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Review

De- and re-differentiation of the melanocytic lineage

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ABSTRACT

Terminally differentiated cells can be reprogrammed by the transient, ectopic overexpression of different sets of genes into induced pluripotent stem cells (iPSCs). This process not only has considerable implications for regenerative medicine but is also highly relevant to multiple stages of oncogenesis, including melanoma. In other settings, the de-differentiation of normal and tumor cells is also responsible for a phenotype switch which completely changes the cell fate. Conversely, iPSCs as well as embryonic stem cells (ESCs) can be differentiated *in vitro* toward specific lineages, for example melanocytes, which offer useful models to investigate the genetic and epigenetic mechanisms involved in cellular differentiation. Here, we summarize recent findings regarding the reprogramming and de-differentiation of melanocytic cells as well as the latest differentiation protocols of pluripotent stem cells into the melanocyte lineage.

Similarities between melanoma cells and pluripotent stem cells

Induced pluripotent stem cells (iPSCs) were first generated by the transient, ectopic overexpression of a certain set of transcription factors (*e.g.* OCT4, SOX2, KLF4 and c-MYC) (Takahashi and Yamanaka, 2006). OCT4 and SOX2 were thought to be strictly required for reprogramming, whereas the other components could be substituted by other genes such as Nanog and LIN28 (Galach and Utikal, 2011; Hanna et al., 2010; Yamanaka, 2012). Lately however, several lineage specifiers involved in mesodermal specification and in ectodermal specification were described as substitutes to OCT4 and SOX2 respectively during mouse somatic cells reprogramming (*e.g.* GATA3, GMNN) (Shu et al., 2013) (Fig. 1).

Interestingly, this groundbreaking work brought new perspectives to cancer biology due to its relevance in cellular transformation. Indeed, several reprogramming transcription factors are known oncogenes (*e.g.* SOX2, c-MYC), whereas many genes that act as barriers to reprogramming are known tumor suppressors (*e.g.* p53 and INK4A/ARF), whose effects on proliferation and apoptosis restrain both reprogramming and transformation (Orkin and Hochedlinger, 2011; Suvà et al., 2013). For example, immortalization of cells by inactivating main regulators of the cellular senescence pathways (p53 or INK4A/ARF) that play also a major role in melanoma pathogenesis leads to significantly increased reprogramming efficiencies (Utikal et al., 2009a,b) (Fig. 2).

Unlimited proliferation and high telomerase activity are also common features of melanoma cells and iPSCs (Horn et al., 2013; Huang et al., 2013; Maherali et al., 2007) and in addition, similar expression profiles, similar epigenetic marks and genomic instability have been frequently shown in both cell types (Baker et al., 2007; Ben-Porath et al., 2008; Sperger et al., 2003).

Moreover, the injection of iPSCs (depending in part on their karyotypic stability) into immunocompromised mice leads to teratoma formation (which contain highly heterogeneous cell populations belonging to the three germ layers and which are benign tumors) but the injection of aneuploid embryonic pluripotent stem cells can also give rise to teratocarcinomas which are highly malignant due to the presence of undifferentiated cells (Evans, 2011; Stevens and Little, 1954).

Furthermore, dynamic cell phenotypes which could explain the intra-tumoral heterogeneity of melanoma have also been described in pluripotent stem cell cultures (Cahan and Daley, 2013; Fukunaga-Kalabis et al., 2011). For example, the H3K4 demethylase JARID1B, which is an epigenetic marker for embryonic stem cells, has recently been described as a marker of a dynamic melanoma subpopulation (Christensen et al., 2007; Klose and Zhang, 2007; Roesch et al., 2008, 2010; Yamane et al., 2007).

Finally, a recent study identified Leukemia Inhibitory Factor (LIF), which regulates the main pluripotency markers (*e.g.* SOX2,

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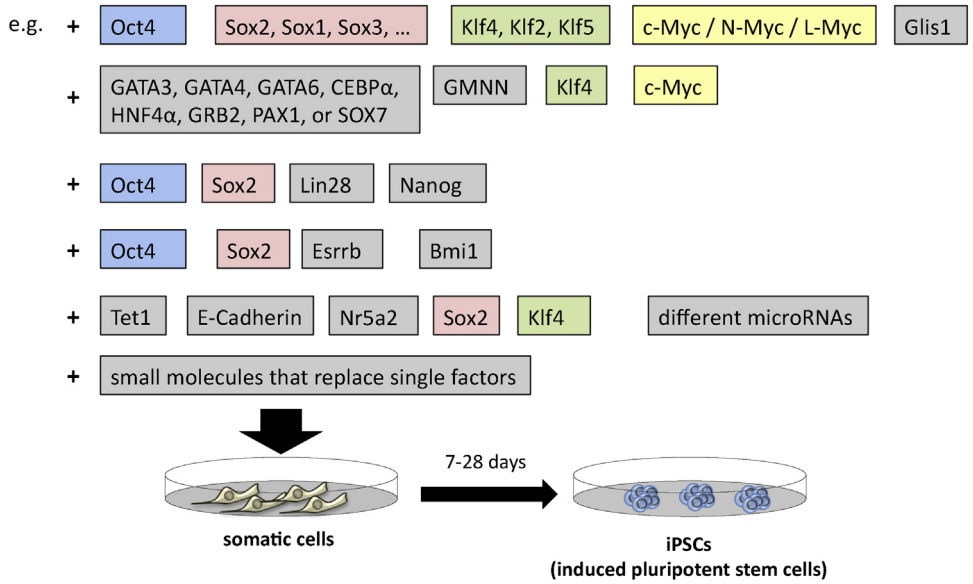

Fig. 1. Schematic overview of factors that can induce a pluripotent state in somatic cells (some factors can be replaced by other factors.

Nanog, OCT3/4 and GBX2) in mouse ESCs and iPSCs, as an important factor for melanoma progression due to its expression in several melanoma cell lines of primary and metastatic origin and in melanoma tissue (Kuphal et al., 2013). LIF signals *via* its binding to a two-part receptor complex involving the LIF receptor and the gp130 receptor, leading to the activation of at least four different signaling pathways involved in self-renewal (*e.g.* JAK/STAT3, MAPK, PI3K and YES). Therefore, LIF has been suggested as an inducer of a "melanoma stem cell"-like behavior. This point will be discussed in paragraph 3.

All together those data suggest that genetic and epigenetic mechanisms involved in cellular reprogramming may be necessary at least in part during transformation of melanoma cells.

Reprogramming of melanocytes

Most human iPSCs have been generated from skin fibroblasts, but alternative sources to derived patient-specific iPSCs, including melanocytes are available (Galach and Utikal, 2011). For example, human keratinocytes have a much higher and faster reprogramming efficiency than human fibroblasts (Aasen et al., 2008; Maherali et al., 2008). Neural progenitor cells and melanocytes express SOX2 endogenously, which makes exogenous SOX2 dispensable for their efficient reprogramming (Eminli et al., 2008; Utikal et al., 2009a,b) (Fig. 2). The pluripotency of melanocyte-derived iPSCs was analyzed by teratoma formation assays (endo-, ecto- and mesodermal derivatives were described)

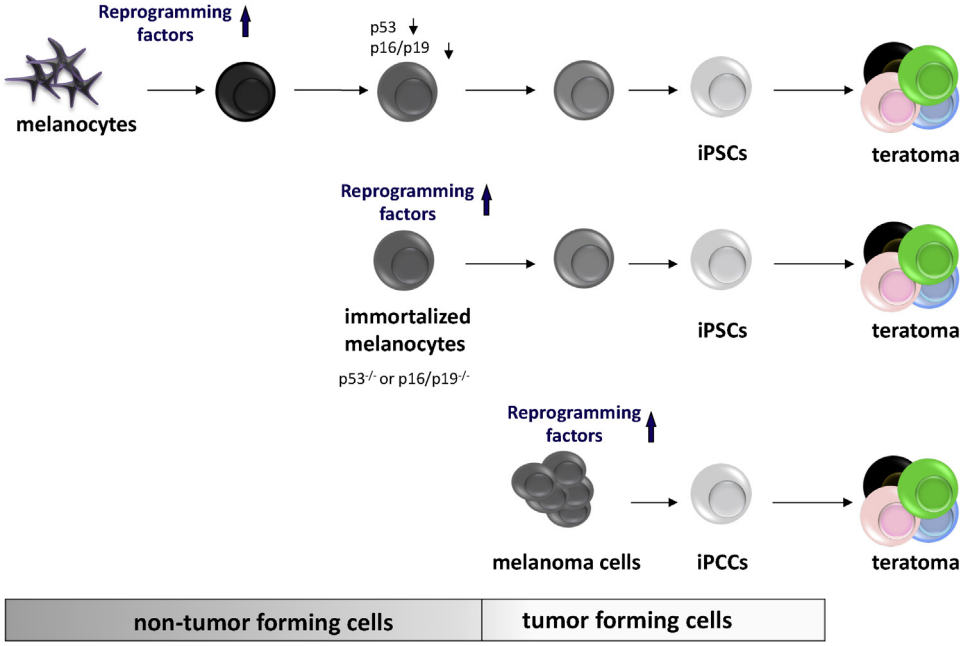

Fig. 2. Melanocytes, immortalized melanocytes and melanoma cells are amenable for reprogramming into a pluripotent state by the transient ectopic overexpression of reprogramming factors. Non-tumor forming cells are converted into tumor-forming cells that can differentiate into all three germ layers.