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TUBB3:
Neuronal marker or melanocyte mimic?

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Dear editor:

Identifying neuronal derivatives of stem cells is essential for both basic research and future applications in regenerative medicine targeting neurodegenerative diseases. Erroneous identification could result in the transplantation of inappropriate cell types, thus directly affecting clinical outcome.

Stem cell and neurobiology researchers widely regard the class III β-tubulin protein (TUBB3), a member of the microtubule family, as being selectively expressed in neuronal cells. Antibodies against this protein are therefore often used to identify cells of the neuronal lineage or to quantify neuronal cells within cell cultures. The most renowned anti-TUBB3 antibody is the monoclonal TUJ1, with other examples including clones TU-20 and 2G10.

Nevertheless, we propose reconsidering the use of TUBB3 as a marker for neurons when melanocytes are potentially present or being formed. Why? Research in our own lab indicated that melanocytes were formed in studies on neuronal differentiation. Intrigued, we investigated and detected high TUBB3 expression during melanocyte differentiation *in vitro* and *in vivo* [1] in both human foetal and adult melanocytes (see Figure 1A for an example in the human skin). In agreement, two other articles [2, 3] have described the expression of TUBB3 in chick and mouse melanoblasts, indicating that TUBB3 expression is clearly not restricted to neurons and should therefore not be regarded as a standard for neuronal identification, but rather as a broader marker of the neural lineage (both neural crest and neural tube derivatives).

Furthermore, double-labelling combinations of microtubule-associated protein 2 (MAP2) and TUBB3 (used to identify cells as *"neurons"*) or tyrosine hydroxylase (TH) and TUBB3 (used to identify cells as *"dopaminergic neurons"*) are not fail-safe as melanocytes can express both MAP2 and TH. Melanocytes can also express nestin (considered to be a neural stem cell marker although its presence in endothelial cells has also been reported), which together with TUBB3 is often used to identify *"neuronal precursor cells"* (Figure 1B and 1C). Thus, using these combinations of markers may result in cells being incorrectly identified as neurons. The same is true for neurotrophins (NGF, BDNF, NT3 or NT4) and neurotrophic receptors (Trks and NGFR (p75NTR)) [4]. Even functional ion channels (sodium, potassium or calcium) of various types have been identified in the melanocyte [5].

Melanocytes and neurons are both derived from the neural plate (more specifically, all neurons from the peripheral nervous system and melanocytes are derived from the neural crest), share morphological features (e.g. an extensive dendritic tree) and to a large extent express the same biomarkers. If melanocytes are potentially present in the experimental system of interest, another method of identification should be sought. One option is to rule out melanocytes, for example by taking advantage of markers present in melanocytes but not in mature neurons such as melan-A or sex-determining region Y box 9 (SOX9) (Figure 1D). The other option is to irrefutably demonstrate successful derivation of neurons by their true neuronal hallmark: the action potential.

Or is it?

References

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