Recent advances in bone marrow biopsy pathology

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Abstract The 2010 has seen steady advances in bone marrow biopsy (BMB) pathology. The following publications are a personal selection of publications that advance the understanding of the pathobiology of the bone marrow and the interpretation of the BMB.

Keywords Bone marrow · Biopsy · Pathology · Myelodysplastic syndromes · Acute myeloid · Leukemia · Lymphoma · Myeloma

Stem cells and myelodysplastic syndromes (MDS)

Hemopoietic stem cells (HSCs) reside within a specified area of the bone marrow (BM) cavity called a “niche” that modulates HSC quiescence, proliferation, differentiation, and migration [1]. Previous studies have identified the endosteal BM region as the site for the HSC niche and demonstrated that hemopoietic stem and progenitor populations (HSPCs, LSK), isolated from different BM regions, exhibit significantly different hemopoietic potential [1]. In a new study, Grassinger et al [1] analyzed subpopulations of LSK cells isolated from different regions of the BM and showed that CD150+ CD48- LSK HSCs within the endosteal BM region have superior proliferative capacity and homing efficiency compared with CD150+ CD48- LSK HSCs isolated from the central BM. A subset of CD150+ CD48+ LSK progenitor cells, previously

defined as B-lymphoid-primed hemopoietic cells, were shown to be capable of multilineage reconstitution; however, only when isolated from the endosteal region. Evidence for an unrecognized role of CD48 in HSC homing was put forward. The authors concluded that there is strong evidence that highly purified HSCs show functional differences depending on their origin within the BM, and that the most primitive HSCs reside within the endosteal BM region. It may be possible to study this phenomenon in the bone marrow biopsy (BMB), and the geographical distribution of HSCs may have practical relevance in post-transplant biopsies and in early transformation in MDS.

A subgroup of MDS, the so-called sideroblastic MDS, shows ring sideroblasts in the bone marrow aspirate that represent mitochondrial iron accumulation. Patients with sideroblastic MDS also develop systemic iron overload and generally have a low-risk MDS. [2] Cuijpers et al [2] reviewed the question of iron overload in sideroblastic MDS. Low levels of the iron-regulatory peptide hepcidin have been found to contribute to body iron overload in β -thalassaemia patients. A similar mechanism may account for systemic iron accumulation in sideroblastic MDS. Mitochondrial iron accumulation is observed in several subtypes of MDS, and predominantly, in refractory anemia with ring sideroblasts, and the presence of ring sideroblasts is also the diagnostic hallmark in patients with inherited forms of sideroblastic anemia. The authors conclude that further insights into the affected pathways in inherited sideroblastic anemia may lead to a better comprehension of the pathogenesis of mitochondrial iron accumulation in MDS patients and lead to novel treatment strategies to reduce both systemic and mitochondrial iron overload, resulting in less tissue damage and more effective erythropoiesis.

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Myeloproliferative neoplasms (MPNs)

Vandyke et al [3] reviewed an unanticipated side effect of imatinib mesylate therapy, hypophosphatemia, and hypocalcemia, which in part, has been attributed to drug-mediated changes to renal and gastrointestinal handling of phosphate and calcium. However, emerging data suggest that imatinib also targets cells of the skeleton, stimulating the retention and sequestration of calcium and phosphate to bone, leading to decreased circulating levels of these minerals. The review highlights current understanding of the mechanisms surrounding the effects of imatinib on the skeleton. Recent studies have suggested that imatinib has direct effects on bone-resorbing osteoclasts and bone-forming osteoblasts through inhibition of c-fms, c-kit, carbonic anhydrase II, and the platelet-derived growth factor receptor; imatinib may thus be clinically useful as an inhibitor of osteoclast activity in diseases associated with excessive bone resorption, such as cancer-induced bone loss. Assessment of bone changes in BMBs from patients treated with imatinib and more recent second-generation tyrosine kinase inhibitors, including dasatinib, nilotinib, and bosutinib is likely to be worthwhile.

Around 40%–50% of patients with chronic myeloid leukemia (CML), who achieve a stable complete molecular response (CMR; undetectable breakpoint cluster region-Abelson leukemia gene human homolog 1 (BCR-ABL1) mRNA) on imatinib, can stop therapy and remain in CMR, at least for several years [4]. Two possible explanations for this observation are: (1) CML has been eradicated, or (2) residual leukemic cells fail to proliferate despite the absence of ongoing kinase inhibition. Ross et al used a highly sensitive patient-specific nested quantitative PCR to look for evidence of genomic BCR-ABL1 DNA in patients who sustained CMR after stopping imatinib therapy. Seven of eight patients who sustained CMR off therapy had BCR-ABL1 DNA detected at least once after stopping imatinib, but none relapsed (follow-up 12–41 months). BCR-ABL1 DNA levels increased in all of the ten patients who lost CMR soon after imatinib cessation, whereas serial testing of patients in sustained CMR showed a stable level of BCR-ABL1 DNA. The authors conclude that their more sensitive assay for BCR-ABL1 provides evidence that even patients who maintain a CMR after stopping imatinib may harbor residual leukemia and that a search for intrinsic or extrinsic (for example, immunological) causes for this drug-free leukemic suppression is now indicated.

MPNs originate from genetically transformed hematopoietic stem cells that retain the capacity for multilineage differentiation and effective myelopoiesis [5]. Tefferi reviewed the current state of understanding of mutations involving Janus kinase 2 (JAK2), Myeloproliferative Leukemia Virus (MPL), TET oncogene family member 2

(TET2), Additional Sex Combs-Like 1 (ASXL1), Casitas B-lineage lymphoma proto-oncogene (CBL), Isocitrate dehydrogenase (IDH) and IKAROS family zinc finger 1 (IKZF1) which have been described in BCR-ABL1-negative MPNs. None of these mutations are MPN specific, display mutual exclusivity, or can be traced back to a common ancestral clone. JAK2 and MPL mutations appear to exert a phenotype-modifying effect and are distinctly associated with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF); the corresponding mutational frequencies are 99%, 55%, and 65% for JAK2 and 0%, 3%, and 10% for MPL mutations. The incidence of TET2, ASXL1, CBL, IDH or IKZF1 mutations in these disorders ranges from 0% to 17%; these latter mutations are more common in chronic (TET2, ASXL1, CBL) or juvenile (CBL) myelomonocytic leukemias, mastocytosis (TET2), MDS (TET2, ASXL1), and secondary acute myeloid leukemia (AML), including blast-phase MPN (IDH, ASXL1, IKZF1). The functional consequences of MPN-associated mutations include unregulated JAK-STAT (Janus kinase/signal transducer and activator of transcription) signaling, epigenetic modulation of transcription, and abnormal accumulation of oncoproteins. Whether and how these abnormalities contribute to disease initiation, clonal evolution or blastic transformation is unclear [5].

AML may follow a JAK2-positive MPN, although the mechanisms of disease evolution, often involving loss of mutant JAK2, remain obscure [6]. Beer et al [6] studied 16 patients with JAK2-mutant (7 of 16) or JAK2 wild-type (9 of 16) AML after a JAK2-mutant MPN. PMF or myelofibrotic transformation preceded all 7 JAK2-mutant but only 1 of 9 JAK2 wild-type AMLs, implying that JAK2-mutant AML is preceded by mutation(s) that give rise to a “myelofibrosis” phenotype. Loss of the JAK2 mutation by mitotic recombination, gene conversion, or deletion was excluded in all wild-type AMLs. A search for additional mutations identified alterations of RUNX1, WT1, TP53, CBL, NRAS, and TET2, without significant differences between JAK2-mutant and wild-type leukemias. In four patients, mutations in TP53, CBL, or TET2 were present in JAK2 wild-type leukemic blasts but absent from the JAK2-mutant MPN. By contrast in a chronic-phase patient, clones harboring mutations in JAK2 or MPL represented the progeny of a shared TET2-mutant ancestral clone. The authors conclude that different pathogenetic mechanisms underlie transformation to JAK2 wild-type and JAK2-mutant AML, and that TET2 mutations may be present in a clone distinct from that harboring a JAK2 mutation, emphasizing the clonal heterogeneity of the MPNs.

Cytogenetic information has established prognostic value in AML and MDS and is incorporated into formal prognostic systems [7]. To investigate the prognostic value

of cytogenetic findings in PMF, Hussein et al [7] investigated 200 patients with PMF and found abnormal karyotype at diagnosis in 42%. Specific cytogenetic abnormalities with more than or equal to five informative cases were identified, and the rest grouped separately as “other abnormalities.” Four cytogenetic risk groups were considered: (1) favorable (sole +9, 20q-, or 13q-), (2) normal, (3) unfavorable (complex karyotype or sole +8), and (4) “other abnormalities.” The respective median survivals for these four groups were 113, 80, 46, and 34 months. Multivariable analysis confirmed the International Prognostic Scoring System (IPSS)–independent prognostic value of both four-way and two-way (ie, favorable/normal vs unfavorable/other abnormalities). The authors conclude that the ability to dissect a specific IPSS category prognostically has major therapeutic implications.

Mutations in the thrombopoietin gene (*THPO*) and in the thrombopoietin receptor gene (*MPL*) can cause hereditary thrombocytosis (HT). HT, like ET, can present with complications, such as thrombosis or bleeding, but progression to malignant disease has not been described for HT. Posthuma et al [8] reported malignant progression to AML in a patient and the development of myelofibrosis in her sister, a progression not previously reported. This report suggests that the *THPO* mutation, which results in elevated TPO serum levels, may increase the risk of progression to myelofibrosis and leukemia, and that careful follow-up of patients with HT is warranted.

Acute myeloid leukemia (AML)

The World Health Organization [9] category “AML with MDS-related changes” (AML-MRC) is separated from “AML not otherwise specified” (AML-NOS) by presence of multilineage dysplasia (MLD), MDS-related cytogenetics, or history of MDS or MDS/MPN. Miesner et al [10] analyzed 408 adult patients categorized as AML-MRC or AML-NOS. Three-year event-free survival (EFS; median, 13.8 vs 16.0 months) and 3-year overall survival (OS; 45.8% vs 53.9%) did not differ significantly between patients with MLD vs those without. However, MLD correlated with preexisting MDS and MDS-related cytogenetics. Patients with MLD as sole AML-MRC criterion (AML-MLD-sole) had less frequently *FLT3* internal tandem duplication and lower median age than AML-NOS. In contrast, patients with AML-NOS combined with AML-MLD-sole had better 3-year EFS (16.9 vs 10.7 months) and 3-year OS (55.8% vs 32.5%) than patients with history of MDS or MDS/MPN or MDS-related cytogenetics. Gene expression analysis showed distinct clusters for AML-MLD-sole combined with AML-NOS versus AML with MDS-related cytogenetics or MDS history. Thus, MLD alone showed no independent clinical

effect, whereas cytogenetics and MDS history were prognostically relevant. The authors [10] suggest that separate categories AML with MRC solely based on MLD and AML-NOS have no fundamental underlying biologic basis. Instead, they suggest restricting the AML-MRC category to cases with a history of MDS or MDS/MPN or MDS-related cytogenetics completely irrespective of MLD findings, whereas cases solely being defined by morphologic criteria of MLD should be combined with the AML-NOS category. As a consequence, this would result in only two distinct groups and avoid the inter-observer variability of grading dysplastic features.

Diagnostic karyotype provides the framework for risk-stratification schemes in AML; however, the prognostic significance of many rare recurring cytogenetic abnormalities remains uncertain [11]. Grimwade et al [11] studied the outcomes of 5876 patients (16–59 years of age) who were classified into 54 cytogenetic subgroups and treated in the Medical Research Council trials. In multivariable analysis, t(15;17)(q22;q21), t(8;21)(q22;q22), and inv(16)(p13q22)/t(16;16)(p13;q22) were the only abnormalities found to predict a relatively favorable prognosis. In patients with inv(16), the presence of additional changes, particularly +22, predicted a better outcome. Various abnormalities predicted a significantly poorer outcome, namely abn(3q) (excluding t(3;5)(q25;q34)), inv(3)(q21q26)/t(3;3)(q21;q26), add(5q)/del(5q), -5, -7, add(7q)/del(7q), t(6;11)(q27;q23), t(10;11)(p11 13;q23), other t(11q23) (excluding t(9;11)(p21 22;q23) and t(11;19)(q23;p13)), t(9;22)(q34;q11), -17, and abn(17p). Patients lacking these favorable or adverse aberrations, but with four or more unrelated abnormalities also exhibited a significantly poorer prognosis, designated “complex” karyotype group. The authors conclude that their data allow more reliable prediction of outcome for patients with rarer abnormalities and may facilitate the development of consensus in reporting of karyotypic information in clinical trials involving younger adults with AML.

Recent developments in AML prompted an international expert panel to provide updated evidence and expert opinion-based recommendations for the diagnosis and management of AML that contain both minimal requirements for general practice, as well as standards for clinical trials. A new standardized reporting system for correlation of cytogenetic and molecular genetic data with clinical data was proposed [12] and is summarized below.

Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data

Favorable

t(8;21)(q22;q22); *RUNX1-RUNX1T1*

inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*

Mutated *NPM1* without *FLT3*-ITD (normal karyotype)

Mutated *CEBPA* (normal karyotype)

Intermediate 1

Mutated *NPM1* and *FLT3*-ITD (normal karyotype)

Wild-type *NPM1* and *FLT3*-ITD (normal karyotype)

Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)

Intermediate-II

t(9;11)(p22;q23); *MLL2-MLL*

Cytogenetic abnormalities not classified as favorable or adverse†

Adverse

inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVII*

t(6;9)(p23;q34); *DEK-NUP214*

t(v;11)(v;q23); *MLL* rearranged

-5 or del(5q); -7; abnl(17p); complex karyotype‡

Response criteria in AML

Complete remission (CR)

Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count $>1.0 \times 10^9/L$ ($1,000/\mu L$); platelet count $>100 \times 10^9/L$ ($100,000/\mu L$); independence of red cell transfusions

CR with incomplete recovery (CRi)

All CR criteria, except for residual neutropenia ($<1.0 \times 10^9/L$ [$1,000/\mu L$]) or thrombocytopenia ($<100 \times 10^9/L$ [$100,000/\mu L$])

Morphologic leukemia-free state

Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required

Partial remission (PR)

Relevant in the setting of phases 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%

Cytogenetic CR (CRc)

Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal

karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow.

Treatment failure

Resistant disease (RD)

Failure to achieve CR or CRi (general practice; phase 2 out of 3 trials), or failure to achieve CR, CRi, or PR (phase 1 trials); only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination

Death in aplasia

Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia

Death from indeterminate cause:

Deaths occurring before completion of therapy, or < 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available.

Relapse

Bone marrow blasts $\geq 5\%$; or reappearance of blasts in the blood; or development of extramedullary disease

This excellent review of AML should be read in its entirety, but strict adherence to the listed criteria when reporting BMBs, would avoid terminological confusion!

Lymphoid lesions in the bone marrow

Chronic lymphocytic leukemia (CLL) and the other low-grade non-Hodgkin lymphomas are among the most common lymphoid malignancies, and recent studies suggest that more than 4% of the general population over age 40 harbor a population of clonal B cells with the phenotype of either CLL or another B-cell malignancy, a condition now designated monoclonal B-cell lymphocytosis (MBL) [13]. Shanafelt et al [13] have reviewed MBL and noted that although all cases of CLL appear to be preceded by MBL, the majority of individuals with MBL will not develop a hematologic malignancy. The biologic characteristics and clinical implications of MBL appear to differ based on whether it is identified during the diagnostic evaluation of lymphocytosis or incidentally discovered through screening of individuals with normal lymphocyte counts as part of research studies using highly sensitive detection methods. The diagnostic criteria and subclassification of MBL were refined:

Diagnostic criteria

- (1) Documentation of clonal B-cell population by one or more of the following:
 - (a) Light chain restriction: Overall kappa:lambda ratio >3:1 or <0.3:1 or >25% of B cells lacking or expressing low-level surface immunoglobulin
 - (b) Heavy chain monoclonal IGHV* rearrangements
- (2) Presence of a disease-specific immunophenotype
- (3) Absolute B-cell count <5×10⁹ cells/l
- (4) No other features of a lymphoproliferative disorder or autoimmune disease
 - (a) Normal physical exam (no lymphadenopathy or organomegaly)
 - (b) Absence of B-symptoms (for example, fatigue, weight loss and night sweats) attributable to NHL
 - (c) No autoimmune/infectious disease

Subclassification

(A) CLL-like phenotype

Co-expression of CD5 with CD19; CD20 (dim); and CD23 Light chain restriction with dim surface immunoglobulin expression (very small MBL clones may be oligoclonal and thus not light chain restricted)

(B) Atypical-CLL phenotype

Co-expression of CD5 with CD19 but CD23 negative or CD20 (bright)

Light chain restriction with moderate-to-bright surface immunoglobulin expression

Exclude t(11;14) to rule out mantle cell lymphoma

(C) Non-CLL phenotype

CD5 negative

Express CD20 Light chain restriction with moderate-to-bright surface immunoglobulin expression

*IGHV, immunoglobulin heavy chain variable

Which MBL cases will progress to CLL in currently unpredictable. In contrast to the measurable risk of progression in patients with clinical CLL-like MBL, progression among patients with population-screening CLL-like MBL is exceedingly rare [13]. The average risk of progression requiring therapy among individuals with clinical CLL-like MBL appears to be approximately 1%–2% per year. The risk of progression among patients with atypical-CLL-phenotype MBL or non-CLL-phenotype MBL is less well defined [13].

Many B-cell chronic lymphocytic leukemia (CLL) monoclonal antibodies (mAbs) can be grouped into subsets based on nearly identical stereotyped sequences. Subset 6 CLL mAbs recognize nonmuscle myosin heavy chain IIA (MYHIIA) [14]. Chu et al [14] report that during apoptosis, MYHIIA becomes exposed on the cell surface of a subgroup of apoptotic cells, allowing sub-set 6 CLL mAbs to bind with it. Because other non-subset 6 CLL mAbs interact with apoptotic cells, 26 CLL mAbs, including 24 not belonging to subset 6, were tested for reactivity with MYHIIA-exposed apoptotic cells (MEACs). More than 60% of CLL mAbs bound MEACs well; most of these mAbs expressed unmutated *IGHV* and belonged to a stereotyped subset. Binding to MEACs inversely correlated with the degree of *IGHV* mutation. High binding to MEACs significantly correlated with poor patient survival, suggesting that the basis of *IGHV* mutation status as a CLL prognostic factor reflects antigen binding. Natural antibodies from human serum also reacted with MEACs. The authors concluded that a large proportion of CLL clones emerge from natural antibody-producing cells expressing immunoglobulins that recognize MEACs, and that this reactivity is associated with poor clinical outcome.

Stamatopoulos [15] in a commentary on the paper concluded that on the available evidence, it seems reasonable to argue that neo/self-antigen/apoptotic cells, acting in synergy with microbial pathogens, may drive CLL progenitors or even the malignant cells themselves by continuously triggering B-cell receptors with distinctive structural features.

Autoimmune lymphoproliferative syndrome (ALPS) is a human genetic disorder of lymphocyte apoptosis resulting in an accumulation of lymphocytes and childhood onset chronic lymphadenopathy, splenomegaly, multilineage cytopenias, and an increased risk of B-cell lymphoma. [16] Diagnosis may be a challenge and the possibility must be borne in mind when investigating obscure cytopenias in childhood. In 1999, investigators at the National Institutes of Health (NIH) suggested criteria to establish the diagnosis of ALPS. Since then, approximately 500 patients with ALPS have been studied worldwide, and significant advances in understanding of the disease have been made. Oliviera et al [16] report revised diagnostic criteria stemming from an international workshop held at NIH in 2009.

Revised diagnostic criteria for ALPS [16]

Required

1. Chronic (>6 months), non-malignant, non-infectious lymphadenopathy or splenomegaly or both.

2. Elevated CD3+ TCRαβ + CD4 - CD8 - DNT cells ($\geq 1.5\%$ of total lymphocytes or 2.5% of CD3+ lymphocytes) in the setting of normal or elevated lymphocyte counts.

Accessory

Primary

1. Defective lymphocyte apoptosis (in two separate assays)
2. Somatic or germline pathogenic mutation in *FAS*, *FASLG*, or *CASP10*

Secondary

1. Elevated plasma sFASL levels (>200 pg/mL) OR elevated plasma interleukin-10 levels (>20 pg/mL) OR elevated serum or plasma vitamin B12 levels (>1500 ng/L) OR elevated plasma interleukin-18 levels >500 pg/mL
2. Typical immunohistological findings, as reviewed by an experienced hematopathologist
3. Autoimmunecytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated immunoglobulin G levels (polyclonal hypergammaglobulinemia)
4. Family history of a non-malignant/non-infectious lymphoproliferation with or without autoimmunity

A definitive diagnosis is based on the presence of both required criteria plus one primary accessory criterion. A probable diagnosis is based on the presence of both required criteria plus one secondary accessory criterion.

Teachey et al [17] recently reviewed the clinical and laboratory manifestations found in ALPS patients, as well as the molecular basis for the disease and new advances in treatment.

On behalf of the International Myeloma Working Group, Kyle et al [18] reviewed the current status of the Monoclonal Gammopathy of Undetermined Significance (MGUS) versus asymptomatic myeloma (AMM). MGUS was identified in 3.2% of 21,463 residents of Olmsted County, Minnesota, 50 years of age or older. The risk of progression to multiple myeloma, Waldenstrom's macroglobulinemia, AL amyloidosis or a lymphoproliferative disorder was approximately 1% per year. Low-risk MGUS was characterized by having an M protein <15 g/l, IgG type and a normal free light chain (FLC) ratio. Risk increased with increasing numbers of abnormalities. It was recommended that patients should be followed with serum protein electrophoresis at 6 months and, if stable, could be followed-up every 2–3 years, or when symptoms sugges-

tive of a plasma cell malignancy arise. Patients with intermediate and high-risk MGUS should be followed-up in 6 months, and then annually for life. The risk of AMM progressing to multiple myeloma or a related disorder was 10% per year for the first 5 years, 3% per year for the next 5 years and 1%–2% per year for the next 10 years. If the patient had both 10% or more PCs and 30 g/l or more of monoclonal protein, the probability of progression at 15 years was 87%; if the patient had 10% or more PCs and 30 g/l or more of monoclonal protein, the risk of progression was 70% at 15 years, whereas those patients with less 10% PCs and 30 g/l or more monoclonal protein had a progression risk of 39%. Testing should be done 2–3 months after the initial recognition of AMM. If the results are stable, the patient should be followed-up every 4–6 months for 1 year and, if stable, every 6–12 months.

Raja et al [19] reviewed the use of flow cytometric immunophenotyping in the diagnosis, classification and monitoring of disease in monoclonal gammopathies (MGUS), and plasma cell myeloma (PCM). The clinical sensitivity of flow cytometry was comparable with advanced molecular methods. Clinical application of flow cytometry in MGUS had various dimensions, such as differential diagnosis of PCM from reactive plasmacytosis, identifying the progression risk in MGUS and asymptomatic PCM, and minimal residual disease detection. Flow cytometry-based clonality assessment with immunophenotyping encouraged and enabled the most stringent method of diagnosis and follow-up. Antigens, such as CD19, CD27, CD28, CD45, CD56, and CD117, play a significant role in the characterization of normal and malignant plasma cells.

Expression of various antigens in plasma cells of MGUS and MM

Normal distribution and functions in brackets.

CD19 (All B cells, including lymphoblasts, mature B-lymphoid cells, and most of plasma cells)

MGUS - normal plasma cells express CD19, malignant plasma cells do not

PCM - only negative or dim CD19 expression on plasma cells

CD20 (During maturation process of pre-B cells and negative expression in plasma cells)

Some subsets of PCM patients express CD20 in plasma cells

CD27 (Aid in differentiation of mature B cells into plasma cells)

MGUS - consistent high expression in plasma cells

PCM - expression is heterogeneous and low intensity comparing to MGUS

CD28 (T cell activation)

MGUS - only very few cases express CD28

PCM - CD28 expressing plasma cells represented as aggressive phenotype. Associates always with tumor expansion

CD33 (Myeloid and monocytic cells)

Only few myeloma patients express CD33 on plasma cells and correlate with lower survival

CD45 (B and T cells), dim expression in precursor cells and some plasma cells

MGUS - equal distribution of CD45+ and CD45- plasma cells

PCM - expression is not well characterized, but survival rate is higher for CD45+ patient group comparing to CD45- group. Also CD45 expression demonstrates proliferating compartment of normal, reactive, and malignant plasma cells

CD56 (NK and NK-T cells)

PCM – most of the cases express CD56.

However, circulating plasma cells and extramedullary myeloma patients lack CD56 expression

CD117 Progenitors of myeloid, erythroid and megakaryocytic lineage

MGUS - 50% of cases express CD117

PCM - one-third of patients express CD117 and have better prognosis comparing to their counterpart

CD138 Plasma cells

MGUS and PCM cases express CD138 on plasma cells, but its expression is dim on malignant plasma cells

A few studies have provided evidence for the systemic relation between genetic aberrations and unique PCM phenotypes. Del (13q), t(4;14) and IGH rearrangements have been more commonly reported in the CD117-phenotype. Translocation t(11;14) has identified most commonly in CD20+ patients. CD56dim/- and lack of CD117 expression have been associated in patients with t(11;14). Non-hyperdiploid myeloma cells are more frequently associated with CD28+ CD20+ CD56- CD117-phenotypes compared to hyperdiploid myeloma cells. CD28+ has been correlated with myeloma cells with t(14;16) and del(17p). A specific association has been reported between phenotype (CD20- CD56- CD117-) and chromosomal abnormality t(11;14) in rare IgM myeloma cases. Del (13) was associated with both CD33+ and CD33- phenotypes. Translocation t(4;14) or t(14;16) were also associated with both CD45+ and CD45-phenotypes. High frequency of CD221 expression on PCs was restricted to specific chromosomal aberration t(4;14) or t(14;16).

Although morphologically similar, several subtypes of PCM have been identified at the genetic and molecular level [20]. Fonseca et al [20] have reviewed the genetic

subtypes and their association with unique clinico-pathological features and dissimilar outcomes. At the top hierarchical level, myeloma can be divided into hyperdiploid and non-hyperdiploid subtypes. Trisomies and a more indolent form of the disease characterize hyperdiploid myeloma. Non-hyperdiploid cases are mainly harboring IgH translocations, generally associated with more aggressive clinical features and shorter survival. The three main IgH translocations in myeloma are the t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23). A number of genetic progression factors have been identified including deletions of chromosomes 13 and 17 and abnormalities of chromosome 1 (1p deletion and 1q amplification). Other key drivers of cell survival and proliferation have also been identified, such as nuclear factor-B-activating mutations and other deregulation factors for the cyclin-dependent pathways regulators. Most myeloma cases may be classified under one of several genetic subtypes. The paper proposed a framework for the classification of myeloma subtypes. The group proposed that genetic testing should be incorporated into daily clinical practice.

Proposed molecular-cytogenetic classification of PCM**Hyperdiploid (45%)**

More favorable, IgG-k, older patients

Non-hyperdiploid (40%)

Aggressive, IgA-λ, younger patients

Cyclin D translocation (18%)

t(11;14)(q13;q32) (16%) Upregulation of CCND1; favorable prognosis; bone lesions. Two subtypes by GEP

t(6;14q)(p21;q32) (2%) Probably same as CCND1

t(12;14)(p13;q32) Rare

MMSET(15%) translocation

t(4;14)(p16;q32) (15%) Upregulation of MMSET; upregulation of FGFR3 in 75%. Unfavorable prognosis with conventional therapy; bone lesions less frequent.

MAF translocation (8%) Aggressive

t(14;16)(q32;q23) (5%) Aggressive

t(14;20)(q32;q11) (2%) Probably aggressive

t(8;14)(q24;q32) (1%) Unknown

Unclassified (other) (15%)

Various subtypes and some with overlap

Conflict of interest The author declares no conflict of interest.

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