Integrated culture and purification of rat Schwann cells from freshly isolated adult tissue

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SUPPLEMENTARY RESULTS

IDENTIFICATION OF FIBROBLAST CULTURES

Fibroblasts were isolated from the epineurial connective tissue of rat sciatic nerves and cultured in basal DMEM medium (DMEM supplemented with 10% (v/v) FBS, as described in Supplementary Methods). Cells cultured at passage 3 were used for experimentation and their positive staining for Thy-1 indicated a fibroblast identity (Supplementary Figure 1a). Isotype controls demonstrated specific staining with Thy-1 antibodies in fibroblast cultures (Supplementary Figure 1b). Thy-1 staining was also used to distinguish fibroblasts from Schwann cells obtained using the present protocol. In contrast, a negative expression of Thy-1 was detected in Schwann cells, confirming Thy-1 fibroblast specificity (Supplementary Figure 1c). Furthermore, the absence of contaminating Schwann cells in fibroblast cultures was confirmed by negative staining for S100β, GFAP and p75NGFR (Supplementary Figure 1d-f). Also, it was evident that the nuclear size of cells found in Schwann cell cultures was homogeneous, whereas that observed in fibroblast cultures varied.

GROWTH INHIBITION OF NERVE FIBROBLASTS CULTURED IN SCHWANN CELL CULTURE MEDIUM

To test the inhibitory effect of the culture medium on fibroblast growth, long term culture of fibroblasts was performed using Schwann cell culture medium was conducted, and separately fibroblasts grown in basal DMEM (DMEM containing 10% (v/v) fetal bovine serum). The growth of fibroblasts under both conditions was also compared with that of Schwann cells. Fibroblasts and Schwann cells were seeded at the same density and maintained for 18 days. It was evident that fibroblasts maintained in Schwann cell culture medium displayed poor growth throughout the cultivation time (Supplementary Figure 2b, e, h), whereas fibroblasts cultured in basal DMEM or Schwann cells approached confluence at day 9 (and were over confluent by
day 18 (Supplementary Figure 2a, d, g, c, f, i)). However, the density of fibroblasts in Schwann cell culture medium was similar to that observed at day 3. In addition, some fibroblast cells developed cellular processes and assumed a stellate shape and were observed to form cytoplasmic vacuoles. It was observed that when medium was changed at day 3 it had a viscous property, indicative of the presence of DNA / protein in the medium and suggestive of fibroblasts in Schwann cell culture medium were undergoing lysis. DAAO expression was compared between Schwann cells grown in DMEM d-valine versus fibroblast cells. Analysis by RT-PCR and western blotting demonstrated that while DAAO was detectible in both cells types, the level of DAAO in Schwann cells was higher than that detected in fibroblasts (as a ration with standard housekeeping markers (Supplementary Figure 2j, k).

SELECTIVE ROLE OF MITOGENIC FACTORS

When developing this protocol, we tested the importance of each mitogen in the DMEM d-valine for supporting Schwann cell growth. Thus, each factor was withdrawn from the culture medium (forskolin, bovine pituitary extract and N2) and growth versus purity measured. Schwann cells displayed an elongated bipolar shape regardless of the culture condition Supplementary Figure 3a-e). A swirling appearance when approaching confluence was found for cells cultured in: i) complete medium; ii) medium without bovine pituitary extract or N2 and iii) medium with forskolin alone (Supplementary Figure 3a, c, d, e). Schwann cell purities revealed that the presence of mitogens were additionally important with values of 98.2 ± 1.3%, 89.9 ± 2.9%, 95.9 ± 1.0%, 97.1 ± 0.4% and 97.6 ± 0.4% (mean ± SD) for Schwann cells cultured in complete medium, medium minus forskolin, bovine pituitary extract or N2 and medium with forskolin alone, respectively (Supplementary Figure 3g). Purities were significantly higher than in cultures containing DMEM-d valine alone (79.1 ± 2.3%, P < 0.001). Moreover, the comparison between DMEM-d valine containing mitogens indicated that the Schwann cell purity from medium in the absence of forskolin was lower than that of any other condition [complete medium (P < 0.001), medium + bovine pituitary extract (P < 0.01) or N2 (P < 0.01) or medium + forskolin alone (P < 0.01)]. Of particular interest was the observation that the purity increased when forskolin was present. Similarly, Schwann cell density (see Supplementary Method for measurement) increased in complete medium (13,735 ± 5294 cells/cm² (mean ± SD), P < 0.05), medium without bovine pituitary extract (16,001 ± 4980 cells/cm², P < 0.05) or N2 (17,035 ± 4217 cells/cm², P < 0.01) and medium with forskolin alone (17,977 ± 3403 cells/cm², P < 0.01), compared to cell densities for DMEM-d valine alone (431 ± 206 cells/cm²) (Supplementary Figure 3h). Interestingly, Schwann cell densities reported for medium lacking forskolin (1,301 ± 918 cells/cm²), while being comparable to that of DMEM-d valine (431 ± 206 cells/cm²), were lower than the densities found in complete medium (13,735 ± 5294 cells/cm², P < 0.05), medium without bovine pituitary extract (16,001 ± 4980 cells/cm², P < 0.01) or N2 (17,035 ± 4217 cells/cm², P < 0.01) and medium with forskolin alone (17,977 ± 3403 cells/cm², P < 0.01).