). In this study, we analyzed the impact of MPS1 inhibition on tumoral cells using three novel inhibitors of MPS1 of two independent structural classes, Mps-BAY1 (a triazolopyridine), Mps-BAY2a and Mps-BAY2b (two imidazopyrazines). By selectively inactivating MPS1, these small inhibitors can arrest the proliferation of cancer cells, causing their polyploidization and/or their demise. Cancer cells treated with Mps-BAY1 or Mps-BAY2a manifested multiple signs of mitotic perturbation including inefficient chromosomal congression during metaphase, unscheduled SAC inactivation, and severe anaphase defects. Videomicroscopic cell fate profiling of histone 2B-GFP-expressing cells revealed the capacity of MPS1 inhibitors to subvert the correct timing of mitosis as they induce a premature anaphase entry in the context of misaligned metaphase plates. Hence, in the presence of MPS1 inhibitors, cells either divided in a bipolar (but often asymmetric) fashion or entered one or more rounds of abortive mitosis, generating gross aneuploidy and polyploidy, respectively. In both cases, cells ultimately succumbed from the mitotic catastrophe-induced activation of the mitochondrial pathway of apoptosis. Of note, low doses of MPS1 inhibitors and paclitaxel synergized at increasing the frequency of chromosome misalignments and missegregations in the context of SAC inactivation. This resulted in massive polyploidization followed by the activation of mitotic catastrophe. A synergistic interaction between paclitaxel and MPS1 inhibitors could also be demonstrated *in vivo*, as the combination of these agents efficiently reduced the growth of tumor xenografts and exerted superior antineoplastic effects as compared to either compound employed alone. Altogether, these results suggest that MPS1 inhibitors may exert robust anticancer activity, either as standalone therapeutic interventions or combined with microtubule-targeting chemicals.

SESSION 3

PAST PRESENT AND FUTURE

CHROMOSOME ABERRATIONS: PAST, PRESENT AND FUTURE

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Spontaneous and induced chromosome aberrations have been studied over more than a century. The involvement of chromosomal changes in "mutations" (de Vries, 1918) and in malignant tumours (Boveri, 1914) was discovered in early 20th century. Spontaneous and radiation induced chromosome aberrations were detected in salivary gland chromosomes of Drosophila (Morgan, 1927, Muller, 1928). The resolution of detection of chromosome has depended on the improvement of available techniques. Squash techniques for plant cells, hypotonic treatment followed by air drying enabled to establish the basic concepts of formation of chromosome aberrations following ionizing radiation (Sax, 1938) and chemical mutagens (Evans & Scott, 1960). The development of chromosome banding techniques in 1970's and the fluorescence in situ hybridization (FISH) in 1980's increased the accuracy of detection of different classes aberrations. An over view on the major highlights in the application of these techniques in different areas of chromosome research in the past, present and the future will be presented.

MICRONUCLEI AND GENE EXPRESSION IN A MARINE BIVALVE, Mytilus galloprovincialis

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Micronucleus (MN) formation is a common evidence after exposure to clastogenic or aneugenic agents and the analysis of micronucleated cells has been widely used to trace the early effects to genotoxins in several organisms. Overall, the MN expression depends on genetic and epigenetic mechanisms, and their spontaneous occurrence has been related to cell defence and differentiation mechanisms (Kirsch-Volders et al. 2011). The biological relevance of significant MN increase in haemocyte and gill cells of *M. galloprovincialis* from the industrial district of Marghera (Ve) was matter of debate in public court, with the late consequences possibly resulting from significant levels of DNA damage in marine mussels remaining unsolved (www.petrolchimico.it). As a matter of fact, *M. galloprovincialis* is a filter-feeding mollusc scarcely affected by typical bivalve diseases and with limited xenobiotic metabolism. Compared to chronic exposure to genotoxic contaminant mixtures in nature, controlled laboratory treatments can improve knowledge on the molecular processes occurring in response to DNA damage. To disentangle complex questions with simplified work plans, we exposed mussels to nanomolar doses of a metal mixture (Cd, Cu, Hg) for two days and then measured the levels of chromosomal damage and gene expression changes in parallel. The data resulting from DNA microarray analysis confirmed previous findings and allowed us to describe specific transcriptional changes. The progressive increase of sequence data on *Mytilus* spp. and evolutionary related species will give us the chance to understand more on mussel genes involved in the control of the cell cycle and DNA repair.

A TOXICOLOGICAL ONTOLOGY FOR THE OECD QSAR TOOLBOX

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We report on a new ontological resource aimed at standardizing and organizing the chemical toxicological databases in the OECD (Q)SAR Toolbox software, which is freely downloadable from www.qsartoolbox.org. The main objective of the Toolbox is to allow the user to use (Q)SAR methodologies to group chemicals into categories and to fill data gaps by read-across, trend analysis and (Q)SARs. In order to group chemicals successfully, robust methods are required which provide mechanistically relevant approaches to form categories. For mutagenicity, robust categories can be formed using different profilers relevant to genetic toxicity:

-DNA binding by OASIS;

-DNA binding by OECD;

-DNA alerts for AMES, MN and CA by OASIS;

-Carcinogenicity (genotox and nongenotox) alerts by ISS;

-*in vitro* mutagenicity (Ames test) alerts by ISS;

-*in vivo* mutagenicity (Micronucleus) alerts by ISS.

In the module "Endpoints" the user can retrieve experimental results from the resident databases (e.g. Bacterial mutagenicity ISSSTY, Micronucleus ISSMIC, Genotoxicity OASIS, Toxicity Japan MHLW).

For the successful toxicity prediction all profilers and datasets need to be standardized and annotated to the common terminology. The definition of ontology and of controlled vocabulary is a crucial requirement for the interoperability between toxicology resources.

The project started in 2012 with ontology development for Carcinogenicity, Repeated Dose Toxicity and Reproductive/Developmental Toxicity. At the beginning several related resources has been identified, including already existing ontologies freely available at the Bioportal ontology depository (e.g. NCI Thesaurus, Clinical Terms Version 3, Mouse pathology and Mouse adult gross anatomy). The OECD harmonised templates available as xml schema have been used as the basis for the development of the ontologies. The Web Ontology Language (OWL DL) supported by the Protégé OWL editor has been used for the ontology implementation.

At the end of the Project each entry of the experimental databases included in the Toolbox software will be associated with the ontology using the OWL hierarchy relationships and restriction rules. A final goal of the Project is the introduction of the ontology as the basis of data exchange and harmonization within the OECD QSAR Toolbox for better integration and standardization of experimental data.

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NEW PERSPECTIVES FOR DETECTING ENVIRONMENTALLY INDUCED GENETIC CHANGES IN THE GERM-LINE

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Before the discovery of the role of mutation in cancer, heritable mutations and genetic risk assessment were the main the focus of environmental mutagenesis. However, despite years of intensive search and strong evidence for the existence of germ cell mutagens in rodents, conclusive evidence for human germ cell mutagens could not be obtained, ostensibly because of technical limitations and the intrinsic complexity of the epidemiological approach.

Recently, new knowledge on the organization of the human genome and technical developments for DNA sequencing have opened new perspectives for investigating de novo heritable changes and the possible impact of environmental mutagens on the germline genome (Singer and Yauk, Environ Mol Mutagen 51, 919-928, 2010). Among the about 40 agents shown to induce mutations in rodent germ cells, a few have been identified that would be amenable to an epidemiological investigation in highly exposed human cohorts such as cigarette smoke, chemotherapeutic drugs and ionizing radiation (DeMarini, Environ Mol Mutagen, 53, 166-172, 2012). A tiered approach has been recommended by an ad-hoc working group on environmentally induced germline mutation analysis (ENIGMA), established by the American Environmental Mutagenesis and Genomics Society (Yauk et al., Mutation Research 752, 6-9, 2013). This approach comprises foundational studies to establish background genomic variability, studies with animals under genetically and environmentally controlled conditions, and accurate exposure assessment. Definitive studies should also aim to include epigenomics, creating an integrated understanding of the epigenetic, genetic, and environmental factors explaining heritable human disease.

It has been proposed that, when genomic data became available, a IARC-type panel could be established, to assess the data and proceed to a classification of exposures for their likelihood of being human germ cell mutagens, by an approach similar to that in place for carcinogens (DeMarini et al., 2012).

Finally, this revived interest on germ-line mutations has also met the attention of the Organization for Economic Cooperation and Development in the frame of its initiative to produce an inventory of documents for the so-called Adverse Outcome Pathways (AOP). This initiative aims at integrating knowledge of the relevant chemicals' interactions with biological systems (i.e. the molecular initiating events) with knowledge of the relevant biological responses or perturbations leading to the apical outcome of interest, to make the best possible use of mechanistic information for risk assessment. In this frame, projects are underway to prepare AOPs for well-characterized mutational pathways in the germ-line, such as the induction of aneuploidy in mammalian oocytes by tubulin binding chemicals, or the induction of gene mutations in spermatogonia by alkylating agents.

The presentation will shortly discuss this novel scenario of opportunities for germ cell mutagenicity studies.

POSTERS

(IN ALPHABETICAL ORDER)

CYTOGENETIC BIOMONITORING ON A COHORT OF PETROLEUM REFINERY WORKERS

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Occupational exposure represents a big concern for modern society because a huge amount of people are employed in industrial factories and despite the use of personal protective devices, they are continually exposed to potentially toxic compounds. In particular, workers of petroleum plants are exposed to a wide range of toxic compounds (benzene, polycyclic aromatic hydrocarbons, heavy metals, etc.) a number of which have been classified as carcinogen for human beings by the International Agency for Research on Cancer (IARC).

Recent data reported no increased mortality from digestive (stomach, large intestine, liver, or pancreas), lung, bladder, kidney, or brain cancer for petroleum refinery workers, but a small increase in skin cancer mortality was found. Significant increases in melanoma mortality were found in some small groups of refinery workers in the United Kingdom and upstream operation workers in Canada (1). Despite many epidemiological data are present in literature, only very few cytogenetic monitoring studies on petroleum workers are still available. Chromosomal aberrations (CA) represent useful biomarkers of genotoxicity, which give information on the persistent damage at the chromatid or chromosome level, identifying irreversible DNA damage. Moreover, several studies demonstrated that CA are involved in tumorigenic processes and that CA frequencies could be predictive of a potential cancer risk (2).

Out of more 500 petroleum refinery workers enrolled in the study, we analyzed chromosomal aberrations on human peripheral blood lymphocytes (PBL) from 78 male subjects (47 non-smokers and 31 smokers) and a total of 51 male control subjects (34 non-smokers and 17 smokers) selected by using very strict selection criteria.

The comparison of total chromosomal aberrations (CA tot%) in PBL between exposed and control populations pointed out a significant increase ($p<0.001$) in the exposed group (4.0±2.5) compared to controls (1.6±1.3). The chromatid-type (CtA%) and chromosome (CsA%) aberrations were also significantly higher in the exposed group in comparison to the control one (2.9±2.2 *vs* 0.9±0.7; $p<0.001$) and (1.1±1 *vs* 0.7±1; $p<0.05$) respectively. As far as the effect of smoking habit and working age on the level of chromosome damage, we didn't observe any significant associations.

In conclusion, our results are indicative of a potential genotoxic risk related to the complex occupational exposure in petroleum refineries, despite the measures adopted in the plants, and corroborate the need to increase safety measures.

References

(1) Wong, O., *et al.* (2000) A critical review of cancer epidemiology in the petroleum industry, with a meta-analysis of a combined database of more than 350,000 workers. *Regul Toxicol Pharmacol*, 32, 78-98.

(2) Norppa, H., *et al.* (2006) Chromosomal aberrations and SCEs as biomarkers of cancer risk. *Mutat Res*, 600, 37-45.

SYNERGIC EFFECTS OF BASE EXCISION REPAIR AND OXIDIZED dNTPs POOL ON (CAG)n/$(CTG)_n$ REPEAT DYNAMIC INSTABILITY

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Several neurodegenerative diseases are caused by trinucleotide repeat (TNR) expansion . Although the mechanism is not fully understood, it has been proposed that TNR expansion can occur during long-patch base excision repair (BER) of the oxidized DNA base 8-oxo-7,8-dihydroguanine (8-oxoG). Recent studies in Huntington's disease (HD) indicate that $(CAG)_n/(CTG)_n$ repeat instability might also depend on the lack of coordination between DNA polymerase β (Polβ)-dependent repair synthesis and flap endonuclease 1 (FEN1) cleavage. This might generate non-B form DNA secondary structures (loop or hairpin) which are difficult to remove and might be integrated into the genome. It has been suggested that this is due to aberrant levels of these repair proteins in the brain of disease R6/1 HD mice.

To further investigate the molecular basis of $(CAG)_n/(CTG)_n$ expansion during BER of 8-oxoG, we chose an experimental strategy based on *in vitro* enzymatic reactions that reproduce the specific conditions of repair synthesis of damaged DNA. We used synthetic 100bp duplex oligonucleotides containing 20 CAG/CTG repeats and a single 8-oxoG located in the first triplet and purified enzymes and/or cell free extracts from different sources (mouse embryo fibroblasts and human cell lines, brain tissues). In addition levels of the BER proteins will be measured in different brain areas of another model for HD, the R6/2 mice.

We hypothesized that $(CAG)_n/(CTG)_n$ expansion might be favoured not only by direct oxidative damage to DNA, but also by incorporation of oxidized triphosphates during repair synthesis. For this purpose the *in vitro* repair assay was performed using a purified polβ and dNTPs supplemented with 8-oxodGTP. Our preliminary results confirm the role of long-patch BER in modulating TNR expansion. Moreover we were able to show that 8-oxodGTP is incorporated opposite either C or A during Polβ-dependent repair synthesis. As expected OGG1 DNA glycosylase was able to repair the newly formed 8-oxoG:C mismatches in TNR. We are still investigating whether the MUTYH DNA glycosylase can remove A from 8-oxoG:A mispairs in this sequence context. A complex scenario might be envisaged because of parallel processing of these mismatches on opposite strands. We propose that remodeling of repair products from subsequent repair events, closely related along the DNA sequence, will establish a 'toxic oxidation cycle' that favours $(CAG)_n/(CTG)_n$ expansion.

TPO GENETIC VARIANTS AND RISK OF DIFFERENTIATED THYROID CARCINOMA (DTC)

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Thyroid cancer risk involves the interaction of genetic and environmental factors. The thyroperoxidase (TPO) has a key role in the iodine metabolism, being essential for the thyroid function. Mutations in the *TPO* gene are common in congenital hypothyroidism, and there are also signs of the implication of *TPO* in thyroid cancer. We performed a case-control association study of single nucleotide polymorphisms (SNPs) in *TPO* (i.e. rs2048722, rs732609 rs1042589), and differentiated thyroid carcinoma (DTC) in 1190 cases and 1290 controls. Multivariate logistic regression analyses were performed separately for each SNP. From the three studied polymorphisms significant associations were detected between DTC and rs2048722 (OR=0.79, 95% CI=0.63-1.00, *P*=0.045) and rs732609 (OR=0.72, 95% CI=0.55-0.94, *P*=0.016). The corresponding associations for the subgroup of the papillary thyroid carcinoma were similar to those for all DTC. No association was detected for the third *TPO* polymorphism. Interestingly, rs732609 encodes for a Threonine to Proline missense change in position 725 within *TPO*, that resides near the complement control protein (CCP)-like gene module (aa 741-795), but the functional significance of this change is unknown. Since the proline residue is conserved in most of the vertebrates, it could be hypothesized that the change affects the conformation of the protein, conferring a reduced flexibility to the carbamidic bond (given its cyclic structure). Thus, present results point to *TPO* as a gene involved in the risk of DTC, and could be of relevance for future studies to understand the role of *TPO* in thyroid tumorigenesis.

TELOMERE DAMAGE INDUCED BY ACUTE OXIDATIVE STRESS AND CHROMOSOME INSTABILITY.

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Telomeres are nucleoprotein structures located at the end of linear chromosomes consisting of non-coding repetitive DNA sequences, such as TTAGGG in human, and telomeres binding proteins (Blackburn, 1991). Their primary role is to maintain chromosome and genome stability. A lot of study demonstrated that short or dysfunctional telomeres of two chromosomes/chromatids can fused together at their ends leading to a dicentric chromosome. Both the bases and the ribose components of DNA have been identified as being susceptible to oxidative damage, however guanine residues have been shown to be particularly sensitive; 7,8-dihydro-8-oxyguanine (8-oxoGua) or the deoxyribonucleoside form of this lesion (8-oxodG) being a common biomarker of oxidative stress. The high incidence of guanine residues in telomeric DNA sequences makes the telomere the preferential target for oxidative damage and the telomeric low efficiency in DNA damage repair, increase the probability of the accumulation of 8oxoGua. In fact oxidative stress was shown to accelerate telomere shortening in replicating fibroblasts "in vitro". This acceleration was attributed to the enhanced induction of telomeric single strand breaks by free radicals, leading to the loss of the distal fragments of telomeric DNA following replication.

With the aim to evaluate if the chromosome instability induced by oxidative stress is related to telomeric damage, we used human primary fibroblasts (MRC-5) treated with two doses of H_2O_2 (100 μM and 200 μM) for 1 hour. The evaluation of the cell cycle by citofluorimetric analysis showed no cell cycle effects after treatments. Our previous studies demonstrated a significant telomere shortening 48 hour after treatment and an increase of micronuclei (MN), nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs) at the same time, leaving us to suppose a correlation between telomere shortening and chromosome instability. To better understand the correlation between chromosome instability and telomeric oxidative damage, we evaluate the persistence of oxidative stress induced damage up to 24 hours after treatment analyzing genomic DNA damage (SSBs) by alkaline comet assay and genomic FPG-sensitive sites by Formamidopyrimidine-DNA-glycosylase-modified comet assay. Furthermore, the measure up to 24 hours of the amount of oxidized residues specifically within telomeric DNA, that are recognized and excised by Formamidopyrimidine-DNA-glycosylase by qPCR, gave us the information about telomeric damage. The results obtained by comparing genomic and telomeric persistent oxidative damage could be interesting to evaluate if telomere dysfunction could be the principal target responsible of chromosome instability induced by oxidative stress.

LACK OF RELATIONSHIP BETWEEN RESISTANCE TO IMATINIB OF MPM CELLS AND MUTATIONS WITHIN TYROSINE-KINASE RECEPTOR GENES

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Imatinib, a tyrosine-kinase inhibitor, selectively induces citotoxicity and apoptosis in Malignant Pleural Mesothelioma (MPM) cell lines. However, in previous clinical trials where imatinib was administered as single agent it was observed a lack of therapeutic effectiveness on MPM patients. Among the targets of imatinib, PDGFR, c-KIT, and c-MET, are candidates with a hypothesized role in MPM. In other types of tumors, it has been shown that mutations in particular sites of tyrosine-kinase receptors domains are associated with chemo-resistance. In particular, in Gastro-Intestinal Stromal Tumors (GIST), the lack of responsiveness to Imatinib is due to mutations within exons 12, 14 and 18 of *PDGFR*, as well as in exons 13, 14 and 17 of *c-KIT*, and exon 18 of *c-MET*.

We undertook a genetic study aimed to ascertain whether mutations within *PDGFR*, *c-KIT* and *c-MET* could explain *in vitro* the lack of response to imatinib of MPM. We induced a long-term resistance to imatinib, in the MPM human cell line MERO-14, and the resistant clone was screened for mutations in the above mentioned genes. One imatinib-resistant clone derivative from the human MPM cell line REN was also screened. According to our observation, no mutations were found. This led to conclude that the lack of response to imatinib should not be ascribed to somatic mutations established in *PDGFR*, *c-KIT*, or *c-MET*.

RUBINSTEIN-TAYBI SYNDROME CELLS AS A MODEL SYSTEM TO INVESTIGATE THE ROLE OF CBP and p300 PROTEINS IN DNA REPAIR MECHANISMS

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The Rubinstein–Taybi syndrome (RSTS OMIM #180849, #613684) is a genetic disorder characterized by postnatal growth deficiency, microcephaly, intellectual disability and increased risk of tumors. Although the exact molecular etiology of RSTS is not clearly understood, it is widely accepted that RSTS is associated with mutations and chromosomal rearrangements - microdeletions and translocations- with breakpoints at 16p13.3, where the gene encoding the cAMP Response Element-Binding Protein (*CREBBP* or *CBP*) resides. About 60% of RSTS individuals carry a heterozygous mutation/deletion of CREBBP, while a small percentage (~3%) of RSTS are caused by mutations in the *EP300* gene (located at 22q13.2) encoding for p300. CREBBP and p300 are highly homologous proteins that possess lysine acetyl transferase (KAT) activity, and play a key role in transcription regulation. However, they have also distinct cellular functions and cannot always replace one another. Both proteins are involved in other aspects of DNA metabolism, including cell cycle checkpoints (by acetylating p53), and DNA repair (by acetylating factors involved in nucleotide (NER) and base excision repair (BER) mechanisms, such as FEN,1 XPG, DNA polymerase β (pol β), some DNA glycosylases (e.g. TDG, NEIL2, OGG1), as well as PARP-1, and WRN and RECQL4 helicases. In agreement with these findings, we have recently shown that depletion of both CBP and p300 by RNA interference, significantly impairs NER. Thus, deficiency in CBP and/or p300 may result in genome instability and cancerogenesis, thereby explaining, at least in part, the higher incidence of malignancies observed in RSTS patients.

To better elucidate the involvement of CBP and p300 in response to DNA damage, and in particular in DNA repair, we have analyzed lymphoblastoid cell lines derived from RSTS patients carrying monoallelic deletion, or mutations of CREBBP gene, in order to define their usefulness for these studies. Western blotting analysis has shown, as expected, a reduced expression of CBP, and in one case also of p300 protein in RSTS lymphoblasts, as compared to the lymphoblastoid cell lines from normal donors. p53 and p21 protein levels were also significantly reduced in RSTS cells, while no significant differences were observed in the protein expression of PCNA, and other related DNA repair proteins, such as XP proteins (A-G), PARP-1, XRCC1 and DNA pol β. Despite a similar proliferation rate, RSTS cells showed signs of histone H2AX phosphorylation, suggesting the presence of DNA damage. In addition, RSTS cells were more sensitive to treatment with the oxidative agent $KBrO_3$. Experiments have been performed to assess the recruitment of XRCC1 and DNA pol β to DNA damage sites, and the efficiency of DNA repair is being analysed by the Comet test. Preliminary results suggest a different and delayed kinetics in the repair of oxidative lesions in RSTS cells, requiring further investigations in order to explain the behaviour of these cell lines.

SEARCH FOR NOVEL COMMON VARIANTS INFLUENCING DIFFERENTIATED THYROID CANCER

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Thyroid cancer is a common endocrine malignancy with a rapidly increasing global incidence in the recent decades. Differentiated thyroid cancer (DTC), arising from follicular cells, includes the most common histological subtypes, papillary and follicular thyroid cancer, representing 80% and 15% of all thyroid cancers, respectively. Genome-wide association studies (GWASs) have identified robust associations with polymorphisms at 9q22.33 (*FOXE1*) and 14q13.2 (*NKX2-1*) and the disease. However, most of the inherited genetic risk factors of DTC remain to be discovered.

To search for new DTC risk variants we performed a GWAS in the high incidence Italian population and followed up the most significant associations in the lower incidence populations from Poland, UK and Spain. After excluding previously identified *loci*, the strongest association was observed for *DIRC3* at 2q35 ($P=6.4\times10^{-10}$). Additionally promising associations were attained for *IMMP2L* at 7q31 ($P=4.1\times10^{-6}$ and $P=5.7\times10^{-6}$), *RARRES1* at 3q25.32 ($P=4.6\times 10^{-5}$) and *SNAPC4/CARD9* at 9q34.3 ($P=3.5\times10^{-5}$).

Our findings provide insights into the genetic and biological basis of inherited genetic susceptibility to DTC. To further improve our knowledge on the disease, new *loci*, selected on the basis of association signals in our GWAS, will be analyzed.

INTER-INDIVIDUAL VARIABILITY OF THE MUTATOR PHENOTYPE IN CELLS DERIVED FROM MUTYH-ASSOCIATED POLYPOSIS PATIENTS

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Oxidative stress causes different kinds of DNA damage, including double and single strand breaks and base modifications; 8-oxo-7,8-dihydroguanine (8-oxodG) is the most extensively studied type of oxidative damage because it is potentially mutagenic and it is implicated in carcinogenesis. DNA 8-oxodG codes ambiguously during replication and directs incorporation of C or A with almost equal efficiency, leading to G:C → T:A transversions. The MUTYH DNA glycosylase counteracts the mutagenic effects of 8-oxodG by removing A opposite the oxidized purine. Biallelic germ-line mutations in *MUTYH* cause the autosomal recessive MUTYH-associated adenomatous polyposis (MAP).

We previously identified a large variability in the spontaneous mutator phenotype associated with inactivation of the *MUTYH* gene in lymphoblastoid cell lines (LCLs) derived from MAP patients harbouring different mutations. Our aim is to investigate whether this variability depended on specific *MUTYH* mutations or the genetic background, so we characterized LCLs derived from MAP patients expressing the same variant. We focused our attention on two mutations, the Y179C and R245H variants. Six LCLs derived from homozygous and four heterozygous carriers were analysed for spontaneous and oxidant-induced mutation frequencies at the *PIG-A* gene. In addition levels of oxidative damage to DNA were provided by measurements of DNA 8-oxodG. Homozygous inactivation of MUTYH by either Y179C or R245H mutations resulted in increased spontaneously or $KBrO_3$-induced mutations frequencies. A certain degree of inter-individual variability was identified in LCLs expressing the same Y179C variant, with increases in spontaneous mutation frequency ranging from 2- up to 7.6-fold over wild-type cells. In contrast the two cell lines expressing the R245H mutation showed a similar 10-fold increase in spontaneous mutagenesis. Once exposed to $KBrO_3$-induced oxidative stress, the increase in mutations was similar in Y179C or R245H expressing LCLs. Finally inter-individual variability in the spontaneous mutator phenotype was also observed in LCLs expressing a single mutant Y179C allele, while both R245H carriers displayed mutation frequencies intermediate between wild-type and homozygous LCLs. We are presently setting up an enzymatic MUTYH assay in cell-free extracts from heterozygous carriers to clarify whether the level of MUTYH DNA glycosylase activity might be influenced by the presence of a mutant protein.

EVALUATION OF MOLECULAR AND CYTOGENETIC DEFECTS IN DYSKERATOSIS CONGENITA

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Dyskeratosis congenital (DC) is a rare multi-system disorder phenotipically characterized by skin pigmentation, nail dystrophy and leukoplakia. DC patients frequently develop bone marrow failure which predisposes them to cancer development. The molecular mechanism involved in tumorigenesis is not yet elucidated. N this study, a cell line of B Lymphocytes from Dyskeratosis congenita GM03650, characterized by X-linked Dyskerin1 gene (DKC1) mutation, was used to evaluate the effects of this gene mutation on chromosome instability. Considering the role of DKC1 gene, telomerase activity and telomere maintenance were taken into account. The results of the telomere length assay (Roche) show that telomeres in mutant cells are not shortened, compared to the telomeres measured in a lymphoblastoid cell line of a health individual, that we used as control. Since the TTAGGG sequences of telomere were intact, a possible involvement of protein dysfunction was considered. The analysis of gene expression highlighted an alteration in the expression of the telomeric cohesin (SA1), the centromeric cohesin (SA2) and the SMC1 gene from the SMC complex of cohesins. All these proteins are involved in the cohesion maintenance between sister chromatids to a proper replication, recombination and segregation of chromosomes. The effects of cohesin up-regulation on DNA replication and recombination have been studied in both cell lines by the Co-FISH, using a PNA telomeric probe fluorescently labelled. The microscopic analysis highlighted a statistically significant increase in the frequency of intra-strand exchanges (2.9 *vs* 0.36; DC *vs wt* cell line), while terminal exchanges were not increased. On the other side, the cytogenetic FISH analysis performed wih a centromeric probe highlighted a significant increase in tetraploidy frequency (7.5 fold in binucleate cells) in the mutant, compared to the *wt* cell line. Interestingly, tetraploidy was increased in both mononucleate and binucleate cells. The molecular mechanism of this cytogenetic defect induction in the DC cell line will be clarified by further studies.

IN VITRO GENOTOXICITY TESTING OF BOTANICAL MATRIXES DERIVED FROM *Foeniculum vulgare* (FENNEL)

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Background - *Foeniculum vulgare* Mill. (fennel), a perennial herb with a characteristic aniseed flavour, belongs to the Apiaceae (Umbelliferae) family, being native to the circum-Mediterranean area, yet naturalized in northern Europe, Australia and North America and cultivated worldwide. A number of beneficial properties have been attributed to fennel fruits. Among the best characterized are anti-inflammatory, analgesic, antibacterial, and antioxidant properties. Fennel infusion, with a mild flavor and good tolerance, is currently regarded as a first-choice treatment in infants and sucklings with dyspeptic disorders. However, estragole, present in this herbal remedy, has been reported to produce hepatic tumors in susceptible strains of mice and a Scientific Committee on Food of the European Commission recommended restrictions in use of estragole-containing remedies.

Methods - The present study was undertaken to evaluate, in the human hepatoma cell line HepG2, the *in vitro* cytotoxic, genotoxic and apoptotic activities of three botanical matrixes derived from *Foeniculum vulgare* Mill. (fennel): (i) fennel seed lyophilised extract (FSLE), (ii) very fine fennel seed powder (VFFSP), and (iii) fennel seed essential oil (FSEO). Estragole was tested as well. To this purpose, the MTT cytotoxicity assay, the trypan blue dye exclusion test, the double staining (acridine orange and DAPI) fluorescence viability assay, the single-cell microgel-electrophoresis (comet) assay, the mitochondrial membrane potential ($\Delta\psi m$) assay and the DNA fragmentation analysis were used. To the best of our knowledge this is the first research aimed at examining the toxicity of naturally multicomponent mixtures (such as FSLE, VFFSP, FSEO) in human cells, as compared to the purified form of the putative toxicant.

Results - As regards genotoxic effects, the comet assay indicated that the compounds tested were not able to induce DNA damage under conditions used in our experiments. FSLE, VFFSP and estragole did not induce apoptosis. Whereas, HepG2 cells treated with FSEO exhibited a marked increase in early and late apoptosis.

Discussion - Our results demonstrate that FSLE, VFFSP and estragole are not cytotoxic, genotoxic and apoptotic in HepG2 cells line. On the basis of these findings we think our data can confirm that fennel decoction (and the exposure to estragole resulting from consumption of food) does not rise significantly the risk of primary liver cancer. Moreover data from this *in vitro* study suggested for FSEO apoptotic (antitumor ?) actions against malignant HepG2 cells. However, prospective cancer-suppressive effects of the tested essential oil should be further evaluated in *in vivo* experiments.

References:

E.C. (2001) Opinion of the Scientific Committee on Food on Estragole (1-Allyl-4-Methoxybenzene). European Commission, Bruxelles.

Mohamad R.H. et al. (2011) Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (*Foeniculum vulgare*). J Med Food. 14, 986-1001.

Raffo A. et al. (2011) Quantification of estragole in fennel herbal teas: Implications on the assessment of dietary exposure to estragole. Food Chem Toxicol. 49, 370-375.

van den Berg S.J. et al. (2011) Safety assessment of plant food supplements (PFS). Food Funct. 2, 760-768.

CONTINUOUS OCCURRENCE OF INTRA-INDIVIDUAL CHROMOSOME REARRANGEMENTS IN THE PEACH POTATO APHID *Myzus persicae*

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Aphids (Hemiptera: Aphididae) are ancient insects that have conquered most of the world's biomes, including the tropics and subarctic regions. Aphids reproduce primarily by apomictic parthenogenesis, a form of reproduction whereby adult females give birth to female progeny in the absence of male fertilization and it has been frequently suggested that no genetic recombination occurs in such parthenogenetic generations so that it has been assumed that the offspring represent a sort of genetically identical clone.

The chromosome number and the structure of the karyotypes of aphids are generally stable within genera, although karyotype variations are relatively common within some species and have been associated to host plant specialization. As a general rule, aphids show more variations in the karyotype than other organisms since they have holocentric chromosomes with kinetic activity spread along the whole chromosome axis. Hence, chromosomal fragments can contact the microtubules and move properly in the daughter cells during cell division. In contrast, fragments of monocentric chromosomes may be lost during mitosis and meiosis in the absence of centromeric activity in the chromosome fragment.

Analysis of the holocentric mitotic chromosomes of the peach-potato aphid *Myzus persicae* (Sulzer), from 4 different clones, revealed diverse chromosome numbers, ranging from 12 to 17, even within each embryo, in contrast to the standard karyotype of this species (2n=12). Chromosome length measurements, combined with fluorescent *in situ* hybridization experiments, showed that the observed chromosomal mosaicisms are due to recurrent fragmentations of autosomes 1 and 3. Contrary to what generally reported in literature, X chromosomes were also frequently involved in recurrent fragmentations, in particular at their telomeric ends opposite to the nucleolar organizer bearing telomere. The four aphid clones showed recurrent fissions of the same chromosomes in the same regions, thereby suggesting that the *M. persicae* genome has a sort of fragile sites that are at the basis of the observed changes in chromosome number.

Experiments to induce males also revealed that the *M. persicae* clones are obligately parthenogenetic, arguing that the reproduction by apomictic parthenogenesis, together with a high telomerase expression that stabilized the chromosomes involved in the observed fragmentations, favoured the stabilization and inheritance of the observed chromosomal fragments.

DDB2 INTERACTIONS WITH NUCLEOTIDE EXCISION REPAIR PROTEINS

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Nucleotide excision repair (NER) is the principal pathway for removal of a broad spectrum of structurally unrelated lesions. In human cells, it is primarily responsible for repair of UV- induced cyclobutane pyrimidine dimers and (6-4)-photoproducts. DNA damaged by ultraviolet light (UV) is recognized by heterodimer complex UV-DDB which comprises two subunits DDB1 and DDB2. Functional defect in UV-DDB activity has a direct relationship to decreased NER efficiency and increased susceptibility to cancer. In particular, DDB2 plays an important role in the recognition step of UV-induced DNA damage in non- transcribed regions (GG-NER), and it is mutated in Xeroderma pigmentosum (group E) patients.

In this study, we have investigated the localization and the interaction between DDB2 and some of the proteins involved in NER process. DDB2 localization was determined in HeLa cells, transiently transfected with pcDNA3.1-DDB2 construct, before and 30 minutes after UV-C irradiation. Confocal analysis showed that DDB2 co-localized with PCNA and p21 proteins recruited to DNA-damaged sites. To study a possible interaction between DDB2 and both these proteins, solubilised chromatin fractions were immunoprecipitated with DDB2 antibody. The results demonstrated that the DDB2-p21 interaction is mediated by PCNA.

MODULATION OF DNA REPAIR AND RADIATION CLASTOGENICITY BY THE HISTONE DEACETYLASE INHIBITOR TRICHOSTATIN A IN ATAXIA TELANGIECTASIA (AT) LYMPHOBLASTOID CELL LINES

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Ataxia Telangiectasia (AT) is an autosomal recessive disorder characterised by acute cancer predisposition and sensitivity to ionizing radiation (IR) revealed with an enhancement of chromosomal instability. Even thought AT cell lines rejoin the majority of double strand breaks (DSBs) with normal kinetics via non homologous end joining (NHEJ) repair pathway, the rejoining of about 15% of IR induced DSBs require ataxia telangiectasia mutated (ATM) protein [Foray et al., Int. J. Radiat. Biol. (1997): 72, 271-283; Riballo et al., Mol. Cell (2004): 16, 715-724]. Moreover, DSBs persisting in the presence of an ATM inhibitor localise to heterochromatin suggesting that ATM is required for repairing DSBs arising within or close to heterochromatic DNA regions [Goodarzi et al., Biochem. Soc. Trans. (2009): 37, 569-576].

In order to study the impact of chromatin compaction on chromosomal instability in AT cells, the response to Trichostatin A (TSA), a histone deacetylase inhibitor, was investigated in normal and AT (carrying different mutations in ATM gene) lymphoblastoid cell lines testing its effect on both DNA repair and chromosomal aberrations (ChA) induced after G_1 and G_2 X-ray exposure. The preliminary results show an enhanced repair capability and a different modulation of chromosomal aberrations in AT cell lines depending on the cell-cycle phase exposed to radiation. These results need to be further investigated in order to better clarify the nature of the modulation of both DNA repair and radiation clastogenicity found in AT cells analysing the epigenetic modifications in presence of TSA. The effect of TSA in AT cells could represent a tool for further research on the possible pharmacological applications of TSA in the A-T therapy.

REPLICATION PROFILE OF THE *FXN* LOCUS IN NORMAL HUMAN CELLS AND IN MUTATED CELLS CARRYING THE GAA/TCC-REPEAT EXPANSION

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Friedreich ataxia (FRDA), the most common inherited ataxia, is transmitted as an autosomal recessive trait and 98% of affected individuals are homozygous for an expanded GAA/TTC trinucleotide repeat in the first intron of the *FXN* gene (9q13), which encodes the mitochondrial protein frataxin. The mutation, resulting from the dynamic instability of the GAA/TCC repeat, causes the transcriptional inhibition of the *FXN* gene. Among several factors contributing to repeat dynamic instability, DNA replication is a key process. By molecular cytogenetic approaches we are currently evaluating the replication profile of the *FXN* locus, in normal cells, and in cell lines derived from FRDA patients and their heterozygous relatives. By interphase FISH we evaluated the proportions of non replicated (single fluorescent spot) and replicated (double fluorescent spot) alleles, both in asynchronous proliferating cells and in 4 (early-to-late) S-phase fractions obtained after FACS sorting; in parallel, for the same nuclei the BrdU staining pattern was recorded. The results indicate that replication of the *FXN* domain occurs during a wide temporal window corresponding to mid-late S-phase patterns. At present, we are considering if the *FXN* domain is replicated with similar or different timing in normal and mutated cells. In parallel, by molecular combing, we attempted to evaluate the position of activated origins, and the fork rates within the *FXN* locus; the global replication dynamics of normal and mutated cells has been also considered. Our preliminary data suggest the lack of activated origins within the *FXN* gene; therefore both the normal and the mutated allele are passively replicated by forks firing in the flanking regions and running at speed in the normal range for human cells. The analysis will be completed by the evaluation of possible altered replication patterns linked to the presence of the expanded repeat.

RADIATION-INDUCED TRANSGENERATIONAL GENOMIC INSTABILITY IN MICE

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Among untargeted effects of ionizing radiation there is the so-called phenomenon of transgenerational genomic instability. This phenomenon encompasses diverse observations in the mouse, including an increased somatic mutation rate and tumor susceptibility in the offspring of irradiated fathers, which cannot be explained by mendelian inheritance. However, some unrepeated experiments and the lack of a mechanistic understanding make the phenomenon still highly controversial.

Here we exploited a tumor susceptible mouse model, heterozygous for a null mutation of the *Ptch1* gene, highly prone to develop spontaneous and radiation-induced medulloblastoma, to test the hypothesis of transgenerational genomic instability induced by irradiation of differentiating spermatogonia.

Ptch1 wild type mice were irradiated with 1 Gy X rays and mated 42 days later with unirradiated *Ptch1*$^{+/-}$ females. Their *Ptch1*$^{+/-}$ progeny was tested for the spontaneous incidence of medulloblastoma and for the incidence of tumors induced by irradiation (1 Gy) at postnatal day 2 (P2), a well-characterized window of cerebellum radiation sensitivity. In parallel, the level of spontaneous and radiation-induced (1 and 2 Gy) DNA damage and repair in P2 cerebellum cells was analyzed by comet assay in the progeny of irradiated and unirradiated fathers. In addition, the possible transmission of chromosomal instability was investigated in bone marrow and spleen cells of adult progeny by comet and micronucleus assays.

Data collected so far showed a borderline statistically significant increase of the incidence of radiation-induced medulloblastoma in the progeny of irradiated fathers with respect to the incidence measured in a matched group of mice born from unirradiated parents, whereas no difference was observed in the spontaneous tumour incidence between the progeny of irradiated and unirradiated fathers.

The results on the spontaneous and radiation-induced level of DNA damage in P2 cerebellar cells, and its repair, did not show an effect of paternal irradiation, suggesting that neither increased DNA fragility nor compromised repair are involved in transgenerational carcinogenicity.

Similarly, the comet assay analysis of baseline DNA damage in bone marrow and spleen cells did not reveal an effect of paternal irradiation. Consistently with these data, the micronucleus test in bone marrow erythrocytes of the F1 progeny did not show an effect of paternal irradiation on either the spontaneous or the radiation-induced (0.1 Gy) level of chromosome instability.

To elucidate the molecular pathways of possible transgenerational carcinogenesis, studies are in progress on the genetic/epigenetic mechanisms of *Ptch1* wild type allele inactivation in tumor samples, and on the characterization of epigenetic changes at the miRNA and DNA methylation level in sperm descendant of irradiated spermatogonia.

EVIDENCE OF ALTERED REDOX BALANCE AND ENERGY METABOLISM IN XPA-DEFECTIVE HUMAN CELLS

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There is mounting evidence that DNA repair/DNA damage response defects are associated with increased intracellular ROS and accumulation of oxidatively generated DNA damage (D'Errico 2013). We have recently shown that also the inactivation of the nucleotide excision repair Xeroderma pigmentosum A (XP-A) gene leads to accumulation of 8-oxoguanine in nuclear DNA likely accounting for the hypersensitivity of XP-A human primary fibroblasts to oxidizing agents (Parlanti 2012).

Here, we show that XP-A defective human cells have an altered redox balance. The steady-state ROS levels were significantly higher both in primary fibroblasts from XP-A donors and in XPA-silenced human cells as compared to normal. This alteration was associated with a shift toward a glycolytic metabolism, as analysed by ^{1}H-NMR. and with a lower levels of endogenous ATP.

To monitor the functionality of mitochondria, cells were grown in the presence of rotenone, a specific inhibitor of the mitochondrial complex 1, and live-cell imaging was conducted by using tetramethylrhodamine ethyl ester (TMRE) as a probe for mitochondrial membrane potential. More active mitochondria were identified in XP-A cells as compared to normal cells.

Moreover, XP-A cells showed increased genetic instability following induction of oxidatively generated DNA damage, as measured by micronucleus frequency.

On the basis of these findings we envisage a model where the lack of XPA, by leading to accumulation of oxidatively generated DNA damage, activates the oxidative stress response with subsequent ROS production. This mechanism might contribute to the increased skin cancer risk and neurodegeneration typical of XP-A patients.

GENETIC INSTABILITY IN BIOMONITORING EXPOSURES TO CARCINOGENS

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Genomic instability and in particular the most common form –- chromosomal instability (i.e. structural or numerical chromosome aberrations) –- is thought to be an early event in tumorigenesis, resulted from a large spectrum of mechanisms including defect in DNA repair and cell cycle control pathways. They may also result from exposure to genotoxic carcinogens. Several genomic instability biomarkers have been used in biomonitoring carcinogen exposure and mostly measured in blood cells. They ranged from chromosomal aberrations, changes in DNA copy number, microsatellite instability, single-nucleotide polymorphisms (SNPs), and telomere shortening, epigenetic changes such as microRNA expression, DNA methylation and histone modifications. This study summarizes biomarkers currently used for carcinogen biomonitoring and highlights their values and limitations, restricting the examples of exposures to those chemicals classified in groups 1 and 2A by the International Agency for Research on Cancer. Several tests to monitor individuals exposed to carcinogens have been introduced, but their clinical and preventive relevance is still uncertain. A new era for biomarkers may result from the application of novel high-throughput techniques.

RADIATION-INDUCED CHROMOSOME DAMAGE ON MICROBEAM FACILITIES: DEVELOPMENT OF AN *IN SITU* PROTOCOL DESIGNED WITHIN THE *"BIOQUART"* PROJECT

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The biological effects of single charged particles cannot be simulated by *in vitro* conventional broad-beam exposures, due to the random Poisson distribution of particle tracks traversing the target cells. Charged-particle microbeam facilities were designed to target the nuclei or cytoplasm of single cells with a predefined number of particles and to analyze the induced damage on a cell-by-cell basis. In such a way, the radiation-induced cell damage can be directly correlated to type and energy of radiation and to the number of ions per cell. Within the *"BioQuaRT"* (Biologically weighted Quantities in RadioTherapy) Project, we developed an *in situ* protocol for the analysis of the unrepaired chromosome damage induced by charged particles irradiations at the PTB (Physikalisch-Technische Bundesanstalt, Braunshweig, Germany) microbeam facility. The development of a special *in situ* assay was required in this microbeam irradiation system because only a very limited number of cells (about 3000 cells/dish) could be seeded on the thin base made from BioFoil (25 μm thick) of the specific irradiation dishes designed at PTB. This method was developed on Chinese Hamster Ovary (CHO) cells, among the most commonly used cell lines in *in vitro* radiobiology experiments.

This protocol has the great advantage of allowing the simultaneous scoring of chromosome aberrations (CA) and micronuclei (MN) on the same irradiated sample. Although this method was developed for single-ion microbeam studies, it could be extended to other radiobiological applications requiring the use of *in situ* cytogenetic assays in case of restricted experimental conditions.

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