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Genetic alterations crossing the borders of distinct hematopoetic lineages and solid tumors: Diagnostic challenges in the era of high-throughput sequencing in hemato-oncology

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ABSTRACT

Owing to the introduction of next-generation sequencing (NGS) new challenges for diagnostic algorithms and the interpretation of the results for therapeutic decision making in hemato-oncology have arisen. Recurrent somatic mutations crossing the borders between different hematological entities and solid neoplasms have been detected. In analogy to mutant *TP53*, the same mutation type may occur in myeloid, B- or T-lymphatic malignancies or solid neoplasms. At the same time, a certain mutation can show different prognostic outcomes in different entities and co-existence of certain mutations may change the prognostic relevance. These insights may spark the investigation of targeted therapies with the same substances across different disease entities. This review article summarizes mutations that can emerge in different hematologic and solid malignancies and summarizes other obstacles in the era of modern molecular diagnostics, such as the phenomenon of "clonal hematopoiesis of indeterminate potential" being difficult to interpret in the individual patient.

1. Introduction

Following the establishment of cytogenetics as well as "traditional" molecular techniques (PCR, fluorescent-based molecular methods such as real-time PCR, and Sanger sequencing) within the last four to five decades, a multitude of genetic alterations have been identified to be typical and crucial for distinct hematologic entities, like t(9;22)(q34;q11)/*BCR-ABL1* in chronic myeloid leukemia (CML) or t(15;17)/*PML-RARA* in acute promyelocytic leukemia (APL), and became relevant for diagnosis, classification, prognostication and therapeutic planning in hematologic and solid malignancies. Unlike traditional molecular assays, which focus on a relatively small panel of commonly mutated sites, next-generation sequencing (NGS) offers the capacity to measure somatic allele frequencies from the complete coding sequences of many genes in the same assay (Cummings et al., 2016; Serrati et al., 2016). Thus, with the advent of NGS around a decade ago, the number of known genetic mutations in patients with hematological and solid malignancies has been continuously increasing.

As a result, multiple recurrent somatic mutations including genes involved in signal transduction (*JAK2*, *KRAS*, *CBL*, *FLT3*), DNA methylation (*DNMT3A*, *TET2*, *IDH1/2*), transcriptional regulation (*EVI1*, *RUNX1*, *GATA2*), chromatin modification (*EZH2*, *ASXL1*), and RNA splicing (*SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*) were explored in various myeloid as well as lymphoid and solid malignancies, assuming some common molecular genetic mechanisms across different entities (Papaemmanuil et al., 2013; Gill et al., 2016; Bejar et al., 2011; Cazzola et al., 2013a). For a long time, mutations in *TP53* had been known to cross the borders of different hematological entities and lineages and to occur also in almost all solid tumors such as breast, lung or ovarian cancer (Olivier et al., 2010). By now, a continuously growing number of

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genetic alterations have been emerging that are crossing the borders between seemingly different hematological entities or lineages. *EZH2* mutations were found to occur in various myeloid malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (Papaemmanuil et al., 2013; Gill et al., 2016; Bejar et al., 2011; Cazzola et al., 2013a; Nebbioso et al., 2015; Herviou et al., 2016) but were also detected in follicular lymphoma (FL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) (Velichutina et al., 2010; Huet et al., 2017; Papakonstantinou et al., 2016; Pawlyn et al., 2017). *IDH2* mutations that are typically associated to myeloid malignancies such as MDS, AML and myeloproliferative neoplasms (MPNs) (Medeiros et al., 2017; Feng et al., 2012; Mondesir et al., 2016; Tefferi et al., 2012; Yonal-Hindilerden et al., 2016) are as well frequent in T-cell angioimmunoblastic lymphoma (T-AITL) and glioma (Cairns et al., 2012; Xia et al., 2015; Rohle et al., 2013). Another striking example is the *BRAF*V600E mutation being documented not only within solid tumors like melanoma, colorectal, papillary thyroid and lung cancer, but also in hematological entities such as hairy cell leukemia or melanoma (Rustad et al., 2015; Falini et al., 2016; Sosman et al., 2012; Hou et al., 2011; Xing, 2005; Tol et al., 2009). Thus, a clear association of these and other molecular markers with distinct disease profiles becomes more demanding and the interpretation of these molecular markers provides more difficulties for clinicians and molecular biologists.

One more diagnostic challenge that has emerged from the NGS era is the phenomenon of “clonal hematopoiesis of indeterminate potential” (CHIP) that shows frequencies up to 10% in individuals aged ≥ 70 years, and up to 20% in those aged ≥ 90 years (Xie et al., 2014; Genovese et al., 2014; Jaiswal et al., 2014). CHIP constitutes detectable clonal somatic mutations in genes recurrently altered in hematologic malignancies in individuals without a known hematologic malignancy and no evidence of another clonal disorder (Steensma et al., 2015; Heuser et al., 2016). Interestingly, CHIP not only predisposes for the development of hematologic malignancies but also increases the risk of atherosclerotic cardiovascular disease (Jaiswal et al., 2017). Although clear strategies for the management of individuals with CHIP are so far missing, it has become clear that the detection of clonal hematopoiesis in the peripheral blood or bone marrow should be interpreted with caution.

The literature provides multiple review articles summarizing the role of distinct somatic gene mutations in distinct hemato-oncological entities. However, a review focusing on the occurrence of somatic gene mutations across the borders of different hematopoietic lineages and eventually even solid tumors to our knowledge is missing so far. This review article provides an overview on molecular alterations that can occur in different types of hematologic entities: in myeloid entities such as AML, MDS, or MPNs, and in lymphomas and acute lymphoblastic leukemia (ALL) of the B- and T-lineage, respectively. Some of these mutations are also found in solid tumors. The review also outlines other difficulties that may arise in the clinical interpretation of molecular hematological results in the NGS era.

2. Molecular background

By Sanger sequencing only specific DNA regions could be investigated at limited sensitivity and only selected genes could be sequenced (Koboldt et al., 2013; Sanger et al., 1977). The advent of rapid DNA sequencing methods like NGS has revolutionized molecular diagnostics in the field of hematology (Gagan and Van Allen, 2015). In general, this method is based on four subsequent steps including library preparation, cluster generation, sequencing and data analysis. The sequencing library is prepared by random fragmentation of the DNA or cDNA sample, followed by adapter ligation. Adapter-ligated fragments are then PCR amplified and purified. For cluster generation, the library is loaded into a flow cell where fragments are captured on a lawn of surface-bound oligos complementary to the library adapters. Each fragment is then amplified into distinct, clonal clusters through bridge amplification, followed by sequencing with proprietary reversible terminator–based method that detects single bases as they are incorporated into DNA template strands. This is followed by bioinformatic data analysis, in which the newly identified sequence reads are aligned to a reference genome (Koboldt et al., 2013). Whereas Sanger sequencing results in sequencing of only a few short fragment of up to 1000 base pairs (bp) in length, NGS allows the simultaneous sequencing of millions of DNA fragments (Kamps et al., 2017). Today, NGS approaches include whole-genome sequencing (WGS), whole-exome sequencing (WES) that focuses on the coding regions, and targeted sequencing. Targeted sequencing, of a selection (= panel) of genes known to be mutated in a certain disease, is the method of choice employed in routine molecular genetic diagnostic settings.

3. Overview on molecular mutations crossing the borders between different hematologic lineages or solid tumors

3.1. TET2 mutation

The *TET*2 (Tet methylcytosine dioxygenase 2) gene encodes a dioxygenase and has a key role in active DNA demethylation (Nakajima and Kunimoto, 2014). Although *TET2* mutations were observed in 20–25% of all MDS cases, their prognostic role within this entity is not yet clear but hints at a rather positive or no prognostic effect. *TET2* mutations were reported to frequently coincide with *ASXL1* and *EZH*2 mutations (Papaemmanuil et al., 2013; Gill et al., 2016; Cazzola et al., 2013a; Kosmider et al., 2009; Haferlach et al., 2016a). Bejar et al. described a trend towards increased response rates of *TET2* mutated MDS patients to hypomethylating agents (HMAs) with better overall response rates (ORR) under azacitidine (AZA) therapy, particularly in case of wild-type *ASXL1* (Bejar et al., 2014). In patients with *de novo* AML, *TET2* mutations show a similar frequency with up to 25%. Again, a relevant prognostic impact could not be determined (Gaidzik et al., 2012). One study described an unfavorable impact of *TET2* mutations in patients with AML and intermediate risk cytogenetics (Chou et al., 2011). Similar to MDS, some studies were suggestive for increased sensitivity of *TET2* mutated AML to HMAs (Kadia et al., 2015; Itzykson et al., 2011). Furthermore, *TET2* mutations were detected in around 10% of MPNs and in around 60% of CMML cases without any clear prognostic impact (Tefferi et al., 2009; Patriarca et al., 2013; Patnaik et al., 2016; Patnaik and Tefferi, 2016; Itzykson et al., 2013). In patients with CMML showing both *TET2* and *ASXL1* mutations, survival was observed to be worse (Patnaik et al., 2016; Patnaik and Tefferi, 2016). In mouse models, *Tet2*-deficient animals were shown to develop a CMML-like disease (Moran-Crusio et al., 2011).

Being initially regarded as a mutation strongly associated with myeloid malignancies, studies in recent years clearly demonstrated that *TET2* mutations may also play a role in T-cell malignant lymphopoiesis, inducing yet unclear epigenetic changes with subsequent development of T-cell lymphoma subtypes that harbor follicular helper T-cell-like features (Muto et al., 2014). *TET2* mutations were detected in around 12% of T-cell lymphomas with an almost exclusive presence in peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), T-AITL and enteropathy-associated T-cell lymphoma (EATL) (38%, 47%–76%, and 20% of all cases, respectively) (Lemonnier et al., 2012; Odejide et al., 2014). Some studies demonstrated coexisting *DNMT3A* (up to 43%) and *IDH2* mutations (up to 23%) in patients with *TET2* mutated AITL. This high selective coincidence of genetic alterations of the DNA methylation machinery may emphasize the critical role of epigenetic changes and these complexity in neoplastic T-cell lymphopoiesis (Lemonnier et al., 2012; Odejide et al., 2014; Couronne et al., 2012). Single reports describe the presence of *TET2* mutations in DLBCL (diffuse large B-cell lymphoma) with frequencies ranging from 3% to 12%. No prognostic impact for this lymphoma subtype was so far detected (Asmar et al., 2013; Kubuki et al., 2017). Yet, the presence of *TET*2 mutations seems to be relevant also for subsets of B-cell