Cancer epigenetics is no Mickey Mouse

Epigenetic changes are the most common alterations in human cancer, but it has been difficult to sort out cause and effect from studies of human tumors. Several recent nonlethal mouse models implicate both hypomethylation and loss of imprinting (LOI) in tumor formation, including a paper in this issue of *Cancer Cell* showing that transient hypomethylation in ES cells causes LOI and liver and intestinal tumors (Holm et al., 2005). Hypomethylation appears to be a critical determinant of cancer, affecting chromosomal stability and specific gene targets.

A few years ago, I found myself in the odd position of having to defend my work to a mouse, actually a costumed Mickey Mouse in Orlando after my daughter gleefully informed him, "My daddy does experiments on mice!" I tried to explain to the shocked Mickey that our community had identified countless epigenetic changes in cancer, and that, while epigenetics might explain part, or half, or most of this horrible disease, nobody else would believe it until we could recapitulate epigenetic changes in a model organism. Had Mickey not run back to his hole in the wall, I would have added that this was a particularly acute problem because epigenetic changes do not involve DNA sequence variation, but heritable alterations such as methylation that are pliable under certain conditions, and thus potentially secondary and not causal to the tumor process. Further, while genes epigenetically activated or silenced in human cancer had been shown by genetic models (transgene activation or suppressor gene silencing) to contribute to neoplasia in mice, we needed models that recapitulated the process of epigenetic derangement itself, in order to determine whether that mechanism could lead to cancer. My explanation earned me only a stern finger waving from Mickey and the opprobrious stares of nearby kids, but the approach was the right one, as illustrated by an article in this issue from Jaenisch and colleagues (Holm et al., 2005) showing that loss of imprinting, induced by hypomethylation in mouse development, causes cancer. Indeed, much of the cancer epigenetics work in mice is from his group, so I think Mickey should cut him a break as well.

Genomic imprinting is a parent of origin-specific relative or complete allelic silencing, caused by epigenetic modifications in the gamete or zygote such as DNA methylation and chromatin modification. Loss of imprinting (LOI) in cancer involves reactivation of the normally silent allele of a growth-promoting gene, and/or silencing of the normally active allele of a tumor suppressor gene. In the present paper, Holm et al. create a clever double transgenic stem cell line with a Cre-inducible *Dnmt1* knockout as well as a Flp-activatable *Dnmt1* rescue gene. By knocking out and then restoring *Dnmt1*, they erase imprinting marks, and probably also nonrecoverable marks in other genes, but then allow de novo methylation during embryogenesis. The resulting mice show loss of imprinting of imprinted genes generally, including variable activation of the silent allele of oncogenes such as *Igf2*, and silencing of the active alleles of tumor suppressor genes such as $p57^{KIP2}$ and *Igf2r*. In addition, chimeric mice develop intestinal adenomas and hepatocellular cancers. LOI appears to confer immortalization on cells and cooperate with *Hras* in in vitro transformation experiments (Holm et al., 2005).

The work that got me in hot water with Mickey also addressed loss of imprinting in cancer. We and others had originally described this epigenetic change in Wilms tumor, an embryonal tumor common in Beckwith-Wiedemann syndrome (BWS), and similar changes are also found in normal cells of Wilms tumor and BWS patients (reviewed in Feinberg and Tycko, 2004), indicating that LOI precedes, and possibly causes, cancer in these patients. LOI is also common in adults and associated with both a positive family history and a personal history of colorectal neoplasia (Cui et al., 2003; Woodson et al., 2004). For an epigenetic change such as LOI, there is also the concern that the alteration arose somehow in association with cancer but did not play a causal role. We therefore developed a mouse model, testing the hypothesis that LOI in combination with genetic mutation at the gatekeeper gene *Apc* in intestinal neoplasia would augment tumor development, i.e., that LOI increases the risk of developing cancer when a mutation occurs, the implication of the human epidemiological studies. That was indeed the case, with a 2- to 2.5-fold increased risk of tumors. Moreover, mice with LOI showed an epigenetically induced change in stem cells, with a shift in the ratio of differentiated to undifferentiated cells (Sakatani et al., 2005).

The present study of LOI mice shares two similarities and one difference with the previous work. First, both models appear to involve a disruption of stem cells that have a delayed effect on tumor incidence. In the present study, the stem cell was the direct target of transient methylation disruption, and in the paper by Sakatani et al. (2005), tissue-specific stem cells were found to be affected. The stem cell is increasingly recognized as the target for oncogenic alteration, because it already has many of the properties that are so vexing in cancer biology and therapy, such as limited replication rate, capacity for multilineage differentiation, and even metastasis.

Second, both models of the associated cancers involve *hypomethylation* rather than *hypermethylation*. While hypermethylation and/or chromatin modification is important in tumor suppressor gene silencing (Jones and Baylin, 2002), oncogene activation can involve loss of methylation, and global hypomethylation, as well as related chromatin alterations, is a defining attribute of cancer genetics (Feinberg and Tycko, 2004). Indeed, three other epigenetic mouse models from the Jaenisch group support the causal role of hypomethylation in cancer. One of these, just published, shows that hypomethylation induced by a *Dnmt1* hypomorphic knockin, and combined with the *Apc* mutation (*Min* mouse), enhances intestinal neoplasia initiation even though it retards later gross tumor formation (Yamada et al., 2005), as shown earlier. This hypomethylation also cooperates with Apc to cause liver cancers (Yamada et al., 2005), a very important result as liver cancer is the leading cancer killer in the world. Hypomethylation was also shown earlier to cause cancer in two other mouse models from the same group, aggressive T cell lymphomas in mice carrying a hypomorphic DNMT1 allele, and accelerated sarcoma forma-

tion in mice mutant for p53 and NF1, both models at least involving increased chromosomal instability caused by the loss of DNA methylation (Eden et al., 2003; Gaudet et al., 2003).

Thus, hypomethylation is a critical determinant of cancer and cancer risk. A difference between the studies by Holm et al. and Sakatani et al. is that the present paper addresses *global* LOI, demonstrating that the targets are not limited to IGF2. Clearly, IGF2 contributes, as its overexpression causes a 6-fold increase in focus formation, but less than that of global LOI. Of course, other nonimprinted genes that could not regain methylation may also be contributing to the cancer phenotype in the model by Holm et al. One should also not overlook the important work being done in mouse knockout models of the DNA methylation recognition machinery (Sansom et al., 2003), or knockout of chromatin modifying genes, such as *Snf5*, which causes rhabdoid tumor (Roberts et al., 2000), or suggestive work involving Polycomb group members in stem cell function (Peters et al., 2001; Valk-Lingbeek et al., 2004).

What studies should follow from these epigenetic mouse models of cancer? First, since we now know that altered methylation affects cancer risk, we should ask what are the downstream effectors and cellular changes that mediate this increased risk? The idea of combining epigenetic with genetic changes should be developed further, as this is likely how epigenetic alterations cause cancer in humans. Second, the models themselves can be used to test whether epigenetic changes can be mitigated therapeutically. This could, but need not, require pharmacological reversal of epigenetic change. One could use agents already thought to be useful, but not tested in this setting, such as dietary modification or nonsteroidal anti-inflammatory drugs for intestinal tumor risk. Similarly, drugs that target downstream effectors of epigenetic change (such as inhibitors of *Igf2* signaling) could be tested. Third, the approach shown by Holm et al. in directed methylation changes can be generalized to tissue-specific modifications (using tissue-specific Cre, for example), and to other epigenetic modifiers. Hopefully, studies such as these will lead to continuing acceptance by the scientific community of the central role of epigenetic changes in cancer, and may even mollify the most critical mouse.

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Selected reading

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