to the importance of CSCs while supporting Tlx as a novel glioma CSC marker and expanding opportunities to investigate regulators of CSCs in a genetic model. The combined use of this powerful model with well characterized human tumor models should inform the discovery of other CSC points of fragility and could provide a useful tool to detect the initial stages of brain cancer. Although the CSC hypothesis does not comprehensively explain all of tumor biology, CSCs as roots of many cancers represent an added level of complexity in tumors, a challenge we must face in trying to develop more effective therapeutics.

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Another Brick in the Wall: RNAi Screens Identify New Barriers in iPSC Reprogramming

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Somatic cells can be reprogrammed to induced pluripotent stem cells via exogenous expression of a small set of transcription factors, but the regulatory mechanisms controlling this cell transition are poorly understood. Two recent reports demonstrate the value of RNAi screens as a tool to uncover roadblocks in this inefficient process.

Groundbreaking work by Takahashi and Yamanka has demonstrated that applying a defined set of transcription factors (Oct4, Sox2, Klf4, and c-Myc; OSKM) can result in the conversion of somatic cells into induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). This method has shown its robustness and reproducibility—it has been applied to a wide variety of species and cell types including human cells (reviewed in Theunissen and Jaenisch, 2014). It is not surprising that since its discovery the processes involved in the generation of iPSCs have been studied intensively. However, in comparison to somatic cell nuclear transfer, direct reprogramming is still a slow and inefficient process, indicating that cellular barriers are hampering the conversion of a committed and specialized cell into an immature and pluripotent iPSC. Different studies in mouse and human reprogramming have highlighted a key role of the epigenetic state in regulating reprogramming. However, other pathways, including TGF-β or p53 signaling, have also been implicated in hampering the generation of iPSCs (reviewed in Theunissen and Jaenisch, 2014), indicating that several different cellular processes can influence the speed and efficiency of iPSC generation. The identification of additional pathways involved in reprogramming would no doubt further enhance our understanding of underlying mechanisms guiding reprogramming. In recent issues of *Cell* and *Cell Reports*, the groups of Ramalho-Santos and Rana report genome-wide RNAi screens to delineate new factors that affect reprogramming efficiency and kinetics using human and mouse fibroblasts, respectively, as the starting cell types (Qin et al., 2014, Yang et al., 2014).

Qin et al. applied the RNAi screen employing an ultracomplex library of shRNAs in combination with next generation sequencing (NGS) to identify roadblocks to human cellular reprogramming. They used a lentiviral library encompassing 600,000 shRNAs targeting almost 20,000 genes and transduced these together with the OSKM factors and p53 RNAi

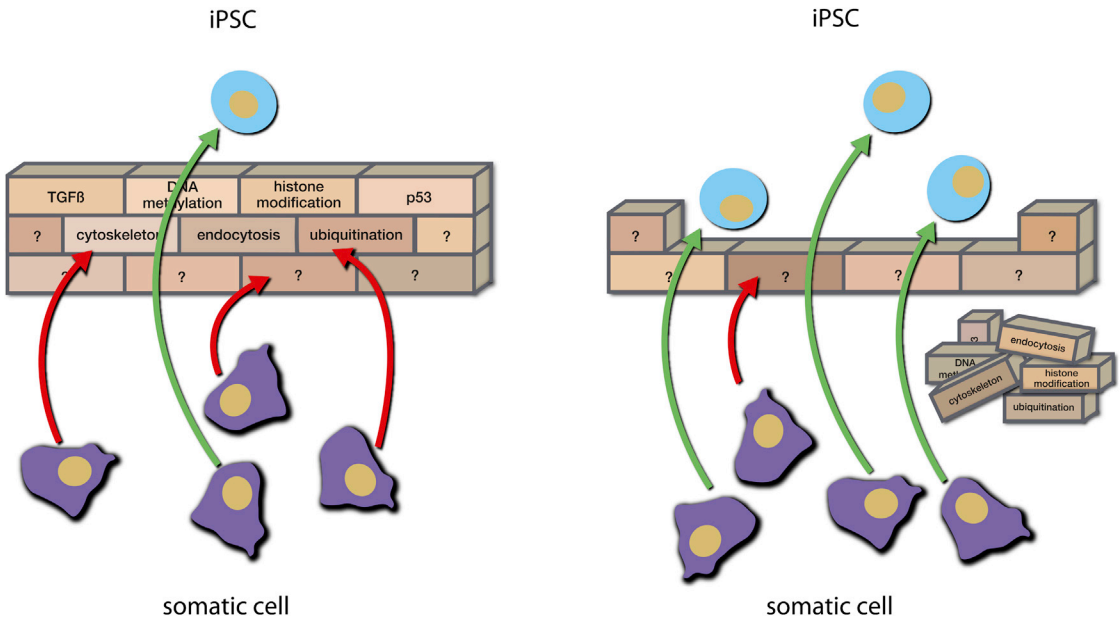

Figure 1. Illustration of the Roadblocks during Reprogramming
Biological processes that constitute barriers prevent successful reprogramming (red arrows). Combinatorial inhibition lowers the barrier, allowing more cells to overcome the roadblocks (green arrows).

into human fibroblasts. FACS sorting after 28 days of Tra-1-81-positive iPSCs and Tra-1-81-negative cells followed by amplification of the shRNA sequences and NGS then revealed enriched shRNAs in the Tra-1-81-positive cells. For hit calling the authors developed a method of combining results from multiple shRNAs targeting the same gene. The advantage of this approach is that the multiobjective optimization algorithm takes into account the collective shRNA activity and the number of enriched shRNAs. The large complexity of the library makes it virtually impossible to test every shRNA sequence in multiple iPSC colonies. On the other hand, the high complexity of the shRNA library with on average 30 shRNA per gene provides a large collection to identify the best working shRNAs for individual genes. However, as a note of caution, the likelihood of identifying two shRNAs with a similar off-target effect increases in step with increasing complexity of the library. Stringent validation with independent silencing triggers, rescue experiments, or both are therefore warranted (Echeverri et al., 2006).

The authors were able to nominate almost 1,000 candidate hits, which they present in an associated web page (http://songlab.ucsf.edu/ipsScreen/index.html). Clustering their screening hits based on comparative analysis together with publicly available time-course gene expression and epigenetic data allowed the authors to nominate ubiquitination, endocytosis, vesicular transport, and cell adhesion as processes implicated in reprogramming (Figure 1). Depletion of factors involved in clatherin-specific endocytosis enhanced reprogramming at an early stage and was linked to TGF-β signaling, a known barrier in the generation of iPSCs. Importantly, enhanced reprogramming was also confirmed with the clathrin-specific small molecule inhibitors Pitstop1 and Pitstop2. Remarkably, the combination of the drugs with some candidate factors synergistically further elevated the reprogramming efficiency up to 15-fold.

In contrast to Qin et al., who performed their study starting with human fibroblasts, Rana and colleagues aimed to identify mechanisms influencing reprogramming in mouse fibroblasts by combining an RNAi screen together with transcriptome analyses (Yang et al., 2014). The authors employed a scarcely specified shRNA library and sorted four populations based on Thy1, SSEA-1, and DsRed marker combinations (Theunissen and Jaenisch, 2014) before amplification and NGS to identify enriched or depleted shRNAs. They discover a variety of sources for induced reprogramming and cell-fate manipulation, including genes implicated in cytoskeleton rearrangements (Figure 1), which seem to be important during late-stage reprogramming (Sakurai et al., 2014). Interestingly, more than 50% of the identified genes do not change their expression levels during the reprogramming procedure, indicating that important factors guiding reprogramming do not necessarily have to be differentially expressed at the transcriptional level. This is an important finding, because many scientists focus their attention on genes that change expression levels in the biological process that they study. However, genes that do not change expression levels might be equally important modulators, which can be uncovered by the unbiased approach of RNAi screening.

Taken together, both studies significantly increase the number of genes that act as roadblocks during cellular reprogramming. Many of the known reprogramming roadblocks have important functions during cancer initiation and progression (Friedmann-Morvinski and

Verma, 2014). Somatic cells likely mount barriers to reprogramming to avoid cellular transformation. Hence, it will be interesting to see whether newly identified roadblocks are also implicated in cancer development. At the moment it is unclear whether the small overlap of hits between the two studies is due to the species difference, the different markers used to isolate cell populations, or the studies not being comprehensive. In support of the latter explanation, it should be noted that several known reprogramming factors were not identified in the screens. For instance, in a similar approach Rais et al. found that Mbd3 RNAi together with OSKM transduction results in deterministic and synchronized iPSC reprogramming (Rais et al., 2013). However, Mbd3 was identified in neither the Qin et al. nor the Yang et al. studies. Therefore, extended RNAi screens will likely uncover even more genes that influence the efficiency and kinetics of iPSC generation. In any event, the presented data should broaden our understanding of the underlying mechanisms of reprograming. The challenging part will now be to combine reprogramming barriers whose combinatorial inhibition will have the largest impact on enhancing reprogramming efficacy and kinetics. In addition, it will be important to see whether the identified factors are fibroblast specific or if they are also roadblocks for reprogramming in other somatic cells. Recent studies have revealed contradicting results for factors implicated in reprogramming, where one group has found that Mbd3 depletion promotes reprogramming (Rais et al., 2013), whereas another group described that Mbd3 is required for efficient reprogramming (Dos Santos et al., 2014). There were a number of differences between the two experimental approaches that might account for this discrepancy. Nevertheless, this example illustrates the necessity to conduct detailed experiments under varying conditions to investigate the molecular mechanisms that operate during reprogramming. Therefore, the development of an optimized protocol demands a careful downstream analysis and a thorough investigation of the reprogrammed iPSCs, including the evaluation of their functionality as well as the verification of their genomic and epigenomic integrity (Liang and Zhang, 2013).

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Tipping the Balance: MTDH-SND1 Curbs Oncogene-Induced Apoptosis and Promotes Tumorigenesis

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Tumorigenesis is a complex and poorly understood process in which oncogenes can activate competing proapoptotic and proneoplastic programs. A recent paper in *Cancer Cell* demonstrates a dual role of the MTDH-SND1 complex in suppressing the apoptotic response and promoting breast cancer development, suggesting a new therapeutic avenue.

Tumorigenesis is a complex process in which cells typically acquire mutations that do not initially alter their biology, but ultimately lead to their transition into a state characterized by the possession of self-perpetuating, malignant properties. Several distinct molecular programs may contribute to this transition, but our knowledge of this aspect of oncogenesis is poor, particularly in epithelial carcinomas that are frequently not detected until after they are well established and often disseminated. Elucidating the relevant events that influence the speed and ability of individual cells to achieve this