EUROPEAN HEMATOLOGY ASSOCIATION

Myelodysplastic syndromes - Section 1

Clonal evolution in myelodysplastic syndromes

Joop H. Jansen

Laboratory Hematology, Radboudumc Nijmegen, The Netherlands

Take-home messages

- Many mutations involved in the pathogenesis of MDS have been identified. These mutations may be used for diagnostic and prognostic purposes and in some selected cases, may contribute to therapeutic decisions.
- Accumulation of genetic mutations leads to the evolution of genetically distinct MDS subclones that may co-exist, or outcompete each other.
- Treatment may create an evolutionary bottleneck and alter the clonal composition of the disease.

Introduction

By the application of whole genome and exome sequencing technology, many genetic mutations underlying the pathogenesis of myelodysplastic syndromes (MDS) have been identified. Most of the recurrently affected genes can be classified in a limited number of biological categories, including transcription factors, signal transduction proteins, epigenetic modifiers, proteins involved in RNA splicing and proteins of the cohesin complex.[1-3] Different patients carry different combinations of mutations, which correlates with the heterogeneity that is seen in MDS. The prognostic value of these mutations is investigated in clinical trials and registries, and in the various risk scoring systems, molecular aberrations are becoming increasingly important. However, the genetic aberrations that are present in a given MDS patient are not stable over time. During the course of the disease, the genetic composition of the MDS cells may evolve due to the acquisition of additional mutations (for an example, see Figure 1). This may lead to altered biological behavior of the MDS cells, including their sensitivity to specific forms of therapy. The assessment of the genetic composition, and the genetic evolutionary patterns will be increasingly important for proper clinical decision making.

Current state of the art

Oncogenesis is a multistep evolutionary process and the successive acquisition of several mutations that confer a selective advantage may result in progression of the disease.[4-6] During life, all cells may acquire genetic mutations, caused by different mechanisms such as irradiation, chemical exposure and DNA-copy errors during cell division. Most of these mutations are non-pathogenic, but when a mutation occurs in a gene that is involved in maturation and growth regulation, this may result in a proliferative advantage and the expansion of a clone of cells carrying this mutation. In addition to this pathogenic mutation (driver mutation), the cells will also carry along any pre-existing non-pathogenic mutation (passenger mutations). The subsequent acquisition of additional mutations may result in a further growth advantage. Recent studies indicate that clonal expansion of hematopoietic cells occurs more often than initially thought.[7-9] In many healthy individuals, expanded clones of hematopoietic cells can be found that carry somatically acquired mutations in genes that have been implicated in hemato-oncological diseases. In most of these cases, only one mutation is found to be present and apparently, these cells do not (yet) carry enough mutations to be completely transformed resulting in clinical symptoms. The incidence of such clonal processes in healthy individuals (termed CHIP, clonal hematopoiesis of indeterminant potential) increases with age, and depending on the sensitivity at which mutations are screened for, above the age of 70 as much as 10% or more of the general population may carry significantly large, clonal hematopoietic populations. When more pathogenic mutations are acquired, the cells may become more malignant. In MDS, mutations in epigenetic regulators including TET2, ASXL1, IDH1/2, EZH2 and DNMT3A are often found, as well as mutations in genes that code for proteins involved in RNA splicing, including SF3B1, SRSF2, U2AF1 and ZRSR2. These mutations are not specific for MDS, but especially the splicing factor mutations are more often found in MDS compared to several other myeloid malignancies. In the case of

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MDS with ring sideroblasts, a very high correlation has been found with the occurrence of SF3B1 mutations. Some mutations are often found together within a certain patient, whereas other mutations are mutually exclusive. Mutations in genes that are involved in the same biological process are not likely to enhance each other's transforming effect; cells that acquire an additional mutation that affects an already activated pathway have no extra growth advantage and are therefore not clonally expanded. In contrast, mutations that affect genes from different biological processes may have an additive or synergistic effect on malignant transformation, and a combination of such mutations may confer a further growth advantage, causing the cells that harbor both types of mutations to clonally expand. In MDS both linear and branching patterns of evolution have been described. Linear evolution is characterized by the successive appearance of dominant clones that overgrow their ancestral clone after the acquisition of additional mutations. Branching evolution is characterized by the emergence of different subclones from one common ancestral clone, leading to the co-existence of related (sub)clones that contain a partially overlapping set of mutations.[10,11] The genetic evolution which may take place over time within a specific patient may result in a change of disease characteristics and progression towards a more aggressive disease, including the development of acute myeloid leukemia. Using exome- and targeted deep-sequencing, clonal evolution can be monitored. The order in which specific mutations are acquired in time differs between patients, but in general, mutations in genes involved in RNA splicing and DNA methylation appear to occur early, whereas mutations in genes involved in chromatin modification and signal transduction tend to occur later. Recent studies demonstrate that therapy can influence clonal evolution by suppressing or selecting particular subclones, while being ineffective towards others.[12-15] For example, treatment with lenalidomide leads to an efficient suppression of malignant cells harbouring a deletion of chromosome 5q. This response may however be lost when an additional *TP53* mutation is acquired and TP53 mutated subclones may emerge dur-

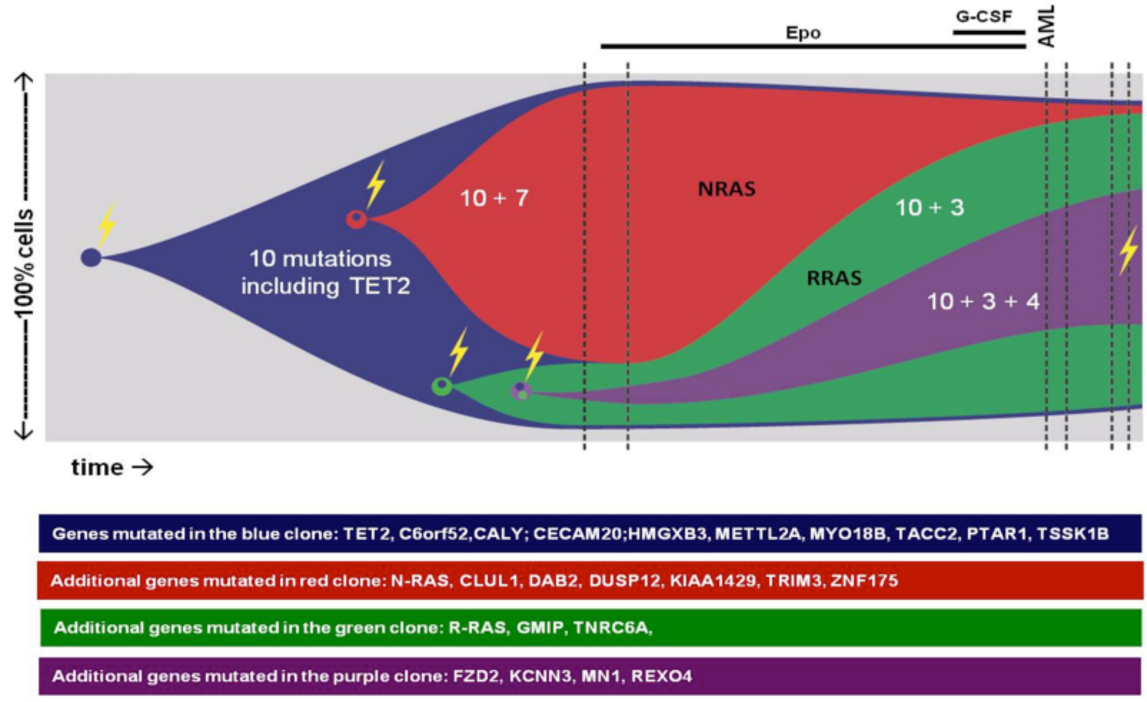

Figure 1. Clonal evolution pattern in a MDS patient over the course of several years. Initially, a clone of cells (blue clone) developed that contained 10 different mutations (passenger plus driver mutations). Subsequently, a branched evolution pattern was observed; from the blue clone, two daughter clones developed that carried an extra 7 (red clone) or 3 (green clone) mutations. Within the green clone another subclone developed (purple) containing yet another 4 mutations. Eventually, the disease progressed into AML. (EPO=erythropoietin, G-CSF= granulocyte colony stimulating factor, AML=acute myeloid leukemia)

ing treatment. In addition to differences between patients, also intra-tumoral differences may be present. Often, genetically different subclones with a different set of mutations are present simultaneously, even before treatment. As the genetic diversity amongst these co-existing subclones may result in a different response to therapy, the genetically complex cases with various subclones may be more difficult to treat. Some subclones may be resistant to one form of therapy, while others are resistant to other treatment modalities, necessitating a multimodal treatment approach. Finally, therapy can create an evolutionary bottleneck, potentially expediting or altering the evolutionary process of the disease. If treatment reduces the hematopoietic compartment but fails to eradicate all the MDS cells from the different subclones, preferential repopulation of the bone marrow may occur by resistant subclones that are able to outcompete the others, including the healthy cells.

Future perspectives

Comprehensive genetic analyses may give insight in subclonal composition and clonal evolution patterns in MDS. Clinically, this information may be used for prognostication at diagnosis, to predict response to specific forms of therapy, to follow and predict disease progression and to monitor the effectiveness of therapy. Sensitivity and specificity of the techniques that are used to simultaneously screen for many different mutations can still be improved. Depending on the sequence context, not all mutations can easily be called reliably when they are present in a small fraction of the cells, and better techniques and bioinformatic algorithms are still under development. Implementation in clinical practice will be enhanced by the decreasing cost of next generation sequencing technology, but most importantly, more extensive studies are still necessary to further establish the true predictive value of the various combinations of mutations, both with and without therapy.

References

1. Bejar R, Stevenson K, Abdel-Wahab O, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med 2011;364:2496-506.
*2. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. Blood 2013;122:3616-27.
*3. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia 2014;28:241-7.

References 2 and 3 describe the genetic landscape in large series of MDS patients.

4. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia.Cell 2012;150:264-78.
5. Walter MJ, Shen D, Ding L, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med 2012;366):1090-8.
6. Klco JM, Spencer DH, Miller CA, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. Cancer Cell 2014;25:379-92.
*7. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med 2014;20:1472-8.
*8. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371:2488-98.
*9. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371:2477-87.

References 7-9 describe clonal hematopoiesis which increases with age.

10. Grove CS, Vassiliou GS. Acute myeloid leukaemia: a paradigm for the clonal evolution of cancer? Dis Mod Mechan 2014;7:941-951.
11. McGranahan N, Swanton C.Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. Cancer Cell 2015;27:15-26.
*12. Jädersten M, Saft L, Smith A, et al. TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. J Clin Oncol. 2011;29:1971-9.

Early paper describing the role of TP53 in disease progression and lenalidomide response.

13. Mossner M, Jann JC, Wittig J, et al. Mutational hierarchies in myelodysplastic syndromes dynamically adapt and evolve upon therapy response and failure. Blood 2016;128:1246-59.
14. Chesnais V, Arcangeli ML, Delette C, et al. Architectural and functional heterogeneity of hematopoietic stem/progenitor cells in non-del(5q) myelodysplastic syndromes. Blood 2017;129:484-96.
15. da Silva-Coelho P, Kroeze LI, Yoshida K, et al. Clonal evolution in myelodysplastic syndromes. Nat Commun 2017;2115099:1-11.