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Programme & Abstracts

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P3.1 - Satellite DNA is responsible for centromere clustering in mammals

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In the 3D architecture of mammalian nuclei, centromeres cluster at the nuclear and nucleoli periphery. It is a matter of debate whether centromere clustering depends on the presence of satellite repeats or on the centromeric function. We discovered that, in equid species (horse and donkey), several centromeres are satellite-free, whereas many satellite DNA loci are not centromeric (Wade et al Science 2009; Piras et al PLoS Genet 2010; Nergadze et al Genome Res 2018). Thus, Equids represent a unique model for investigating the basis of centromere clustering.

By studying their 3D distribution, we showed that centromeric and non-centromeric satellite DNA loci form clusters, indicating a tendency of satellite sequences to coalesce irrespectively of the centromeric function. On the other hand, satellite-less centromeres, although localizing mainly at nucleoli and nuclear periphery, do not associate to the satellite-based centromeres.

These observations are in agreement with the notion that in the mammalian nucleus, chromosomal loci tend to associate with each other according to their repeat enrichment as a result of mutual repeat recognition (Solovei et al Curr Opin Cell Biol 2016).

P3.2 - Two immortalized rat astrocyte cell lines as *in vitro* model for specific cell proliferation studies: cytogenetic and epigenomic characterization and diversification

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Here we report differences between: 1) a heterogeneous population of primary rat brain astrocytes (Primary), in culture since several years ago, and 2) a cloned cell line (Clone), obtained from the Primary cells. Both populations maintain astrocyte morphology but, according to cytogenetic and epigenomic characterization, differ for the chromosomal asset from rat normal cells (42 chromosomes): Primary cells show mostly a bimodal karyotype with 41 or 43 chromosomes, and Clone has a unique-modal karyotype of 43 chromosomes. Interestingly, we also found that both cell lines show genome-wide DNA hypomethylation, with Clone showing even more pronounced demethylation respect to Primary cells. These features, together with a faster doubling time, confer to Clone an altered proliferation control phenotype. Conversely, the Primary cell population is more similar to normal cells. Used together the two cell populations are a promising model to investigate in vitro modifications of genome, epigenome and others 'omics', mimicking tumor clonal evolution-derived heterogeneity, particularly useful in studies on CNS cancers, which derive mostly from glial cells.

P3.3 - The tumor suppressor p14^{ARF} hampers proliferation of aneuploid cells induced by CENP-E partial depletion

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The Spindle Assembly Checkpoint (SAC) is a cellular surveillance mechanism that ensures

faithfully segregation of chromosomes. Reduced expression of some of its components weakens the SAC and induces chromosome instability and aneuploidy, both hallmarks of tumor cells. Centromere Protein-E (CENP-E) is a crucial component of the SAC and facilitates kinetochore microtubule attachment required to achieve and maintain chromosome alignment. To investigate the possible role of $p14^{ARF}$ on aneuploid cells proliferation we induced aneuploidy in primary human fibroblasts (IMR90) and in near diploid tumor cells (HCT116) by partial depletion of CENP-E obtained by RNA interference. Our results show that in contrast to IMR90 aneuploid cell number that was drastically reduced tending toward wild type condition, HCT116 aneuploid cells were slightly decreased at late time points. This euploidy restoration was accompanied by increased $p14^{ARF}$ expression in IMR90 cells and followed ectopic $p14^{ARF}$ re-expression in $p14^{ARF}$-null HCT116 cells. Collectively, our results strongly suggest that hampering proliferation of aneuploid cells is an additional role of the $p14^{ARF}$ tumor suppressor.

P3.4 - Identifying new genes linked to cell division and regulation of brain size

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During evolution, the human brain increased in size and complexity when compared to other Hominidae. In particular, changes in brain globularization and skull shape took place during the transition from *H. neanderthalensis* to *H. sapiens*. These changes are thought to be due to fixed mutations in genes involved in brain development. In order to identify regulators of human brain size, we selected candidate genes that carry fixed sequence changes in *H. sapiens* compared to *H. neanderthalensis*, and performed siRNA based phenotypic screening in human hTERT-RPE-1 cells. The screening identified several genes with roles in mitosis, in particular in spindle assembly and chromosome segregation. siRNA-induced mitotic defects, resulted in p53 activation and cell cycle arrest or apoptosis, depending on the target. Moreover, we observed defects in serum starvation-induced ciliogenesis following gene silencing. To better understand the consequences of the defects that we observed in cultured cells on brain development, we are performing experiments *in vivo* in chicken embryos with selected candidates. The results of the screening and current characterization of candidate genes will be presented.

P3.5 - Alternative Lengthening of Telomere (ALT) activated by telomere damage in human primary fibroblasts

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The large majority of cancers (80–85%) maintain telomere length by expressing telomerase whereas the other ones (15-20%) utilize the Alternative Lengthening of Telomeres (ALT) pathway. Although the telomerase mechanism is well known, the molecular details of ALT remain poorly described. Previous studies provided evidence that X-rays modulate telomere length at 15 days after exposure in human primary fibroblasts (HFFF2). In order to understand the mechanism responsible for such modulation, we treated HFFF2 with 4Gy of X-rays and analyzed telomere length/dysfunction, telomerase activity, ALT markers and epigenetic changes at different times after irradiation. Results demonstrated that irradiation modulates telomere length with ALT mechanism instead of telomerase activity. Furthermore the analysis of Telomere Induced Foci (TIFs), indicated a telomere dysfunction and ChIP results support also a epigenetic modification after telomere damage. These data confirm our previous hypothesis that ALT is a mechanism