Common Themes of Dedifferentiation in Somatic Cell Reprogramming and Cancer

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With its hallmarks of unregulated cell proliferation and compromised differentiation, cancer represents a derangement of normal tissue homeostasis. A common set of pathways are activated in the transformed state, through either mutation or altered epigenetic regulation, and both heritable effects sustain the tumor. Classical views of cancer have invoked tissue dedifferentiation in the oncogenic process, whereas modern views embodied in the cancer stem cell hypothesis hold that cancer emerges from primitive tissue stem cells or specific progenitor populations that through mutations assume the self-renewal properties of stem cells. Recently, somatic tissues have been reprogrammed to a pluripotent state resembling embryonic stem (ES) cells by ectopic expression of a cocktail of transcription factors. The factors that drive reprogramming are oncogenes or have been linked to cellular transformation, suggesting that tumorigenesis and somatic cell reprogramming might indeed share common mechanisms of dedifferentiation.

Cancer is the quintessential clonal disorder, arising in a single cell that over time sustains a series of mutations in genes that govern cellular growth control, survival, and cell–cell interactions. During the last several decades, cancer researchers have focused on the nature of oncogenic mutations and their effects on the function of signal transduction pathways and states of cellular differentiation. The assembly of a catalog of oncogenic mutations has led to a host of effective anticancer agents directed against specific targets essential to the cancer cell.

More recently, a major goal of cancer research has been to define the specific cell of origin of cancer among the hierarchy of cells that constitute a specific tissue. It is well documented that tissues such as the skin, gut, and blood arise from a rare stem cell that self-renews and whose daughters undergo differentiation into a variety of specialized progeny that constitute the tissue mass. Cancers in these tissues might thus arise from mutations in the tissue stem cell itself, which already possess the machinery for continuous proliferation, or by alterations in the physiology of various stages of progenitor cells that have limited self-renewal potential and would otherwise be committed to differentiation. The “cancer stem cell hypothesis” suggests that the cancer is sustained by a cell with stem-like properties of self-renewal and multilineage differentiation (Reya et al. 2001). The implication is that only rare cells within the tumor are critical to the maintenance of the tumor, and this has considerable implications for understanding the origins and treatment of malignancy.

The disease chronic myelogenous leukemia (CML) exemplifies many aspects of the cancer stem cell hypothesis (Daley 2004). The initial phase of the disease arises in the hematopoietic stem cell itself, by virtue of sustaining

the Philadelphia chromosome translocation, but the more aggressive blast crisis stage appears to arise by mutation in more differentiated cells such as the granulocyte-macrophage progenitor (GMP) (Jamieson et al. 2004). Whereas normal GMPs lack self-renewal potential, the leukemic GMPs of blast crisis CML have activated a gene expression program that mimics that of hematopoietic stem cells, which entail heightened function of β -catenin. Analysis of cases of human acute myeloid leukemia (AML) identifies the hematopoietic stem cell as the likely cell of origin of the initiating oncogenic translocations, but this population is essentially nonleukemic; the leukemic population is a more committed progenitor that has acquired additional cooperating oncogenic lesions (Miyamoto et al. 2000). Transformation by the MLL-AF9 oncogene associated with AML represents another model wherein expression in GMPs induces a self-renewal gene expression signature typical of hematopoietic stem cells, meaning that the tissue progenitors assume the replicative properties of the tissue stem cells (Krivtsov et al. 2006). Accumulating evidence in animal models of solid tumors suggests that the transit-amplifying cells of a tissue may frequently be the target population for oncogenic mutations, and the resulting cancer stem cell acquires features not only of self-renewal, but of a dedifferentiated phenotype. Unraveling the molecular mechanisms of tumorigenesis, in the context of the specific target cells involved, will be critical for developing therapies that target cancer stem cells while salvaging the function of normal tissue stem cells.

The traditional notion that differentiated cells become “dedifferentiated” in cancer has been controversial, even more so since the wider appreciation that rare stem cells persist in many adult tissues and that these stem cells can

act as a reservoir of oncogenic mutations. A rich literature has documented the reactivation of primitive programs of tissue gene expression, most notably the “cancer-testis antigens” that characterize a number of human tumors and that represent novel targets for cancer immunotherapy (Simpson et al. 2005). Recent studies of a number of highly malignant, poorly differentiated solid tumors have identified similarities in gene expression programs with embryonic stem cells (Ben-Porath et al. 2008), highlighting the fact that tumorigenesis can hijack embryonic pathways of tissue development. Indeed, morphogenetic changes exemplified by the epithelial-mesenchymal transition (EMT) of gastrulation are replayed in the evolution of primary tumors toward metastatic phenotypes and typically invoke the same players: the transcription factors snail, slug, and twist (Yang et al. 2004; Huber et al. 2005; Baum et al. 2008; Natalwala et al. 2008). In the context of mammary epithelial cells, EMT is associated with acquisition of stem cell markers and stem cell properties, including enhanced mammosphere formation and the capacity to form tumors, establishing a link between the EMT and acquisition of epithelial stem cell qualities (Mani et al. 2008).

Pathologists examining tumor tissue have long described the histologic appearance of various tumors as “dedifferentiated,” reflecting the expansion of primitive less-specialized malignant cell types. In cases where cancers arise from primitive tissue stem cells that are otherwise rare but become morphologically apparent after extensive proliferation or from immature transit-amplifying progenitors, the description of dedifferentiation in fact reflects expansion of an undifferentiated cell population, rather than a dedifferentiated cell population that resides normally within the tissues. Dedifferentiation would apply only in cases where a more specialized cell type literally extinguishes expression of lineage-specific genes of specialized tissue function, in favor of expression of the more primitive program of tissue development, perhaps accompanied by a block in differentiation that makes the transition apparent. Indeed, it is likely, at least in some cancers, that alterations in differentiated progenitor populations result in reversion to the stem cell phenotype. Evidence for such a tissue transition must come from experimental systems that prospectively identify the cell of origin of the tumor as having already acquired markers of tissue specialization.

Insights into common mechanisms of dedifferentiation and cancer have arisen unexpectedly from studies of direct cellular reprogramming to the pluripotent state. Yamanaka’s pioneering work has demonstrated that ectopic expression of a set of transcription factors (Oct4, Sox2, Myc, and Klf4) can reset the epigenetic state of differentiated somatic cells to pluripotency (Takahashi and Yamanaka 2006; Takahashi et al. 2007). The alternative pluripotency factors Nanog and Lin28 can be substituted for Myc and KLF4 to reprogram human somatic cells (Yu et al. 2007b). Of great interest, these reprogramming factors have all been implicated in tumorigenesis, raising the provocative hypothesis that cellular transformation and reprogramming entail common pathways and thus represent variations on similar biological themes. Examining the factors that mediate somatic cell reprogramming shows how dedifferentiation may be a more

prominent element of tumorigenesis than previously appreciated.

Somatic cell reprogramming entails the erasure of gene expression programs characteristic of differentiated somatic cells and the reactivation of embryonic patterns of gene expression that are characteristic of the pluripotent state (Boyer et al. 2005, 2006). This genome-wide epigenetic transformation appears to be initiated by the Oct4 and Sox2 transcription factors, which bind to a large common set of target genes and alter chromatin structure. Aberrant expression of either Oct4 or Sox2 is associated with deranged tissue homeostasis and tumorigenesis (Hochedlinger et al. 2005; Chen et al. 2008), and nanog misexpression is characteristic of germ cell tumors (Clark 2007). Myc is among the most well-documented oncogenes, known for its role in promoting immortalization of tumor cells in part through direct actions on telomerase (Wang et al. 1998). Klf4 is associated with colorectal tumors (Wei et al. 2006), and a close relative of Lin28, Lin28B, is associated with hepatocellular cancer (Guo et al. 2006). Activation targets of Oct4, Sox2, Nanog, and Myc are frequently expressed in poorly differentiated tumors (Ben-Porath et al. 2008), again revealing a common molecular circuitry in reprogramming and cancer.

The case of *lin-28* in particular highlights a common pathway of reprogramming, dedifferentiation, and cancer. *lin-28* was originally identified in *C. elegans* as a gene regulating the developmental timing of larval stages (Ambros and Horvitz 1984). Later, *lin-28* was shown to reverse cell fates during vulval development, directly linking it to reprogramming (Euling and Ambros 1996). Recently, in studies aimed at understanding why certain microRNAs (miRs) were transcribed in embryonic stem cells but not expressed as functional miRs, we and other investigators showed that a prominent function of *lin-28* is to inhibit the *let-7* family of miRs by binding to the loop domain and inhibiting processing to the mature form (Newman et al. 2008; Piskounova et al. 2008; Rybak et al. 2008; Viswanathan et al. 2008). *let-7* has been shown to function as a tumor suppressor in a variety of cancers, including breast, lung, and colon (Takamizawa et al. 2004; Akao et al. 2006; Yu et al. 2007a), likely by inhibiting the expression of known oncogenes such as Ras, Myc, and HMGA2 (Johnson et al. 2005; Sampson et al. 2007; Yu et al. 2007a). To date, only a closely related homolog of *lin-28* (*lin-28B*) has been directly linked to hepatocellular carcinoma (Guo et al. 2006), a tumor type that has long been described as showing histopathologic dedifferentiation (Abelev 1971; Abelev and Lazarevich 2006). In unpublished studies, we have considerably extended the association of *lin-28* and *lin-28B* with a number of human malignancies, strongly suggesting that modulation of processing of the *let-7* family of tumor suppressors is a common feature of malignancy (S.R. Viswanathan et al., in prep.). *lin-28* is the only nontranscription factor to date to be implicated in reprogramming of human somatic cells to pluripotency (Yu et al. 2007b). Understanding precisely how *lin-28* functions in somatic cell reprogramming and tumorigenesis, whether by direct effects on *let-7* processing or perhaps other mechanisms such as mRNA stability (Bagga et al. 2005; Balzer and Moss 2007; Chendrimada et al. 2007), will shed light on how the dedif-

ferentiation that is so evident in somatic cell reprogramming might also underlie tissue metaplasia, dysplasia, and tumor formation.

The predominant target cell for reprogramming studies has been the fibroblast, a motile mesenchymal cell type. Following ectopic expression of reprogramming factors, fibroblasts assume properties of epithelial cells, with tight cell–cell association through gap junctions and expression of E-cadherin reminiscent of mesenchymal–epithelial transitions in embryonic organ development. Interestingly, cells derived from gastric epithelia and keratinocytes appear to be reprogrammed more efficiently than fibroblasts (Aoi et al. 2008; Maherali et al. 2008). It will be revealing to probe how mediators of mesenchymal–epithelial transitions influence the reprogramming process.

Although there are common pathways activated during reprogramming and tumorigenesis, pluripotent stem cells and tumorigenic cells have important differences. A cardinal feature of pluripotent stem cells is the capacity to form teratomas, a distinct type of highly differentiated benign tumor that differs in fundamental ways from a malignancy (Lensch and Ince 2007; Lensch et al. 2007). Teratomas, by virtue of their predisposition to differentiation and tissue complexity, remain encapsulated and do not metastasize. Thus, whereas pluripotent cells are immortal, self-renew, and can form tumors, they fundamentally remain dependent on mitogenic signals in their environment and responsive to exogenous cues to differentiate. Teratocarcinomas, the malignant counterparts of teratoma, typically retain a degree of pluripotency but show more limited degrees of differentiation and behave more like malignant tumors in their potential for metastasis and tissue invasion. The critical distinctions between true cancer cells and reprogrammed somatic cells may be that reprogrammed cells remain genetically intact. Unraveling the critical distinctions—both genetic and epigenetic—between the pathways of tumorigenesis and somatic cell reprogramming promises to illuminate fundamental aspects of dedifferentiation that will be essential to understanding the safety of cell replacement products derived from pluripotent stem cells.

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