currently in development to target aberrant spliceosome function in MDS and other malignancies, and these models will be invaluable for pre-clinical testing of these drugs.

REFERENCES

Brooks, A.N., Choi, P.S., de Waal, L., Sharifnia, T., Imielinski, M., Saksena, G., Pedamallu, C.S., Sivachenko, A., Rosenberg, M., Chmielecki, J., et al. (2014). PLoS ONE *9*, e87361.

Colla, S., Ong, D.S.T., Ogoti, Y., Marchesini, M., Mistry, N.A., Clise-Dwyer, K., Ang, S.A., Storti, P., Viale, A., Giuliani, N., et al. (2015). Cancer Cell *27*, this issue, 644–657.

Daubner, G.M., Cléry, A., Jayne, S., Stevenin, J., and Allain, F.H. (2012). EMBO J. *31*, 162–174.

Kim, E., Ilagan, J.O., Liang, Y., Daubner, G.M., Lee, S.C.W., Ramakrishnan, A., Li, Y., Chung, Y.R., Micol, J., et al. (2015). Cancer Cell *27*, this issue, 617–630.

Nikoloski, G., Langemeijer, S.M., Kuiper, R.P., Knops, R., Massop, M., Tönnissen, E.R., van der Heijden, A., Scheele, T.N., Vandenberghe, P., de Witte, T., et al. (2010). Nat. Genet. *42*, 665–667.

Papaemmanuil, E., Cazzola, M., Boultwood, J., Malcovati, L., Vyas, P., Bowen, D., Pellagatti, A., Wainscoat, J.S., Hellstrom-Lindberg, E., Gambacorti-Passerini, C., et al.; Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium (2011). N. Engl. J. Med. *365*, 1384–1395.

Rossi, D., Bruscaggin, A., Spina, V., Rasi, S., Khiabanian, H., Messina, M., Fangazio, M., Vaisitti, T., Monti, S., Chiaretti, S., et al. (2011). Blood *118*, 6904–6908.

Shirai, C.L., Ley, J.N., White, B.S., Kim, S., Tibbitts, J., Shao, J., Ndonwi1, M., Wadugu1, B., Duncavage, E.J., Okeyo-Owuor, T., et al. (2015). Cancer Cell *27*, this issue, 631–643.

Wahl, M.C., Will, C.L., and Lührmann, R. (2009). Cell *136*, 701–718.

Yoshida, K., Sanada, M., Shiraishi, Y., Nowak, D., Nagata, Y., Yamamoto, R., Sato, Y., Sato-Otsubo, A., Kon, A., Nagasaki, M., et al. (2011). Nature *478*, 64–69.

Functionally Relevant RNA Helicase Mutations in Familial and Sporadic Myeloid Malignancies

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In this issue of *Cancer Cell*, Polprasert and colleagues identified recurrent mutations in the DEAD/H-box RNA helicase gene *DDX41* in familial and acquired cases of myelodsyplasia and acute myeloid leukemia. These mutations induce defects in RNA splicing and represent a new class of mutations in myeloid malignancies.

The familial myelodysplastic (MDS)/acute myeloid leukemia (AML) syndromes belong to a group of rare inherited disorders that represent a unique resource to study the initial steps toward leukemia progression. Recent findings indicate that AML occurs after a stepwise process of genetic and epigenetic changes, some of which are ancestral and are found in a preleukemic clone that precedes the fully transformed AML cells bearing additional alterations (Pandolfi et al., 2013). In familial MDS/AML, the initial germline mutation can be considered a preleukemic event, required but not sufficient for leukemia initiation. Among the most studied ones are familial AML with mutated *CEBPA*, familial MDS/AML with *GATA2* mutations, and familial platelet disorder with predisposition to AML (FPD/AML) due to *RUNX1* mutations (West et al., 2014). Interestingly, mutations in these transcription factors also play important roles in sporadic AML, showing that, in principle, both germline and acquired mutations of the same genes can deregulate the hematopoietic compartment and lead to AML development. Thus, the study of patients with familial MDS/AML has proven to be extremely valuable for understanding the multi-step leukemogenic process (Antony-Debré et al., 2015), including in sporadic leukemias.

The advent of whole exome sequencing technology is of great interest for the identification of new mutations in familial MDS/AML syndromes, because the genetic origin of many of them remains unknown. A better knowledge of these syndromes would be beneficial not only for research, but also for clinical care, as individuals harboring familial mutations could be identified, e.g., for more frequent follow-up examinations or for exclusion when a bone marrow allograft is considered. In this issue of *Cancer Cell*, Polprasert et al. (2015) identified functionally relevant mutations in the gene coding for the DEAD/H-box RNA helicase DDX41 in both familial and sporadic MDS/AML cases. Initially, they discovered inherited *DDX41* mutations in several MDS/AML pedigrees and showed that additional somatic *DDX41* mutations of the remaining wild-type allele were present at MDS/AML diagnosis in half of the cases (Figure 1A). This is similar to what has been observed in MDS/AML with *CEBPA* or *RUNX1* mutations, where biallelic mutations are found with AML progression (Pabst et al., 2008; Preudhomme et al., 2009). The clonal architecture of leukemic clones revealed that acquired *DDX41* mutations could be a founder event in some patients. Familial DDX41 MDS/AML syndrome was characterized by a long latency, an advanced stage at diagnosis (high risk MDS/AML), normal

karyotype, and a lack of "typical" AML mutations. This phenotype, the presence of biallelic mutations, as well as their recurrence, were in support of an oncogenic functional role of *DDX41* mutations.

In addition to familial cases, the authors found recurrent *DDX41* mutations in acquired myeloid neoplasms (at a frequency of around 1.5%) and showed an association with inferior overall survival and advanced stage of disease (high risk MDS and AML). Interestingly, in half of these patients, the *DDX41* mutation was germline. Conceptually similar findings were previously described for *CEBPA* mutations (Pabst et al., 2008). These findings suggest that germline initiation/predisposition of MDS/AML is currently likely underestimated. Mutations in AML-associated genes such as *RUNX1* or *CEBPA,* and now *DDX41* and possibly others, are detected at AML diagnosis, but their germline character may not be identified in a considerable number of cases. Moreover, germline gene mutations can easily be missed because of the applied analysis strategies in many major studies, because mutations found in both germline and leukemic samples are often excluded as polymorphisms, (falsely) assumed functionally irrelevant. Somatic *DDX41* mutations have been reported in one previous study of sporadic AML (Ding et al., 2012). Overexpression as well as mutations/deletions of other members of the DEAD/H-box RNA helicase family have been described in several other cancers, where they can act both as tumor suppressors and oncogenes (Fuller-Pace, 2013). Interestingly, Polprasert et al. (2015) identified mutations in other members of the DEAD/H-box RNA helicase family in about 4% of patients in their cohort, suggesting that RNA helicase mutations represent an entire new family of mutations in myeloid neoplasms. All helicase mutations, including *DDX41*, were found to be mutually exclusive. Interestingly, *DDX41* is located on chromosome 5 and is deleted in ~25% of MDS with del(5q). Patients harboring mutations or low levels of *DDX41* showed a better response to lenalidomide, even in the absence of del(5q), suggesting an important role of *DDX41* in treatment response and a potential targeted therapy for this group of patients.

The reduced expression as well as the presence of *DDX41* deletions and the presence of frameshift mutations in familial MDS/AML indicated a tumor suppressor function for DDX41 in MDS/AML. Indeed, the knockdown of DDX41 resulted in increased proliferation and a differentiation block, whereas its overexpression decreased cell growth and enhanced cellular differentiation in cell lines as well as primary patients' diseased cells. Furthermore, DDX41 knockdown led to an increase in tumor growth in a xenotransplantation model in vivo.

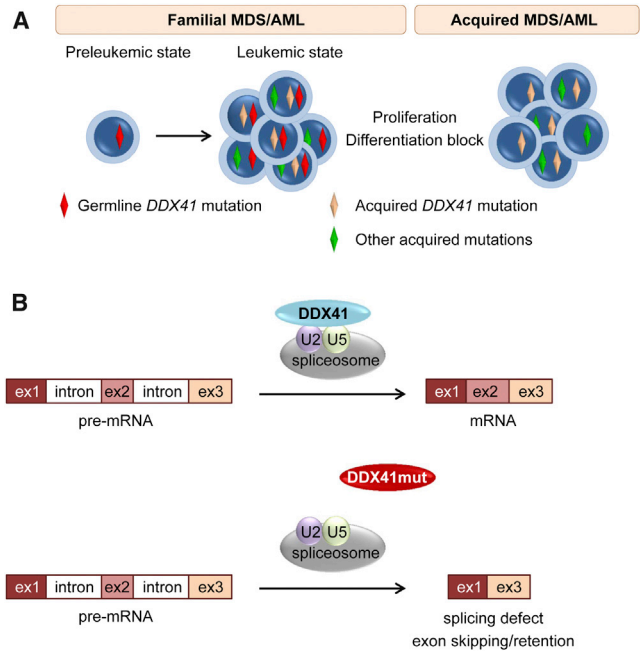

Figure 1. *DDX41* Mutations in Myeloid Neoplasms

(A) *DDX41* mutations in familial and acquired MDS/AML. Germline mutations are consistent with a preleukemic state, whereas acquired mutations, including in the second allele of *DDX41* (found in half of the patients), are necessary for leukemia initiation. Analysis of the clonal architecture at the leukemic stage revealed that acquired *DDX41* mutations can be ancestral or be present in a subclone. Abbreviations: AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

(B) Association of DDX41 with the spliceosome. Wild-type DDX41 physically interacts with components of the U2 and U5 small nuclear ribonucleic protein complexes, and mutation of *DDX41* results in disruption of these interactions and splicing defects, including aberrant exon skipping or exon retention. Abbreviations: ex, exon; DDX41 mut, DDX41 mutated; mRNA, messenger RNA.

Little is known about the molecular mechanism/function of DDX41, and its role in hematopoiesis is unclear. Other RNA helicase members are involved in several processes, including in ribosome biogenesis, pre-mRNA splicing, and translation (Jankowsky, 2011). For example, one member of the DEAD/H-box protein family, Prp5, is required for spliceosome assembly and physically interacts with the U1 and U2 small nuclear ribonucleic proteins (snRNPs) (Xu et al., 2004). Thus, Polprasert et al. (2015) investigated more closely a possible association of DDX41 with spliceosomal proteins. They identified a direct interaction of DDX41 with spliceosomal components and showed that *DDX41* mutations impaired interactions with several components of the U2 snRNP, including SF3B1, and the U5 snRNP, including PRPF8. Investigation of RNA splicing revealed defects associated with *DDX41* mutations with aberrant exon skipping or exon retention (Figure 1B). It remains to be determined exactly how and when DDX41 interacts with the spliceosome during the splicing process and whether this new class of mutations could be part of the spliceosomal-related defects that were recently discovered in MDS/AML. Spliceosomal mutations can result in the inactivation of tumor suppressor genes as well as alterations in the balance of isoforms of genes, with increased expression of isoforms promoting cell proliferation. It remains to be explored whether mutations in DEAD/H-box protein family members may have similar consequences on RNA splicing and promote leukemia development and progression through similar mechanisms.

In summary, the study by Polprasert et al. (2015) shows that functionally relevant mutations of *DDX41* and other members of the DEAD/H-box RNA helicase family genes represent a novel family of mutations in myeloid malignancies with possible implications for

prognosis and treatment of MDS/AML. They can be founder events and are associated with a preleukemic state. Further studies of the mechanisms and functions of DDX41 and other members of the RNA helicases family in hematopoiesis and leukemogenesis are warranted and may ultimately lead to approaches of therapeutic targeting of these proteins.

REFERENCES

Antony-Debré, I., Manchev, V.T., Balayn, N., Bluteau, D., Tomowiak, C., Legrand, C., Langlois, T., Bawa, O., Tosca, L., Tachdjian, G., et al. (2015). Blood *125*, 930–940.

Ding, L., Ley, T.J., Larson, D.E., Miller, C.A., Koboldt, D.C., Welch, J.S., Ritchey, J.K., Young, M.A., Lamprecht, T., McLellan, M.D., et al. (2012). Nature *481*, 506–510.

Fuller-Pace, F.V. (2013). RNA Biol. *10*, 121–132.

Jankowsky, E. (2011). Trends Biochem. Sci. *36*, 19–29.

Pabst, T., Eyholzer, M., Haefliger, S., Schardt, J., and Mueller, B.U. (2008). J. Clin. Oncol. *26*, 5088–5093.

Pandolfi, A., Barreyro, L., and Steidl, U. (2013). Stem Cells Transl. Med. *2*, 143–150.

Polprasert, C., Schulze, I., Sekeres, M.A., Makishima, H., Przychodzen, B., Hosono, N., Singh, J., Padgett, R.A., Gu, X., Phillips, J.G., et al. (2015). Cancer Cell *27*, this issue, 658–670.

Preudhomme, C., Renneville, A., Bourdon, V., Philippe, N., Roche-Lestienne, C., Boissel, N., Dhedin, N., André, J.M., Cornillet-Lefebvre, P., Baruchel, A., et al. (2009). Blood *113*, 5583–5587.

West, A.H., Godley, L.A., and Churpek, J.E. (2014). Ann. N Y Acad. Sci. *1310*, 111–118.

Xu, Y.Z., Newnham, C.M., Kameoka, S., Huang, T., Konarska, M.M., and Query, C.C. (2004). EMBO J. *23*, 376–385.

Convert and Conquer: The Strategy of Chronic Myelogenous Leukemic Cells

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Emerging evidence is contributing to explain how leukemias disrupt normal blood cell production. In this issue of *Cancer Cell*, Welner and colleagues show that, during the development of chronic myeloid leukemia, mutated cells transform normal hematopoietic progenitors into "leukemic like" cells through IL-6 secretion, proposing a new cellular target.

Classical cancer therapies target the malignant cells. However, the environment surrounding the tumors, referred to as "niche," during cancer development and progression is gaining increased attention. Many efforts are now directed toward understanding the relationships between the tumor and its supporting cells (e.g., stromal cells), which have become a novel target for therapies.

This also applies to blood proliferative disorders, such as myeloproliferative neoplasms (MPNs), in which the interactions between tumoral and stromal cells are critical for disease progression both at the hematopoietic stem cell (HSC) and progenitor level (Arranz et al., 2014; Krause et al., 2013; Schepers et al., 2013). However, leukemic stem cell niches might be different in chronic and acute myeloid leukemias. The Scadden group has demonstrated that activation of osteoblastic cells by parathyroid hormone attenuates chronic myelogenous leukemia (CML), but enhances instead MLL-AF9 oncogene-induced acute myeloid leukemia (Krause et al., 2013).

Using a $JAK2^{V617F}$ murine model, we have recently shown that $nestin^{+}$ mesenchymal stem cells (MSCs) play a pivotal role in regulating the proliferation of mutant MPN HSCs. IL-1β secreted by mutant HSCs induces neural damage to the niche, leading to a marked reduction in $nestin^{+}$ MSCs, uncontrolled mutant HSC proliferation, and subsequent disease progression (Arranz et al., 2014). Bone marrow stromal cells have been proposed to secrete factors that protect $JAK2^{V617F}$ cells from JAK2 inhibitors (Manshouri et al., 2011). Additionally, the Passegué group has recently demonstrated that CML cells induce expansion of osteoblastic precursors that poorly support normal hematopoietic progenitors (Schepers et al., 2013).

CML is an MPN that originates in a mutated hematopoietic stem/progenitor cell (HSPC) carrying the t(9;22)(q34;q11) translocation. This mutation leads to the expression of the BCR/ABL fusion protein, a constitutively active tyrosine kinase that promotes the growth of mutated HSCs and their progeny. In humans, CML presents first with a chronic phase in which differentiated myeloid cells accumulate. This is followed by a short accelerated phase that precedes a rapid blast phase when symptoms aggravate as myeloid cells fail to mature and fully differentiate (Savona and Talpaz, 2008).