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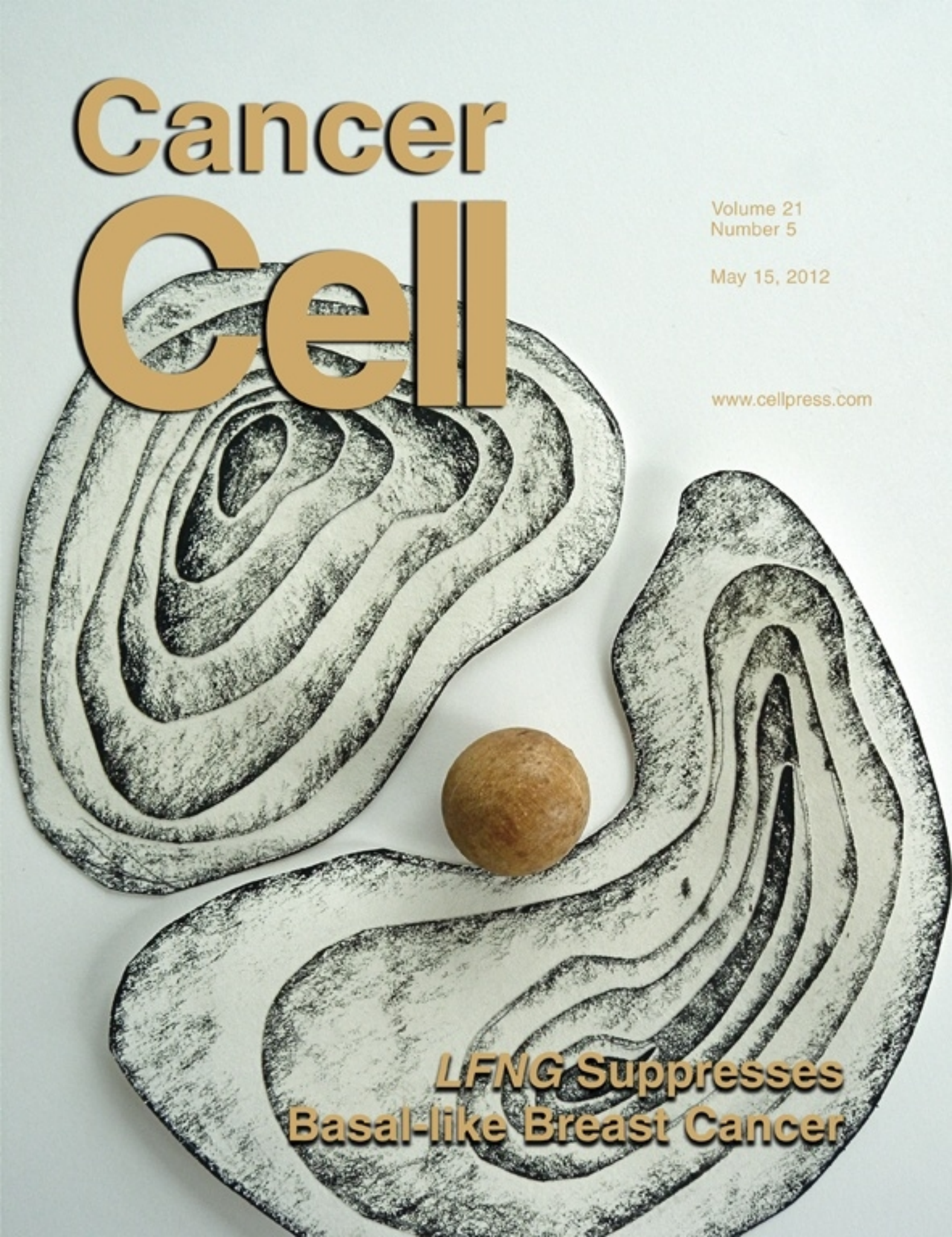

LFNG Suppresses Basal-like Breast Cancer

There's a Time and a Place for *MYCN*

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Brain tumors display extensive diversity. In this issue of *Cancer Cell*, Swartling et al. provide evidence that the temporal and spatial transcriptional programs in neural stem cells underlie a diverse response to the MYCN oncogene, potentially contributing to cancer diversity.

The billions of neurons and glia that form the central nervous system (CNS) arise from neural stem cells (NSCs) derived from the neural tube (Kriegstein and Alvarez-Buylla, 2009). Throughout development, the daughters of NSCs follow topographically and temporally discrete cellular programs; spinal neurons are distinct from those that form in the cortex, and neurogenesis precedes gliogenesis in the forebrain. These programs are controlled by a combination of cell intrinsic and extrinsic factors e.g., chromatin marks and morphogens (Gräff et al., 2011; Ihrie and Alvarez-Buylla, 2011). Recent evidence suggests that highly specific developmental programs might render cells peculiarly susceptible to particular oncogenic events, resulting in the formation of discrete subgroups of cancer (Gilbertson, 2011). For example, robust subgroups of the brain tumors ependymoma and medulloblastoma contain global gene expression profiles that reflect their developmental and molecular origins in the CNS (Gibson et al., 2010; Johnson et al., 2010; Kawauchi et al., 2012; Pei et al., 2012).

In this issue of *Cancer Cell*, Swartling et al. (2012) investigate how different mouse NSC populations respond to aberrant expression of *MYCN*—an oncogene commonly deregulated in various human brain tumors. Through a series of elegant in vitro and in vivo studies, the authors challenged cells from different regions of the developing CNS with wild-type or mutant *MYCN*. They show that distinct populations of NSCs display different cellular response to this oncogene, including transformation capacity, and ultimately the type of tumor produced (Figure 1).

First, using their previously reported GTML mouse model of *MYCN*-driven medulloblastoma (Swartling et al., 2010), the authors show that MYCN drives Sonic Hedgehog (SHH) independent proliferation of GTML cells and that tumor growth likely correlates with *MYCN* expression in these tumors. Switching to the developing CNS, the authors then surveyed potential susceptibility to N-myc driven transformation by assessing the response of NSCs from different neurogenic regions to wild-type (N-Myc^{WT}) or stabilized mutant N-Myc^{T58A}. N-Myc^{T58A}, but not N-Myc^{WT}, significantly increased the proliferation of cerebellar and forebrain NSCs isolated from either embryonic day 16 (E16) or postnatal day 0 (P0) mice. In contrast, E14 rather than E16 lower rhombic lip progenitors (LRLPs), proliferated in response to N-Myc^{T58A}; confirming previous reports that these cells are susceptible to transformation early in development (Gibson et al., 2010) and revealing temporal differences in susceptibility to N-Myc.

In addition to inducing proliferation, N-Myc^{T58A} also appeared to subvert intrinsic cell signals in certain NSCs. Once again, this effect varied with the time and the place from which NSCs were isolated. Prompted by changes in Sox9 expression that acts downstream of SHH signaling in NSCs (Scott et al., 2010), the authors noted that while N-Myc^{T58A} promoted SHH-independence in E16 cerebellar and P0 forebrain cells, this did not appear to occur in P0 cerebellar and E16 forebrain cells.

Armed with these data, the authors asked whether cell context might dictate the capacity of N-Myc^{WT} or N-Myc^{T58A} to drive tumorigenesis in the CNS. In keeping with their observations in vitro, N-Myc^{WT}-transduced NSCs failed to form tumors when orthotopically implanted in mice. But N-Myc^{T58A} drove transformation of E16 and P0 cerebellar and forebrain NSCs as well as E14, but not E16 or P0, LRLPs. Interestingly, N-Myc^{T58A}-transduced P0 cerebellar NSCs formed tumors in the cerebellum that resembled human and mouse GTML medulloblastoma, while N-Myc^{T58A}-transduced P0 forebrain NSCs generated gliomas in the cerebrum. To clarify the influence of intrinsic versus extrinsic factors on the type of brain tumor formed in these models, the authors transplanted P0 forebrain and cerebellar NSCs, each transduced with N-Myc^{T58A}, into the cerebellum and forebrain, respectively. Their results suggest that the cell of origin plays a dominant role in determining tumor phenotype, as forebrain cells still made gliomas in the cerebellum, and cerebellar cells made primitive neuroectodermal tumors in the forebrain. The only difference noted was a modest reciprocal change in Sox9 and Olig2 within "misplaced" tumor implants. So while programs intrinsic to particular NSCs may primarily determine the course of tumorigenesis, a role for environmental influence in tumor specification remains a possibility.

Together, these data support a growing body of evidence that the cellular origin of cancers heavily dictates disease phenotype and biology and that this lineage relationship is often revealed by a cancer's transcriptome (Gilbertson, 2011). Indeed, using Affymetrix exon arrays, Swartling et al. (2012) identified a close alignment between the gene expression patterns of N-Myc^{T58A} tumors and the corresponding, originating, cerebellar or forebrain NSC. In an additional twist on this theme, the authors showed that NSCs derived from different developmental stages of the cerebellum generate distinct forms of medulloblastoma. Medulloblastomas derived from

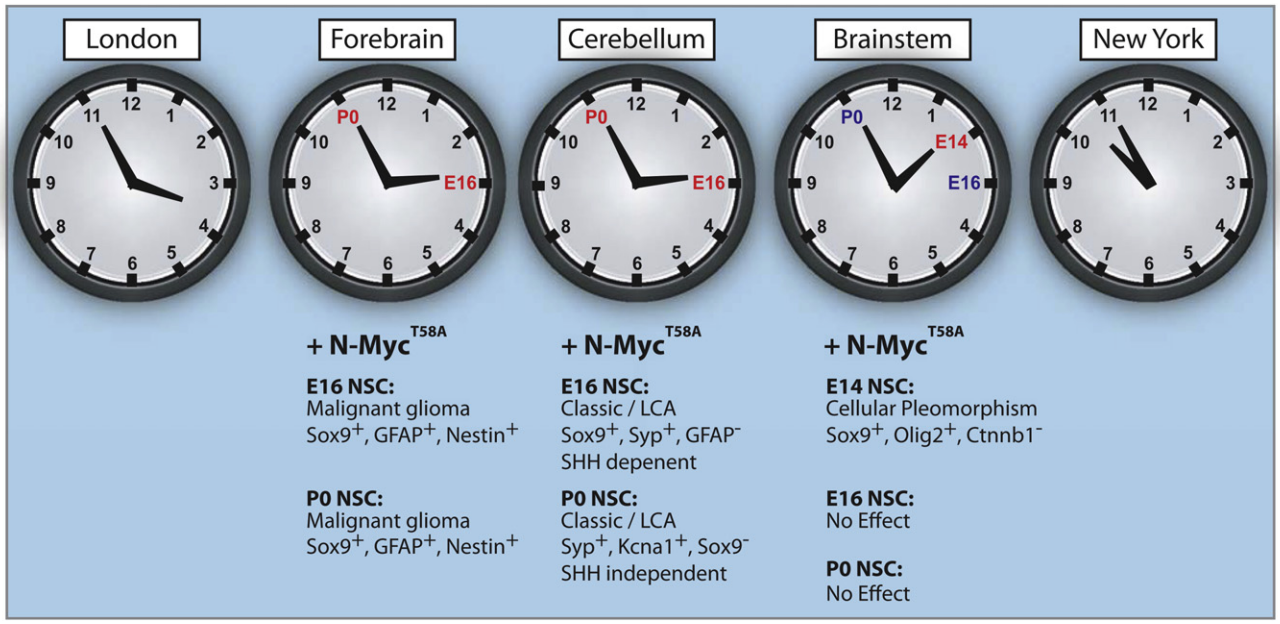

Figure 1. There Is a Time and a Place for N-Myc-Driven Tumorigenesis in the CNS
Swartling et al. (2012) show that different NSCs in the mouse CNS are differentially susceptible to transformation by N-Myc, driving time and location-specific tumors.

E16 cerebellar cells expressed high levels of *Sox9*, *Mycn*, and the granule neuron precursor marker *Math1*, suggesting a Shh-dependent form of the disease. In contrast, medulloblastomas derived from P0 cells displayed lower expression of *Sox9*, *Mycn*, and *Math1* compatible with a SHH-independent origin. Forebrain N-Myc^{T58A} P0 tumors (P0 glioma) also showed high levels of *Sox9* and *Mycn* with no *Math1*, consistent with transformation of a forebrain cell type that shows Shh-dependence independently of Math1.

Finally, the authors used their model systems to begin to unravel some of the cell signals that drive tumorigenesis in concert with N-Myc. Focusing on Sox9, the authors investigated whether this putative effector of SHH signaling plays a functional role in transforming P0 cerebellar NSCs. Overexpression of Sox9 with N-Myc^{T58A} in P0 cerebellar NSCs suppressed proliferation but enhanced self-renewal, ultimately causing tumors to arise with shorter latencies and higher penetrance. Tumors driven by Sox9 with N-Myc^{T58A} also showed elevated levels of *Gli2*, and focal expression of GFAP, not seen in those driven by N-Myc^{T58A} alone. These studies reveal potential cooperative interactions between N-Myc and Sox9 in the development of brain tumor subtypes.

This work by Swartling et al. (2012) represents an important step forward in understanding the mechanisms that contribute to tumor diversity in the CNS. Using a single oncogene, *MYCN*, the authors were able to generate different types of CNS tumor by varying the age and location of the originating NSC. These data not only confirm a role for *MYCN* in forebrain and hindbrain tumorigenesis but also underscore the emerging importance of the originating cell type in dictating the cancer phenotype. The observation that N-Myc^{T58A} drives anaplastic medulloblastomas from E14 LRLPs independent of WNT signaling is noteworthy, because these same cells were shown to generate WNT-subgroup medulloblastomas (Gibson et al., 2010). Thus, LRLPs that reside outside the cerebellum might be a source of multiple forms of medulloblastoma. Comparison of Swartling's *MYCN*-driven model with recently reported tumors driven by *MYC* should also help unravel the different roles of these "sister" oncogenes in medulloblastoma (Kawauchi et al., 2012; Pei et al., 2012). The authors note appropriately that the originating cell types in their models are yet to be defined formally as NSCs, but this does not detract from the importance of their work that demonstrates time and location specific tumorigenesis in the CNS. The data and models generated by Swartling et al. (2012) should provide an extremely useful resource to the brain tumor research community as we seek to unravel the diversity of these diseases and develop curative therapies for all patients.

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What a Difference a Phosphate Makes: Life or Death Decided by a Single Amino Acid in MDM2

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In this issue of *Cancer Cell*, Gannon and colleagues create genetically engineered mice to test the role phosphorylation plays in the modification of one serine long thought to play a critical role in controlling the activity of MDM2, one of p53's main negative regulators.

The tumor suppressor p53 is activated by numerous stressors and results in expression or repression of hundreds of genes that elicit a broad range of biological responses culminating in effective tumor suppression. However, p53 activation must be controlled with exquisite care because as little as a 2-fold reduction in its activity can cause radio resistance and increased tumorigenicity (Bond et al., 2004; Wang et al., 2009). Conversely, a 2-fold increase in p53 activity such as in $p53^{7KR/7KR}$ mice (a knock-in model in which seven conserved C-terminal lysine residues were replaced by arginine) can lead to myeloblation and death by heart failure (Wang et al., 2011).

Ubiquitin-mediated proteolysis is central to controlling p53's protein level and activity. In unstressed cells, the E3 ubiquitin-ligase MDM2 recruits E2 ubiquitin-conjugating enzymes to transfer ubiquitins onto p53 and MDM2 itself, resulting in proteasomal degradation of both proteins. MDM4 (also known as MDMX), a protein related to MDM2 but lacking intrinsic E3 ubiquitin-ligase activity, hetero-oligomerizes with MDM2 to modulate MDM2's E3 ligase activity (Wade et al., 2010). MDM2 and MDM4 play non-overlapping and tissue-specific roles to precisely control p53 levels and activity (Wade et al., 2010). Deleting *Mdm2* typically elicits a more extreme phenotype than deleting *Mdm4*, but eliminating p53 rescues both. This demonstrates that both MDM2 and MDM4 are critical nodes in p53 regulation. *MDM2* is a p53-induced gene, and in vitro studies show that increasing MDM2 abundance can attenuate p53 activation, leading p53 to return to low basal levels upon resolution of the inducing stress. The importance of this negative feedback loop for p53 regulation in vivo in different tissues remains unclear.

Correct temporal control of p53 responses is critical, but how this is achieved in vivo remains to be resolved. Posttranslational modifications play critical roles in p53 regulation, so the residues of p53, MDM2, and MDM4 that are modified by damage-activated kinases, phosphatases, and other modifying enzymes are prime candidates for temporal regulators. The acceptable thresholds for p53 regulation have been dramatically revealed by studies showing that mice heterozygous for *Mdm2* or *Mdm4*, with reduced expression of *Mdm2* or *Mdm4*, or with blocked posttranslational modification at damage-modifiable residues have profoundly altered radiation responses (Bondar and Medzhitov, 2010; Wang et al., 2009). Ionizing radiation activates DNA damage-activated kinases such as ATM and CHK2, resulting in phosphorylation of multiple residues on MDM2, MDM4, and p53 (Wade et al., 2010). Studies in human cancer cell lines first suggested that preventing MDM2 Ser395 (mouse Ser394) phosphorylation could impair its damage-dependent degradation and consequently attenuate p53 activation (Maya et al., 2001). DNA damage also induces MDM4 phosphorylation at serine 341,367, and 402, resulting in its MDM2-dependent degradation. Mice expressing Mdm4 3SA, an MDM4 mutant with alanine substitutions at these three positions, are remarkably resistant to ionizing radiation due to attenuated radiation-induced p53 responses in the hematopoietic system but are very sensitive to c-Myc induced lymphomagenesis (Wang et al., 2009). Both phenotypes result from a modest 2-fold reduction in p53 basal and induced activity. These data suggest the importance of regulating MDM4 stability in vivo for controlling p53 activity.

In this issue of *Cancer Cell*, Gannon et al. (2012) demonstrate the importance of MDM2 Ser394 phosphorylation in regulating the responses of mice to irradiation by making S394A (non-phosphorylatable) and S394D (phosphomimetic) mutations. They show that this amino acid can swing the pendulum