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Finding a needle in a haystack: whole genome sequencing and mutation discovery in murine models

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Commentary

Acute promyelocytic leukemia (APL) is a malignancy of the bone marrow, in which there is a deficiency of myeloid cells and an excess of immature cells called promyelocytes. APL is most commonly caused by a translocation (15:17) and expression of the promyelocytic leukemia and the retinoic receptor α (PML-RARA) fusion product; however, the events that cooperate with PML-RARA in APL pathogenesis are not well understood. In this issue of the *JCI*, Wartman and colleagues use an innovative approach to find other relevant mutations in APL. They performed whole genome sequencing and copy number analysis of a well-characterized APL mouse model to uncover somatic mutations in *Jak1* and lysine (K)-specific demethylase 6A (*Kdm6a*, also known as *Utx*) in mice with APL and validated the ability of*Jak1* mutations to cooperate with *PML-RARA* in APL. The findings implicate the JAK/STAT pathway in the pathogenesis of APL and illustrate the power of whole genome sequencing to identify novel disease alleles in murine models of disease.

blast niche? Finally, studies are needed to better characterize the biological effects of the osteoblast niche on the survival, quiescence, and sensitivity to chemotherapy of cancer cells. Ultimately, identification of the niche signals that regulate cancer cell phenotype may provide targeted strategies to render metastatic bone cancers more susceptible to chemotherapy.

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Finding a needle in a haystack: whole genome sequencing and mutation discovery in murine models

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Acute promyelocytic leukemia (APL) is a malignancy of the bone marrow, in which there is a deficiency of myeloid cells and an excess of immature cells called promyelocytes. APL is most commonly caused by a translocation (15:17) and expression of the promyelocytic leukemia and the retinoic receptor α (PML-RARA) fusion product; however, the events that cooperate with PML-RARA in APL pathogenesis are not well understood. In this issue of the *JCI*, Wartman and colleagues use an innovative approach to find other relevant mutations in APL. They performed whole genome sequencing and copy number analysis of a well-characterized APL mouse model to uncover somatic mutations in *Jak1* and lysine (K)-specific demethylase 6A (*Kdm6a*, also known as *Utx*) in mice with APL and validated the ability of *Jak1* mutations to cooperate with *PML-RARA* in APL. The findings implicate the JAK/STAT pathway in the pathogenesis of APL and illustrate the power of whole genome sequencing to identify novel disease alleles in murine models of disease.

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Acute promyelocytic leukemia (APL) is a clinically and molecularly distinct subtype of acute myeloid leukemia that is distinguished by a recurrent chromosomal translocation fusing chromosomes 15 and 17. The t(15:17) translocation results in the fusion of the promyelocytic leukemia (*PML*) gene and the retinoic receptor α (*RARA*) gene (*PML-RARA*). The PML-RARA fusion protein is thought to contribute to APL pathogenesis by dimerizing and binding DNA and repressing the transcription of RARA target genes through recruitment of corepressors. More recent work indicates that PML-RARA also is able to bind to alternate DNA sites and to interact with chromatin remodeling complexes involved in stem cell maintenance and that the PML-RARA protein undergoes posttranslational modifications (sumoylation and phosphorylation) that are required for APL initiation (1, 2). A detailed understanding of the role of the PML-RARA

Table 1
JAK mutations in human malignancies

JAK	Mutation	Human disease	Ref.
JAK1	T478S	AML	15
	V623A	AML	15
	G871E	Uterine leiomyosarcoma	18
	V658F	T-ALL, APL, pediatric ALL	14, 16, 10
	L783F	T-ALL	16
	T782M	NSCLC	16
	H647Y	Breast cancer	16
	I62V	B-ALL, T-ALL	17
	K204M	B-ALL	17
	A634D	B-ALL, T-ALL	17
	R724H	B-ALL, T-ALL	17
	R360W	T-ALL	17
	S512L	T-ALL	17
	R879S	T-ALL	17
	R879C	T-ALL	17
	R879H	T-ALL	17
	L653F	Pediatric ALL	17
	L624_R629>W	Pediatric ALL	14
	S646F	Pediatric ALL	14
JAK2	V617F	MPN	18
	K539L	PV	18
	T875N	AML	18
	del682–686	ALL	18
	R683G	Pediatric ALL/Down syndrome ALL	14
	R683S	Pediatric ALL	14
	I682F	Pediatric ALL	14
	QGinsR683	Pediatric ALL/Down syndrome ALL	14
	R867Q	Pediatric ALL	14
	D873N	Pediatric ALL	14
	P933R	Pediatric ALL	14
JAK3	A572V	AML	18
	V722I	AML	18
	P132T	AML	18
	V715I	Breast cancer	16
	S789P	Pediatric ALL	14

B-ALL, B cell ALL; NSCLC, non–small cell lung cancer; PV, polycythemia vera; T-ALL, T cell ALL.

oncogenic fusion in APL pathogenesis has allowed investigators to elucidate the molecular basis by which retinoic acid and arsenic trioxide offer dramatic efficacy in human patients with APL. Importantly, current therapeutic approaches combining retinoic acid/arsenic with anthracyclines allow as many of 90% of patients with APL to be cured (1).

The incidence of APL is reported to be constant over a human life span, which has led to models suggesting there is a single genetic rate-limiting step involved in the development of APL (3). However, mouse models of APL suggest that PML-RARA expression in vivo leads to APL with long latency and incomplete penetrance. This suggests that the acquisition of other genetic alterations in addition to PML-RARA contributes to the development of APL (4). Indeed, data from transgenic mouse models expressing PML-RARA indicate that mice that progress to APL acquire additional genetic alterations (4–6). Studies in patients with APL have also demonstrated the occurrence of activating *FLT3* mutations and *NRAS*, *KRAS*, and *MYC* mutations (2). Collectively, these studies indicate that other genetic alterations may contribute to APL pathogenesis, even if *PML-RARA* is the disease-initiating event.

The utility of mouse models of APL

Although there exist key genetic and biologic differences in the development of malignancies between mice and humans, mouse models of APL have proven to be very useful in both modeling APL pathogenesis and in investigating specific therapies. Such models have been used to elucidate the effects and mechanisms of retinoic acid and arsenic trioxide therapy, with mouse APL cells demonstrating similar in vivo responses to both compounds when compared to human APL cells (7).

Several approaches have been used to generate mouse APL models, including transgenic PML-RARA expression, xenograft models, and adoptive transfer strategies. Transgenic models placing *PML-RARA* under the control of cathepsin G or migration inhibitory factor-related protein 8 (MRP8) promoters resulted in APL; however, in each case, APL developed with relatively long latency (6 months or longer) with incomplete penetrance (8). These data suggest additional mutations are required for the development of APL. The role of cooperating disease alleles in APL pathogenesis is underscored by mutational studies of primary human APL samples. Candidate gene studies have shown that mutations in *NRAS*, *KRAS*, *FLT3*–internal tandem duplication (*FLT3-ITD*), and *FLT3*–tyrosine kinase domain (*FLT3-TKD*) as well as trisomy 8 are observed in a subset of patients who present with APL (2). The functional significance of the *Kras* mutation in particular was elucidated by Chan et al., who found that coexpressing oncogenic Kras from the endogenous *Ras* locus with PML-RARA resulted in APL with shorter latency and near complete penetrance (9). Taken together, the human and murine genetic data suggest that additional disease alleles cooperate with PML-RARA in APL pathogenesis.

To elucidate potential cooperating events in murine APL models, previous studies performed karyotypic analysis. Zimonjic et al. used spectral karyotype analysis to identify recurrent abnormalities in murine APL cells, including interstitial or terminal deletion of one copy of chromosome 2, gains of chromosome 15, and loss of chromosome 11, X, and Y (5). Le Beau et al. performed spectral karyotyping analysis in hMRP8-PML-RARA mice and identified trisomies 8, 15, and 16 and monosomies X or Y as recurrent somatic alterations in murine APL cells (4). These results collectively indicate that PML-RARA fusion is necessary but not sufficient to produce APL in murine transgenic models. However, in the majority of humans with APL and in most murine APL models, the identity of cooperating disease alleles has not been revealed.

Whole genomic sequencing

In this issue of the *JCI*, Wartman and colleagues used massive parallel DNA sequencing in an effort to perform systematic mutational analysis of the murine APL genome (10). Until recently, whole genome sequencing of primary murine and human tumors was not feasible due to cost and the requirement for large amounts of tumor material. However, these limitations have largely been overcome due to improved sequencing technology and analytic tools (11). Indeed, previous efforts by the current investigators using whole genome sequencing in human patients with acute myelogenous leukemia (AML) has allowed them to identify novel clinically and biologically relevant AML mutations, demonstrating the power of massive parallel sequencing (11, 12).

Wartman et al. used an innovative strategy to find additional mutations in this APL model (10). An inbred mouse strain was used in an attempt to reduce the number of variants, as many of the variants found in sequencing a murine genome may not be relevant to disease pathogenesis. Mice expressing the *PML-RARA* transgene under the control of the murine cathepsin G promoter were backcrossed to the Black 6/Taconic background for 10 generations. These mice developed an APL-like disease with a relatively long latency (9–12 months), suggesting the acquisition of additional genetic events is required for APL development in this model. In previous human studies, tumor whole genome sequencing data were compared with sequencing data from matched germline DNA to assess whether candidate mutations were present in the germline or were bona fide somatic mutations acquired during tumorigenesis. In contrast, here the authors compared the spectrum of single nucleotide variants present in murine APL cells with a sequenced genome of the initial mouse strain. An alternative, and perhaps more discriminating, strategy might have compared the APL mutational data with DNA from littermate controls or compared the murine APL genome with hematopoietic DNA from the same mouse from an earlier time point, before APL development.

Six nonsynonymous mutations were identified and validated as being present in the APL genome; the authors then performed secondary mutational analysis of 89 additional mouse APL samples for these 6 mutations. Importantly, this approach allowed them to identify that one mutation, *Jak1* V658F, was present as a recurrent alteration in murine APL. Of note, the *Jak1* V658F mutation occurs at the homologous position to JAK2 V617, which is commonly mutated in patients with myeloproliferative neoplasms (MPNs) (13), and has been observed previously in patients with high-risk acute lymphoblastic leukemia (ALL) (14). They then ectopically expressed Jak1 V658F in mCG-PML-RARA bone marrow, followed by transplantation into lethally irradiated recipients, which resulted in a short latency, completely penetrant APL phenotype. In addition, they also performed high-resolution copy number analysis of the murine tumors and identified a somatic deletion of a histone demethylase, lysine (K)-specific demethylase 6A (*Kdm6a*, also known as *Utx*), in the same murine APL genome, a deletion also observed in human APL. However, its functional contribution to APL pathogenesis and to APL development in this mouse model has not been elucidated.

Mutations in the JAK/STAT pathway and targeted therapies

The JAKs are involved in the transduction of cytokine receptor signaling. These kinases (JAK1, JAK2, JAK3, and Tyk2) bind to the cytosolic domains of cytokine receptors. Conformational changes occur in the cytokine receptor as a result of binding to cytokines, allowing them to recruit JAKs. Somatic mutations in JAKs have been described in human malignancy (Table 1). Most notable has been the elucidation of the role of *JAK2* V617F mutation in MPN pathogenesis. Somatic *JAK1* mutations have been recently described in AML (15, 16) and in ALL (17). These genetic observations have led to the development of JAK inhibitors, including inhibitors with JAK1 inhibitory activity; these agents have now entered late-stage clinical trials in patients with MPN. Here, the investigators used a pan-JAK inhibitor in the context of a methylcellulose assay to show that JAK1 inhibition exhibited similar efficacy to all-trans retinoic acid (a standard treatment for APL) in reducing APL colony formation. Furthermore, the use of the pan-JAK inhibitor decreased STAT5 phosphorylation, indicating on-target effects with regards to the JAK/STAT pathway. Collectively, these data indicate a role for the JAK/STAT pathway in the pathogenesis of APL in this model that extends beyond the presence of a *JAK* mutation. The role of the JAK/STAT pathway in APL and in other AML subtypes thus warrants further investigation. In addition, whether *JAK1*-mutant APL constitutes a specific clinicopathologic subtype of APL with prognostic or therapeutic relevance remains to be delineated.

Conclusions

Wartman and colleagues successfully employed an elegant use of whole genome sequencing as a dragnet to ensure broad coverage of the genome. It is possible that this strategy may not identify all relevant disease alleles, due to the sequencing approach, the analytic platform, or the presence of large mutations that may be missed by short-read parallel sequencing. However, the strength of this approach is demonstrated in this report, as the authors show how whole genome sequencing can be used to provide pathogenetic insight in murine cancer models. We predict that subsequent utilization of this approach will allow investigators to add further to the list of recurrent driver mutations that contribute to malignant transformation.

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Dietary nitrate, nitric oxide, and restenosis

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Endothelium-derived NO controls the contractility and growth state of the underlying vascular smooth muscle cells and regulates the interaction of the vessel wall with circulating blood elements. Acute injury of the vessel wall denudes the endothelial lining, removing homeostatic regulation and precipitating a wave of events leading to myointimal hyperplasia. In this issue of the *JCI*, Alef and colleagues provide evidence that in the injured vessel wall, the disruption of the NOS pathway is countered by induction of xanthine oxidoreductase, an enzyme capable of producing NO from nitrite. In addition, they link low dietary nitrite levels to increased severity of myointimal hyperplasia following vessel injury in mice.

The role of endothelium-derived NO in vascular homeostasis

The endothelium is a diaphanous film of tissue comprising a single-cell monolayer that lines the luminal surface of all blood vessels. Despite its fragility, this delicate membrane exerts dramatic control over vascular homeostasis. Endothelium-derived factors control the contractility and growth state of the underlying VSMCs, and regulate the interaction of the vessel wall with circulating blood elements. NO is arguably the most influential of these paracrine substances.

NO is derived from the metabolism of L-arginine by NOS. NO activates soluble guanylate cyclase to produce cGMP, which participates in signaling pathways involving phosphorylation (1). Furthermore, NO regulates the activities of enzymes and signaling proteins by S-nitrosylation of thiol moieties (2). The endothelial synthesis of this potent molecule is highly regulated, in response to hemodynamic (e.g., shear stress) as well as humoral (e.g., serotonin) factors (3). Endothelium-derived NO suppresses platelet adhesion, leukocyte infiltration, and VSMC proliferation and migration (3). Finally, endothelium-derived NO suppresses the endothelial expression of adhesion molecules and chemokines that would otherwise initiate vascular inflammation (3). Cardiovascular risk factors (e.g., hypercholesterolemia, diabetes mellitus) impair the synthesis and bioactivity of endothelium-derived NO (4, 5). Their adverse effect on the NOS pathway is mediated by asymmetric dimethylarginine (ADMA; the endogenous antagonist of NO synthesis) and by the vascular generation of superoxide anion (O_2^-), which degrades NO to peroxynitrite anion ($ONOO^-$) (4, 5).

Myointimal hyperplasia: response to vascular injury and loss of endothelial homeostasis

Acute injury of the vessel wall (as occurs with balloon angioplasty) denudes the endothelium, fractures the internal elastic lamina, and damages the underlying VSMCs, removing homeostatic regulation and precipitating a wave of events that can lead to myointimal hyperplasia. Platelets adhere to collagen in the damaged vessel wall, and secrete PDGF and other proliferative factors. Infiltrating immune cells secrete inflammatory cytokines that contribute to cellular proliferation, and injured VSMCs release FGF. Vascular progenitor cells circulating in the blood may also contribute to the injury response (6). In the absence of the moderating influence of the endothelium, VSMCs from the media proliferate and migrate into the intimal space, and secrete extracellular matrix. This phenotypic modulation is transcriptionally regulated by CArG box DNA sequences within promoter chromatin of VSMC genes (7).

At the site of vascular injury, arginase is induced, converting arginine to urea and ornithine (8). Ornithine serves as a precursor for polyamines that are known to stimulate VSMC proliferation. Although the endothelial source of NO generation is lost, the activated VSMCs and the infiltrating immune cells express the inducible form of NOS (iNOS). Unfortunately, this enzyme is an imperfect replacement for endothelial NO synthase (eNOS). Unlike eNOS, which produces small amounts of NO in a compartmentalized and regulated fashion, iNOS is constitutively active, with a V_{max} that is a thousand-fold that of eNOS. As a result, iNOS quickly outstrips the reduced supply of arginine and donates electrons to oxygen, generating O_2^- (9). Superoxide anion combines with NO to form the cytotoxic radical $ONOO^-$. Subsequent activation of oxidant-sensitive transcriptional pathways increases VSMC proliferation and migration (10), contributing to the myointimal lesion.

Conflict of interest: John P. Cooke is an inventor on Stanford University patents related to therapeutic modulation of the NOS pathway.

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