Review Article

Frequent Chromatin Rearrangements in Myelodysplastic Syndromes – What Stands Behind?

(myelodysplastic syndromes / chromosomal rearrangements / chromosome 5 deletions / chromatin structure / architecture of the cell nucleus / chromothripsis)

E. PAGÁČOVÁ[1], M. FALK[1], I. FALKOVÁ[1,2,3], E. LUKÁŠOVÁ[1], K. MICHALOVÁ[4], A. OLTOVÁ[3], I. RAŠKA[5], S. KOZUBEK[1]

[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, v. v. i., Brno, Czech Republic
[2]Department of Medical Technology, St. Elisabeth University of Health and Social Sciences, Bratislava, Slovak Republic, Signatory of Magnae Chartae Universitatum Bologna, European Union
[3]Department of Internal Medicine – Haematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4]Centre of Oncocytogenetics, General University Hospital in Prague, Prague, Czech Republic, and The Institute of Haematology and Blood Transfusion (IHBT), Prague, Czech Republic
[5]Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Abstract. Myelodysplastic syndromes (MDS) represent a clinically and genetically heterogeneous group of clonal haematopoietic diseases characterized by a short survival and high rate of transformation to acute myeloid leukaemia (AML). In spite of this variability, MDS is associated with typical recurrent non-random cytogenetic defects. Chromosomal abnormalities are detected in the malignant bone-marrow cells of approximately 40–80 % of patients with primary or secondary MDS. The most frequent chromosomal rearrangements involve chromosomes 5, 7 and 8. MDS often shows presence of unbalanced chromosomal changes, especially large deletions [del(5), del(7q), del(12p), del(18q), del(20q)] or losses of whole chromosomes (7 and Y). The most typical cytogenetic abnormality is a partial or complete deletion of 5q- that occurs in roughly 30 % of all MDS cases either as the sole abnormality or in combination with other aberrations as a part of frequently complex karyotypes. The mechanisms responsible for the formation of MDS-associated recurrent translocations and complex karyotypes are unknown. Since some of the mentioned aberrations are characteristic for several haematological malignancies, more general cellular conditions could be expected to play a role. In this article, we introduce the most common rearrangements linked to MDS and discuss the potential role of the non-random higher-order chromatin structure in their formation. A contribution of the chromothripsis – a catastrophic event discovered only recently – is considered to explain how complex karyotypes may occur (during a single event).

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Corresponding author: Martin Falk, Institute of Biophysics, Academy of Sciences of CR, v. v. i., Královopolská 135, 612 65 Brno, Czech Republic. Phones: (+420) 541515116/165; (mobile) (+420) 728084060; e-mails: mfalk@seznam.cz, falk@ibp.cz

Abbreviations: AML – acute myeloid leukaemia, CA – chromosomal aberrations, C-CA – complex chromosomal aberrations, CDR – commonly/critical deleted region, DSB – DNA double-strand break, MDS – myelodysplastic syndrome, RIDGE – regions of increased gene expression, RS – replication stress.

I. Myelodysplastic syndromes – a brief introduction

Myelodysplastic syndromes (MDS) represent a diverse group of heterogeneous clonal bone marrow diseases (Vardiman et al., 2009; Ades et al., 2014) that are associated with ineffective haematopoiesis, peripheral blood cytopoenias and increased risk of progression to acute myeloid leukaemia (AML) (Lindsley and Ebert, 2013). Typical morphologic features of MDS involve, among others, defects in maturation in the myeloid series and rising amounts of blasts or ringed sideroblasts (Nimer, 2006). The annual incidence of MDS is about four cases per 100,000 people (Ades et al., 2014).

Although MDS may also appear in childhood as a consequence of various inherited predispositions, such

as Fanconi anaemia (Liew and Owen, 2011; Ades et al., 2014; West et al., 2014), most cases burst sporadically and patients are diagnosed in their late 60s or early 70s, with a median age at diagnosis being 65–70 years; less than 10 % of patients are younger than 50 years. This might indicate that MDS originates from accumulation of unrepaired DNA defects caused by normal physiological cellular processes (Kryston et al., 2011; Ghosal and Chen, 2013; Behrens et al., 2014). The life style, history of various diseases, and exposures to stress are therefore expected to contribute to MDS initiation. On the other hand, chromothripsis – a still mysterious process of chromosome "explosion" (Stephens et al., 2011; Forment et al., 2012) – has recently been discovered as a single-step alternative to this multi-step development of complex cancer karyotypes and cancer disease.

II. Recurrent chromosomal abnormalities in MDS

At the molecular level, MDS syndromes arise due to various types of genetic aberrations (Table 1) (Fernandez-Mercado et al., 2013; Huret et al., 2013); hence, different subtypes of MDS can be distinguished with a different molecular pathogenesis and various propensity for development of acute myeloid leukaemia (AML). On average, AML occurs in 10–15 % of MDS patients (reviewed e.g. in Ades et al., 2014; Visconte et al., 2014).

The most frequent initiating aberration in MDS is a large, unbalanced chromosomal deletion that can include even whole chromosome arms (Fig. 1) (Zemanova et al., 2008). This fact seriously complicates identification of genes that are critically involved in MDS pathogenesis. The deletions typically include long arms of chromosome 5 (Fig. 1), 7, and 20 but can affect different parts of other chromosomes as well, such as chromosomes 3q, 12p, 13q, 16q, 17p, 18q, and 20q (Haase et al., 2007). Except deletions, trisomies (8, less frequently 11 and 21) monosomies (21 and 10), and other unbalanced chromosomal changes (Haase et al., 2007) were reported. Chromosomes 5, 7, and 17 also frequently participate in rearrangements that involve more chromosomes (Zemanova et al., 2008, 2014). Simple chromosomal aberrations (CA) are typical of primary MDS (Fig. 1), while secondary MDS are frequently characterized by very complex genomic rearrangements (C-CA) (similar to an AML karyotype in Fig. 2).

Table 1: Cytogenetic abnormalities in myelodysplastic syndrome (Greenberg et al., 1997; Bernasconi et al., 2006; Olney and Le Beau, 2007)

Recurring cytogenetic abnormalities	
Abnormality	**Incidence**
De novo MDS	
- 5/del (5q)	6-20%
- 7/del (7q)	1-10%
Trisomy 8	5-10%
Y	1-10%
del(20q)	2-5%
del(17q)	< 1-7%
Complex (≥ 3 abnormalities)	10-20%
Treatment-related MDS	
- 5/del (5q)	40%
- 7/del (7q)	40%

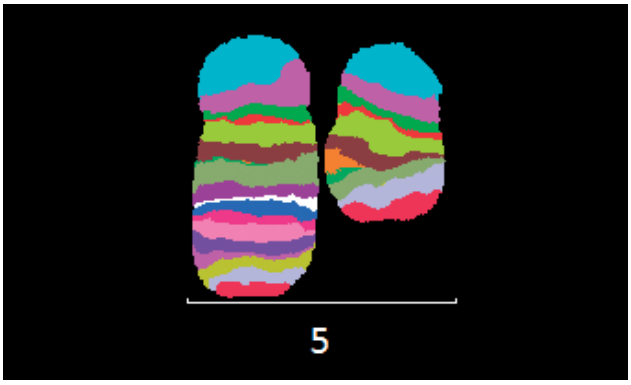

Fig. 1. An illustrative example of large recurrent deletions of the long arm of chromosome 5 in MDS. Figure shows the deletion del(5)(q13.3q33.3) detected by multicolour banding (m-band) in the karyotype of a patient suffering from MDS.

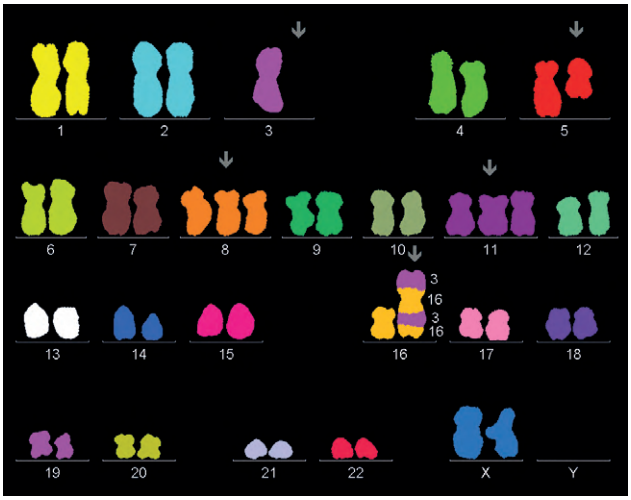

Fig. 2. An illustrative example of complex karyotypes associated with MDS/AML. Figure shows the karyotype 47,XX,-3,del(5)(q13q33),+8,+11,der(16)ins(16;3)(q22;?)t(3;16)(?;p13) that was discovered in an AML patient by multicolour fluorescence *in situ* hybridization (m-FISH). Each chromosome is identified by a specific colour.

II.1. Chromosome 5

Interstitial deletions of 5q (Fig. 1) represent one of the most frequent cytogenetic aberrations in myeloid malignancies and can be found in the majority of all *de novo* MDS cases (about 10–20 %) – either as an isolated abnormality (in 14 % of patients with clonal abnormalities) (Fig. 1), together with one other abnormality (5 %), or as a part of a more complex karyotype (11 %) (Bernasconi et al., 2005; Haase et al., 2007; Fernandez-Mercado et al., 2013). Patients carrying the interstitial deletion of 5q as a single defect are classified as a distinct MDS subcategory (5q- syndrome). Interstitial de-

letions of 5q also appear with a similar frequency in acute myeloid leukaemia (AML) (Fig. 2). Interestingly, no differences in the breakpoints were noticed for these different diseases, which suggests the same origin of the rearrangements (Giagounidis et al., 2004). However, the mechanisms responsible for this specific impairment of the bone marrow in MDS and AML patients are still largely unknown, as discussed later.

The position and size of 5q deletions depend on the study, methods used, and patients involved, but two commonly deleted regions (CDR) were identified: CDR1, which includes chromosomal bands 5q32-5q33.2 (8.5 Mb), and CDR2, which encompasses bands 5q31.2--5q31.3 (1.92 Mb). While deletions of 5q32-q33 were mostly linked with the milder form of MDS (5q- syndrome), the region 5q31 was absent in many MDS patients with a high risk of progression into AML (Le Beau et al., 1993). Boultwood et al. (2010) demonstrated that the majority of all reported interstitial deletions of chromosome 5 fall into one of the three following types: del(5)(q13q31), del(5)(q13q33), and del(5)(q22q35). In most cases, the deletions include all the three or two of these regions.

For the description of other frequent rearrangements and CDR on the remaining chromosomes, the reader is referred to the following original works: chromosome 7 (Stephenson et al., 1995; Le Beau et al., 1996; Bernasconi et al., 2006; Olney and Le Beau, 2007; Haase, 2008; Adema et al., 2013) chromosome 20 (Dewald et al., 1993; Bench et al., 2000; Bernasconi et al., 2006; Douet-Guilbert et al., 2008; Huh et al., 2010; Okada et al., 2012; Bacher et al., 2014); and chromosome 8 (Greenberg et al., 1997; Mishima et al., 1998; Paulsson and Johansson, 2007).

III. Speculations on the mechanism responsible for formation of recurrent and complex chromosomal rearrangements in MDS

If we could better understand MDS at the molecular level, we could more efficiently develop the disease treatment and diagnostics. Nowadays, researchers can scrutinize genomes by modern methods of molecular cytogenetics. Although this methodological progress helped us to reveal some genes and functions of their products involved in MDS pathogenesis (Visconte et al., 2014), we still poorly comprehend how the most frequent aberrations form in MDS, and what is the relationship between single and complex rearrangements.

The existence of recurrent chromosomal aberrations in MDS points to important roles of the affected regions in the disease pathogenesis, which is probably associated with clonal selection of these particular aberrations. In addition, this may also indicate that some chromosomes and their loci are more prone to chromatin damage and rearrangements. As described, deletions of the q-arms of chromosomes 5 (Fig. 1), 7, and 20 markedly predominate in MDS. In addition to deletions, the same chromosomes can often also be affected by other types of aberrations, such as translocations. Multiple rearrangements of these chromosomes are detected in almost all patients with complex genotype changes. On the other hand, some other chromosomes or their parts, e.g. the short arms of chromosome 10, do not participate in MDS-associated chromatin rearrangements at all. Importantly, the most frequent chromosomal abnormalities described above are characteristic not only for MDS, but also for some other blood malignancies (Fig. 2).

These facts suggest that both the formation and clonal selection of recurrent aberrations might be driven by more general cell conditions that are not limited to MDS. Concerning the formation of chromosomal lesions and rearrangements, we propose that a cell type-specific or even individual cell-specific chromatin structure could play a role, potentially in combination with some other still unspecified/unknown factors.

For instance, a chromatin structure that allows fragile sites to appear at specific chromosomal loci may simplify "directed" chromatin damage and result in preferential formation of *sui generis* aberrations that may be consequently selected during clonal evolution of the cancer genome (Wang et al., 2008; Burrow, et al., 2009; Dillon et al., 2010; Monyarch et al., 2013). Indeed, the FRA5C and FRA5G fragile sites were discovered at q31 and q35 loci of chromosome 5, respectively, and put into context with cancer development (Calin et al., 2004; Monyarch et al., 2013).

However, the size and breakpoints of interstitial deletions at chromosome 5, chromosome 7, and chromosome 20 largely vary among patients, although some common chromosomal regions (CDR) are deleted in most cases. Hence, the locus-specific chromatin structure at higher levels of organization, together with global nuclear chromatin architecture, could also be reasonably suspected to participate in the formation of some typical chromosomal aberrations in MDS. Likely, various hierarchical levels of chromatin organization might contribute to an additive or even synergistic effect.

Contrary to the older hypothesis, the cell nucleus is now considered as a highly organized organelle (reviewed in Manuelidis and Chen, 1990; Münkel et al., 1999; Kozubek et al., 2002; Cremer and Cremer, 2010). Many researchers confirmed that genes are distributed non-homogeneously along the genome (Caron et al., 2001) and that the dynamic chromatin structure regulates its function (Kozubek et al., 2002; Goetze et al., 2007). Historically distinguished chromatin domains are euchromatin and heterochromatin, which can be stained with Giemsa on metaphase chromosomes and recognized as the G-light and dark bands, respectively. While heterochromatic G-dark bands contain only about 9.3 genes per megabase (Mb) of DNA and are tightly condensed, gene-rich G-bands (G-light) and very gene-rich sub-telomeric T-bands (in humans) are largely decondensed and estimated to include 20 and 78 genes/Mb, respectively (Bernardi, 1993). Genetically active chromatin and inactive chromatin also differ in their

protein composition. We have recently shown that inactive condensed chromatin, abundant in heterochromatin-binding proteins, is better protected by these proteins from induction of DNA double-strand breaks (DSB) by free radicals coming from water radiolysis (Falk et al., 2008, 2010, 2014). On the other hand, repair of DSB in heterochromatin is more difficult and slower, and requires extensive chromatin decondensation to proceed (Kruhlak et al., 2006; Falk et al., 2007, 2008). This decondensation may locally increase chromatin mobility at the sites of heterochromatic DSB, which is followed by protrusion of these lesions into the nuclear subcompartments of low chromatin density or interchromatin space (Falk et al., 2007). This behaviour may increase the probability of chromatin translocations between originally more distant partner loci (reviewed in Falk et al., 2010).

Genetically active chromosomal regions locate preferentially closer to the nuclear centre, while the inactive ones mostly appear around the nucleolus and nuclear envelope (Cremer and Cremer, 2010). Importantly, the same rules also apply to chromatin organization inside chromosomal territories (Falk et al., 2002; Kozubek et al., 2002; Lukasova et al., 2002) where the centromere and heterochromatic loci usually occupy the envelope-oriented part of the territory, while telomeres and active chromatin "protrude" to its inner part facing the nuclear centre (Falk et al., 2002; Kozubek et al., 2002; Lukasova et al., 2002). This causes functional and structural polarization of genetically active chromosomal territories, such as in chromosomes 17 and 19 (Kozubek et al., 2002; Lukasova et al., 2002), which can potentially introduce some tension in specific chromatin loci.

The polarization is less prominent or absent in territories with only low overall expression, like chromosomes 18 and X (Falk et al., 2002; Kozubek et al., 2002). Therefore, chromosome-specific polarization forces may create chromatin loops that could perhaps contribute to preferential deletions of large chromatin blocks that contain specific CDR regions but arise at variable breakpoints; in contrast, more precise breakage hotspots may be expected if MDS deletions appear due to a simple presence of chromatin fragile sites.

Highly expressed loci, e.g. those containing clusters of co-regulated genes or so called Regions of Increased Gene Expression (RIDGE; Caron et al., 2001), may even protrude outside of their maternal territory, into the interchromatin space (Pombo et al., 1998; Volpi et al., 2000; Branco and Pombo, 2006). Evidently, this phenomenon in general might simplify formation of chromosome breaks at specific loci as well.

The radial distribution of the whole chromosomal territories in interphase nuclei also reflects their overall transcription levels; the active territories preferentially inhabit central concentric shells of the nucleus and *vice versa* (Kozubek et al., 2002; Cremer and Cremer, 2010). The width of radial shells occupied by particular chromosomes is chromosome-specific (Kozubek et al., 2002). The higher-order chromatin structure therefore also determines the probability of mutual chromatin interactions and potentially chromosomal translocations between individual chromosomes (Kozubek et al., 1997; Lukasova et al., 1999; Neves et al., 1999; Falk et al., 2010; Kenter et al., 2013).

The chance that particular loci would be involved in a translocation may further increase with their localization in the outer zone of the territory, characterized by more or less extensive intermingling between chromatin of neighbouring chromosomes (Branco and Pombo, 2006). Although the nuclear positions of specific loci are in general dictated by the location of their maternal chromosome territories, some chromatin loops can protrude even outside the territory, as already discussed. Whether and to what extent the described observations can explain formation of frequent chromosomal aberrations in MDS is under investigation (Falk et al., unpublished).

Advanced MDS are accompanied by very complex chromosomal rearrangements. For instance, Zemanova et al. (2013, 2014) discovered that the true monosomy of chromosome 5, frequently reported in MDS, *de facto* does not exist. Rather, chromosome 5 seems to undergo extensive pulverization followed by translocation of the generated chromatin fragments to the "surrounding" chromosomes (Zemanova et al., 2013, 2014). What triggers such chromosome "explosion" and why it affects only specific chromosomes or chromosomal loci represents an exciting subject of current research. Zemanova et al. (2014) suggest that initial deletion at the long arm of chromosome 5 destabilizes the chromosome, which is consequently easily prone to further damage. However, chromosome fragmentation by chromothripsis has recently been described as a new and probably more common phenomenon in carcinogenesis (Stephens et al., 2011; Forment et al., 2012). Contrary to the currently accepted theory of the multi-step tumour development (Righolt and Mai, 2012; Burrell et al., 2013; Korbel and Campbell, 2013; Pihan, 2013; Zhang et al., 2013), chromothripsis presupposes sudden multiple chromosome rearrangements that can result in complex karyotypes in a single step. What fraction of cancers can be initiated by chromothripsis is under investigation; nevertheless, it is already evident that the mechanism of chromothripsis must also be applicable to other cancer types, not always associated with large deletions. Hence, although chromosomal deletions might decrease the chromosome stability, chromothripsis is probably initiated by a more general process in cancer cells.

A frequent and early event during the tumour genesis is replication stress (RS). RS is a dynamic chain of events that starts from acutely arrested replication forks with fully assembled replisomes. If RS persists, stalled forks are converted into collapsed forks (Lambert and Carr, 2005), specific nucleases cleave problematic DNA, and finally transform collapsed forks into DSBs (Fekairi et al., 2009; Forment et al., 2012). Recently, Toledo et al. (2013) suggested that long-lasting RS causes a replication catastrophe and cell death due to exhaustion of RPA proteins. RPA bind to ssDNA in replication forks and protect them from DNA breakage. Hence, the lack

of these proteins initiates massive and synchronized fragmentation of chromatin loops that are associated in the affected replication factory/factories and may originate from one or more chromosomes. The authors propose that this chromosome destruction mostly brings about complete disintegration of the nucleus, but may also represent a precursor of cancer-related genomic abnormalities. This may happen when DNA, previously "pulverized" by chromothripsis, is erratically reassembled (Stephens et al., 2011; Forment et al., 2012). Nevertheless, various mechanisms of chromothripsis have been put forward, so that further research is necessary to shed more light on the processes by which complex MDS karyotypes are formed.

IV. Conclusion

MDS is associated with various chromosomal aberrations among which interstitial deletions of the q arms of several chromosomes are the most prevalent. The same chromosomes also participate in other types of rearrangements that frequently form very complex MDS karyotypes. Some chromosomal abnormalities typical of MDS are also recurrent in other haematological malignancies. The cause of preferential selection or formation of these specific aberrations is not yet known. We propose that the higher-order chromatin structure, cell type-specific or even individual cell-specific, might represent one of important cellular factors that influence formation of MDS-associated deletions, translocations, and other genomic lesions. Complex MDS karyotypes may potentially arise as a consequence of chromothripsis, which allows formation of complicated multiple rearrangements in a "single" step. However, more experiments are needed to support the above-presented theoretical speculations.

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