for discussions. This work was supported by the California Institute for Regenerative Medicine (to J.-A. Lee) as well as by Pfizer, the American Federation for Aging Research, and the NIH (to F.-B. Gao).

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1. Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. 2008. Autophagy fights disease through cellular self-digestion. *Nature.* **451**:1069–1075.
2. Komatsu, M., et al. 2006. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature.* **441**:880–884.
3. Hara, T., et al. 2006. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature.* **441**:885–889.
4. Engelender, S. 2008. Ubiquitination of alpha-synuclein and autophagy in Parkinson's disease. *Autophagy.* **4**:372–374.
5. Sarkar, S., et al. 2007. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat. Chem. Biol.* **3**:331–338.
6. Yu, W.H., et al. 2005. Macroautophagy — a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. *J. Cell Biol.* **171**:87–98.
7. Lee, J., Beigneux, A., Ahmad, S.T., Young, S.G., and Gao, F.B. 2007. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. *Curr. Biol.* **17**:1561–1567.
8. Yu, W.H., et al. 2004. Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease. *Int. J. Biochem. Cell Biol.* **36**:2531–2540.
9. Nixon, R.A., et al. 2005. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J. Neuropathol. Exp. Neurol.* **64**:113–122.
10. Pickford, F., et al. 2008. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice. *J. Clin. Invest.* **118**:2190–2199.
11. Liang, X.H., et al. 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature.* **402**:672–676.
12. Cao, Y., and Klionsky, D.J. 2007. Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. *Cell Res.* **17**:839–849.
13. Diskin, T., et al. 2005. Closed head injury induces upregulation of Beclin 1 at the cortical site of injury. *J. Neurotrauma.* **22**:750–762.
14. Palop, J.J., et al. 2003. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proc. Natl. Acad. Sci. U. S. A.* **100**:9572–9577.
15. Rockenstein, E., Mallory, M., Mante, M., Sisk, A., and Masliaha, E. 2001. Early formation of mature amyloid-beta protein deposits in a mutant APP transgenic model depends on levels of Abeta(1-42). *J. Neurosci. Res.* **66**:573–582.
16. Shibata, M., et al. 2006. Regulation of intracellular accumulation of mutant huntingtin by beclin 1. *J. Biol. Chem.* **281**:14474–14485.
17. Qu, X., et al. 2003. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J. Clin. Invest.* **112**:1809–1820.
18. Mizushima, N., and Yoshimori, T. 2007. How to interpret LC3 immunoblotting. *Autophagy.* **3**:542–545.
19. Klionsky, D., et al. 2008. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy.* **4**:151–175.
20. Kihara, A., Noda, T., Ishihara, N., and Ohsumi, Y. 2001. Two distinct Vps34 nphosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y msorting in Saccharomyces cerevisiae. *J. Cell Biol.* **152**:519–530.
21. Yue, Z., Jin, S., Yang, C., Levine, A.J., and Heintz, N. 2003. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc. Natl. Acad. Sci. U. S. A.* **100**:15077–15082.
22. Selkoe, D.J. 2001. Clearing the brain's amyloid cobwebs. *Neuron.* **32**:177–180.

A signaling pathway AKTing up in schizophrenia

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The serine/threonine protein kinase AKT (also known as PKB) signaling pathway has been associated with several human diseases, including schizophrenia. Studies in preclinical models have demonstrated that impaired AKT signaling affects neuronal connectivity and neuromodulation and have identified AKT as a key signaling intermediary downstream of dopamine (DA) receptor 2 (DRD2), the best-established target of antipsychotic drugs. A study by Tan et al. in this issue of the *JCI* strengthens links among AKT signaling, DA transmission, and cognition in healthy individuals and offers potential avenues to explore in an effort to find more effective pharmacotherapies for schizophrenia and related disorders (see the related article beginning on page 2200).

AKT (also known as PKB) is a key mediator of signal transduction processes mediated by protein phosphorylation and dephosphorylation (1) and a central node in cell signaling downstream of growth factors, cytokines, and other external stimuli. The *AKT* gene family includes three members (*AKT1*, *AKT2*, *AKT3*), which possess partially redundant functions and contribute to several cellular functions including cell growth, survival, and metabolism. AKT has several substrates, most notable among them glycogen synthase kinase 3α (GSK-3α) and GSK-3β, both of which are inhibited by AKT in response to various external cellular stimuli (1).

AKT signaling and susceptibility to schizophrenia

Gain or loss of AKT activity has been associated with several human diseases, including cancer and type 2 diabetes (1). Since the initial report in 2004 from Emamian et al. (2), accumulating evidence suggests that impaired AKT signaling also plays a role in the pathogenesis of schizophrenia. First, an association between schizophrenia and *AKT1* genetic variants (Figure 1A) has been reported in several case/control samples and family cohorts (an updated compilation of such studies is now available; see ref. 3). Second, a number of studies have provided convergent evidence of a decrease in *AKT1* mRNA, protein, and activity levels (reflected by changes in substrate phosphorylation) in brains of some individuals with schizophrenia (2, 4, 5). Negative findings have also been reported in some patient cohorts (3), but overall, an increasingly strong link between a dysregulation of AKT signaling and schizophrenia is emerging. Importantly, pharmacological evidence indicates that drugs used in the management of psychosis, such as the typical antipsychotic haloperidol as well as several atypical antipsychotics, can act as enhancers of AKT signaling in vivo or in vitro by directly activating AKT or by increasing the phosphorylation of its substrates GSK-3α and GSK-3β (reviewed in ref. 6).

Nonstandard abbreviations used: COMT, catechol-O-methyltransferase; DA, dopamine; DRD2, dopamine receptor 2; GSK-3α, glycogen synthase kinase 3α; PFC, prefrontal cortex.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:2018–2021 (2008). doi:10.1172/JCI35931.

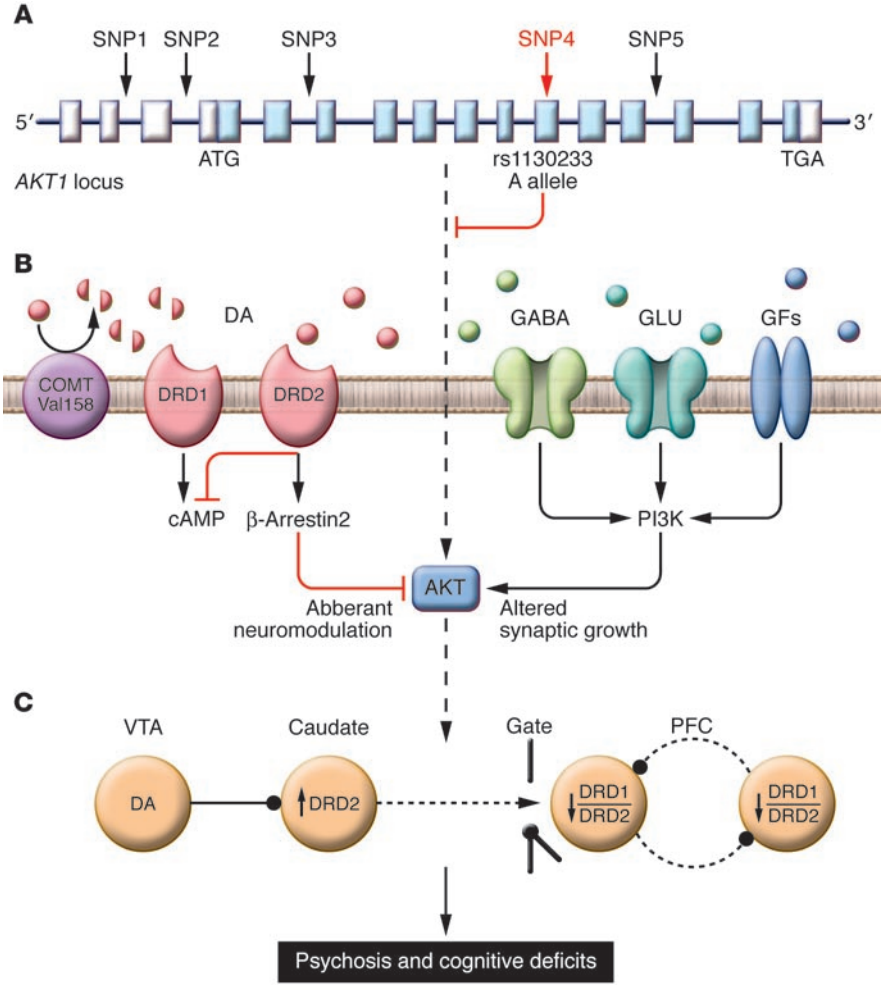

Figure 1

AKT1 genetic variation and its impact on multiple levels of neuronal function. (**A**) Indicated are the 5 SNPs within the *AKT1* locus that were examined by Emamian et al. (2), as well as by Tan et al. in their current study in this issue of the *JCI* (16). The rs1130233 SNP that reduces expression of *AKT1* appears in red. *AKT1* has 16 exons (boxes). Coding sequence (blue boxes) flanked by the start (ATG) and stop (TGA) codons as well as expressed noncoding sequence (white boxes) are indicated. (**B**) AKT is a key node for various signaling systems. DA in the cortex is inactivated by COMT and activates DRD1-coupled cAMP signaling whereas DRD2 decreases cAMP levels and inhibits AKT activity via β-arrestin2. GABA, glutamate (GLU), and various growth factors (GFs) also modulate AKT activity via PI3K, resulting in alterations in synaptic growth and transmission. (**C**) These combined effects on DA modulation and synaptic connectivity may alter the function of cells (circles) within neuronal circuits important for cognitive function. The caudate of the basal ganglia plays a critical role in gating information and restricting access to working memory, which relies on proper connections among cortical neurons in the PFC. The gating of information itself is heavily dependent on DA transmission, which originates from the ventral tegmental area (VTA). One possible, although probably oversimplified, scenario is that sensitization to DA via DRD2 may lead to psychotic symptoms and, coupled with altered neuronal connectivity and decreased DRD1 signaling, also contribute to cognitive dysfunction. Dashed lines represent the functional impairments resulting from the SNP4-dependent attenuation of *AKT1* expression and signaling.

AKT signaling and dopamine transmission

In elucidating the molecular basis of the link between AKT signaling and schizophrenia, one of the first clues came from the observation that mice lacking the *Akt1* gene show enhanced sensitivity to disruption of sensory motor gating by amphetamine (2). One action of this drug is to cause the release of dopamine (DA) at nerve terminals and thus increase DA transmission via G protein–coupled DA receptors. The two major classes of DA receptors, the DA receptor 1 (DRD1) class and the DA receptor 2 (DRD2) class, signal through *increases* and *decreases*, respectively, in cAMP levels within nerve cells. Sensitivity to amphetamine is widely used to model psychosis in rodents and is efficiently prevented by blocking DRD2, the best-established target of antipsychotic drugs (6–9). Moreover, *Akt1*-knockout mice show a more pronounced deficit in response to DRD2 class but not DRD1 class agonists in working memory tests that depend on proper function of prefrontal cortex (PFC) (10). In a series of elegant experiments, Caron and colleagues have established that AKT is a novel and key signaling intermediary downstream of DRD2 class receptors (6, 8, 11). These experiments demonstrate that DRD2 class receptors are essential for the inhibition of AKT by DA and that AKT function is important for normal dopaminergic transmission and expression of DA-associated behaviors in a manner distinct from cAMP-dependent signaling. Thus, a hypothesis has been formulated according to which a partial loss of function of AKT1 in schizophrenia, mimicking DA overactivity, results in exacerbated responses to DRD2 class receptor stimulation (Figure 1B). Antipsychotics acting by blocking DRD2 class receptors could correct this imbalance by preventing further reductions of AKT activity by these receptors (10, 11).

AKT signaling and neuronal connectivity

AKT signaling has also been implicated in the downstream cellular effects of GABA and glutamate released at nerve terminals (12, 13). In addition, AKT signaling is known to modulate the development of neuronal connectivity in vitro (14) as well as in the human brain (15). Notably, *Akt1*-deficient mice show changes in neuronal connectivity of PFC layer V neurons (10), the output neurons of the cortex. Transcriptional profiling in the PFCs of these mice identified concerted changes in genes controlling synaptic function, neuronal development, myelination, and actin polymerization. Thus, impaired AKT and, in particular, AKT1 signaling may have an additive effect on the properties of neural networks, resulting in neuronal disconnectivity as well as aberrant neuromodulation. How these effects interact at the circuit level and neural systems levels to modulate the input/output function of specific brain areas is the focus of active investigation.

From neurons to neural systems and cognition

Along these lines, in the current issue of the *JCI*, Tan and colleagues report on their investigations of whether genetic variation in the *AKT1* gene influences structural and functional measures sensitive to changes in cortical DA signaling (16). This approach investigated discrete and diagnostically independent disease components rather than clinical entities. The authors started with 5 *AKT1* SNPs previously associated with schizophrenia and investigated in healthy human subjects which of these markers was associated with a cognitive test battery designed to measure a wide range of cognitive processes such as attention, memory, language comprehension, and processing speed. They found the strongest associations with the A allele of the *AKT1* SNP rs1130233 (Figure 1A) and certain indices of frontal lobe function, such as IQ and executive function. In line with animal research implicating reduced AKT function in cognitive deficits (10), the A allele of rs1130233 was also associated with reduced *AKT1* expression in B lymphoblasts of healthy individuals. Examining *AKT1* expression in these peripheral cells offers a compromise because it is not possible to directly assess the impact of genetic variants in live human brain tissue. These observations thus provide a possible link between reduced AKT1 function and cognition in humans.

In order to examine more closely how AKT may influence cognition, the authors administered an explicit task of working memory and executive function, the N-back task, which measures the ability to remember and manipulate information in short-term memory. Healthy human subjects who varied in their genotypes at the *AKT1* locus completed the N-back task while undergoing functional MRI (16). Importantly, optimal execution of this task critically depends on proper levels of DA within the PFC: either too much or too little DA can impair performance (17). Interestingly, those individuals who carried the *AKT1* rs1130233 A allele had relatively inefficient information processing in the PFC. Thus, in order to achieve the same level of performance, these volunteers required increased activation of their PFCs. This may result from the impact of AKT on DA function, its role in establishing cortical connections, or both. To begin addressing this question, the authors also examined a functional polymorphism (Val158Met) of the catechol-O-methyltransferase (*COMT*) gene, which is responsible for breaking down DA, particularly within the PFC; the presence of the Val158 allele leads to lower DA levels. Previous work in preclinical models has shown that variation in the activity of this gene can modulate the effects that other schizophrenia susceptibility loci have on cortical DA transmission and related behaviors (18). Tan et al. (16) found that carriers of the *AKT1* rs1130233 A allele and two *COMT* Val158 alleles had disproportionately inefficient prefrontal cortical processing, suggesting perhaps a genetic interaction between these two DA-related genes. Interestingly, they also found *AKT1* rs1130233 A allele carriers had reduced gray matter volumes of the caudate nucleus of the basal ganglia and the right PFC, with the latter again disproportionately reduced in those individuals also carrying two *COMT* Val158 alleles. These results may indicate how genetic variation of *AKT1* may increase risk for psychiatric disorders, such as schizophrenia, which is linked to DA and PFC dysfunction. Indeed, the authors provide further evidence for association of the *AKT1* rs1130233 A allele and the diagnosis of schizophrenia.

Unresolved questions and therapeutic prospects

Several questions remain, however, as to exactly how impaired AKT signaling leads to impaired cognition. There is a clear link between DA and AKT signaling (11), and the current study links AKT-dependent DA signaling with working memory in humans (16). However, AKT requires a timescale of minutes to achieve its effects on DRD2 signaling, whereas working memory operates on a timescale of seconds (11). It is interesting that the basal ganglia play a critical role in regulating information flow within working memory networks (19), but this gating is also rapid, much faster than the immediate influence of AKT on DRD2 signaling in either the caudate or PFC. It is possible, however, that long-term AKT deficiency reorganizes cortical and even perhaps basal ganglia networks and their functional modulation by DA. Indeed, DRD2 sensitization resulting from reduced AKT function coupled with decreased cortical DRD1 signaling resulting from increased COMT activity may alter the DRD1/DRD2 activation ratio and affect how information is processed in the PFC (Figure 1C) (17). This may ultimately contribute to cognitive deficits and predisposition to schizophrenia.

The observation that both typical and atypical antipsychotics can compensate for impaired AKT signaling in schizophrenia coupled with the more recent observation that lithium (a pharmacological agent used for the management of bipolar disorder, schizophrenia, and depression) also directly regulates AKT signaling (7) suggest that this pathway may be a prime target for the design of effective targeted therapies for patients with specific genetic predisposition to schizophrenia or related disorders. However, it also highlights concerns regarding current schizophrenia pharmacotherapies, which primarily target DRD2 class receptors yet are inadequate in improving cognition (20) and produce serious side effects. At this point, it remains unclear how therapies that target AKT signaling will benefit the schizophrenia-associated cognitive measures linked to this pathway (16). On the other hand, the gene expression changes associated with *AKT1* risk variants are low and the development of serious side effects due to the multifunctionality of the AKT pathway may be avoided if directed therapies only need to provide relatively modest functional enhancement in order to reach an effective threshold. Moreover, because the *AKT1* risk variants are common, a low disease risk corresponds to a large population attributable risk (if the population were monomorphic for the nonrisk allele, the prevalence of the disease would be considerably lower). Therefore, AKT signaling pathway–directed therapies may be beneficial to a large group of patients.

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1. Woodgett, J.R. 2005. Recent advances in the protein kinase B signaling pathway. *Curr. Opin. Cell Biol.* **17**:150–157.
2. Emamian, E.S., Hall, D., Birnbaum, M.J., Karayiorgou, M., and Gogos, J.A. 2004. Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia. *Nat. Genet.* **36**:131–137.
3. Schizophrenia Research Forum. 2008. Gene overview of all published schizophrenia-association studies for AKT1. http://www.schizophreniaforum.org/res/sczgene/geneoverview.asp?geneid=6.
4. Thiselton, D.L., et al. 2008. AKT1 is associated with schizophrenia across multiple symptom dimensions in the Irish study of high density schizophrenia families. *Biol. Psychiatry.* **63**:449–457.

5. Zhao, Z., Ksiezak-Reding, H., Riggio, S., Haroutunian, V., and Pasinetti, G.M. 2006. Insulin receptor deficits in schizophrenia and in cellular and animal models of insulin receptor dysfunction. *Schizophr. Res.* **84**:1–14.
6. Beaulieu, J.M., et al. 2007. Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. *J. Neurosci.* **27**:881–885.
7. Beaulieu, J.M., et al. 2008. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell.* **132**:125–136.
8. Beaulieu, J.M., et al. 2005. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell.* **122**:261–273.
9. Beaulieu, J.M., et al. 2004. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. U. S. A.* **101**:5099–5104.
10. Lai, W.S., et al. 2006. Akt1 deficiency affects neuronal morphology and predisposes to abnormalities in prefrontal cortex functioning. *Proc. Natl. Acad. Sci. U. S. A.* **103**:16906–16911.
11. Beaulieu, J.M., Gainetdinov, R.R., and Caron, M.G. 2007. The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol. Sci.* **28**:166–172.
12. Wang, Q., et al. 2003. Control of synaptic strength, a novel function of Akt. *Neuron.* **38**:915–928.
13. Qin, Y., et al. 2005. State-dependent Ras signaling and AMPA receptor trafficking. *Genes Dev.* **19**:2000–2015.
14. Jaworski, J., et al. 2005. Control of dendritic arborization by the phosphoinositide-3′-kinase-Akt-mammalian target of rapamycin pathway. *J. Neurosci.* **25**:11300–11312.
15. Boland, E., et al. 2007. Mapping of deletion and translocation breakpoints in 1q44 implicates the serine/threonine kinase AKT3 in postnatal microcephaly and agenesis of the corpus callosum. *Am. J. Hum. Genet.* **81**:292–303.
16. Tan, H.-Y., et al. 2008. Genetic variation in *AKT1* is linked to dopamine-associated prefrontal cortical structure and function in humans. *J. Clin. Invest.* **118**:2200–2208.
17. Winterer, G., and Weinberger, D.R. 2004. Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. *Trends Neurosci.* **27**:683–690.
18. Paterlini, M., et al. 2005. Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. *Nat. Neurosci.* **8**:1586–1594.
19. McNab, F., and Klingberg, T. 2008. Prefrontal cortex and basal ganglia control access to working memory. *Nat. Neurosci.* **11**:103–107.
20. Green, M.F. 2007. Cognition, drug treatment, and functional outcome in schizophrenia: a tale of two transitions. *Am. J. Psychiatry.* **164**:992–994.

Is CD133 a marker of metastatic colon cancer stem cells?

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The concept of the so-called cancer stem cell (CSC) holds that only a minority of cells within a tumor have the ability to generate a new tumor. Over the last decade, a large body of literature has implicated the protein CD133 as a marker of organ-specific adult stem cells and in some cancers as a bona fide CSC marker. In this issue of the *JCI*, Shmelkov et al. challenge the view that CD133 is a marker of CSCs in colon cancer (see the related article beginning on page 2111). CD133 was thought previously to have a very restricted distribution within tissues; the authors have used genetic knock-in models to demonstrate that CD133 in fact is expressed on a wide range of differentiated epithelial cells in adult mouse tissues and on spontaneous primary colon tumors in mice. In primary human colon tumors, all of the epithelial cells also expressed CD133, whereas metastatic colon cancers isolated from liver had distinct $CD133^+$ and $CD133^-$ epithelial populations. Intriguingly, the authors demonstrate that the $CD133^+$ and $CD133^-$ populations were equally capable of tumor initiation in xenografts. In light of these new findings, the popular notion that CD133 is a marker of colon CSCs may need to be revised.

Until now, there has been little controversy over whether the protein CD133 is a marker of cancer stem cells (CSCs). Originally described as a marker of normal hematopoietic stem cells (1, 2), it has gained more prominence as a marker of CSCs in solid primary tumors such as medulloblastomas and glioblastomas (3, 4) and subsequently of CSCs in a growing number of cancers of epithelial tissues. In their study in this issue of the *JCI*, Shmelkov et al. challenge this increasingly influential dogma; first, on the basis of CD133's wide distribution in many epithelial tissues, and second, because CD133 expression does not necessarily correlate with the ability of colon tumors to metastasize (Figure 1) (5).

Nonstandard abbreviations used: CSC, cancer stem cell; EpCAM, epithelial cell adhesion molecule.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:2021–2024 (2008). doi:10.1172/JCI36046.

Normal distribution of CD133 in vivo

CD133 (also known as prominin-1) is a surface protein with five transmembrane domains. Still mysterious in its function, this pentaspan has drawn a lot of attention since its discovery in 1997 on normal human hematopoietic stem cells (1, 2). The simultaneous discovery of the mouse homolog and its implications in neurogenesis opened up new directions for studying human neural stem cells (6, 7); CD133 has been linked in several reports to multiple organ-specific stem cells and referred to as "the molecule of the moment" (8).

Prior to the current report by Shmelkov et al. in this issue of the *JCI* (5), studies of CD133 as a normal stem cell marker or a CSC marker have used primarily one monoclonal antibody against CD133, the clone known as AC133, which marks an epitope of CD133 at the cell surface (1, 2). Using a single monoclonal antibody to define a stem cell marker is usually not sufficient, and it is not clear to us why such a practice was so widely accepted. The current study by Shmelkov et al. is, to our knowledge, the first that uses a knock-in reporter mouse to track expression of CD133, both temporally and spatially, in normal tissues and during tumorigenesis in vivo. In their previous studies, Shmelkov et al. dissected the regulatory region of the human *CD133* gene, demonstrating that CD133 expression was regulated in a tissue-specific manner by multiple alternative promoters (9). Importantly, they identified similarities among the mouse and human regulatory regions of *CD133*, which paved the way for the design of their CD133 reporter