EDITORIAL

‘mi’-nimal interference: Somatic cell reprogramming in cancer and therapy

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The discovery by Yamanaka and Takahashi (2006) that over-expression of key embryonic stem cell transcription factors can drive the emergence of a pluripotent cell population from adult fibroblasts has generated new excitement in the field of stem cell therapy, offering to overcome technical and ethical hurdles in generating patient specific cells for therapeutic and research applications.

Induced pluripotent stem-like (iPS) cells have now been derived from multiple source tissues, and demonstrated to contribute to all tissue lineages when injected into mouse blastocysts - the practical definition of pluripotency (Reviewed in Jaenisch and Young, 2008). Proof of principle experiments from Jaenisch and colleagues have demonstrated the potential to reprogram and genetically correct subject-derived tissue in a humanized sickle cell anemia mouse model, achieving replacement of the diseased hematopoietic progenitor niche by transplantation with hematopoietic progenitors obtained by *in vitro* differentiation from autologous iPS cells (Hanna et al, 2007). Refinement of this technology promises to provide new opportunities for development of more accurate models of human disease for research, and potentially new therapeutic technology to repair tissue damage and genetic deficiencies in human patients.

Cell fusion and nuclear transfer experiments suggested that differentiated cells can be reprogrammed to an embryonic-like state using components present in pluripotent cells (Do et al, 2004). The concept of reprogramming also exists in accumulated research into cancer development; tumor formation appears to involve a ‘de-differentitation’ of post-mitotic cells into a progenitor, cycling state. Higher grade tumors appear less differentiated morphologically and genetically, and begin to resemble embryonic stem cells in their transcriptome content (Ben-Porath et al, 2008). Proto-oncogene c-Myc is also a key driver of stem cell proliferation, and the re-activation of embryonic gene expression programs underlies much of the metastasis of tumors *in vivo* (Yang et al, 2006).

Tumor development involves the gradual attainment of multipotency, the ability to differentiate into multiple lineages. A small population of CD44-high/CD24-low cells can be isolated from mammary epithelial tumors that are capable of seeding new tumors when injected into mice (Mani et al, 2008). These ‘cancer stem cells’ may be responsible for much of the tumor capacity to metastasize, and perhaps repopulate after chemotherapy. Conspicuous similarities between reprogramming and tumor development seem likely to provide new insights into how cancers might arise, be tackled, and how reprogramming therapies may avoid enhancing tumor risk.

A range of protein factors have now been identified that can be combined to reprogram somatic cells into iPS cells. Key proteins appear to be embryonic stem cell transcription factors POUF51 and SOX2, with MYC and KLF family members enhancing the efficiency of reprogramming, although the exact combination of factors necessary may vary with the somatic tissue chosen. Common technical difficulties remain however in that these proteins must be expressed at high copy number and often integrated into the somatic cell genome to achieve expression over the necessary timeframe. Given the proliferation inducing properties of this combination of factors, much focus has converged upon means to transiently express and remove the necessary proteins in order to leave the iPS genome as pristine as possible and to prevent the anticipated increased tumor propensity in cells containing multiple copies of oncogene cDNA (Okita et al, 2008; Lyssiotis et al, 2009). New reports suggest that the expression of microRNAs can efficiently reprogram somatic cells, potentially without requiring genomic integration (Lin et al, 2008; Judson et al, 2009).

miRNAs are broad regulators of messenger RNA stability, interacting with many mRNA targets within the cellular transcriptome. The breadth of miRNA regulation makes these small RNAs powerful regulators of cell state; tumor formation often involves down regulation of the cellular miRNA component, avoidance of miRNA targeting by mRNAs and in some cases selection for expression of particular miRNAs that enhance proliferation or cell survival (reviewed in Ventura and Jacks, 2009). The small footprint of miRNAs and siRNAs, and the ability to effectively deliver transient functionality without need for genomic integration, makes them good candidates for use in reprogramming. Indeed an early cDNA screen identifying factors for reprogramming human fibroblasts identified LIN28B as a reprogramming protein, the primary function of which (in embryonic stem cells) appears to be in the inhibition of miRNA processing for the anti-proliferation Let-7 family of miRNAs (specifically those containing the trinucleotide 'CCC' motif within the miRNA terminal loop)(Yu et al, 2007). This early indication of the importance of miRNAs to reprogramming has further been expanded upon through the observation that c-MYC activity can be substituted by members of the mouse specific miR-290-295 cluster that contain the seed sequence AAGUGCA (which is partially shared by members of the miR-17-92 family)(Judson et al, 2009). Accumulating evidence indicates that the common seed of the miR-17-92 cluster drives the G1 to S transition that is fundamental for ES self-renewal and cell proliferation (Wang et al, 2008). Additionally, miR-302 (seed AGUGCUU;) enhances the expression of reprogramming factors in human cancer cell lines and appears to reprogram these cells into a stem-like state (Lin et al, 2008). Mir-145, in contrast, has been demonstrated to repress key reprogramming genes OCT4, SOX2 and KLF4 in vivo, but notably is often down regulated in colorectal tumor samples, indicating a further potential route to a stem-cell like state through intervention at the miRNA level (Xu et al, 2009).

The efficiency of reprogramming techniques is low (commonly less than 0.001% of cultured cells are capable of forming embryonic stem cell-like colonies), and much remains to be learned about the stability of re-differentiated reprogrammed cells over time *in vivo*. Key in developing this technology will be an understanding of the core biology of how reprogramming factors impact upon cellular gene expression networks, and the role of small RNAs in regulating these gene expression networks.

Antagomirs directed against key miRNAs in immortalized cells have demonstrated researcher's ability to modulate proliferation networks in tumors through delivery of small oligonucleotides (*e.g.*, Fontana et al, 2008). Delivery of multiple small oligonucloetides that mimic miRNAs may more efficiently modulate cellular gene expression so as to both inhibit proliferation genes and enhance non-proliferation gene expression – in essence both taking the foot off the gas, and applying the brakes. Such combinatorial therapy might potentiate the effect of cooperative miRNAs, and be more resistant to 'escape' by tumor cells that can overcome a single chemical agent. Furthermore, miRNA-orientated approaches may be necessary to overcome the inherent canalization of differentiated cells prior to full induction of progenitor gene expression programs – releasing the brakes to allow the car to be pushed. Annotation of important miRNA targets will aid in the rational design of such approaches, but will also require a re-evaluation of much existing interaction data with regards to identifying targets that are functionally relevant nodes in specific tissues and biological networks.

Research into the basic biology of cancer, and an understanding of embryonic stem cells, has synergized in the burgeoning field of somatic cell reprogramming. The rapid advancements in miRNA research over the past ten years represent one of the first applications of truly holistic systems biology approaches to representing the biological networks of a cell. There exist now stirring opportunities to apply these advances to the specific manipulation of cellular gene expression programs, and towards genuinely groundbreaking therapeutic technologies.

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