

## Melanoma pathogenesis and Nodal: a partial picture?

### To the editor:

After reading the article by Topczewska *et al.*<sup>1</sup> implicating the role of the embryonic morphogen Nodal in melanoma pathogenesis, which I found to be extremely interesting, I was struck by the lack of scientific and historical perspective displayed by the authors. In particular, there is no mention or citation within their article of the possible expression of Cripto-1 in melanomas and its potential interactions with Nodal. Cripto-1 is an essential co-receptor for Nodal and is critical for Nodal's ability to function in a biological context through a Smad2/3 and FoxH1 signaling pathway. Nearly 20 years of research have demonstrated an important and essential role for Nodal in conjunction with Cripto-1 in early vertebrate development. In addition, the expression of Cripto-1 has been documented in a number of different types of human carcinomas, thereby exemplifying the importance of an early embryonic gene in the development of cancer where Cripto-1 may function to regulate epithelial-mesenchymal transition and tumor cell invasiveness<sup>2,3</sup>. Finally, there is evidence that Cripto can function through a Nodal-independent pathway via glypican-1 and src and that Nodal can function through a Cripto-1-independent signaling pathway during early development where it can act as a bone morphogenetic protein (BMP) antagonist. A more detailed

examination as to the mechanism by which Nodal can regulate melanoma tumorigenesis and invasiveness is warranted.

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1. Topczewska, J.M. *Nat. Med.* **12**, 925–932 (2006).
2. Strizzi, L. *et al. J. Cell Physiol.* **201**, 266–276 (2004).
3. Ebert, A.D. *et al. Exp. Cell Res.* **257**, 223–229 (2000).

### Topczewska *et al.* reply:

We agree with Dr. Salomon that it will be interesting to further explore the mechanisms by which Nodal regulates melanoma cell behavior. We did not intend to slight his work linking Cripto-1 to tumorigenesis and therefore cited his seminal paper<sup>1</sup> highlighting the expression of Nodal in testicular and breast carcinoma cells of epithelial origin. However, our paper is the first to report Nodal in melanoma, a mesenchymally derived tumor that does not undergo epithelial-to-mesenchymal transition and contains fewer than 20% Cripto-1-positive cells. Thus, we did not wish to suggest the involvement of a Cripto-1-dependent pathway without more compelling evidence. A role for Cripto-1 in melanoma pathogenesis also remains

uncertain because during development, Nodal apparently signals in part through stimulation—rather than inhibition—of a Cripto-1-independent bone morphogenetic protein (BMP) pathway<sup>2</sup>. The intent of our study was to use the zebrafish embryo as a biosensor for metastatic melanoma cells expressing a plastic, stem cell-like phenotype to modulate an embryonic microenvironment, which ultimately revealed Nodal as a mediator of melanoma plasticity and progression. As a corollary to our findings, we agree that it will be interesting to explore the possible role of Cripto-1 in Nodal-mediated tumorigenesis, tumor progression and metastasis.

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1. Adkins, H.B. *et al. J. Clin. Invest.* **112**, 575–587 (2003).
2. Ben-Haim, N. *et al. Dev. Cell* **11**, 313–323 (2006).

## Challenges to the report of Nogo antibody effects in primates

### To the editor:

The conclusion that Nogo-A-specific antibodies enhance sprouting and functional recovery after spinal cord lesions in primates is not supported by the data presented in a recent *Nature Medicine* article<sup>1</sup>.

First, the control animal CP was excluded from certain statistical analyses on the basis

of the incompleteness of its corticospinal tract lesion. However, 8 of 12 experimental subjects exhibited incomplete corticospinal tract lesions based on Supplementary Figure 1 of ref. 1. Further, two Nogo-treated animals, AA and AS, had partial corticospinal tract lesions similar to that of animal CP, yet were not excluded. This selective elimination of

subjects was not conceptually or statistically valid and skewed the results. Second, spared ventral pathways can contribute to functional recovery. Supplementary Figure 1 indicates that there was slightly more ventral sparing in Nogo-treated animals compared to control-lesioned subjects, which could have favored functional recovery in the Nogo-treated group. Third,

one control-lesioned and two Nogo-treated animals exhibited functional recovery within 2 to 10 days of the lesion. This rapid post-lesion improvement suggests that recovery was mediated by spared rather than regenerating axons. Fourth, some statistical analyses seemed to have been conducted using pairwise comparisons of specific individuals in different groups, for reasons that are unclear. The inappropriate use of pairwise comparisons would lower the rigor for establishing statistical significance. Fifth, the anatomical data are primarily presented in camera lucida format, prohibiting the reader from assessing whether enhanced sprouting or

regeneration in fact occurred, which can only be assessed from clear, high-quality images.

Finally, I do not understand the explanation for the peculiar triplicate figures published in the original online article, which the authors provided as “corrigendum.” How could the same rostral half of a figure be attributed to three different animals while the corrected manuscript still uses the original drawing for one of the animals? If the original error occurred because a schematic was inserted as ‘place holder’ in the three different figures, the schematic should be replaced by the actual anatomy in all cases. Instead, the place holder is still there in one of

the animals. The explanation of this anomaly requires further clarification.

Thus, the effects of Nogo antibodies in primates with spinal cord injury have not been established.

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1. Freund, P. *et al.* *Nat. Med.* **12**, 790–792 (2006).

#### To the editor:

We were surprised to read the title, abstract and conclusions of a recent article in *Nature Medicine* by Freund *et al.*: “Nogo-A specific antibody treatment enhances sprouting and functional recovery after cervical lesion in adult primates” (ref. 1), as no clear anatomical evidence of regeneration is provided in the paper.

The authors performed spinal cord lesions on monkeys, then quantified the “normalized cumulated axonal length” beyond the lesion in six monkeys, three treated with Nogo-A antibody and three with control. In the text, the authors report that the difference between the two groups “was not statistically significant ( $P = 0.12$ ),” a conclusion that can be verified directly from the specific values reported in Supplementary Table 1 of ref. 1 (48.8  $\mu\text{m}$ , 36.7  $\mu\text{m}$  and 8  $\mu\text{m}$  of regeneration for controls, compared to 59.3  $\mu\text{m}$ , 46  $\mu\text{m}$  and 79.9  $\mu\text{m}$  in monkeys treated with Nogo-A antibody).

The authors do show a statistically significant increase in “the number of axonal swellings.” However, this parameter was not validated as a measure of regeneration. Swellings are just as likely to be signs of incipient degeneration as they are of any anatomical improvement. Unless evidence is provided to the contrary, these data cannot be taken as evidence of enhanced repair.

Finally, the authors refer in the text to Supplementary Figure 1 as showing “sprouting for a total distance of 10–12 mm,” but that figure does not in fact contain those data, nor could we find any data on the extent of sprouting in any of the other figures or tables, nor any indication of the statistical significance of this purported effect.

We were therefore surprised that the concluding paragraph states that “Neutralization of Nogo-A promotes regrowth of cortico-

spinal (and possibly other) axons around the lesion and into the denervated spinal cord in macaque monkeys,” as the data on regrowth (that is, regeneration) showed no statistically significant difference. We were equally surprised that the title and abstract claim that the Nogo-A antibody produces “enhanced sprouting,” as sprouting was not documented.

Because the conclusions of the paper do not match the data that are presented, it would seem appropriate for the authors to revise their conclusions in an erratum.

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1. Freund, P. *et al.* *Nat. Med.* **12**, 790–792 (2006).

#### Freund *et al.* reply:

In an experimental injury situation, the key prerequisite for studying regeneration is the completeness of the transection of the studied fiber tract. In our paper, the crucial criterion for a complete lesion was not only the extent of the lesion as it appeared in reconstructed cross-sections for each monkey, but the full interruption of the corticospinal tract (CST) as assessed by anterograde transport of the tracer biotinylated dextran amine (BDA) from the hand area of the motor cortex.

As stated in our paper and on the basis of this criterion, 8 of 12 monkeys had a complete lesion of the CST in the dorsolateral funiculus (and not only 4 as claimed by our colleagues; see Supplementary Table 1 of ref. 1). Furthermore, among the animals that we considered for the tracing analysis of axonal

arbors caudal to the lesion, only a single monkey—Mk-CP—had an incomplete CST lesion, as shown by the presence of a few BDA-labeled CST axons that were not transected at the level of the lesion.

Notably, manual dexterity using the Brinkman board test (Fig. 1 of ref. 1) was significantly ( $P = 0.037$ ) different between the groups of anti-Nogo-A antibody-treated monkeys and of control antibody-treated monkeys. The conclusion of enhanced functional recovery in the monkeys treated with antibody to Nogo-A (anti-Nogo-A) is therefore justified.

Spinal cord injuries trigger a cascade of secondary tissue reactions as a result of bleeding, ischemia and inflammation, which lead to substantial variability within groups of animals. In this regard, the outcomes of the experimental injuries in our monkeys resemble the vari-

ability seen in human patients. In spite of the interindividual variability, however, the mean of the extent of the ventral column lesions in the control antibody-treated monkeys (28.5%) was almost identical to that in the anti-Nogo-A antibody-treated monkeys (28%; see Supplementary Table 1 of ref. 1). Note also that the only monkey in which both the dorsolateral and ventral funiculi were fully sectioned recovered better than any of the control monkeys, all of which had some portion of their ventral funiculi intact. The statement that there was slightly more ventral sparing in anti-Nogo-A antibody-treated monkeys is therefore inaccurate, and the assertion that the improved functional recovery of the anti-Nogo-A antibody-treated monkeys can be explained by the spared ventral funiculus is not supported by the data.