Review Article

Genes and Mechanisms Responsible for Expansion of Acute Myeloid Leukaemia Blasts

(acute myeloid leukaemia / bone marrow / leukaemic stem cells / leukaemic blasts / recurrent gene mutations / mechanisms of malignant transformation)

M. DVOŘÁK, M. DVOŘÁKOVÁ

Department of Cell Differentiation, Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic

Abstract. Acute myeloid leukaemia (AML) is the leading form of fatal acute leukaemia in adults. AML is a heterogeneous disease with respect to responsible mutations and chromosomal abnormalities as well as to their clinicopathological image. In recent years, great progress has been made in techniques allowing detection of genetic changes in both *de novo* AML and in secondary AML induced by other haematological disorders or therapy, and in detection of residual disease after therapy. Accumulated knowledge allowed better understanding of the molecules and mechanisms involved not only in the formation and expansion of a primary leukaemia-founding clone, but also of a temporal order of changes leading to the fully malignant phenotype. The recent knowledge of bone marrow (BM) compartments and interrelations among various BM resident and recruited cell types helps in understanding the AML development. The progress in the techniques and knowledge will result in the development and use of molecularly targeted therapies tailored to individual patient needs.

Received January 17, 2019. Accepted February 4, 2019.

This work was supported by the Ministry of Education of the Czech Republic (Programme NPU I – LO1220).

Corresponding author: Michal Dvořák, Department of Cell Differentiation, Institute of Molecular Genetics of the ASCR, v. v. i., Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: mdvorak@img.cas.cz

Abbreviations: AML – acute myeloid leukaemia, Asx – Additional sex combs, BM – bone marrow, CBF – core binding factor, *CBFB* – CBF β subunit gene, CAR – (CXCL12)-abundant reticular, FL – Fms-like tyrosine kinase 3 ligand, HIFs – hypoxia-inducible factors, HSCs – haematopoietic stem cells, HSPCs – haematopoietic progenitor cells, $LepR^+$ – leptin receptor-expressing, LSCs – leukaemic stem cells, MLL – mixed lineage leukaemia, MSCs – mesenchymal stem cells, SMC – structural maintenance of chromosomes, NADPH – nicotinamide adenine dinucleotide phosphate, TET – Ten-eleven translocation, VEGF – vascular endothelial growth factor, WHO – World Health Organization.

Introduction

The bone marrow (BM) is the site of haematopoiesis in mammals. Billions of cells comprising populations of erythrocytes, thrombocytes, granulocytes, macrophages, and lymphoid cells are generated every day in mammalian bodies to transport oxygen, prevent bleeding, and fight against pathogens and abnormal self-cells. All these blood cells are derived from a rather small population of multipotent stem cells – haematopoietic stem cells (HSCs), which can gradually leave their multipotent state and step by step specialize into myeloid or lymphoid precursors of the above-mentioned cell types. These precursors intensely proliferate to give rise to terminally differentiated cells. HSCs are also endowed with self-renewal potential, ensuring the long-term existence of a pool of stem cells and thus providing a virtually unlimited source of multipotent cells necessary for proliferation and differentiation of their committed progeny. HSCs can also adopt a state of quiescence. Quiescence is a basic property of stem cells. It is the specific non-proliferative G0 state, actively controlled and reversible, in which stem cells can persist as a reserve for a long time. Upon physiological stimuli the idle quiescent stem cells can resume proliferation, differentiate, and thus replenish their progeny to the damaged tissue (Cheung and Rando, 2013).

Bone marrow microenvironment – home of early leukaemic cells

Mammalian HSCs reside in the marrow mainly of long bones, sternum, ribs, and vertebrae and in the bones of the pelvis. BM is a complex compartment consisting, in addition to stem and differentiated haematopoietic cells, of multiple non-haematopoietic cell types, namely, endothelial cells, osteoblasts, osteoclasts and mesenchymal stem cells (MSCs), which further differentiate into adipocytes, chondrocytes, myocytes and fibroblasts, non-myelinating Schwann cells and nerve fibres (Yin and Li, 2006; Birbrair and Frenette, 2016). BM is supplied with oxygen and nutrients by arteries. From arteries

branch arterioles, which further branch into a labyrinth of sinusoids, thin vessels with fenestrated walls that ensure the direct contact of the bone marrow space with the circulation.

It was proposed some forty years ago that HSCs live in and are protected and regulated by other non-haematopoietic cells within specific BM compartments called the niche (Schofield, 1978). The niche was supposed to provide the signals that regulate the degree of proliferation and differentiation of stem cells. The following years confirmed the idea of a stem cell niche and brought extensive information on the niche composition and properties of the stem cell-niche cooperation. It was suggested that the HSC niche is spatially bound to distinct BM structures. Initially, BM osteoblasts lining the endosteal bone surface were identified as HSC niche cells (Nilsson et al., 2001; Calvi et al., 2003).

However, recent observations based chiefly on experiments with genetically modified mice and advanced imaging techniques led to the conclusion that the HSC niche is localized to the abluminal side of the BM sinusoids, in close association with vascular endothelial cells, which are the principal component of blood vessel walls (Kiel et al., 2005; Ding et al., 2012; Ding and Morrison, 2013; Greenbaum et al., 2013; Acar et al., 2015). Sinusoidal vascular endothelial cells accommodate at the abluminal side the specific types of perivascular MSCs that appeared crucial for the regulation and maintenance of HSCs. These are mainly the CXC motif chemokine ligand 12 (CXCL12)-abundant reticular cells (CAR cells) or leptin receptor-expressing ($LepR^+$) cells, nowadays called CAR/$LepR^+$ cells (Ding et al., 2012; Ding and Morrison, 2013). Their projections form the network creating a physical space for HSCs. CAR/$LepR^+$ cells express stem cell factor and CXCL12 cytokines necessary for the self-renewal and maintenance of HSCs. CAR/$LepR^+$ cells can also differentiate into adipocytes and osteocytes. However, their undifferentiated stage, which appears to be dependent on the expression of transcription factor Ebf3 (Seike et al., 2018), is required for their niche functions.

BM-resident non-myelinating Schwann cells as well as nerve fibres were for some time considered candidates for HSC niche cells. Recent observations, however, document only rare contacts of these cells with HSCs (Acar et al., 2015). Similarly, refinement of the experimental techniques led to the conclusion that the endosteal niche, formerly considered as the HSC niche, rather serves as a home for lymphoid progenitors (Ding and Morrison, 2013) and that arterioles likely do not provide the framework for the HSC niche at homeostasis (Acar et al., 2015; Shimoto et al., 2017). Nevertheless, the question of the HSC niche composition and function has not been definitively solved to this day and novel concepts can be expected in the future.

The most frequent mutations in AML

As in other tissues, mutations also accumulate in the BM and immature haematopoietic cells. Upon a particular set(s) of mutations, a stem or progenitor haematopoietic cell acquires tumour-forming potential and leukaemia may develop. AML is one of the deadliest forms of leukaemia. According to the popular definition, AML is a rapidly progressing blood cancer, characterized by an abnormally large and growing amount of immature and non-functional cells belonging to the myeloid lineage of blood cells, which gradually more and more interferes with the normal function of blood and other organs. At the outset of leukaemia clone formation, there is a mutation(s) which predisposes the affected HSC to become a founder of the leukaemic population (Jaiswal et al., 2014; Shlush et al., 2017). These HSCs, upon acquisition of additional specific mutations that get them out of control of normal homeostasis, become leukaemic stem cells (LSC) (Reinisch et al., 2015; Pollyea and Jordan, 2017). Many mutations do not manifest themselves in karyotypic changes, so that approximately 45 % of AML cases display normal karyotype. The studies aimed at identifying mutations in *de novo* adult AML patients found an average of only 13 mutations in the genes of a patient (Cancer Genome Atlas Research et al., 2013), which is a relatively low amount in comparison with solid tumour mutations. For instance, lungs or breast tumours often have several hundreds of mutations.

Mutations in tumour cells are divided into driver and passenger mutations. Driver mutations are decisive for the malignant phenotype as they lend a significant growth advantage to the cell and are recurrently detected in individual cases. Driver mutations in AML comprise both chromosomal rearrangements and point mutations. The identification of driver mutations in AML patients allowed definition of several cytomorphological groups characterized by particular combinations of mutations (Metzeler et al., 2016; Papaemmanuil et al., 2016; Rose et al., 2016). Data concerning specific mutations combined with morphologic, cytochemical, immunophenotypic, and clinical information enabled physicians and researchers to categorize AML cases into eight groups defined by recurrent genetic deviations to which clinicopathological features and prognostic outcomes were assigned. These categories are listed in the World Health Organization (WHO) classification of myeloid neoplasms and leukaemia, whose latest update was published in 2016 (Arber et al., 2016). The French, American, and British (FAB) classification system for AML has been incorporated into the WHO system and brings specific information within the bounds of "AML not otherwise specified" category (Canaani et al., 2017).

AML is often referred to as a heterogeneous disease. This heterogeneity is likely due to the nature of mutations and chromosomal abnormalities that enable a HSC to attain a certain degree of independence in relation to the specific environment of the BM. In these interrelations, the unusual plasticity of LSCs and their progeny is behind the heterogeneous clinicopathological presentations. Here we will discuss the most often occurring driver mutations and their contribution to the ability of

leukaemic cells to overcome the regulatory network of the BM.

Early changes present already in premalignant AML clone(s) comprise mutations mainly in factors imposing large-scale and essentially reversible changes in the genome. Early mutations were found in chromatin topology organizer cohesin, epigenetic regulators such as chromatin modifiers *ASXL1* and *MLL*, and in genes encoding or affecting epigenetic modifiers of DNA such as *DNMT3A*, *TET2*, and *IDH1/2*. Such mutations are likely compatible with the quiescent state of a HSC, and can thus persist after therapy and cause relapsed disease. During further evolution, pre-leukaemic clones can acquire additional mutations in signalling molecules such as *FLT3, KIT*, and *RAS* family members, tumour suppressors (*TP53, WT1*), transcription factors (*RUNX1, GATA2, CEBPA*), and multifunctional organizer *NPM1*. These mutations convert initially premalignant clones into the fully malignant ones.

The cohesin complex is an evolutionarily conserved multi-protein suite, which belongs to the family of the structural maintenance of chromosomes (SMC) protein complexes. It brings together distant DNA domains, thereby participating in DNA replication, repair and transcription regulation. It also controls cohesion and accurate separation of sister chromatids during cell division. The core of this complex is encoded by *STAG1/2, RAD21, SMC3* and *SMC1A* genes (Villa-Hernandez and Bermejo, 2018)). Missense and frame-shift mutations in cohesin complex genes are among the recurrent mutations in AML (Cancer Genome Atlas Research et al., 2013; Thol et al., 2014; Thota et al., 2014; Fisher et al., 2017). The known cohesin mutations result in enhanced self-renewal potential of HSCs and haematopoietic progenitor cells (HSPCs). The precise mechanism through which cohesin mutants increase self-renewal is not known. For the fully developed leukaemic phenotype, cohesin complex mutations likely cooperate with other mutations (Duployez et al., 2016).

The Additional sex combs (*Asx*) gene of Drosophila is a regulator of homeotic genes. Mammalian homologues – Additional sex combs-like *Asxl1, Asxl2,* and *Asxl3* genes – are expressed as multiple transcripts at varying levels. *Asxl1* regulates transcription through disruption of chromatin. Mutations of *ASXL1* are frequently found in clinicopathological variants of AML with poor prognosis (Gelsi-Boyer et al., 2012). Specific *ASXL1* mutants were shown to affect chromatin structure and cause changes in the expression of genes controlling self-renewal and differentiation of HSCs (Yang et al., 2018).

The mixed lineage leukaemia (*MLL*) gene is located in the human genome at 11q23. The gene encodes lysine-specific methyl transferase 2A (*KMT2A*), which adds three methyl groups to lysine 4 of histone H3 (H3K4me3). This modification regulates accessibility of genes for transcription. In AML, the *MLL* gene is often found in chromosome translocations that result in in-frame fusions of *MLL* with other genes – e.g., *AF4, AF9, ENL, AF10* (Rubnitz et al., 1996; Meyer et al., 2009). The most prevalent fusion partners, *AF4* and *AF9*, belong to the super elongation complex that regulates the transcriptional elongation checkpoint stage of transcription (Luo et al., 2012). Incorrect regulation of an elongation checkpoint may directly relate to cancer pathogenesis. Indeed, these MLL fusion proteins were found to cooperate with other factors to deregulate the *RUNX1* gene programme in 11q23 AML (Prange et al., 2017).

DNA methylation is a key epigenetic mechanism controlling gene expression, and changes in the methylation pattern can radically change behaviour of a cell. Therefore, also mutations in enzymes directly engaged in DNA methylation/demethylation can contribute to malignant transformation of cells. The so far accumulated data indicate that almost one half of patients with AML show recurrent mutations in genes that control methylation of genomic DNA (Ley et al., 2010). DNA methyltransferases such as DNMT3A and DNMT3B create the *de novo* methylation pattern on DNA during gametogenesis and embryogenesis (Okano et al., 1999). These enzymes convert cytosine to 5-methylcytosine and in this way create or eliminate binding sites for regulatory proteins in regulatory DNA sequences (Jones, 2012). *DNMT3A* mutations are associated with poor outcome of *de novo* AML (Ley et al., 2010). Different expression levels of the DNMT3A variants affect proliferation of myeloid cells and are also implicated in the pathophysiology of AML (Lin et al., 2017).

On the other hand, the Ten-eleven translocation (TET) proteins (Fe^{2+}- and 2-oxoglutarate-dependent dioxygenases) catalyse the initial step in 5mC demethylation in DNA via oxidation of the methyl group on cytosine to 5-hydroxymethylcytosine (5hmC) and to other cytosine derivatives (Iyer et al., 2009; Tahiliani et al., 2009; Ito et al., 2011; Tan and Shi, 2012; Pastor et al., 2013). As 5hmC alone appears to be a significant epigenetic mark, TET proteins are also involved in epigenetic regulation of gene expression. The loss of the *Tet2* gene in mice led to increased repopulation capacity of haematopoietic stem cells and a differentiation bias toward the myeloid lineage (Li et al., 2011). Similarly, loss-of-function somatic mutations of the human *TET2* gene cause enhanced self-renewal (Moran-Crusio et al., 2011) and confer a myeloid bias on HSCs (Cimmino et al., 2017; Buscarlet et al., 2018). In accordance with that, mutations causing the loss of *TET2* result in hyper-mutagenicity of haematopoietic stem/progenitor cells and can significantly participate in the pathogenesis of haematological malignancies (Pan et al., 2017).

Metabolic enzymes isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) generate reduced nicotinamide adenine dinucleotide phosphate (NADPH) from $NADP^+$ through oxidative decarboxylation of isocitrate to α-ketoglutarate outside of the Krebs cycle. IDH enzymes play an important role in the cellular defence against oxidative stress, and cells with low levels of IDH are sensitive to oxidative damage (Lee et al., 2002). Specific

mutations in IDH1 and IDH2, which were found in several tumour types including AML, result in the ability of mutant IDH enzymes to synthesize D-2-hydroxyglutarate. Accumulated oncometabolite D-2-hydroxyglutarate inhibits α-ketoglutarate-dependent histone and DNA demethylases. This results in epigenetic dysregulation due to hypermethylation of DNA and histones, which ends up blocking differentiation of the affected immature cell (Rakheja et al., 2012; Medeiros et al., 2017).

Recurrent chromosomal translocations t(8;21)/(t(8;21)(q22;q22)) (Rowley, 1973), and inv(16) (inv(16)(p13q22)/t(16;16)(p13;q22))(Arthur and Bloomfield, 1983; Le Beau et al., 1983) are behind the core binding factor (CBF)-acute myeloid leukaemia CBF-AML. CBF is a multi-subunit transcription factor complex that regulates genes controlling normal haematopoiesis (Speck and Gilliland, 2002). In t(8;21), the 5´-end of the *CBFα* subunit gene, *RUNX1* (*AML1*), is fused to the 3´-end of the *RUNX1T1* (*ETO*) gene, and the resulting chimeric fusion protein blocks haematopoietic differentiation and supports maintenance of the stem cell phenotype (Nimer and Moore, 2004). In inv(16) translocation, the CBF β subunit (*CBFB)* gene is fused to the smooth muscle myosin heavy chain gene *MYH11* (Liu et al., 1993). The resulting fusion protein binds DNA in a RUNX1-dependent manner and is possibly engaged in the maintenance of stem cell phenotype of the affected cells (Mandoli et al., 2014). It appears, however, that translocations affecting CBFs per se are not sufficient to induce high-grade AML and that cooperating mutations, such as mutations in genes activating tyrosine kinase signalling (*KIT*, *N/KRAS*, and *FLT3*) or affecting members of the cohesin complex, are also required (Goemans et al., 2005; Qin et al., 2014; Duployez et al., 2016).

The GATA transcription factor family is of crucial importance for haematopoiesis (Briegel et al., 1996; Katsumura et al., 2017). GATA2 is indispensable to the proliferation and maintenance of haematopoietic stem and progenitor cells. Various monoallelic *GATA2* mutations have been present in AML patients. In acute cases, mutations in the zinc-finger region of GATA2 are found in combination with mutation of other genes usually involved in AML (Vicente et al., 2012; Collin et al., 2015). However, the detailed knowledge of the GATA2 role in AML is missing yet.

RUNX1, a homologue of the Drosophila runt segmentation gene, is a transcription factor that is in vertebrates necessary for definitive haematopoiesis. Its function is dependent on cooperation with other factors in complexes exerting both activation and repression function within chromatin (Bonifer et al., 2017). *RUNX1* mutations, both translocations and point mutations, resulting mainly in inactive or dominant negative *RUNX* alleles, have been found in acute myeloid leukaemia, acute lymphoblastic leukaemia, and chronic myelomonocytic leukaemia and *RUNX1* has been recognized as one of key genes in these neoplasias (Sood et al., 2017). Inactivation mutations appear to be required for growth and survival of some leukaemic cells. Changes in the cell metabolism caused by inactivated *RUNX1* result in relative stress resistance, which allows the affected HSPCs to expand and overcome normal HSPCs in the bone marrow (Cai et al., 2015). On the other hand, functional *RUNX* alleles support the growth of leukaemic cells (Goyama et al., 2013; Wilkinson et al., 2013). Thus, it is likely a delicate balance between wild-type and mutant *RUNX* alleles that provide the affected immature myeloid cell with the growth advantage necessary to avoid BM microenvironment regulation and emerge as a leukaemic clone (population).

Translocation t(15;17) found in acute promyelocytic leukaemia cases generates *PML-RARA* and *RARA-PML* fusion genes and results in the protein with transcriptional regulator activity fused from promyelocytic leukaemia and retinoic acid receptor-α proteins (Larson et al., 1984). The fusion protein blocks differentiation and activates proliferation of immature blasts.

The C/EBP α transcription factor, the founding member of the C/EBP family of basic region leucine zipper factors, binds to the CCAAT motif in DNA regulatory sequences (Graves et al., 1986). C/EBPα plays an important role in tissue homeostasis (Wang et al., 1995; Flodby et al., 1996; Zhang et al., 1997). Moreover, C/EBPα is a key factor controlling maturation of the myeloid lineage. It significantly contributes to the formation of granulocytes. The absence of *CEBPA* in mice results in enhanced repopulation of HSCs and accumulation of myeloblasts in the bone marrow at the expense of granulocytes (Zhang et al., 2004). It is evident that inactivation of *CEBPA* in a stem cell might contribute to myeloid leukaemia. Indeed, inherited germ-line mutations or acquired inactivation of *CEBPA* strongly enhance the susceptibility of affected individuals to myeloid leukaemia. Evidence has accumulated that also in a variety of solid tumours CEBPA displays a tumour suppressor role (Lourenco and Coffer, 2017).

Mutants of the splicing factor gene *U2AF1* frequently occur in myeloid malignancies including AML with complex morphology (e.g., myeloid, erythroid, megakaryocytic dysplasia). The S34F U2AF1 mutant induces splicing alteration of genes often mutated in AML (Ohgami et al., 2015). Experiments with a S34F U2AF1 knock-in allele affected haematopoiesis at several levels, but did not reveal the detailed mechanism of U2AF1 mutant involvement in leukaemia development (Fei et al., 2018).

Nucleophosmin (NPM1) is a nucleolar phosphoprotein (Yung and Chan, 1987), a member of the nucleophosmin/nucleoplasmin family, which can be found in all metazoans (Eirin-Lopez et al., 2006) and displays the activity of a molecular chaperone (Szebeni and Olson, 1999). It is engaged in several cellular processes including chromatin remodelling, centrosome duplication and closely related genome stability, mRNA transport, ribosome biogenesis and apoptosis. *NPM1* has been implicated both in proliferation and growth-suppression pathways. Abnormal *NPM1* expression or localization correlates with uncontrolled cell growth/enhanced self-

renewal of HSPCs and cellular transformation in haematopoietic and solid tumours (Box et al., 2016).

Approximately 50-60 % of patients with normal karyotype-AML carry *NPM1* mutations and are characterized by cytoplasmic dislocation of the NPM1 protein. The cytoplasmic localization appears to be crucial to the differentiation block of leukaemic cells (Brunetti et al., 2018).

The FLT3 tyrosine kinase receptor is the essential differentiation and survival factor of dendritic cells. It is also expressed in other haematopoietic cells, where in cooperation with its cognate ligand it activates proliferation and colony formation of normal progenitor hematopoietic cells as well as proliferation of various leukaemia cell lines (Drexler, 1996). Binding of its ligand (FL) induces FLT3 auto-phosphorylation, which generates binding sites for downstream signal transduction molecules and activation of several signal transduction molecules including kinases of the SRC family and subsequently RAS/ERK pathways. Specific *FLT3* mutations, including internal tandem duplication (ITD) of the juxtamembrane FLT3 domain (Nakao et al., 1996), result in the constitutively active FLT3 receptor and permanent activation of downstream pathways resulting in deregulated growth of mutant cells. Oncogenic constitutively active FLT3 mutants, found in approx. 30 % of AML patients, drive proliferation of AML cells and their appearance correlates with poor prognosis (Cauchy et al., 2015; Hirade et al., 2016). Other FLT3 ITD mutations found in the kinase domain (Breitenbuecher et al., 2009) similarly support expansion of AML clones (Marhall et al., 2018).

Proto-oncogene *EVI1* encodes a transcription factor necessary for the maintenance of HSCs (Goyama et al., 2008). Its overexpression has been observed in high-risk AML (Lugthart et al., 2008). It has been reported that *EVI1* is activated by chromosomal rearrangements in AML patients (Morishita et al., 1992; Suzukawa et al., 1994) by both translocation t(3;3)(q21;q26) and inversion inv(3)(q21;q26). Further research revealed that in inv(3)(q21;q26), the *EVI* coding sequence gets under the influence of the GATA2 enhancer. This arrangement was shown to be required for leukaemia formation (Yamazaki et al., 2014). In a group of AML patients, the translocation/inversion-activated *EVI1* was found to cooperate with mutations in *KRAS, NRAS, NF1* and *RUNX1* (Haferlach et al., 2011).

SALL4, a human homologue of Drosophila *spalt* gene, is a transcription factor found constitutively expressed in blasts of some AML patients. Experiments with transgenic mice revealed that constitutive expression of *SALL4* induces AML in experimental animals (Ma et al., 2006).

AML with complex karyotype is often associated with mutations and deletions of *TP53. TP53* is a well-known tumour suppressor gene. It encodes several transcripts. The main form has the properties of a transcription regulator. *TP53* is essential for the control of genome stability, participates in DNA repair and control of cell cycle progression. It can also activate apoptosis of a damaged cell. Thus, the loss of the *TP53* gene very often results in genome destabilization and malignant transformation. In AML, *TP53* inactivation or deletion is always associated with poor prognosis (Fenaux et al., 1992; Haferlach et al., 2008; Bowen et al., 2009; Rucker et al., 2012).

In addition to *TP53*, the Wilms tumour 1 (*WT1*) tumour suppressor (Haber and Buckler, 1992) is also mutated in AML cases (Krauth et al., 2015; Rampal et al., 2014; Sardina and Graf, 2015; Wang et al., 2015). Its mutation was critical for AML relapse following stem cell transplantation (Vosberg et al., 2018).

Another important regulation of haematopoiesis involves small regulatory RNAs. microRNAs are a class of small non-coding RNAs consisting of 19–24 nucleotides. They participate in a variety of biological processes, including cell proliferation, differentiation and apoptosis (Bartel, 2004). They display a specific expression pattern in tumours and are also used, with certain reservations, in classification of tumours (Lu et al., 2005; Peng et al., 2009). MiR135a, for example, was found to function as a tumour suppressor in AML by targeting HOXA10. In AML patients, downregulation of miR135a was linked to poor prognosis. AML cells with downregulated miR135a displayed enhanced proliferation capacity (Xu and Wen, 2018).

AML cells modulate the BM microenvironment

A growing population of leukaemic cells disrupt normal BM homeostasis in several ways. Normal HSPCs and AML cells compete for the same functional niche (Glait-Santar et al., 2015). Due to that, the growing population of leukaemia stem cells can displace normal HSPCs and weaken normal haematopoiesis. However, leukaemic cells affect all other BM components as well and induce deep changes in the BM microenvironment also through their metabolic products, e.g., lactate. High production of lactate by cancer cells results from their reprograming of metabolism, privileging glycolysis over oxidative phosphorylation (Warburg, 1956). AML cells, similarly to many other tumour cells, preferentially exploit glycolysis for energy production and generate lactate. This lactic acidosis accompanies some leukaemia cases and always means poor prognosis (Sillos et al., 2001; Friedenberg et al., 2007; De Raes et al., 2012; Brault et al., 2018). The metabolic changes in tumour cells resulting in lactate production and acidification of the microenvironment are critical for tumorigenesis and support further tumour progression (Kraut and Madias, 2014). For instance, lactic acid produced by tumour cells acts as a signal molecule that induces vascular endothelial growth factor (VEGF) expression and M2-like polarization of tumour-associated macrophages (Colegio et al., 2014).

Hypoxia within the tumour microenvironment, caused by the enhanced oxygen consumption of proliferating tumour cells, has been found to be an important factor in tumour progression. Hypoxia activates expression of

hypoxia-inducible factors (HIFs), which mediate metabolic switches in cells exposed to hypoxic conditions. In tumour cells, they can activate self-renewal, survival and anti-apoptotic pathways. Hypoxia in the BM was also proposed to support leukaemia progression (Mimeault and Batra, 2013; Tong et al., 2018). However, the impact of hypoxia on AML within naturally low-oxygen BM has been challenged (Rieger and Fiegl, 2016).

Nevertheless, the abnormal ambient conditions created by LSC and its progeny can change the expression pattern of normal haematopoiesis-supporting niche cells. For instance, the affected (CXCL)12-abundant reticular (CAR) cells, a population of mesenchymal stem cells producing the chemokine ligand (CXCL)12, engaged in the cross-talk between HSCs and niche cells, can support leukaemogenesis at the expense of regular haematopoiesis (Colmone et al., 2008; Kim et al., 2015). Mesenchymal stem cells in AML were found to exhibit altered gene expression profiles, an aberrant phenotype, and abnormal functional properties. These changes may contribute to the "reprogramming" of the stem cell niche into a leukaemia-permissive microenvironment (Pleyer et al., 2016) in which tumour cells are protected from being restricted or eliminated by the resident and infiltrating cells. Changes in the BM microenvironment caused by leukaemic cells may also result in changes in the expression profile of endothelial cells and abnormalities in vascular architecture and function (Passaro et al., 2017). The remodelled vasculature in endosteal regions have reduced capacity to support non-leukemic HSCs. This insufficiency impairs normal haematopoiesis (Duarte et al., 2018).

Leukaemic cells can disturb the BM microenvironment not only by metabolites and other soluble factors, but also by liberated particles. Some of them can cause direct destruction of the microenvironment. It was observed that blasts of experimental monoblastic leukaemia destroy the BM structures and kill stromal cells. The *in vitro* experiments showed that the cytotoxic agent released by leukaemic cells is the fragmented DNA derived from their genome and occurring in nucleosome-like complexes. This DNA entered nuclei of BM or other cells, where it induced changes similar to double-strand break-inducing agents. There was a correlation between large amounts of acquired DNA and death of recipient cells. Moreover, the DNA integrated into chromosomal DNA of recipient cells (Dvorakova et al., 2013). Primary human AML cells also released fragmented DNA that penetrated the nuclei of other cells both *in vitro* and *in vivo*. It was suggested that DNA fragments released from leukaemic and also perhaps other types of tumour cells can activate DNA repair mechanisms or death in recipient cells of a tumour microenvironment, depending on the amount of the acquired DNA. This can impair DNA stability and viability of tumour stromal cells, undermine homeostatic capacity of the tumour microenvironment and facilitate tumour progression (Dvorakova et al., 2013).

It was also shown that AML blasts remodel the BM niche into a leukaemia growth-permissive and normal haematopoiesis-suppressive microenvironment through exosome secretion (Kumar et al., 2018).

AML cells dissipate the immune response

During the initial phases of tumour development, clones of transformed cells are selected under the pressure of homeostatic mechanisms of their microenvironment. Clones insensitive to these mechanisms overgrow and can acquire additional "advantageous" attributes. The resulting established clones of tumour cells literally teem with abilities to evade control mechanisms, among them immune control. They were found e.g. to re-program the microenvironment to suppress the infiltration and function of immune cells (Garrido and Algarra, 2001; Lardner, 2001; Altieri, 2003; Wang and El-Deiry, 2003; Zou, 2005; Godin-Ethier et al., 2011; Leone et al., 2013; Schlesinger and Bendas, 2015). Many of these mechanisms pertain to cells of myeloid leukaemia and are considered in connection with a therapy. Leukaemic cells and the ambience they generate were found to induce e.g. T-cell dysregulation/dysfunction, immune tolerance, T-cell apoptosis, M2-like polarization of tumour-associated macrophages, recruitment of regulatory T-cells, and impairment of natural killer cell function (Lion et al., 2012; Dolen and Esendagli, 2013; Yao and Chen, 2013; Colegio et al., 2014; Riether et al., 2015; Austin et al., 2016; Kursunel and Esendagli, 2017; Masarova et al., 2017; Acheampong et al., 2018; Ramapriyan et al., 2018; Wang et al., 2018; Zhu et al., 2018).

Conclusions

It has often been observed that AML therapy with current anti-cancer drugs is largely characterized by rapid clearance of peripheral blasts, but only marginal reduction of BM blasts. The BM microenvironment appears to provide resident blasts with significant protection (Weisberg et al., 2008; Wilson et al., 2012; Yang et al., 2014; Long et al., 2017). Thus, in order to improve the outcomes of AML therapy, it is necessary to better understand the properties of LSCs and their communication with BM stroma.

The properties of LSCs can be summarized as follows:

- AML stem cells develop either from a normal stem cell biased towards the myeloid lineage or from a normal committed myeloid progenitor, provided the mutations reactivate in it the self-renewal programme of stem cells (Krivtsov et al., 2006).
- Driver mutations do not seem to impair LSC quiescence. The ability of an LSC to temporize in a quiescent state may be responsible for the often observed relapse of AML following treatment by drugs interfering with DNA synthesis of proliferating cells. A quiescent state would efficiently make LSCs resistant to such drugs and would provide time for LSCs to acquire further mutation(s).

– Mutations do not impair the self-renewal potential of an LSC. This is critical to the long-term production of the leukaemic progeny.
– LSC and its progeny are able to overcome the suppressive action of the stroma but still enjoy its protective power.
– LSC and its progeny are able to evade immune control, although many of them may synthesize unusual proteins.
– LSCs display a distinct metabolic profile.

As the majority of LSC properties overlap with those of normal HSCs, an improved strategy of anti AML therapy should focus on rescue of the immune control (targeting immune checkpoints in T cells) and on specific metabolic requirements of LSCs. These approaches are ideally combined with the detailed knowledge of a patient´s haematopoietic stem/progenitor cell mutation status. This knowledge, along with the expected successful efforts to find new therapeutics interfering very specifically only with abnormal LSC pathways, might form the background of future AML therapy.

Acknowledgements

The authors thank Petr Bartůněk for inspiration, consultations and long-term support. The authors are indebted to Trevor Epp for critical reading of the manuscript.

Disclosure statements

The authors declare that there are no competing financial interests.

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