The Fluorescent Intensity from the Transgenic Thy1–Yellow Fluorescent Protein 16 Mouse Correlates with the Amount of Regenerated Axons

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Sir:

Neural regeneration has been electrophysiologically, histomorphometrically, and immunocytochemically evaluated.[1] However, these techniques only evaluate the regeneration state at specific time points. Development of transgenic mice with axons constitutively expressing fluorescent proteins has enabled the serial imaging of regenerated axons in the same mice.[2,3,4] However, it is unclear whether in transgenic mice, the intensity of axonal fluorescence correlates with the number of regenerated axons.

We investigated the correlation between the intensity of fluorescence from regenerated axons and the histological number of regenerated axons in transgenic mice.

We used Thy1–yellow fluorescent protein (YFP) 16 mice with all motor and sensory nerves constitutively expressing YFP fluorescence. They were housed in a central animal facility, and all procedures complied with protocols approved by the Juntendo University School of Medicine. After isoflurane anesthetization, the right sciatic nerve was exposed and crushed for 5 seconds using fine forceps.

After 2 weeks, the mice were reanesthetized to reexpose the crushed sciatic nerve. Fluorescent images were obtained using a fluorescence stereomicroscope (Leica MZ 16; Leica DFC 300FX, Wetzalr, Germany) and evaluated using the Leica Application Suite Advanced Fluorescence image acquisition software.

The distance of the regenerated axons in Thy1–YFP mice was evaluated based on the most advanced fluorescent point. We quantified the intensity 3 mm distal and proximal (▼) to the site where the nerve fluorescence intensity started to decrease (↓) (Fig. 1). The intensities of points A and B were evaluated as a ratio to the intensity of the undamaged initial nerve fluorescence (defined as 1). We obtained the nerve tissue samples from points A and B and conducted histological evaluations, such as axonal count, myelin thickness, and axon minor axis dimension at both points.

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The correlation coefficient r between the pixel fluorescence ratio and each histological parameter was calculated, and the following results were obtained: axon numbers in every field, $r = 0.9673$; mean myelin thickness, $r = 0.8487$; and mean axon minor axis, $r = 0.905$ (Fig. 2).

In general, Thy1–YFP mice were evaluated for the position of the fastest growing fluorescence along the time axis, and sometimes, regeneration speed was calculated.[2,3,5]

Thus, we investigated the consistency between the intensity of fluorescence and histological evaluation of regenerated axons in the entire nerve.

Correlation was noted for all histological values; it was stronger with the number of regenerated axons than the axon and myelin thickness. Therefore, the evaluation of intensity of fluorescence under specific conditions may be useful for the overall number of regenerated axons.

However, the conditions of fluorescence imaging could be easily changed. Therefore, imaging must be performed under a uniform condition in a dark room, and the collagenous scar (thin but opaque) around the nerve should be atraumatically removed without any damages to the axons.[5] Such technical aspects are important to obtain stable results.

In conclusion, serial in vivo imaging in Thy1–YFP mice provides real-time insight into peripheral nerve regeneration, and it is useful for evaluating the overall number of regenerated axons that can be correlated with histological values.

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DISCLOSURE

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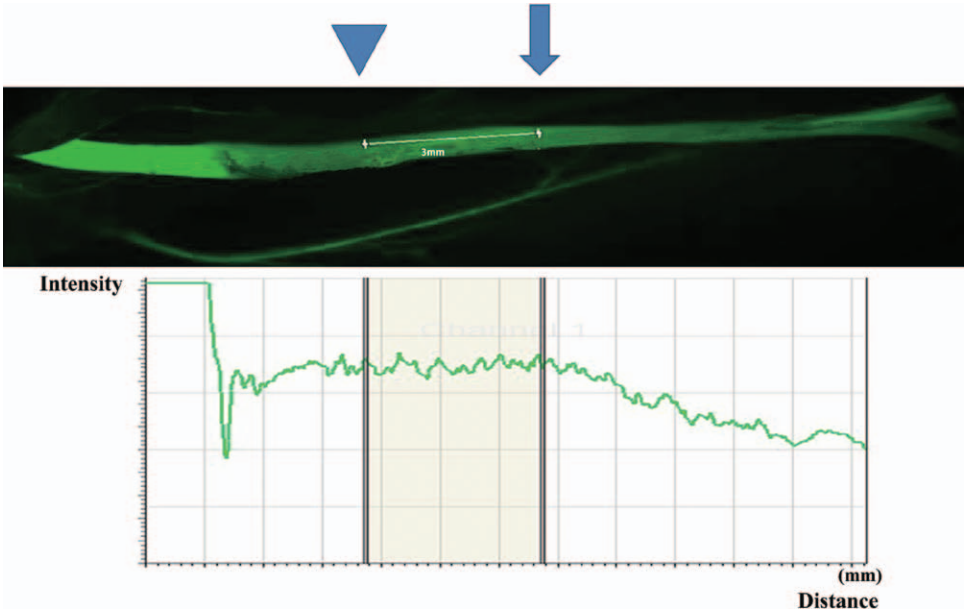

Fig. 1. The site where the fluorescence decrease began is shown by an arrow (↓). Point B was 3 mm proximal to the crushed site, which is shown by a triangle (▼).

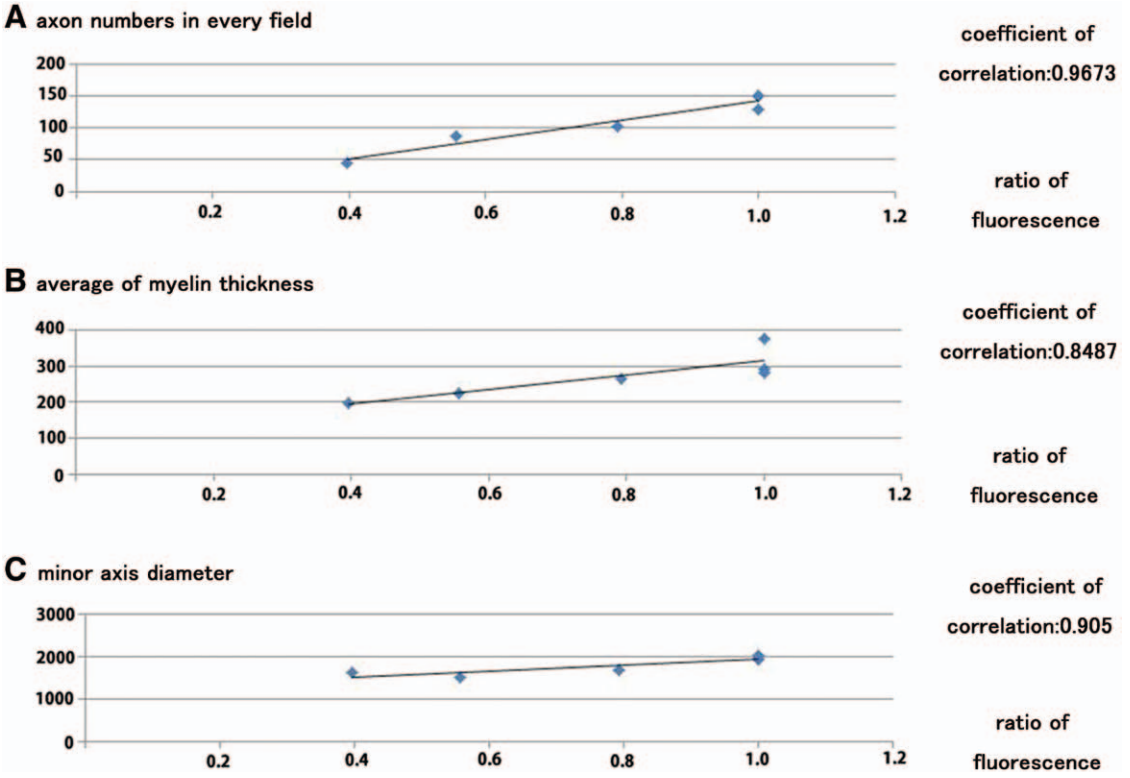

Fig. 2. A, The ratio of the intensity of fluorescence to the number of axons in every field had a correlation coefficient of 0.9673. B, The ratio of the intensity of fluorescence to the mean thickness of myelin had a correlation coefficient of 0.8487. C, The ratio of the intensity of fluorescence to the mean axon minor axis had a correlation coefficient of 0.905.