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ABSTRACT

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RHPS4 induces mitotic DNA synthesis (MiDAS) in ALT positive osteosarcoma cell lines

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Most of the tumors maintain their telomeres by a telomerase dependent mechanism, whereas a fraction of them (10-15%) uses a mechanism known as alternative lengthening of telomeres (ALT). The telomeric G-quadruplex (G4) ligand RHPS4 is known for its potent antiproliferative effect as shown in many different telomerase-positive cell lines and i*n vivo* models whereas few data are available in ALT cells. Interestingly, our data indicate that RHPS4 sensitivity was comparable in ALT-positive (U2OS and SAOS-2) and telomerase-positive (HOS) osteosarcoma cell lines. This prompted us to investigate the possibility that telomeric G4 stabilization may interfere with ALT mechanism impeding telomeric recombination. ALT hallmarks, such as ALT-associated PML-bodies (APBs) and telomeric repeat containing RNA (TERRA), were not modified by RHPS4, on the contrary telomere sister chromatid exchanges (t-SCE) showed a significant upregulation after treatment in U2OS and SAOS ALT-positive cells. However, since t-SCE may also be the product of delayed telomeric replication, our hypothesis is that RHPS4 did not affect ALT mechanisms but rather induced replicative stress, which in turn may trigger mitotic DNA synthesis (MiDAS) at telomeres. Interestingly, RAD51, which is involved in replication fork reversal, was found significantly downregulated upon RHPS4 treatment. A detailed analysis of MiDAS by cytofluorimetric techniques is in progress.

Epigenetic regulation of RA-induced *Zscan4* in early-embryonic-like cells

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Embryonic stem cells (ESCs) are a heterogeneous population that include several cell intermediates that presenting different degree of potency. Recently, it was identified an intermediate population marked by *Zscan4* expression, so-called 2C-like, that resembles molecular and epigenetic 2 cell stage preimplantation signature. Interestingly, 2C-like metastate is enhanced from Retinoic Acid treatment. Here, we aim to detect the minimal *Zscan4* promoter region responsive to epigenetic stimuli induction and the proteins that bind and regulate RA-induced *Zscan4* activation. To this goal we first focused on the identification of the minimal *Zscan4* promoter region responsive to stimuli (AZA and TSA) using ESCs transgenic cell lines stably transfected with different deletions of the *Zscan4* promoter regions. We thus identified a minimal promoter region responsive to epigenetics stimuli, demonstrating that *Zscan4* activation is related to the histone acetylation status and DNA demethylation. The identified region (about 300 bp near to start transcription sequence) contains binding motifs for the DUX and TBX transcription factors and three specific CpG sites. We now intend to identify the proteins that bind and regulate the RA-induced *Zscan4* activation by making use of a dCAS9 system, followed by Mass Spec analysis.

Past human migrations account for the origins of the major Eurasian linguistic families

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Demographic events in human history leave traces in languages and genes, hence Darwin's intuition that the best possible description of linguistic relationships among populations would be their phylogenetic tree.
Studies based on genetic and linguistic data have investigated the question of the origin of Indo-European (IE) and Uralic (UR) languages. At the basis of these studies lies Cavalli-Sforza's hypothesis that a major demographic shift with a massive population turnover across a large geographic area can be accompanied by the introduction of a new culture/language.
In this study, we combined linguistic and genomic data to shed light on the origin and spread dynamics of the IE and UR linguistic families in Eurasia. We investigated the congruence between linguistic traits inferred from syntactic comparisons and human genome diversity, finding a general correlation with a few exceptions. Then we used genome-wide data to characterize the genetic background and phylogenetic relationships of modern populations in Eurasia speaking IE and UR. Finally, we compared modern and ancient DNA data to investigate the genetic ancestry of these populations.
We found that modern populations speaking UR in Europe are genetically closer to the modern and Bronze-Age populations from the Pontic steppes, than present-day IE speakers are. Our preliminary results suggest that the distribution of grammatical diversity of most languages in Europe is largely related to past human migrations, and to the different impact of their culture and genetic legacy during their expansion.

Lgals3: a possible modulator for Arrhythmogenic Cardiomyopathy?

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Arrhythmogenic cardiomyopathy (AC) is a genetic heart muscle disorder characterized by fibrofatty replacement of the myocardium, leading to ventricular arrhythmias. Despite the discovery of AC causative genes, the pathogenesis remains elusive. To identify molecular mechanisms underlying early stages of AC, a comprehensive gene expression profiling in both AC patients and transgenic mice models was performed, identifying an early dysregulation of *Lgals3*, a member of the lectin gene family. The purpose of this study is to understand the role of Lgals3 in AC by analyzing Crispr/Cas9-generated zebrafish models, mutated in *lgals3* (*a* and *b*) genes. The mutant lines were characterized by confocal and TEM microscopy, and functionally tested for alterations in different pathways, using signaling reporter lines. At embryonic and larval stages, KD and KO of Lgals3 function induces a general developmental delay, microcephaly, pericardial effusion, altered heart rate and defects in cell-cell adhesion molecules. Analysis of juvenile mutant hearts show cell detachments and highly disorganized junctions. Moreover, the analysis of signaling pathways detects a cardiac-specific reduction of Wnt signaling responsiveness in all Lgals3 models. Overall, these zebrafish Lgals3 models appear suitable tools to identify early molecular events leading to AC, exploitable for a better diagnosis of those patients not easily classifiable by ECG, and for the development of more targeted therapies for AC.

G-quadruplex ligand RHPS4 radiosensitizes glioblastoma xenograft *in vivo* through a differential targeting of bulky differentiated- and stem-cancer cells

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Glioblastoma is the most aggressive and most lethal primary brain tumor in the adulthood and novel therapeutic options are urgently required. Sustained glioblastoma growth and recurrence is determined by glioblastoma stem-like cells (GSCs), which display self-renewal, tumorigenic potential, and increased radio- and chemo-resistance. As previously shown, the G-quadruplex ligand RHPS4 displayed radiosensitizing effect in GBM radioresistant cells through the targeting and dysfunctionalization of telomeres. In the present work, RHPS4 and ionizing radiation (IR) combined effect were tested *in vivo* in a heterotopic mice xenograft model and *in vitro* in stem-like cells derived from U251MG and from four GBM patients.
Data showed that RHPS4 administration in combination with IR exposure was very effective in blocking tumor growth *in vivo* up to 65 days. The tumor volume reduction and the long-term tumor control suggested the targeting of the stem cell compartment. Interestingly, RHPS4 treatment was able to strongly reduce cell proliferation in GSCs but, unexpectedly, did not synergize with IR. Remarkably, RHPS4 as single agent determined a strong reduction of RAD51 and CHK1 protein and transcript levels. This suggested that the potent anti-proliferative effect observed in GSCs is not determined by telomeric dysfunction but rather by the RHPS4-induced replicative stress and the concomitant depletion of CHK1 and RAD51, leading to DNA damage and cell death.

Population genomic analyses of a highly mobile large carnivore, the gray wolf (*Canis lupus*), across Eurasia

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Large carnivores, such as the gray wolf (*Canis lupus*), are wide-ranging and can disperse hundreds of kilometers, yet can show population genetic structure consistent with ecological and environmental gradients. Within Eurasia we expect E-W gene flow in such species to be higher than for similar N-S distances, owing to generally more similar environmental conditions across latitudinal than longitudinal gradients. We thus expect limited isolation-by-distance in the E-W direction in gray wolf populations from across Eurasia.

Our analyses include > 700 wolves sampled in Europe from Scandinavia to the southern Balkans, Iberia, and Italy, plus Caucasus, central and eastern Russia and genotyped on the Illumina CanineHD BeadChip with > 170K SNP loci. Population genetic structure was analysed by PCA and maximum-likelihood methods.

The Italian population was highly divergent. Population clusters were also observed in central and eastern Russia, Caucasus, Iberia, northcentral Europe, Scandinavia, the Carpathian Mountains, and the Dinaric-Balkan region. The population clusters extending through northcentral Europe and Russia suggest relatively high gene flow across these broad geographic regions against the finer-scale genetic structure found in other areas, including Italy, the Dinaric-Balkan region and the Carpathian Mountains. Over time, wolf recolonization of historic ranges could increase gene flow between currently divergent population such as Dinaric-Balkan and Italian wolves.

Defining the circadian transcriptome of the Antarctic krill

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Antarctic krill (*Euphausia superba*) is a high latitude pelagic organism which plays a central role in the Southern Ocean ecosystem. *E. superba* shows daily and seasonal rhythms in physiology and behaviour, which are synchronized with the environmental cycles of its habitat. Recently, the main components of the krill circadian machinery have been identified and characterized. However, the exact mechanisms through which the endogenous timing system operates the control and regulation of the overt rhythms remains only partially understood. Here we investigate the involvement of the circadian clock in the temporal orchestration of gene expression by using a newly developed version of a krill microarray platform. The analysis of transcriptome data from krill exposed to both light-dark cycles (LD 18:6) and constant darkness (DD), has led to the identification of 1,564 putative clock-controlled genes. A remarkably large proportion of such genes, including several clock components (*clock*, *period*, *cry2*, *vrille*, and *slimb*), show oscillatory expression patterns in DD, with a periodicity shorter than 24 hours. Energy-storage pathways appear to be regulated by the endogenous clock in accordance with their ecological relevance in daily energy managing and overwintering. Our results provide the first representation of the krill circadian transcriptome under laboratory, free-running conditions.

HLXB9 gene expression is independent from its nuclear location in breast cancer cells

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The nuclear location of genes and chromosomes in healthy human cells is non-random, therefore understanding the mechanisms that regulate nuclear genome organization is an emerging field of interest. *HLXB9* (also called *MNX1*), a homeobox gene that is located on human chromosome 7q36.3, is expressed during foetal development, and can be considered an early specific marker of differentiation of pancreatic cells in the initial steps of beta cell specification. We previously reported the overexpression of *HLXB9* in a specific subset of childhood leukaemia, and this overexpression corresponded to a mis-location of the gene to the inner part of the nucleus. Overexpression of HLXB9 has been also reported in various cancers including hepatocarcinoma, insulinomas and colorectal cancer. Here, we show expression data, and nuclear localisation of *HLXB9* gene in breast cancer. We observed a down-regulation of *HLXB9* in breast cancer cell lines compared to the controls, and a nuclear location more internally in the nucleus. These findings indicate that *HLXB9* expression in breast cancer is not correlated with its nuclear position, as observed in leukaemia cells. Further studies aimed to investigate the structure of the nuclear envelop in breast cancer cells might shed some light into the mechanisms of nuclear gene positioning and expression in these diseased cells.

miR-130a controls amino acids availability to affect melanoma metastasis

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Metastatic melanoma is the most aggressive form of skin cancer and is refractory to therapies when in metastasis. During last years, a substantial number of reports about the involvement of miRNAs in the melanoma progression have been published strongly suggesting their importance in this tumor. Interestingly, these non-coding RNAs could represent therapeutic compounds in the treatment of pathologies because stable and deliverable *in vivo*.
Our aim was to identify miRNAs functionally involved in melanoma metastatization. Via High-Throughput Sequencing of RNA isolated by Crosslinking ImmunoPrecipitation analysis (HITS-CLIP) we evidenced that the interaction of miR-130a with SLC7A5 was present only in non-metastatic melanoma. We evaluated the effects of this interaction overexpressing the miR-130a in melanoma cells and modulating SLC7A5 by RNAi or using different inhibitors.
Our results demonstrate that miR-130a is able to alter the amino acids transport in the cells inducing a decreased metastatic capacity and an increased cell death via the apoptotic and autophagic pathways both *in vitro* and *in vivo*.
This represent an important step forward for understanding the mechanisms used by melanoma cells to proliferate by escaping growth controls and producing metastases. These results can be used to counteract those mechanisms in order to ameliorate care outcome.

YB-1: a bridge between the circadian clock and cell cycle control

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The cold-shock Y-box binding protein 1 (YB-1) is a multifunctional protein that regulates a variety of fundamental biological processes. The functions played by YB-1 are strictly dependent on its subcellular localization. In resting cells, YB-1 localizes mainly to the cytoplasm where it is a component of ribonucleoprotein particles (mRNPs). Under stress conditions, YB-1 contributes to the formation of stress granules (SGs), cytoplasmic foci where untranslated messenger RNAs (mRNAs) are sorted or processed for reinitiation, degradation, or packaging into mRNPs. Using zebrafish as a model, we have shown that the nuclear localization of YB-1 is robustly regulated by the circadian clock. We implicate clock-controlled changes in YB-1 SUMOylation as one of the mechanisms regulating its periodic nuclear entry at the beginning of the light phase. Furthermore, we have demonstrated that nuclear YB-1 is able to downregulate cyclin A2 mRNA expression in zebrafish via its direct interaction with the cyclin A2 promoter. YB-1 can also be secreted and we have shown that acute oxidative stress causes sustained release of YB-1 which in turn controls proliferation of receiving cells. Extracellular YB-1 (exYB-1) significantly inhibited proliferation of receiving cells causing a G2/M cell cycle arrest, induced p21WAF and reduced ΔNp63α protein levels. Together, these data show that YB-1 can serve as a mediator of circadian-dependent regulation of cell proliferation.

Analysis of DNA methylation age in organ transplantation

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The global shortage of human organs for transplantation represents one of the most dramatic crises facing biomedicine today. A critical re-examination of donor's criteria has been addressed, extending the age limit to older donors (>65 years)[1]. A great heterogeneity in aging trajectories occurs in coeval individuals. And therefore chronological age could not be a reliable indicator of the body's/organ's physiological decline. Genetic and environmental factors can differently contribute to biological aging. Age-related DNA methylation levels at specific CpG sites have been used to create an "epigenetic clock" able to appraise the DNA methylation age (DNAmAge)[2], an emerging estimator of biological aging.
We determined DNAmAge by analyzing the methylation levels of five selected genes (ELOVL2, C1orf132, KLF14, TRIM59, FHL2) after DNA extraction[3], in blood, right (RA) and left atrium (LA) of 17 donors [median 54 years (16-65)] collected during heart transplantation. The discrepancy between DNAmAge and chronological age (ΔDNAmAge) was determined in donors' organs and blood.
ΔDNAmAge revealed that RA and LA are younger than their chronological age (>14 years, $p<0.001$), and ΔDNAmAge of blood and heart don't correlate.
Our findings show that organ's biological age is different than chronological age, cardiac tissue is remarkably younger, and provide insight into the role biological age might play in widening the donors' pool and counteract the organ shortage.

References

1. Khush KK. Donor selection in the modern era. Ann Cardiothorac Surg 2018; 7:126–134.
2. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013; 14:R115.Zbieć-
3. Zbieć-Piekarska R, Spólnicka M, Kupiec T, et al. Development of a forensically useful age prediction method based on DNA methylation analysis. Forensic Sci Int Genet 2015; 17:173–179.

Survival protection mechanisms and genetic variability induction after stress: two sides of the same Hsp70 coin

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Previous studies have shown that heat shock stress may activate transposable elements (TEs) in Drosophila and other organisms. Such an effect depends on the disruption of a chaperone complex that is normally involved in biogenesis of Piwi-interacting RNAs (piRNAs), the largest class of germline-enriched small noncoding RNAs implicated in the epigenetic silencing of TEs. However, a satisfying picture of how chaperones could be involved in repressing TEs in germ cells is still unknown. Here we show that, in Drosophila, heat shock stress increases the expression of TEs at a posttranscriptional level by affecting piRNA biogenesis through the action of the inducible chaperone Hsp70. We found that stress-induced TE activation is triggered by an interaction of Hsp70 with the Hsc70−Hsp90 complex and other factors all involved in piRNA biogenesis in both ovaries and testes. Such interaction induces a displacement of all such factors to the lysosomes, resulting in a functional collapse of piRNA biogenesis. This mechanism has clear evolutionary implications. In the presence of drastic environmental changes, Hsp70 plays a key dual role in increasing both the survival probability of individuals and the genetic variability in their germ cells. The consequent increase of genetic variation in a population potentiates evolutionary plasticity and evolvability.

Methylomic signature and molecular modelling to better understand autophagy induced by phytochemical in Caco-2 cells

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The binomial "autophagy-cancer" is intricate and methylomic studies can help to understand it by changing point of view from a gene level to an -omic one. Recently, autophagy-modulating properties of several phytochemicals have attracted attention in anticancer research.
We evaluated whether Indicaxanthin (IND), the peculiar known beneficial phytochemical of prickly pear, seasonally available in the southern Italy, could induce autophagy in Caco2 cells, and whether it results from an epigenomic modification and/or a direct molecular interaction.
IND increased autophagy in Caco-2 cells; the methylomic signature, obtained by Reduced Representation Bisulfite Sequencing (15 million of clusters) reported that 14 main genes of autophagy, showed a different methylation consistent with the induction of this phenomenon. Among these: *MTOR*, *ATG13*, *BECN1*, *TFEB*, *ATG3*, *WIPI2*, *TECPR1*, *SNAP29*, *VPS11*, *VPS16*. By traditional approaches we confirmed the demethylation of *BECN1* gene and the increase of Beclin1 levels. By *in-silico* molecular modelling, we displayed a possible interference of IND, by competitive mechanisms, in the Beclin1-Bcl2 interaction.
Methylomic signature and molecular modelling has been helpful to understand autophagy IND-induced in intestinal epithelial tumour cells. Our results suggest that the pro-autophagic action promoted by this phytochemical involves both epigenomic modulation and post-translation mechanisms by direct interaction with key targets of autophagy pathway.

Inversion variants in human and primate genomes

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For many years, inversions have been proposed to be a direct driving force in speciation since they suppress recombination when heterozygous. Inversions are the most common large-scale differences among humans and great apes. Nevertheless, they represent large events easily distinguishable by classical cytogenetics, whose resolution, however, is limited. We performed a genome-wide comparison between human, great ape, and macaque genomes using the net alignments for the most recent releases of genome assemblies. We identified a total of 156 putative inversions, between 103 kb and 91 Mb, corresponding to 136 human loci. Combining literature and experimental analyses, we analyzed 109 of these loci and found 67 regions inverted in one or multiple primates, including 28 newly identified inversions. These events overlap with 81 human genes at their breakpoints, and 7 correspond to sites of recurrent rearrangements associated with human disease. This work doubles the number of validated primate inversions larger than 100 kb, beyond what was previously documented. Our data serve two aims: (i) we generated a map of evolutionary inversions in these genomes representing a resource for interrogating differences among these species at a functional level; (ii) we provide a list of misassembled regions in these primate genome references, involving over 300 Mb of DNA and 1978 human genes. Accurately annotating these regions has immediate applications for evolutionary and biomedical studies.

The novel long non-coding RNA RP1X cooperates to N-Myc stabilization and poor prognosis in high-risk neuroblastoma

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Neuroblastoma is the third most common neurogenic solid cancer occurring in childhood. The genetic aberration most consistently associated with neuroblastoma is MYCN gene amplification, encoding for the N-Myc protein, a transcriptional regulator of several pro-tumorigenic coding and non-coding genes. Long non-coding RNAs (lncRNAs) exert key functions for the regulation of several processes like protein synthesis, RNA maturation, as well as gene silencing and chromatin modification, which critically influence cell condition. In this study, we have investigated how N-Myc regulates transcription of lncRNAs by comparing transcriptional profiles between non-amplified and MYCN-amplified neuroblastoma cell lines. This study allowed us to identify RP1X, a lncRNA that in addition to being selectively highly expressed in high MYCN cells only, is almost uniquely transcribed in neuroblastoma. Our data showed that N-Myc activates transcription of RP1X, which accumulates in the cytoplasm to physically interact with ribosomal protein L35 (RPL35). This interaction enhances translation of E2F-1, whose accumulation in the nucleus up-regulates in particular DEPDC1B gene expression, which downstream stimulates ERKs to increase N-Myc half-life. Our findings show that RP1X can instruct a complex network of interactions resulting in N-Myc oncogenetic program reinforcement. The regulatory levels, herein identified, are novel and may become relevant targets for therapeutic interventions.

The extra-telomeric role of the human protein TRF2

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TRF2 is a small protein that coats the length of all human telomeres as a component of the shelterin complex. At the telomere TRF2 ensures the chromosome stability. However, this protein can also bind to non-telomeric DNA sequences, where it takes part in the DDR and is also involved in the resolution of topological stress. In particular, TRF2 ensures the replication fork progression through hard-to-replicate regions such as the pericentromeric heterochromatin. Therefore, these new roles have been extensively analyzed in replicative stress conditions of cervical cancer cells (HeLa) that have been silenced for TRF2. Collected data revealed that the level of TRF2 at the telomeres before and after the silencing was not altered, proving that the residual protein is sufficient to ensure telomere protection even after the treatment with aphidicolin, a DNA-polymerase inhibitor. In fact, no significant telomeric effects were detected neither in the control sample nor in the TRF2 silenced one as evaluated by the analysis of telomeric DDR foci. On the contrary, an increased number of genomic DSBs was detected and specifically at the pericentromeres, especially in the shTRF2 sample where it is aphidicolin dose-dependent. These data were confirmed by m-FISH analysis that showed an increased number of aberrations in the shTRF2 samples, with specific chromosomes involved, probably due to the presence of extra-telomeric TRF2 binding sites. Further experiments are in progress.

NBS1 interacts with HP1 to ensure genome integrity

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Heterochromatin Protein 1 (HP1) and the Mre11-Rad50-Nbs1 (MRN) complex are conserved factors that play crucial role in genome stability and integrity. Despite their involvement in overlapping cellular functions, ranging from chromatin organization, telomere maintenance, to DNA replication and repair, a tight functional relationship between HP1 and the MRN complex has never been elucidated. Here we show that the Drosophila HP1a protein binds to the MRN complex through its Chromoshadow domain (CSD). In addition, loss of any of the MRN members reduces HP1a levels indicating that the MRN complex acts as regulator of HP1a stability. Moreover, overexpression of HP1a in nbs (but not in rad50 or mre11) mutant cells drastically reduces DNA damage associated to the loss of Nbs suggesting that HP1a and Nbs work in concert to maintain chromosome integrity in flies. We have also found that human HP1α and NBS1 interact with each other and that, similarly to Drosophila, siRNA-mediated inhibition of NBS1 reduces HP1αlevels in human cultured cells. Surprisingly, fibroblasts from Nijmegen Breakage Syndrome (NBS) patients carrying the 657del5 hypomorphic mutation in NBS1 and expressing the p26 and p70 NBS1 fragments, accumulate HP1α indicating that, differently from NBS1 knockout cells, the presence of truncated NBS1 extends HP1α turnover and/or promotes its stability. Remarkably, a siRNA mediated reduction of HP1α in NBS fibroblasts decreases the hypersensitivity to irradiation, a characteristic of the NBS syndrome. Overall our data provide an unanticipated evidence of a close interaction between HP1 and NBS1 that is essential for genome stability and point up HP1α as a potential target to counteract chromosome instability in NBS patient cells.

Genomics of Pregnancy Loss

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Pregnancy Loss (PL) is the spontaneous demise of a pregnancy before 24 weeks of gestation and occurs in 10-15% of pregnancies. PL is often the result of chromosomal aneuploidies of the gametes but it can also be caused by small-size mutations, both de-novo or inherited from parents. Comparative genomic hybridization (CGH) is the most accurate method for the genetic analysis of PL but it detects variants of several thousand base pairs, leaving unexplored smaller variants.
We aim to identify genetic variants likely to cause PL not seen by current diagnostic tools, either because of size or because they are located in non-coding regions not considered in medical diagnostics. We will build a predictive model that integrate genomic variation and functional annotations, based on the analysis of whole-genome sequences (WGS) of miscarried embryos.
In a pilot study based on 96 cases, we estimate that 18% of samples are euploid after CGH analysis, and, therefore suitable for WGS while the rest present aneuploidies (47%), or are not resolvable by CGH (25%), or drop out due to quality issues and maternal contamination (10%). Preliminary WGS analysis of six samples identifies 1M of potentially deleterious variant per sample. We are developing a model to prioritize deleterious variants in the hypothesis that PL is a complex trait with tens of variants acting in homozygosis or compound heterozygosis. Meanwhile, raw filtering of deleterious variants based on simple criteria identified homozygous non-synonymous mutations shared by three samples, showing the potential of developing the full project.

High-fat diet, oxidative damage and susceptibility to chronic-degenerative diseases in transgenic mice

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Several evidence show that an increase in body fat mass is linked to oxidative stress and that the accumulation of radical oxygen species contributes to metabolic syndrome development.
Mice defective in DNA damage processing genes, such as *ogg1*, are highly susceptible to obesity upon high-fat diet (HFD) feeding, suggesting a potential role of DNA damage repair proteins in metabolic dysfunction.
Previous studies demonstrated that transgenic mice (hMTH1-Tg) which overexpress human MutT homologue (hMTH1), the hydrolase responsible for dNTPs pool sanitization, showed a decrease in oxidative DNA damage, protection against neurodegeneration and increased longevity.
In this study hMTH1-Tg mouse was used as a model to investigate the potential role of this protein in obesity and metabolic syndrome susceptibility. In order to address this question, hMTH1-Tg mice have been exposed to HFD.
To test DNA integrity and detect oxidative DNA damage, a modified version of the Comet assay, which implies the use of formamido-pyrimidine glycosylase, will be performed in blood samples collected at different feeding time.
Preliminary data, obtained after 4 weeks of HFD, show a lower level of oxidative DNA damage accumulation in hMTH1-Tg mice under both normal and HFD regime when compared to the wild type (wt) counterpart. After 11 weeks of HFD, a strong increase of DNA damage has been exclusively observed in wt mice.
Insulin and adiponectin levels and blood chemistry profiles will be shown.

Integrating genetics screening approaches to identify chromatin key factors of epidermal cell plasticity

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The epidermis, the upper most layer of the skin, is a physical, chemical and immunological barrier from the outside environment. When altered, such as during wound healing, different epidermal stem and differentiated cells participate to restore the integrity of the skin through the acquisition of an unexpected plasticity.
The concept that inflammatory events occurring during skin regeneration, such as the immune cell infiltration, can contribute to trigger skin cancer onset, is well established. However, recent studies also highlighted a striking common chromatin conformation and gene expression profiles between neoplastic epidermal cells and epidermal cells involved in skin repair. Together with the identification of a surprisingly high frequency and co-occurrence of cancer-causing mutations in physiologically normal epidermal cells, these data suggest the existence of common, but yet unknown, chromatin key factors of epidermal cell plasticity that control and regulate wound healing and skin cancer onset.
To identify these factors, including the essential genes, we optimised multiple in vitro pooled shRNA screenings in primary epidermal stem cells using several reporter systems, driven by wound responsive promoters, that impact on cell viability. As predicted, enrichment analysis of protein complexes confirmed the ability of our new RNAi-based approach to identify transcriptional activators and repressors. The integration of the results using multiple promoters is ongoing.

MYH9 as a novel therapeutic target of miR199b5p in pediatric brain fossa tumours

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MiR199b-5p was reported with an anti-tumorigenic action in Medulloblastoma (MB) by targeting HES1 by our team. The reduction of miR-199b-5p levels in MB was mediated by epigenetic regulation through HES1.
Here, we found decreased levels of miR-199b-5p in our cohort of pediatric Ependimoma/Glioma patients. We show in Glioblastoma the anti-tumorigenic action of miR-199b-5p *in vivo* by impairing tumor growth in an orthotopic xenograft mice models using U87-MG-LUC cells. We then applied the Multidimensional Protein Identification Technology proteomic tool on brain tumor tissues derived from orthotopic mice and we found a network of proteins significantly down-regulated by miR-199b-5p, consisting of cytoskeletal components. I*n silico* analyses predict mRNAs with conserved seed sites for miR-199b-5p, suggesting MYH9 as a direct *target* of miR-199b-5p in Glioma. MYH9 mRNA levels of expression are higher in Ependimoma/Glioma affected patients thus indicating an inverse correlation to miR-199b-5p expression. We show an anti-migratory action of miR199b-5p due to alteration of focal adhesion mostly mediated by FAK and MYH9 down-regulation. Of importance, miRNA miR199b-5p is epigenetically regulated in Glioma after treatment with epigenetic drugs currently used in clinics for the treatment Glioma pediatric patients.
Altogether, we show that miR-199b5p retains an anti-tumourigenic role in Glioma by targeting the cytoskeletal MYH9 regulating migration and invasion processes during tumour progression.

References

[1] Rupaimoole R and Slack FJ.. Nat Rev Drug Discov. 2017 Mar;16(3):203-222
[2] Garzia L, Andolfo I, Cusanelli E, Marino N, Petrosino G, De Martino D, Esposito V, Galeone A, Navas L, Esposito S, Gargiulo S, Fattet S, Donofrio V, Cinalli G, Brunetti A, Vecchio LD, Northcott PA, Delattre O, Taylor MD, Iolascon A, Zollo M. PloS one (2009). 4 (3):e4998
[3] Andolfo I, Liguori L, De Antonellis P, Cusanelli E, Marinaro F, Pistollato F, Garzia L, De Vita G, Petrosino G, Accordi B, Migliorati R, Basso G, Iolascon A, Cinalli G, Zollo M. Neuro Oncol. 2012 May;14(5):596-612
[4] de Antonellis P, Liguori L, Falanga A, Carotenuto M, Ferrucci V, Andolfo I, Marinaro F, Scognamiglio I, Virgilio A, De Rosa G, Galeone A, Galdiero S, Zollo M. Naunyn Schmiedebergs Arch Pharmacol. 2013 Apr;386(4):287-302.

Genome-wide mapping of the 8-oxodG reveals accumulation of oxidatively-generated damage at the promoter regions of transcribed genes in human genome.

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The 8-oxodG (8-Oxo-7,8-dihydro-2'-deoxyguanosine) is one of the major DNA modifications. It is a pre-mutagenic lesion prone to mispair with 2'-deoxyadenosine (dA). Recently, we developed the OxiDIP-Seq technique and reported the genome-wide distribution of 8-oxodG in proliferating DDR-proficient mammary cells (MCF10A and MEFs). We found that, even though the central tenet of the evolutionary theory claims that mutations occur randomly, endogenous 8-oxodG is regio-selectively distributed across the mammalian genome. Here, we show that human promoters accumulates oxidative DNA Damage in Transcription-dependent manner. Our study helps to dissect the molecular mechanisms underlying local mutation rate heterogeneity and to understand why certain regions appear to be oxidatively-targeted or protected.

microRNA-133b appears to epigenetically affect muscle mass

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Micro RNAs (miRNAs) are endogenous small, non-coding RNAs that epigenetically modulate the expression of mRNAs. Many reports have revealed that several miRNAs are up- or down-regulated in skeletal muscle during aging, suggesting that they may be related to the reduced age-related muscle functioning. In particular, a pivotal role in muscle physiology is presently assigned to a family of miRNAs (miR-1, miR-133a, miR-133b, miR-206, miR-208b and miR-499), designated myomiRs.
Aim of this study was to assess the relationship between the expression of myomiRs and sarcopenia, the age related loss of muscle mass that adversely impacts quality of life and survival. Quantitative real-time PCR (QPCR) was used to detect the miRNA levels in plasma samples of healthy subjects (N=99, age range 59-95 years) and sarcopenic patients (N=88, age range 65-97 years). Only three (miR-206, miR-133a and miR-133b) of the 6 miRNAs examined were detectable in our cohort. Data analysis indicated that the plasma levels of miRNA-133b in patients with sarcopenia were significantly reduced (0.66-fold lower) in comparison with healthy controls (p=0.042). MiR-133b is upregulated during myogenesis, a process that is essential for muscle regeneration and growth; therefore, the downregulation of this miRNA might contribute to the impaired myogenesis linked to sarcopenia.
The characterization of miR-133b targets will be explored for a better understanding of the molecular mechanisms behind sarcopenia.

The mitogenome portrait of the "heart of Italy" depicted by modern Umbrians and pre-Roman remains

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It was recently shown that the Italian peninsula is characterized by a high degree of genomic variability. However, specific microgeographic analyses of some regions are still missing, including Umbria in the "heart of Italy". Its name derives from the ancient Umbrians (or Umbri), traditionally considered an indigenous and very old Italic population, whose origins are still debated. In order to provide answers to this issue, at least from a maternal perspective, we investigated the mitochondrial DNA haplogroup affiliation of 545 present-day Umbrians. Eventually, the entire mitogenome of 198 randomly selected individuals was also sequenced pointing to a quite homogeneous distribution of western Eurasian lineages across the region. A notable exception is represented by the eastern part of Umbria, which shows a high incidence of haplogroup J (30%) and a peculiar proximity to eastern Europeans in a western Eurasian population context.
The concomitant analysis of 19 mitogenomes from ancient remains, excavated at the necropolis of Plestia (eastern Umbria) and dated from the early 9th to the late 3rd century BCE, attests mitochondrial haplogroup continuity with the same overrepresentation (30%) of J lineages as in modern inhabitants of the same area. Likewise, the phylogenetic proximity of one of our J1c3 pre-Roman samples to ancient mtDNAs from Ukraine and Hungary corroborates the hypothesis of an eastern European origin of Umbri.

Retrotransposon activation and genomic instability participate to Huntington Disease pathogenesis in a *Drosophila melanogaster* model

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Huntington's disease (HD) is a late-onset, autosomal dominant disorder characterized by progressive motor dysfunction, early death and psychiatric disturbances. The disease is caused by a CAG repeat expansion in the IT15 gene, which elongates a stretch of polyglutamine (polyQ) at the amino-terminus of the HD protein, huntingtin (Htt). Despite the accumulated data on the molecular basis of neurodegeneration, no cure is still available. It is therefore important to keep investigating potential previously unnoticed pathways that may be altered in HD and target of therapeutic treatments.
Transposable elements (TEs) are mobile genetic elements that constitute a large fraction of eukaryotic genomes. Retrotransposons represent approximately 40% and 30% of the human and *Drosophila* genomes. Mounting evidences suggest mammalian L1 elements are normally active during neurogenesis. Interestingly, recent reports show that unregulated activation of TE is associated with neurodegenerative diseases.
Our experimental results show that retrotransposon transcripts are up-regulated in HD brain and that their inhibition determines the block of polyQ-dependent neurodegeneration. Moreover, we found a high rate of DNA damage and chromosomal abnormalities in HD brains. Taken together, these data suggest that TE activation and genomic instability represent two important pieces in the complicated puzzle of polyQ-induced neurotoxicity.

Reconstructing the Phylogeny of the Barn Swallow (*Hirundo rustica*) using Mitogenomes

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The barn swallow (*Hirundo rustica*) is the most widespread species of swallow in the world; this can be linked to its habit of building nests in man-made structures like barns, hence the name. It is a polytypic species consisting of at least eight subspecies, seven of which are found between Africa and Eurasia and one in America. In this study the mitogenomes of eleven barn swallows from Italy (*H.rustica* ssp. *rustica*) were completely sequenced. This survey revealed eleven distinct haplotypes that phylogenetically cluster into four haplogroups. In our phylogenetic analyses we also included mitogenomes from *H.r. erythrogaster* from North America, *H.r. savignii* from Egypt and *H.r. gutturalis* from East Asia, thus allowing a detailed reconstruction of the matrilineal genetic relationships between *Hirundo rustica* subspecies.

Common Fragile Sites: how the impaired replication timing promote their tissue-specific expression

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DNA replication stress is one of the main causes for genomic instability at Common Fragile Sites (CFS), chromosome regions prone to breaks or constrictions involved in different type of diseases. CFS are characterized by late replication timing due to A/T nucleotides enrichment which tend to form secondary structures, to the paucity of active replication origins and to the presence of long genes.
In order to demonstrate that the CFS induction is tissue specific, we performed our experiments on two human lung fibroblast cell lines, whereas our previous data reported regards CFS characterization in human lymphocytes. After the induction of CFS through aphidicolin, the most expressed CFS in fibroblasts are 1p31.1 and 3q13.3, not expressed in lymphocytes. Moreover, these sites have the typical features of CFS and in their most fragile region are present late-replicating long genes.
To better understand the causes of their instability, it was determined the replication profile of these two sites both in normal and stressful condition through the combination of Fluorescent *in situ* Hybridization and immunofluorescence on interphase nuclei. Lymphocytes' metaphases and nuclei, in which the regions are non-fragile, were used as control. The replication timing analysis showed alleles not completely replicated in normal and stressful conditions only in fibroblasts, thus suggesting a prominent role of DNA replication in promoting chromosomal instability at CFS in a tissue specific manner.

Metabolic alterations of obesity: in a search for predictive biomarkers

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Obesity is a complex disorder characterized by chronic low-grade sterile inflammation, increase of radical oxygen species, mitochondrial dysfunction and gut microbiome dysbiosis.
The main goal of this study is the identification of a predictive biomarker of metabolic syndrome. A cohort of 36 severe obese patients, unaffected by obesity-related pathologies, has been enrolled and different biological samples have been collected both at the enrolment time and 6 and 12 months after sleeve gastrectomy. Several analyses at molecular level have been carried out. Mitochondrial markers and gut microbiome profile will be analyzed.
Preliminary data show that *sirt3* mRNA expression level, involved in the fatty acid oxidation pathway and antioxidant response, is significantly downregulated in adipose tissue of obese patients vs what observed in lean control specimens. A significant increase of *sirt3* expression level has been observed in peripheral blood mononuclear cells (PBMC) after weight loss compared to what measured at enrolment time, suggesting a restoration of mitochondrial function. Remarkably, the blood chemistry analyses show an improvement of clinical features after weight loss. The expression levels of mitochondrial proteins and mitochondrial DNA content in PBMC will be shown.
The gut microbiome profile has been also characterized in stool samples by 16s rRNA region sequencing and analyzed by Galaxy platform, showing a rearrangement of the bacterial population after weight loss.

Apurinic/Apyrimidinic endonuclease 1 (APE1) is released in extracellular vesicles by cancer cells and exerts paracrine effects in modulating SASP factors expression upon genotoxic damage

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Apurinic/apyrimidinic endonuclease 1 (APE1), an enzyme involved in the DNA base excision repair (BER) pathway, is also known for its role in different non-repair activities such as the cell response to oxidative stress, the regulation of gene expression and miRNA processing. We recently demonstrated that serum APE1 (sAPE1) could be considered as a diagnostic biomarker in hepatocellular carcinoma (HCC), because its higher expression was found in sera of HCC patients compared to healthy controls. Both full length and APE1 N-Term cleaved form were also detected in HCC tissue analyses. We provided indications about sAPE1 biological role in HCC, elucidating sAPE1 paracrine function in the regulation of IL-6 and IL-8 mRNA expression. Here, we wanted to elucidate the mechanisms responsible for APE1 secretion using HCC cell line. We here demonstrated that i) APE1 is abundantly present $2.1/10^7$ molecules/exosomal vesicle and is enzymatically active; ii) its accumulation in the exosomal particles is increased upon different genotoxic treatments; iii) the main accumulated protein upon genotoxic treatment lacks the first 33 N-terminal sequence; iv) the secreted protein triggers IL-6 and IL-8 expression in a paracrine mechanisms, suggesting that it could play important roles in chemoresistance processes and tumor development. These findings suggest a role of extracellular APE1 as a paracrine pro-inflammatory molecule and provide a characterization of the APE1 exogenous function.

Role of telomerase in hTERT stable transfected fibroblasts, upon genotoxic stress.

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Telomerase is a reverse transcriptase consisting of two components: the functional RNA component (hTERC) and the catalytic subunit with reverse transcriptase activity (hTERT). The enzyme telomerase counteracts the shortening at the end of linear chromosomes and prevents the onset of replicative senescence and genetic instability. Previous studies provide evidence that human primary fibroblasts (HFFF2) after X-rays are able to activate Alternative Lengthening of Telomeres (ALT) in response to telomere shortening. For this reason, we used HFFF2 hTERT-transfected cells to evaluate if X-rays activate ALT also in the presence of active telomerase enzyme. Differently from the normal counterpart, X-rays exposed HFFF2-hTERT does not present any ALT activation, telomere dysfunction and oxidative stress induction. Because the end points analyzed are associated with oxidative stress induced by radiation, this evidence indicates that hTERT could have an essential role to protect against oxidative stress. In the last decade several studies suggest that TERT exerts functions independently from its telomeric role. In fact, hTERT under stress condition, shuts in the mitochondrial protecting mitochondrial DNA from damage and contributing to the better respiratory chain activity. Our hypothesis is that hTERT could contribute to the antioxidant function by enhancing mitochondria metabolism. Preliminary results will be showed to describe the role of hTERT on effects induced by genotoxic agents.

A new portrait of constitutive heterochromatin: lessons from *Drosophila*

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Constitutive heterochromatin represents a significant portion of the eukaryotic genomes, but its functions still need to be elucidated. Even in the most updated textbooks of Genetics, constitutive heterochromatin is still regarded mainly as the "silent" component of eukaryotic genomes. However, there may be more complexity to the relationship between heterochromatin and gene expression. In the fruit fly *Drosophila melanogaster*, a model for heterochromatin studies, about one-third of the genome is heterochromatic and map to the centric, pericentric and telomeric regions of the chromosomes. Recent findings indicate that hundreds of *D. melanogaster* genes can "live and work" properly within constitutive heterochromatin. They are generally larger than euchromatic genes and account for a significant fraction of the entire constitutive heterochromatin. Thus, constitutive heterochromatin in spite of its ability to induce silencing, as the general view supports, has in fact the means fro being quite dynamic. In conclusion, euchromatin and costitutive heterochromatin may be regarded as two different and yet dynamic components of genomes, both of which can be active or silent during developmental stages or cell cycle phases, as a consequence of different regulatory strategies they have acquired to control gene expression.

A genomic survey of Tc1-mariner transposons in Nematodes and their involvement in Horizontal Transposon Transfer

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As the genomes of more metazoan species are sequenced, reports of horizontal transposon transfers (HTT) have increased. Our understanding of the mechanisms of such phenomenon is still at an early stage. The Nematodes constitute an animal phylum successfully adapted to almost every ecosystem and, for this reason, they could potentially contribute to spreading the genetic information through horizontal transfer. To date, few studies only focused on horizontal transfer of retrotransposons, report HTT events involving nematodes.
Since DNA transposons, especially those belonging to the *Tc1/mariner* superfamily, are the best horizontal traveller among mobile sequences, we have started a survey of DNA transposons and their possible involvement in HTT in sequenced nematode genomes. Here, we describe 81 new families of the *Tc1/mariner* superfamily distributed in 16 nematode species. Among them, nine families are possibly involved in HTT events, engaged with the most diverse non-nematode species.
The results obtained suggest that, as expected, HTT events involving nematodes *Tc1/mariner* elements are not uncommon, and that nematodes could have a possible role as transposon reservoir that, in turn, can be redistributed among animal genomes.

Comparative genomics and deep phenotyping of the plant-associated genus *Ensifer*

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The *Rhizobiaceae* family of the Alphaproteobacteria includes species able to perform nitrogen fixation during symbiotic interactions with plants. However, not all species within this family present these abilities. In fact, horizontal transfer of symbiotic plasmids/islands has played a crucial role in the evolution of symbiotic phenotypes. Within the genus *Ensifer* (syn. *Sinorhizobium*), it is possible to find both symbiotic and non-symbiotic species.
The aim of this work is to characterize the phylogenetic relationships inside the genus *Ensifer* by comparative genomics and Phenotype MicrorrayTM, in order to shed light on the different features of the symbiotic and non-symbiotic species.
Comparative genomics identified two distinct clades that clearly separate the symbiotic and non-symbiotic species. Genes involved in nodule formation and nitrogen fixation (*nodA, nodB, nodC, nifD, nifK, nifH*) were almost exclusively present in the "symbiotic" clade. The separation into two groups was also noted in the Phenotype MicrorrayTM data. In particular, the non-symbiotic species (originally isolated from soil and rhizospheres) showed higher utilization of carbon sources (e.g. Stachyose) and tollerance to extreme pH (e.g. pH 9.5) compared to the symbiotic organisms.
In conclusion, these preliminary data suggest that several genomic and phenotypic features are associated with the presence/absence of symbiotic phenotypes among species of the genus *Ensifer.*

Beneficial effects of a curcumin-supplemented diet on central and peripheral dysfunctions in a mouse model of Huntington disease

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Huntington's disease (HD) is a neurodegenerative disorder caused by a polyQ expansion in the huntingtin (htt) protein. The disease is notoriously described as brain disorder, however peripheral dysfunctions, such as an unintended weight loss that occurs even before neurological symptoms appear and that correlates with disease progression, is also seen. Although the cause of weight loss in HD is still unknown, a possible link with intestinal dysfunctions has been hypothesized. Weight loss represents a hallmark of HD, it significantly impairs patient's quality of life, however no targeted treatment is currently available to mitigate it.
Curcumin, a natural compound with a variety of therapeutic properties, has been shown to exert beneficial effects in both neurodegenerative and intestinal disorders.
Here, we investigated whether treatment with curcumin may represent a preventive measure for some phenotypic features in HD R6/2 mouse model. R6/2 mice were treated with curcumin from conception to explore it potential effect in modifying the overall HD onset and progression. Our results show that curcumin is beneficial in HD. It significantly mitigates disease progression and preserves body weight as well as normal intestinal homeostasis. Molecularly, curcumin triggers activation of pro-survival pathways and reduced mutant htt aggregates in the brain of HD mice, and preserves normal villi length as well as the expression of tight junction proteins in the intestinal tract.

Mitochondrial Genome Diversity in Collembola

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Collembola (springtails) is an early diverging Class of apterygote hexapods and marks the first substantial radiation of these latter on land. Despite extensive work, relationships among major lineages are still debated and the time frame of their differentiation is unknown. We reanalyse all known mitochondrial genomes, alongside 2 new ones and data from a recent metagenomic study, to produce an improved phylogenetic hypothesis for the group, develop a tentative time frame for their differentiation, and provide a comprehensive overview of gene order diversity. Our analyses support most taxonomically recognized entities, with support for an Entomobryomorpha + Symplypleona and possibly a Poduromorpha + Neelipleona clade. A Silurian time frame for their basal diversification is recovered, with an indication that divergence times may be fairly old overall. The distribution of mitochondrial gene order models indicates the pancrustacean arrangement as plesiomorphic and dominant in the group with the exception of family Onychiuridae, that presents an alternative model, and multiple instances of different arrangements in individual genomes or small clusters. We suggest that gene order rearrangement may not be totally independent, as we observe at least two instances of multiple rearrangements along a single evolutionary line. We further discuss the opportunities and drawbacks associated to the inclusion of metagenomic data in a classic study on mitochondrial genome diversity.

DNA Damage Response, oxidative stress and GAA-repeat instability in Friedreich's Ataxia disease: in search of the link

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Friedreich's Ataxia (FRDA) is an autosomal recessive disease, caused by transcriptional inhibition of the *Frataxin* (*FXN*) gene, after expansion of an intronic GAA-repeat. Depletion of frataxin, a mitochondrial protein, leads to unbalanced production of reactive oxygen species (ROS), a condition that could affect the DNA damage response (DDR) and contribute to somatic GAA-repeat instability[1]. How FRDA cells respond to induced oxidative stress remains unexplored. By microscopy analyses with differentially labelled fluorogenic probes, we quantified ROS in nucleus/mitochondria or in the cytoplasm of FRDA and normal lymphoblastoid cells. Unexpectedly, FRDA cells were less responsive than normal ones to induced oxidative stress. In agreement, lower induction of SOD1 and catalase, and less efficient PARP1 cleavage were found, by western blot, in FRDA than normal cells. Instead, these cells display comparable Caspase-3 activation. Thus, PARP1-mediated apoptosis, or possibly PARP1-mediated DDR, might be affected in FRDA. PARP1 has key roles in replication stress response and DDR; its involvement in FRDA is attractive, as the expanded GAA-repeat is a source of replication stress[2,3]. Also remarkable is that *in vitro* physical interaction between frataxin and SOD1 has been recently reported[4].

In parallel, by RNA-FISH we are investigating *FXN* transcription along the cell cycle, with the goal to clarify the relevance of replication/transcription conflicts for FRDA mutation.

References

1.Yau, W. Y., O'Connor, E., Sullivan, R., Akijian, L. & Wood, N. W. *FEBS J.* **285**, 3669–3682 (2018).
2.Stevanoni, M., Palumbo, E. & Russo, A. *PLOS Genet.* **12**, e1006201 (2016).
3.Gerhardt, J. *et al. Cell Rep.* **16**, 1218–1227 (2016).
4.Han, T. H. L. *et al. Biochim. Biophys. Acta - Gen. Subj.* **1863**, 883–892 (2019).

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Enzymatic activity of Drp1 link mitochondrial dysfunction to neurodegenerative features in Cockayne Syndrome A cells

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A growing body of evidence indicate that the neurological defects in Cockayne Syndrome (CS) may be due to loss of mitochondrial function, whereas the impaired transcription-coupled repair could account for the skin photosensitivity. Evidence has been provided that human CS cells present an altered redox balance and excessive mitochondrial fission due to hyper-phosphorylation of the mitochondrial fission protein (Drp1)(Pascucci et al.; 2012, 2016).
Since we found that pDRP1 and CSA localize at mitochondria after mitochondrial damage, we wondered if DRP1 inhibition was capable to rescue the CSA pathological phenotype.
When enzymatic activity of DRP1 is inhibited by MDVI, a mitochondrial fission inhibitor, the high ROS levels are reduced, and the dysfunctional mitochondrial, apoptotic phenotype of CS-A cells is recovered.
Moreover, CS-A cells present a significant reduction of nitric oxide (NO) levels respect to WT fibroblasts and interestingly, CS-A cells are characterized by an overexpression of the S-nitrosoglutathione reductase, suggesting for the first time that nitrosative stress might play a role in CS.
All these preliminary data clearly indicate that the modulation of enzymatic activity of DRP1 is critical in CS-A cells, suggesting DRP1 as a potential therapeutic target as well as its inhibitors as potential therapeutic tools.

Diauxie and co-utilization are not exclusive during growth in nutritionally complex environments

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The classic view of microbial growth strategy when multiple carbon sources are available states that they either metabolize them sequentially (diauxic growth) or simultaneously (co-utilization). This perspective is biased by the fact that this process has been mainly analysed in over-simplified laboratory settings, i.e. using a few model microorganisms and growth media containing only two alternative compounds. Models concerning the mechanisms and the dynamics regulating nutrients assimilation strategies in conditions that are closer to the ones found in natural settings (i.e. with many alternative carbon/energy sources) are missing.
We show that bacterial co-utilization and sequential uptake of multiple substrates can coexist in the same growth experiment, leading to an efficient exploitation of nutritionally complex settings. The order of nutrient uptake is determined by the actual biomass yield (and growth rate) that can be achieved on the same compounds when these are used as single carbon sources. Finally, using two alternative theoretical models we show that this rather complex metabolic phenotype is most likely the outcome of a tight regulation process that allows microbes to actively modulate the different assimilatory pathways involved

Maremmano mitogenomes to improve the horse mtDNA phylogeny and develop an automatic classification tool (HorseGrep)

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The horse is one of the domestic species whose mitochondrial DNA (mtDNA) phylogeny was best studied. The mitogenome data contributed to clarify mode and times of domestication, confirming the central Asian "Steppe Hypothesis" (~5,500 years ago), but also proposing a more complex scenario with different domestication centers including one in western Europe, as recently confirmed by the analysis of modern and ancient genomes.
As for the European context, the climatic and cultural diversity of the Italian Peninsula has triggered the development of a great variety of horse breeds over time. Therefore, the horse phylogeny was recently used to classify the mtDNA of ten Italian breeds, highlighting a remarkable mitochondrial variability in the Maremmano horse. In this work, we pushed the analysis to the entire mitogenome including for the first time representatives of all known matrilineal lineages registered in the Maremmano studbook. The comparison with other worldwide breeds allowed enriching the horse mitochondrial tree with novel branches (haplogroups). The updated phylogenetic information primarily allowed to clarify the origin of the Maremmano horse, suggesting multiple female contributions to the final breed. Furthermore, the first version of "HorseGrep" was developed, a companion tool of the human-specific software "HaploGrep", that enables quality assessment and fast automatic classification of horse mtDNA sequences into phylogenetic haplogroups.

Chromatin Fiber Analysis for the Epigenetic Characterization of Equid Centromeres

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Most vertebrate centromeres are defined by the presence of highly repeated DNA sequences (satellite DNA). Species belonging to the genus *Equus* represent an unprecedented model for the analysis of mammalian centromere birth, evolution and complete maturation. Indeed, an exceedingly high number of centromere repositioning events occurred during their evolution leading to the formation of immature centromeres void of satellite DNA. As a consequence, equid karyotypes are characterized by the presence of both canonical satellite-based and evolutionarily young satellite-free centromeres (Wade et al, Science 2009 326:865-7; Piras et al, PLoS Genet 2010 6:e1000845; Nergadze et al, Genome Res 2018 28:789-99).

By means of high resolution analysis on chromatin fibers, here we compared the architectural organization of different post-translational histone marks at the functional centromere core of satellite-based and satellite-free horse and donkey centromeres.

We demonstrated that, at satellite-free as at satellite-based centromeres, typical heterochromatic marks are present. However, quantitative analysis of fluorescence intensity profiles (ImageJ and Plot2 software) on chromatin fibers demonstrated that satellite-free centromeres show a much lower amount of heterochromatic marks with respect the satellite-based ones.

Our results strongly suggest that a heterochromatic environment favors centromere seeding independently from sequence composition.

Evaluation of homologous recombination rate induced by Diepoxibutane

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Some of the mechanisms associated with Homologous Directed DNA Repair (HR) are not completely elucidated. It is known that HR is elicited in correspondence of stalled DNA replication forks, such as those induced by DNA-DNA crosslinking agents. Diepoxybutane (DEB) can cause crosslinks DNA-DNA and the occurrence of HR can be observed by the dramatic increase of sister-chromatid exchanges in mitotic metaphases. In order to investigate whether HR systems act only on the specific spots of damaged DNA or they act globally once activated, we performed *in vitro* experiments using the HCT116 cell line and measuring the mutation rates with a modified *HPRT* Assay. Namely, we compared the number of colonies resistant to 6-thioguanine (6-TG) under different combinations of treatments (control or DEB) and transfections (control reagents, unspecific donor vector, or a mutagenic donor vector with homologies for *HPRT* locus).
Preliminary results showed that DEB increases the mutation frequency over the background level and that the subsequent administration of the *HPRT*-specific vector can further enhance this frequency. The vector alone does not induce mutations. These data suggest that exogenous DNA homologous to *HPRT* locus could be specifically incorporated/integrated within the *HPRT* locus without the presence of damaged DNA on the same locus. If this hold true, the HR machinery could be exploited for the modification of genes following its activation by small chemical compounds or drugs.

LSD1 depletion activates senescence in Glioblastoma in a SASP-independent manner

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An important mechanism for preventing proliferation in tumor cells is to activate senescence which is a stress response that leads to a stable exit from the cell cycle. Cellular senescence is a tumor suppressor response that acts as a barrier to cancer development and progression. It is accompanied by changes in DNA methylation, histone-associated epigenetic processes, chromatin remodeling and ncRNA expression. Accumulating evidences show also that senescent cells may have deleterious effects on the tissue microenvironment. The most significant of these effects is the acquisition of a senescence-associated secretory phenotype (SASP) that converts senescent cells into proinflammatory cells which have the ability to promote tumor progression. We investigated the role of the epigenetic master regulator Lysine-specific demethylase 1 (LSD1) that is found to functions as a regulatory hub controlling different aspects of cellular senescence, as DNA damage, proliferation and cellular migration. In our recent studies we elucidate a mechanism whereby LSD1 controls senescence in Glioblastoma tumor cells (GBM) through the regulation of HIF-1α.
In addition we also demonstrate that LSD1 depletion or treatment of cells with the senescence conditional medium, SCM, following LSD1 inhibition does not result in interleukin (IL6 and IL8) upregulation in glioblastoma cells suggesting that LSD1 depletion activates Senescence in a SASP-independent manner. The mechanism underlying this behavior is under investigation. Additionally, exposure of GBM cells to SCM derived by cells treated with drugs for LSD1 inhibition, inhibits cell migration suggesting a reduction of tumor aggressiveness.
Collectively our results have important implications for the use of drugs that target chromatin and epigenetic regulators for GBM cancer therapy. Overall the inhibition of LSD1 can be exploited in the future as adjuvant for GBM therapy.

The male determining factor of the Medfly, *Maleness-on-the-Y* (*MoY*) acts by a short protein during early embryogenesis: a single embryos molecular analysis of its male determining regulatory effects

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Very recently, we have reported with other collaborators, the identification of the Y-linked master gene for male sex determination, *MoY*, in the Medfly *Ceratitis capitata,* a major agricultural pest [1]. *MoY* is at the top of a partially defined genetic pathway controlling sexual differentiation. It encodes a short protein lacking any significant similarity with those reported in NCBI databases but it is conserved in other Tephritidae, as the olive fly and the oriental fruit fly. The observed effects of *MoY* gene during XY embryonic development is a rapid change toward male-specific splicing of the female determining *transformer* RNA (*Cctra*), switching it into the OFF mode. Here, to investigate whether *MoY* transiently reduces the maternal and zygotic RNA quantity of *Cctra* functional transcripts (female-specifically spliced) in XY versus XX, we performed a quantitative real time PCR on single embryos at 4-8 hours after oviposition. As we have previously observed that MOY recombinant protein induces partial masculinization of XX individuals at adult stages, we are currently injecting MOY into XX embryos also to investigate its direct or indirect molecular action on *Cctra* splicing during early embryogenesis.

[1] Meccariello et al., *Science,* 27 Sep 2019: eaax13182019.

Molecular analyses of sex determination genes in *Leishmania* vector sand fly species: discovery of evolutionary conservations and novelties and implication for the development of innovative eco-friendly control methods

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Phlebotomine sand flies (Diptera, Nematocera) are important vectors of several pathogens, including Leishmania parasites, causing serious diseases of humans and dogs. Despite their importance as disease vectors, most aspects of sand fly biology remain unknown including the molecular basis of their reproduction and sex determination, aspects also relevant for the development of novel vector control strategies. Using a comparative genomics/transcriptomics approach, we identified all the main sex determining genes in phlebotomine sand flies, including the *transformer* gene which exhibits both conserved and novel features, and we proposed the first sex determination model for these important vector species. The *tra* gene represents the key gene for female sex determination in insects and to date it has not been identified in any species of the Diptera suborder Nematocera, which comprises important vector species such as mosquitos, sand flies and black flies. Our results permit to fill the gap about sex determination in sand flies, contribute to a better understanding of this developmental pathway in Nematocera and could help for the identification of sex determining orthologs in other Nematocera species. Furthermore, the sex determination genes identified in our work also open the way to future development of novel biotechnological applications, including *in vivo* RNAi and Crispr-Cas9 gene targeting to control natural population of sand flies, reducing their impact on public health.

Explaining the Bantu expansion through a new Approximate Bayesian Computation (ABC) framework using linguistic and complete genomes data

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One of the most significant moments in African history is the expansion of Bantu-speaking populations 5000 year BCE. Bantu languages represent the largest African language family occupying a vast territory and spoken by a high number of people.
Multidisciplinary studies associated this expansion with the transition from hunter-gatherer societies to food producers that allowed populations to accumulate stored food and to increase in size, resulting in the expansions of populations. However, the dynamics of this expansion are matter of debate. Two main hypotheses have been proposed: an early-split of Bantu farmers into Western and Eastern, at the north of the rainforest; or a later-split, in which the Eastern group branches off the Western group at south of the rainforest. Recent studies have tried to shed light on the modality of the Bantu expansion combining data from different fields.
In this project, we propose a new ABC framework in which genomic and linguistic data would be simultaneously considered in the analysis of demographic models. Linguistic evolutionary models will be integrated in the classical ABC framework. Preliminary analysis were performed on this conceptual model, assessing its validity through a power analysis, and obtaining satisfactory results. In the end, we will test both Early and Late-Split models using for the first time whole-genome data, from Bantu-speaking individuals, together with linguistic data. With these two extended datasets, combined with the preferential power produced by the present ABC method, we expect to reveal details of the past history of Bantu population with an unprecedented definition.

Pathogenic variants in *EP300* and *ANKRD11* in patients with phenotypes overlapping Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS), Rubinstein -Taybi syndrome (RTS) and KBG syndrome are three distinct developmental human disorders. Mutations in five genes belonging to the cohesin pathway, *NIPBL*, *SMC1A*, *SMC3*, *HDAC8* and *RAD21*, were identified in about 70% of CdLS patients, suggesting that additional causative genes remain to be discovered. Two genes, *CREBBP* and *EP300*, have been associated with RTS whereas KBG results from mutations in *ANKRD11*. By whole exome sequencing we identified pathogenetic variants in three patients with diagnosis of CdLS but negative for mutations in CdLS-causative genes by Sanger sequencing. In particular, one patient carried a heterozygous pathogenic variant in *NIPBL* whereas two mutations in *EP300* and *ANKRD11* were identified in the other two CdLS patients. *NIPBL* pathogenic variant had no effect on protein expression, whereas variants in both *EP300* and *ANKRD11* caused low levels of the respective proteins suggesting a role of P300 and ANKRD11 haploinsufficiency in a CdLS-like phenotype. These findings highlight the clinical overlap between CdLS, RTS and KBG and support the notion that these rare disorders are linked to abnormal chromatin remodelling, which in turn affects the transcriptional machinery.

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The influence of COMT Val158Met polymorphism on tinnitus

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Tinnitus is a common debilitating condition defined by the perception of phantom sounds in the absence of physical stimuli and for which little knowledge on the molecular mechanisms exists. Recent evidence has pointed towards a genetic contribution in the familial transmission of tinnitus. Furthermore, several variants have been recently found to be associated with tinnitus. However, these were based on small-sized studies, and lacking replication in an independent group of people. The catecholamine-O-methyltransferase (COMT) gene Val158Met polymorphism is mainly expressed in the prefrontal cortex (PFC), interacts with stressful life events and has been associated with pain sensitivity and auditory gating. Recently the COMT Met/Met variant has been associated with greater tinnitus loudness. Here we hypothesize that tinnitus people carrying this COMT variant display greater tinnitus distress. To test our hypothesis, we took advantage of the Swedish Tinnitus Outreach Project (STOP) and genotyped 376 tinnitus subjects and 2161 controls. While we confirmed that the COMT Val158Met polymorphism impacted on tinnitus loudness, it did not influence tinnitus distress or tinnitus severity. In non-tinnitus controls, Met/Met carriers did not display increased stress or anxiety, contrasting with what has been suggested in the literature. Our study emphasizes the need of creating large biobanks in ENT clinics in order to improve genetic knowledge on clinically relevant tinnitus patients.

Effect of novel RHPS4-derivative ligands in combination with ionizing radiations in breast cancer cell lines

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G-quadruplex (G4) interacting agents are a class of ligands able to bind to and stabilize secondary structures located in genomic G-rich regions such as telomeres and promoters. RHPS4 is one of the most effective telomeric G4 ligand though its potential as a therapeutic agent is compromised by off-target effects on cardiovascular physiology. Therefore, non-toxic analogues (named 190, 761, 785) recently synthetized to overcome this problem, have been tested as single agent and in combination with ionizing radiation (IR) in epithelial (MCF10A) and breast cancer cell lines (MCF7, Tamoxifen-resistant-MCF7-Y537S). Data collected indicated that RHPS4 and 190 were the most effective compounds in cell growth inhibition, although no significant telomeric effects were detected for any of the derivative compounds. All the compounds strongly downregulated CHK1 and RAD51 proteins that are well known players in replication stress response and homologous recombination repair whereas only RHPS4 and 190 were able to reduce estrogen receptor alpha (ERα). Since these proteins were associated with increased response to IR we performed colony-forming assay to evaluate radiosensitization potentiality of the tested compounds. Although no marked radiosensitization effect was detected for any of the compounds, the most effective were 190 and 761, which preferentially affected MCF7 and Y537S, respectively.

Investigating the role of PCNA acetylation in DNA replication and repair processes

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Proliferating Cell Nuclear Antigen (PCNA) plays a critical role in DNA metabolism, by interacting with DNA replication and repair factors. Its spatio-temporal function is also determined by post-translational modifications such as acetylation, which was shown to promote PCNA removal and degradation after UV. In fact, PCNA stability can be influenced by the activity of two lysine acetyl transferases (KATs), CBP and p300, that prevents deleterious PCNA accumulation at the DNA damage sites and fosters the DNA repair pathway progression. To elucidate the role of PCNA acetylation during replication, firstly we evaluated the DNA synthesis in HeLa cells expressing an acetylation-mimetic PCNA mutant (2KQ) and the PCNA accumulation in $p300^{-/-}$ tumor cells. Then, we performed an immunoprecipitation assay to assess the interaction between non-ubiquitinable PCNA mutants (2KR and 5KR) and Cul4A, which is required for PCNA ubiquitination. Moreover, we studied the influence of $PCNA^{K77,80R}$ knock-in mice to evaluate whether PCNA stability is influenced *in vivo*. Lastly, the recruitment and release kinetics of $PCNA^{2KQ}$ after localized DNA damage were determined by live cell imaging analysis of microirradiated cells. The results showed that PCNA acetylation is a crucial step in DNA repair pathway as well as during replication, indeed the PCNA mutants accumulation lead to the DNA synthesis impairment *in vitro*, H2AX phosphorylation *in vivo* and alteration of the PCNA dynamics at the DNA damage sites.

Paired genomic and transcriptomic profiling of Small Cell Lung Cancer with high-copy number amplifications of the *MYC* family genes.

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Gene amplification in small cell lung cancer (SCLC) often leads to high copy number gain of *MYC*, *MYCN* or *MYCL*. Since the architecture and expression pattern of such amplifications as dmin/hsr in SCLC were poorly investigated so far, we studied 7 patients and 12 cell lines by an integrated genomic/transcriptomic NGS approach. Our evidences poorly matched chromothripsis criteria, supporting a step-wise mechanism of genesis for these amplicons, often arranged as head-to-tail repeats. Furthermore, amplified genes (mainly *PVT1* and *RLF*) frequently participated in fusion transcripts lacking a corresponding DNA template. Among them, the *RLF/MYCL* chimera was found as recurrent in both patients and cell lines. *In vitro* functional studies indicated its causative role in tumorigenesis, supported by clinical investigations in a cohort of 28 SCLC patients, showing its correlation with a shorter OS (11 vs 17 months). We also evaluated the expression of the circular isoform of *PVT1* (*circPVT1*) and explored *in vitro* its tumorigenic role, as well as its clinical impact. Our results showed that *circPVT1* expression was associated with a better OS (14 vs 8 months). In conclusion, our results, to be confirmed in a larger patient cohort, indicated that both transcripts, obtained by amplified genes, might be used as potential prognostic markers in SCLC. This is of relevance, considering that the therapies against SCLC are still unchanged since 40 years, leaving this disease as largely incurable.

Genotoxic effects of occupationally-relevant doses of X-ray on lens epithelial cells

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As part of the project "Evaluation techniques of lens doses for workers exposed to ionizing radiation in the medical field, modelling of biological effects and radio-induced risk reduction strategies", we realized in-vitro dose-effect curves for the different types of radiation and compared these curves with the doses to which operators are exposed. The identified endpoints were proliferation, cellular senescence and DNA damage. The biological model consisted of lens epithelial cell (LEC) cultures. So far, the effects of two dose rates (0.28 and 0.55 Gy/min) of X-rays (170 kVp) have been studied. Effects on proliferation and senescence have been found only at high doses (1 and 2 Gy, respectively, for the two dose/rates), to which, realistically, no operator can be exposed. By means of the micronuclei test, instead, a statistically significant DNA damage was detected starting from the lowest dose (27.5 mGy). This result has important implications for radioprotection, given the established role of DNA damage as an initiator of the etiology of cataracts.

Conservation of the DDR regulatory module composed by three MYB transcription factors in orchids

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Floral zygomorphy is an important trait evolved many times from the ancestral condition of radial symmetry. In the snapdragon *Antirrhinum majus*, the interaction of the MYB transcription factors DIVARICATA (DIV), DIV-and-RAD-Interacting-Factor (DRIF) and RADIALIS (RAD) is responsible for the establishment of floral dorsoventral asymmetry (DDR regulatory module). The molecular basis of floral symmetry has been studied in a few other dicot species outside *Antirrhinum*, and it is almost completely unknown in monocots. The orchids are monocot plants with zygomorphic flowers. In order to verify if the molecular basis of floral zygomorphy is conserved between orchids and snapdragon, we analysed the protein interactions among the orchid DIV, DRIF and RAD. As observed in *A. majus*, the orchid DRIF can interact both with the orchid DIV and RAD, whereas DIV and RAD do not directly interact. In addition, we found snapdragon transcriptional *cis*-regulatory elements of the *DIV* and *RAD* loci conserved within the corresponding orchid orthologues. Finally, the analysis of the expression pattern revealed that in *Orchis italica* and *Phalaenopsis equestris* (with zygomorphic flowers) the *DIV*, *DRIF* and *RAD* genes are expressed at higher levels in the lip than in lateral inner tepals, whereas in orchid flowers with radial symmetry they show similar expression levels. These results support the conserved role in floral symmetry of the orchid *DIV*, *DRIF* and *RAD* genes, enriching the orchid developmental code.

Dyskerin silencing induces autophagy in human colorectal cancer and human embryonic kidney cells by inhibiting the AKT/mTOR signaling pathway

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Human dyskerin is an evolutionarily conserved protein whose deficiency causes the X-linked dyskeratosis congenita disease. Dyskerin, encoded by the DKC1 gene, is a multifunctional nucleolar protein involved in multiple functions related to cell growth, proliferation, vesicular trafficking and telomere maintenance. Starting from the finding that dyskerin downregulation causes alteration in translational fidelity, we wondered if dyskerin depletion affected the correct folding of proteins. To this aim, using human colorectal cancer (RKO) and human embryonic kidney cells (HEK 293T), we generated two stable and inducible cellular models able to silence the DKC1 gene following administration of tetracycline or its more stable derivative doxycycline. By using these cellular systems, it was possible to analyse the events occurring immediately following dyskerin knockdown, in a short time frame, well before the eventual occurrence of telomere erosion. We found that dyskerin downregulation causes an accumulation of unfolded/misfolded proteins in the endoplasmic reticulum and consequent activation of the unfolded protein response. Unfolded protein response culminates in activation of autophagy via inhibition of the AKT/mTOR signalling pathway. In conclusion, our data shed more light into the comprehension of molecular mechanisms underlying the dyskeratosis congenita disease.

Genotoxicity of particles debris generated from passenger and truck tires on Raw 264.7 cell line

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Particulate matter (PM) is constantly associated with morbidity and mortality, can reach the pulmonary alveoli when give acute respiratory symptoms. We evaluated the *in vitro* effects of passenger and truck tires on the RAW 264.7 cell line. We have analyzed the tire particles with SEM-EDX analysis for the chemical and semi-quantitative characterization; viability was determined by MTS-assay, after exposure at different concentrations of particles. The genotoxicity was evaluated using the cytokinesis-block micronucleus (CBMN) assay. We detected the release of TNFα evaluate the inflammatory response.
MTS test at 24h shows a reduction in metabolic activity in both tire particles, while at 48h, shows an increase in metabolic activity. The CBMN assay show an increase in the number of micronuclei in all conditions in the cells treated with passenger tire, on the contrary truck tire shows slight increase. We have a significant increase in the TNFα release at different concentrations in both treatments. Size of the PM and qualitative properties influence the ability of the particles to induce inflammatory response on macrophages. Both tire particles induce an inflammatory response but the cyto and genotoxic response varies according to the chemical composition and is higher in the passengers. Less toxicity of the trucks could allow the development of tires with an ecological composition due to the greater presence of natural rubber.

p14ARF phosphorylation modulates autophagy in cancer cells

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The CDKN2a/ARF locus is one of the most frequently mutated sites in human cancers. It encodes two completely unrelated proteins, p16INK4a and ARF, both tumor suppressors. In particular, ARF halts cell growth by both p53-dependent and independent pathways being involved in cellular stress response, autophagy, mitochondrial homeostasis, and differentiation. In the last years, quite unexpectedly, a pro-oncogenic ARF function has been observed. Cancer cell lines expressing high ARF levels showed that its expression, far from being dispensable, is instead required to guarantee tumor cell survival. We now provide evidence that ARF functions can be regulated through its phosphorylation on a conserved Threonine 8. Interestingly, the expression, in both HeLa and H1299 cells, of an ARF mutant mimicking a constitutively phosphorylated status of the protein (ARF-T8D) appears to inhibit autophagy. Several observations underlined how this pathway could serve a dual role in cancer progression, either protecting healthy cells from damage or aiding cancerous cells to survive. We observed that the hyper-expression of ARF-T8D in HeLa cells, in contrast to the wt protein, induces nuclear re-localization of the LC3 protein, a well-known crucial player of autophagy. The experiments suggest that ARF mediated autophagy could explain its role in cell adhesion and anoikis protection. Our data provide for the first time the evidence that ARF dual role in cancer could mirror its involvement in autophagy.

Complete human genome data account for multiple dispersals of anatomically modern humans out of Africa

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There is a wide consensus in considering Africa as the birthplace of anatomically modern humans, but the dispersal pattern and the main routes followed by our ancestors to colonize the world are still matters of debate. It is still an open question, indeed, whether early modern humans left Africathrough a single major process, dispersing almost simultaneously over Asia and Europe, or in two main waves, first through the Arab Peninsula into southern Asia and Australo-Melanesia, and later through a northern route crossing the Levant. In this work, we test the two main out-of-Africa hypotheses through an Approximate Bayesian computation (ABC) approach, based on the recently developed Random Forest algorithm. We first explicitly evaluated the ability of the method to discriminate between the alternative evolutionary models (i.e. single vs multiple exits) using simulated data. Once assessed that the two competing models are distinguishable through ABC, we compare simulated data with real genomic variation, from worldwide modern and archaic populations.