Chromothripsis after Stumbling through DNA Replication

Most structural genome variations (SVs), such as chromosome translocations and inversions, are readily detected by classical chromosome banding in combination with molecular cytogenetic techniques. With steadily improving levels of resolution, the frequency of detection of SVs increases. At nucleotide resolution, these techniques will also allow us to investigate the sequences of the break points of the SVs and eventually their possible mechanism of origin. Using FISH, array CGH, Southern blot hybridization and MLPA, the break points of SVs were mapped, which then allowed us to determine the sequences surrounding the fusion points of these breaks [Higgins et al., 2008; Sobreira et al., 2011; Chiang et al., 2012]. Only one out of 17 fusions was perfectly balanced at the nucleotide level [Higgins et al., 2008]. The overwhelming majority, roughly three quarters, involved insertions, deletions and stretches of microhomology of a few base pairs (bp), which is consistent with nonhomologous end joining (NHEJ). The remainder may have resulted from microhomology-mediated break-induced replication (MMBIR), since they involved more extensive DNA synthesis [Higgins et al., 2008; Sobreira et al., 2011; Chiang et al., 2012]. In a study of 8 de novo unbalanced translocations, which most likely occurred postzygotically, 5 indicated nonallelic homologous recombination between paralogous repeats as the predominant mechanism [Robberecht et al., 2013]. Apparently, at least 3 distinct pathways of ‘repair’ of chromosome breaks exist.

Analysis of 51 reciprocal translocations and 6 SVs with more than 3 break points involving 2–5 chromosomes showed even more complex patterns [Weckselblatt et al., 2015]. Sequencing 37 of the fusion points of reciprocal translocations revealed microhomologies between 0 and 4 bp ($n = 26$), short inserted sequences ($n = 8$), or paralogous repeats ($n = 3$), consistent with NHEJ and MMBIR. In 3 reciprocal translocations, fusion genes were predicted, which may lead to gain of function, but this was not confirmed by mRNA sequencing. In 2 out of 3 cases of complex translocations with associated inversions and insertions, 18 and 33 fusions involving up to 5 different chromosomes were found. The large number of break points and their relatively close spacing suggests that these SVs arose after chromothripsis. All fusion points had 0–4 bp of microhomology, consistent with NHEJ, and both SVs occurred as de novo events on paternal alleles. Together with other studies, these data suggest that germline chromothripsis arises in the paternal genome and may be transmitted maternally [Kloosterman et al., 2011, 2012; Chiang et al., 2012; Nazaryan et al., 2014; Pellestor et al., 2014; Poot and Haaf, 2015; Weckselblatt et al., 2015].

While all of these studies indicated possible mechanisms of fusions, they did not reveal the mechanism(s) of breakage that produced the SV. Thus far, mechanisms such as excessive levels of double-strand breaks (DSBs) during meiosis, mismatching DSB repair during DNA

replication or during meiosis, or postzygotic rearrangements have been suggested [Hart and O'Driscoll, 2013; Robberecht et al., 2013; Pellestor et al., 2014; Poot and Haaf, 2015]. The most extreme form of SV is germline chromothripsis, i.e. shattering of one or a few chromosomes and stitching together the resulting fragments in random order and orientation [Kloosterman and Hochstenbach, 2014; Pellestor et al., 2014; Poot and Haaf, 2015]. The chromosome shattering step of chromothripsis is poorly understood, but in a recent study, chromothripsis has been 'caught in the act' [Zhang et al., 2015].

After release of retinal pigment epithelial cells in culture from nocodazole arrest, micronuclei, containing one or several chromosomes, were formed [Zhang et al., 2015]. Upon entry into the S phase of the cell cycle, as demonstrated by 5-ethynyl-2'-deoxyuridine (EdU) labeling, these underwent DNA damage as shown by fluorescence labeling for the phosphorylated form of histone H2AX. Since their level of EdU labeling was lower than that of the mother nucleus, replication of the chromosomes in the micronuclei was incomplete. After completion of one cell cycle, the EdU-positive micronuclei reincorporated into the mother nucleus. These cells were then isolated and their genomes sequenced. Some cells lost and some cells gained one or several chromosomes. To determine what has happened to the chromosomes in the micronuclei, the authors studied nuclei which had gained a chromosome or part of it. Nuclei with chromosome gains showed 12.5-fold higher rates of SVs. Thus, rearrangements >150 kb and interchromosomal rearrangements, such as insertion translocations, and inversions within 150 kb from a break point were significantly more frequent in nuclei with gains, which is in agreement with chromothripsis only. By SNP haplotyping, the authors found that all rearrangements had exclusively taken place on the missegregated and reincorporated chromosome. More than 50% of the fusion points of the rearrangements showed extensive microhomologies and contained insertions ranging from 50 to 500 bp, which is consistent with MMBIR as the fusion mechanism. This mechanism, which requires one round of DNA replication, is in agreement with several, but not all of the thus far described cases of germline chromothripsis [Kloosterman et al., 2011, 2012; Nazaryan et al., 2014].

These observations also bear on chromosomal instability disorders, such as Werner syndrome (WRN) and Bloom syndrome (BLM). WRN is associated with a variegated translocation mosaicism, a prolongation of the S phase of the cell cycle, hypersensitivity to DNA cross-linking and DSBs provoking agents, and a reduction in recombina-

tional DSB repair [Salk et al., 1981; Poot et al., 1992, 2001, 2002; Ogburn et al., 1997; Melcher et al., 2000; Dhillon et al., 2007]. In BLM, rates of sister chromatid exchanges and hypersensitivity to DSB-causing agents are elevated [Bischof et al., 2001]. Cultured lymphoblastic cells from both WRN and BLM patients show spontaneously elevated levels of micronuclei, which further increased after treatment with compounds that interfered with DNA replication [Honma et al., 2002]. Most micronuclei accumulate DSBs, which contain phosphorylated H2AX and the RAD51 protein [Haaf et al., 1999; Crasta et al., 2012]. The latter is mobilized by the BLM protein to sites of DSBs [Wu and Hickson, 2003; Haber, 2015]. Thus, these observations link the chromosomal instability of WRN and BLM to this novel micronuclei-related form of chromothripsis.

In families in which SVs have resulted from germline chromothripsis, this appeared to be an isolated event [Poot et al., 2009; Kloosterman et al., 2011, 2012]. In somatic cells, chromothripsis is particularly frequent in malignancies of mesenchymal tissues and increases with the age of the patient [Cai et al., 2014]. A strongly increased risk of malignancies of mesenchymal tissues, likely due to the continuous genomic instability of somatic cells, is also a hallmark of WRN [Salk et al., 1981; Poot et al., 1992, 2001, 2002; Ogburn et al., 1997; Melcher et al., 2000; Honma et al., 2002; Dhillon et al., 2007]. While lymphoid cells from WRN patients respond to DNA damage with apoptosis, mesenchymal cells show growth arrest leading to a state of cellular senescence [Poot et al., 2002]. The latter ties in with the premature ageing of WRN patients and suggests that DNA damage-induced perturbation of DNA replication, somatic cell chromothripsis, risk of malignant disease of mesenchymal tissues, and ageing are somehow interconnected.

While in the study of Zhang et al. [2015], cultured epithelial cells, which were made to 'stumble' at the G1/S border of the cell cycle by nocodazole treatment, gave us a novel insight into how chromothripsis may arise, it also prompted numerous novel questions. Some cases of chromothripsis show evidence of MMBIR, in agreement with micronucleus-dependent chromosome shattering, while others are consistent with NHEJ. This suggests that at least one other mechanism of chromosome shattering may exist. In addition, the here described studies indicate that several methods of structural genome analysis have to be applied concomitantly to grasp the full complexity of SVs in general and chromothripsis in particular [Kloosterman et al., 2011, 2012; Nazaryan et al., 2014; Weckselblatt et al., 2015].

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