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RESEARCH HIGHLIGHT

Epithelial plasticity, stemness and pluripotency

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Embryonic stem cells derived from the inner cell mass of blastocyst stage embryos (ES cells) are capable of differentiating into any cell type, offering the possibility of their use in cell transplantation therapies. However, the risk of rejection by the immune system and the bioethical issues inherent to the use of embryonic cells prompted the search for a mechanism of obtaining pluripotent cells from adult cells and thus, potentially self tissues. In 2006, Takahashi and Yamanaka succeeded in reprogramming adult fibroblasts to induced pluripotent stem cells (iPSCs) by the forced expression of four transcription factors, namely Sox2, Klf4, Oct4, and Myc, now called the "Yamanaka Factors" or SKOM [1]. Despite the subsequent successful generation of iPSCs derived from various sources, the understanding of the mechanism by which these four factors act has remained unclear, as well as the steps that progressively suppress the somatic cell program and activate ES cell marker genes. Two recent papers published in *Cell Stem Cell* shed light on these issues by dissecting both the individual role of the SKOM factors [2] and the sequential order of the full reprogramming process into initiation, maturation and stabilization phases [3].

The two studies show that the conversion of fibroblasts into an intermediate epithelial cell is crucial during the initial stages of reprogramming into iPSCs [2, 3]. This phenotypic change occurs through the induction of a mesenchymal to epithelial transition (MET) and is compatible with the fact that the morphology of the ES cells is more epithelial-like than mesenchymal-like and that mammary epithelial cells can be reprogrammed faster and with higher efficiency than fibroblasts [2]. The MET and the reverse process, epithelial to mesenchymal transition (EMT) are at the centre stage of epithelial plasticity and play a central role during embryo development. Moreover, the EMT is crucial during the progression of organ fibrosis and cancer [4].

In the first study, Li *et al*. [2] analyzed the effect of each reprogramming factor on the expression of EMT-MET inducing genes. They showed that Sox2/Oct4 suppresses the transcription of the EMT mediator Snail1, c-Myc downregulates the TGFβ signaling pathway and Klf4 induces the expression of E-cadherin among other epithelial markers and decreases Snail1 protein levels (Figure 1). In agreement with these findings, TGFβ1 treatment or Snail1 overexpression, both of which inhibit MET, reduces the formation of iPSCs [2], also consistent with recent results showing that TGFβ1 inhibitors promote nuclear reprogramming [5, 6]. The combined action of SKOM factors in the recipient fibroblasts is directed towards the repression of the EMT program. As such, Snail1 transcription factor is a potent EMT inducer, E-Cadherin repressor and TGFβ1 is the main signaling pathway for the triggering of the EMT and the major activator of *Snail1* transcription in physiological and pathological conditions [4]. Thus, while Sox2/Oct4 repress EMT inducers, Myc prevents them from being reactivated by inhibiting TGFβ, and Klf4 reinforces the epithelial program by directly inducing E-cadherin expression, altogether leading to the epithelialization of fibroblast or MET.

Based on gene expression profiles during fibroblasts reprogramming, Samavarchi-Tehrani *et al.* [3] also concluded that the MET is one of the initial changes that fibroblasts undergo during cellular reprogramming and found that MET concurs with the inhibition of TGFβ and the activation of BMP7. BMP7 induces epithelialization and MET by reverting the TGFβ-induced EMT in a mouse model of renal fibrosis [7]. This indicates that TGFβ and BMP7 can act as antagonistic agents in epithelial plasticity, supporting that the SKOM factors together with BMP7 favor the progression towards the epithelial phenotype and reprogramming, and that BMP-7 administration during reprogramming significantly increases the number of iPSCs [3] (Figure 1). Interestingly, BMP7 activates the expression of the miR200 family, which in turn, are strong repressors of the EMT inducers and can also repress TGFβ (see [8] for a review).

The initiation phase of reprogramming to iPSCs is not accompanied by

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the expression of ES cells markers [3]. In addition, the expression of genes associated with MET induction is reverted and cells return to the parental fibroblastic profile after SKOM removal, indicating that the initiation phase is unstable and reversible [3]. The maturation phase leads to irreversible commitment to reprogramming and it is associated with the expression of ES cell markers including Nanog, which is recently described as a mediator of embryonic and induced pluripotency [9]. Therefore, irreversibility and pluripotency seem to be linked and dependent on the acquisition of Nanog expression during the maturation phase of the reprogramming process (Figure 1).

Stem cell properties have been associated not only with ES cells or iPSCs but also with immortalized mammary epithelial cells that have undergone EMT, the reverse of the MET process [10, 11]. This seems to be at odd with the finding that MET is a crucial initial step towards pluripotency. The combination of EMT and stemness is particularly relevant during cancer progression, as cells delaminate from the primary tumor through a process of EMT that allows cell migration and dissemination to form metastasis. This is reminiscent of the EMT process that the embryo uses for similar purposes, the formation of different tissues and organs which cells originate far from their final destination [4]. The migratory cancer cell thus combines the mesenchymal phenotype necessary for efficient dissemination together with stem cell properties, as CSCs are defined by their ability to seed new tumors, to self-renew and to produce non-stem differentiated cells [12]. Interestingly, CSCs have not been shown to be pluripotent, making them different from iPSCs. Indeed, CSCs do not need pluripotency, as during metastasis formation, cancer cells do not

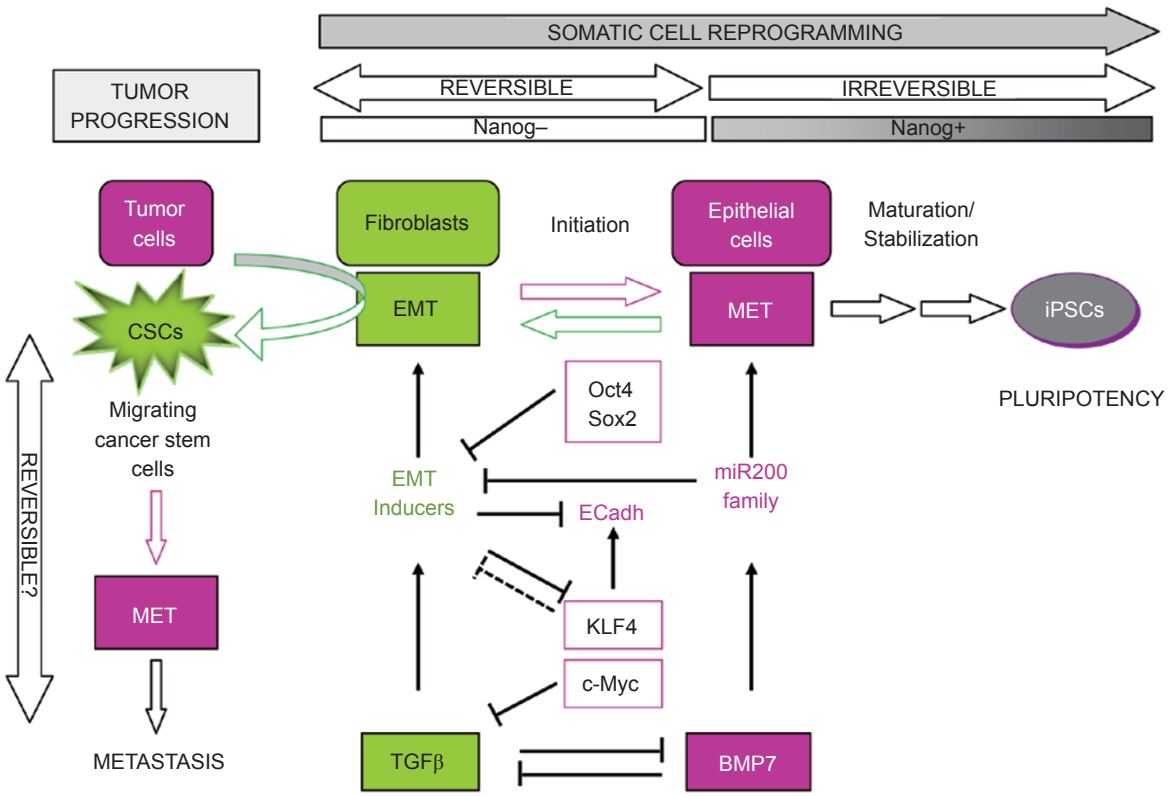

Figure 1 Schematic representation of the regulatory network for the generation of iPSCs and CSCs. During the initiation phase of fibroblast reprogramming the SKOM factors in combination with BMP7 and miR200 family members induce a mesenchymal to epithelial transition (MET) mainly through the repression of the EMT program. As such, Sox2/Oct4 repress the inducers of the epithelial to mesenchymal transition (EMT), c-Myc downregulates TGFβ signaling and Klf4 induces E-cadherin expression and decreases Snail1 protein levels (dotted line). After the MET, these intermediate epithelial cells do not express stem cell markers and maintain the ability to revert to the mesenchymal phenotype. The irreversible maturation/stabilization phase is marked by the onset of Nanog expression, key for the generation of pluripotency and iPSCs. During tumor progression, epithelial carcinoma cells or CSCs undergo EMT to acquire the ability to disseminate while maintaining some stem cell properties, but not pluripotency. These migrating CSCs generate distant metastasis upon suffering a MET and reverting to the differentiated state of the primary tumor (see text for details). BMP7, bone morphogentic protein; CSCs, cancer stem cells; iPSCs, induced pluripotent stem cells; TGFβ, transforming growth factor β.

generate any cell type but revert to the phenotypes of the primary carcinoma [13]. If Nanog expression defines the irreversible commitment to full reprogramming and pluripotency, the prediction would be that the migrating CSCs [14] should be Nanog negative or low, and their progeny in the metastasis is the result of a MET that produces epithelial cells resembling the primary tumor. Of note, this MET has some similarities with the reversible initiation phase during iPSCs reprogramming. The lack of Nanog could also allow these metastastic cells to maintain plasticity so that they could subsequently undergo another round of EMT that would allow the formation of new CSCs, in line with the proposed bidirectional interconvertibility between CSCs and non CSCs [12]. In summary, pluripotency and stemness are not equivalent terms, which may explain the paradox in the dynamics of the EMT/MET processes occurring during reprogramming and cancer progression.

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