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Meeting Report

Chromosome instability: From molecular mechanisms to disease

ABSTRACT

The International University of Andalusian (UNIA) held on the 13th to 15th of November 2017 was a meeting oriented to the concept of Chromosomal Instability and related diseases. The meeting was part of the renowned UNIA workshops programme "Current Trends in Biomedicine", held in the UNESCO World Heritage awarded city Baeza, located in the south of Spain.

The goal of this Workshop was to bring together experts in DNA repair and chromosome segregation in order to address the phenomenon of Chromosomal Instability as a whole, facilitating the communication between scientists from different fields to foster cross-disciplinary approaches.

This report summarizes a selection of the many interesting results and data presented during the meeting.

1. Introduction

The fidelity in chromosome maintenance and segregation are indispensable to maintain genomic stability and the perpetuation of life. Cells with defects in those processes will end up with an aberrant set of chromosomes, and this may lead to different types of diseases. Chromosomal Instability (CIN) is the gain and/or loss of whole chromosomes or chromosomal segments. Remarkably, there are a variety of human diseases directly related to Chromosomal Instability. The study of the causes and consequences of CIN has become one of the leading fields in biomedical research in the past years.

There are several mechanisms that can give rise to CIN. On one hand, alterations during the process of DNA replication, known as Replication Stress may lead to incomplete DNA replication. Incomplete replicated DNA generates entangled sister chromatids that eventually promote segregation defects and CIN [1]. In addition, the induction of DNA double strand breaks (DSBs), by exogenous or endogenous sources of DNA damage, may also end up generating chromosomal rearrangements [2].

On the other hand, mitosis is a key process related to Chromosomal Instability. Any mitotic alteration can eventually lead to an Aberrant Chromosome Segregation. Alterations in the spindle assembly, inefficient Spindle Assembly Checkpoint (SAC), problems in chromosome cohesion/condensation or alterations in cytokinesis may lead to aneuploidy in the resulting daughter cells [3]. Although aneuploidy interferes with cell proliferation in non-transformed cells, paradoxically it is a hallmark of cancer, a disease characterized by an increased proliferative potential. More intriguing, aneuploidy can either promote or inhibit cancer cell growth, depending on the levels of aneuploidy generated [4].

Importantly, DNA replication and mitosis are closely related. Alterations in DNA replication often lead to perturbations in the following mitotic round. If a cell reaches mitosis with the DNA not properly replicated, the non-replicated DNA cannot be segregated during anaphase, therefore generating Chromosomal Bridges. These chromosome bridges are another classical hallmark of CIN. Finally, the circle can be closed by the effect that mitotic alterations can exert on DNA replication. Mitotic errors can lead to lagging chromosomes, which in turn can be partitioned into Micronuclei. This can produce DNA damage either as a direct consequence of aberrant DNA replication, or due to chromosome pulverization because premature chromosome compaction can occur if a cell enters mitosis with micronuclei still undergoing DNA replication [5].

There are a variety of human diseases directly related with Chromosomal Instability. Most diseases related to CIN share a high susceptibility to develop cancer, and in certain cases, premature ageing.

Mutations in several genes of the DNA repair/damage response pathway lead to Chromosomal Breakage Syndromes (Ataxia telangiectasia, Bloom syndrome, Fanconi anemia, Nijmegen breakage syndrome, Xeroderma pigmentosum). These are a group of genetic disorders that are typically transmitted in an autosomal recessive mode of inheritance [6]. These disorders are characterized by defects in DNA repair mechanisms, and patients show increased predisposition to cancer. In addition, patients harbour other traits, such as cerebellar ataxia, growth retardation, microcephaly, skeletal abnormalities, hypogonadism, pancytopenia, or abnormal pigmentation.

Another type of chromosome associated syndromes are the so-called Cohesinopathies. These genetic instability syndromes are due to defects in the Sister Chromatid Cohesion, a process that keeps the newly replicated chromosomes together from the time of their synthesis in S phase until they separate during mitosis. Typical cohesinopathies are Cornelia de Lange syndrome and Roberts syndrome [7].

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Finally, and probably the disease more intimately linked to CIN is Cancer. A classical hallmark of cancer is that tumors present a high ratio of aneuploidy and miss-segregate chromosomes at very high rates, leading to CIN. High aneuploidy tumors correlate with poor patient prognosis, indicating that reduced mitotic fidelity contributes to cancer progression by increasing the genetic diversity among the tumor cells [3]. Therefore, there is an intense research effort on depicting the role of Chromosomal Instability in specific cancers. This is crucial for understanding the disease pathogenesis and may also lead to new avenues for treatment [8].

The idea of the workshop was to bring together the topics of DNA Repair (replicative stress and DNA damage) and Mitosis (chromosome segregation alterations) in order to study and discuss the two main sources of Chromosomal Instability.

2. Implications of DNA replication, damage and repair in CIN

Prof. **Ian Hickson** from the Center for Chromosome Stability at the University of Copenhagen gave the inaugural talk at the meeting. Prof Hickson talked about various cellular tools that generate a block in DNA replication in mammalian cells, such as the Tus/Ter system [9] and the Lac Operator/Repressor [10]. His latest findings suggest that the Lac O/R array behaves to some extent as a common fragile site, but with clear differences. For example, although they observe Mitotic DNA synthesis (MiDAs) as for common fragile sites [11], they depend on a different set of factors. In a related topic, Dr. **Ying Liu** from the Centre for Chromosome Stability at the University of Copenhagen (Denmark) reported the presence of UFBs −which has been reported to be associated to common fragile sites in the rare fragile site FRAXA [12]. Why rare fragile sites are particularly sensitive to folate deficiency whereas common fragile sites are sensitive to low doses of aphidicolin is still an intriguing open question in the field.

Dr. **Andrés J López-Contreras** from the Centre for Chromosome Stability at the University of Copenhagen (Denmark) presented his findings on the study of a mouse model deficient for the DNA translocase PICH. PICH KO cells and embryos exhibit chromosomal instability and, consequently, KO mice die during embryonic development. Current investigations in López-Contrera's lab are focused on the impact of PICH deficiency on cancer and its potential use as a therapeutic target. Preliminary data indicate that PICH is largely dispensable for the homeostasis of adult tissues, whereas it is required for rapidly proliferating tumor cells. This could provide a therapeutic window to treat certain types of cancer by promoting CIN targeting PICH.

In the past years, there is an increasing interest on the study of DNA/RNA hybrids or R-loops and its relation to chromosomal stability [13,14]. **Andrés Aguilera** at the CABIMER (Sevilla, Spain) is a leading expert in this area. Dr. **Belén Gómez-Gonzalez**, a senior researcher in Aguilera's lab, presented some of their recent discoveries on this area. They have found a relevant crosstalk between the presence of R-loops and the landscape of chromatin modifications [15,16]. Interestingly, treatments with histone acetyltransferase inhibitors suppress phenotypes derived from R-loop accumulation. On the other hand, taking advantage of a siRNA screen, they have observed that a number of genes related to DNA damage checkpoints prevent the accumulation of R-loops. Dr. **Ralf Wellinger** from the University of Sevilla presented his latest work on R-loop metabolism in yeast. He showed that cells with mutations in RNAse H, an enzyme that degrades R-loops, exhibit an accelerated S phase transition and defects in mitosis and chromosomal segregation.

Prof. **Andre Nussenzweig** from the NIH (Bethesta, USA) talked about Replication Fork Collapse (reviewed in [17]); *when*, *where* and *how* replication forks collapse. Using 10 mM HU treatments in B cells Nussenzweig's lab observed that DSBs mainly occur during S phase and in regions flaking active genes, likely at replication origins.

Dr. **Travis Stracker** from the IRB (Barcelona, Spain), discussed the role of Tousled-like kinases (TLKs) in the maintenance of a proper chromatin structure after replication. Moreover, his data suggested an increase replication stress in the absence of TLK activity. Finally, he discussed how this molecular function might account for the overexpression of TLK in many cancer types and the prognostic value of such amplification.

Prof. **Oscar Fernández-Capetillo** from the CNIO (Madrid, Spain) and Karloninska Institute (Stockholm, Sweden) brought up a provocative discussion about the actual existence of the cell cycle G2 phase. Data from his lab suggest that what is defined as G2 is actually late S-phase, and that the termination of DNA replication is coordinated with the entrance in mitosis. This observation is consistent with the presence of DNA replication in G2 and even in mitotic chromosome previously reported by Prof Ian Hickson [11]. Fernández-Capetillo's data support that active replication prevents mitotic entry through a signaling pathway involving SUMO and ubiquitin. They are able to induce the ubiquitinilation of replisome components and mimic DNA replication termination using USP7 inhibitors, which they previously showed interfere with DNA replication [18], by affecting the ubiquitination of SUMO molecules at the replication fork. Interestingly, the use of USP7 inhibitors leads to a concomitant activation of mitotic kinases such as CDK1 and AURKB, supporting a potential coordination between DNA replication termination and mitotic entry.

An important topic in the field of DNA repair is why and how different DNA breaks are repaired differently reflecting cellular status, chromosome position, chromatin configuration, etc [19]. Therefore, it is not surprising that along the meeting there was an open discussion on this issue. Along those lines, Dr. **Pablo Huertas**, from CABIMER (Sevilla, Spain), discussed recent both published and unpublished findings from his lab about how the repair of broken chromosomes is intimately linked with the stemness status of the cells. Indeed, during cell reprogramming cells change the balance towards a more error free repair of DNA breaks [20]. In a related issue, **Teresa Anglada**, from the Autonomous University of Barcelona (Barcelona, Spain), described how DNA repair in human mammary epithelial cells depends on the age of the donor.

Moreover, such effect does not simply rely on the global status of the cell but is directly controlled by specific pluripotency factors. But not only cell status affect the repair of broken chromosomes. Dr. **Evi Soutoglou** from the IGBMC (Strasbourg, France) described the behaviour of DNA breaks when they are located in heterochromatin, how they are mobilized in or out heterochromatic structures to be repaired, and how the repair factors themselves might play a role in this changes in localization. Interestingly, their data suggest intrinsic differences between mouse and human cells that might reflect basic differences in chromosome architecture between those organisms.

Dr. **Fernando Gómez-Herreros**, from IBIS (Sevilla, Spain), showed how topoisomerase II poisons induce translocations, a relevant clinical problem as those treatments are commonly used in cancer therapy but often cause the appearance of secondary cancers. His data pointed out toward a transcription-induced event of DNA repair that is counteracted by the enzyme TDP2 [21].

3. Impact of mitotic and chromosome segregation alterations in CIN

One of the most prominent sources of CIN are alterations in the mitotic fidelity, such as defects in the Spindle Assembly Checkpoint, deficiencies in the microtubule-kinetochore binding, defects in chromosome compaction, etc. During the workshop, several speakers brought up interesting biological questions regarding this topic.

Dr. **Helder Maiato**, from the Cellular and Molecular Biology Institute (Porto, Portugal), raised the conceptual question of how kinetochore size can impact on chromosome segregation and subsequently be related with CIN. Kinetochore size differs not only in between different species, but also differ between chromosomes from the same species (including humans). Maiato uses a very particular cell line