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'Spotlight' commentary

OCT4B1 isoform: The novel *OCT4* alternative spliced variant as a putative marker of stemness

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A novel *OCT4* alternative spliced variant (i.e. *OCT4B1*) has recently been sequenced and identified (Atlasi *et al.*, 2008); in their valuable study, the authors report that the novel *OCT4* isoform is sharply down-regulated during the course of all-trans retinoic acid induced differentiation of human embryonic stem cells (hESCs) and suggest a potential involvement of *OCT4B1* in maintaining the pluripotent state of hESCs. Herein we provide tenable evidence towards the establishment of *OCT4B1* as a marker of stemness (Mikkers and Frisén, 2005).

In 2007 the International Stem Cell Initiative (ISCI), aiming in providing a more comprehensive assessment of the hES phenotype, analyzed the expression profile of a large set of cell-surface antigens and genes in 59 independently derived hESC lines (Adewumi *et al.*, 2007). Gene profiles were assessed according to the transcription factors *NANOG*, *OCT4* and teratocarcinoma-derived growth factor,(*TDGF)*, which "can be regarded as archetypal pluripotent stem cell markers"; Regarding *OCT4*, the stem cell marker description corresponds to the long isoform of the *OCT4* gene i.e. *OCT4A* [NM_002701.4] (Lee *et al.*, 2006).

However, in a way analogous to that described earlier [Zangrossi *et al.*, 2007, Kotoula *et al.*, 2008], the data obtained by the ISCI in this study do not correspond to *OCT4A* but to *OCT4B* related sequences. This can be readily deduced from the Taqman assay used by the ISCI to quantify *OCT4* expression (Hs00742896_s1 [http://www.appliedbiosystems.com]), which targets a sequence in the 1st exon of *OCT4B* (NM_203289.3). Consequently, it can detect *OCT4B*, the non-stemness factor (Lee *et al.*, 2006) and *OCT4B1* but not *OCT4A* (Atlasi *et al.*, 2008; Lee *et al.*, 2006). However, the expression level of *OCT4B* in hESCs is only basal (Atlasi *et al.*, 2008); hence, it can be argued that the *OCT4* transcripts detected in the ISCI study corresponded in fact to *OCT4B1*.

Looking at the ISCI study data from this standpoint, it seems that in 59 hESC lines: (a) *OCT4B1* expression levels were high and remarkably consistent across all undifferentiated hESC samples tested; (b) *OCT4B1* expression presented significant correlation with *NANOG* expression ($r \geq 0.75$) in all undifferentiated and differentiated hESC samples tested; (c) *OCT4B1* expression presented strong negative correlation with alpha-fetoprotein (*AFP)* and the transcription factor *NEUROD1* expression levels, genes that are upregulated during cell differentiation. In the same context, when the ISCI suggested that *OCT4*, *NANOG*, *TDGF*, gamma-aminobutyric acid A receptor (*GABRB3)*, growth and differentiation factor (*GDF3)* and DNA(cytosine-5-)methyltransferase (*DNMT3B)* genes constitute a core set of markers that could be used to define undifferentiated hESCs, according to current knowledge, they were referring to *OCT4B1*. Of note, all the above do not preclude *OCT4A* from being a marker of stemness and pluripotency; *OCT4A* expression may well be present but was simply not addressed in the 59 hESC cell line panel.

As shown by Atlasi *et al.* (2008), *OCT4B1* expression was very low in most somatic cancer cell lines being more prominent in those derived from highly aggressive bladder transitional carcinomas. However, *OCT4B1* expression by somatic cancer cells should not be an argument against its potential value as a stemness marker, since the transformation events before the derivation of stable cancer cell lines are largely unclear, while the hypothesis of *OCT4B1* reactivation in certain types of non-tumorigenic somatic cells inducing transformation into the cancerous state can not be excluded with certainty. Reinforcing the above, a recent publication (Mizuno and Kosaka, 2008) reports the existence of several *Oct4* spliced variants in mice; among them, the variant orthologous to the human *OCT4A* isoform, as well as a previously unknown alternative transcript (i.e. *Oct-3/4C*), induce significant transformation

activity *in vitro*. At this point it seems intriguing to raise the challenging question of which *OCT4* isoform, exactly, is related to oncogenesis and could subsequently serve as a cancer stem cell marker, since isoform-specific valid experimental data are yet unavailable for human somatic cancer cells (Liedtke *et al.*, 2008).

Overall, the existing data strongly suggest that *OCT4B1* is a marker of stemness; of note, there are currently no studies reporting the existence of neither *OCT4B1* nor *OCT4B* highly homologous pseudogene sequences that could potentially confuse *OCT4*-related stem cell research (Liedtke *et al*., 2007). In this context, *OCT4B1* may in future studies prove to serve as a more reliable stemness marker than *OCT4A*, the longwise established regulator of stemness and pluripotency.

"The voyage of discovery is not in seeking new landscapes but in having new eyes" (Marcel Proust)

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