Research Journal of Medical Sciences 5 (6): 320-323, 2011
ISSN: 1815-9346

Preferencial Culture of Schwann Cells from Primary Cultures of Human Neurofibromatosis Type 1 (NF1)

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Abstract: Neurofibromatosis type 1 (NF1)-Von Recklinghausen disease is the most frequent single-gene disorder that affects the nervous system. NF1 is inherited in an autosomal dominant manner with an estimated incidence of about 1 in 3000. The *NF1* gene is mapped to chromosome 17, (17p21). Its product neurofibromin reduces cell proliferation. The apparent lack of a suitable *in vitro* model of human Neurofibromatosis type 1 (NF1) has directly limited the progress of research on its tumourigenesis and therapy. The problems of establishing pure NF1 culture include the control of fibroblasts proliferation. At the moment, efforts put in place in order to extend the life span of NF1 Schwann cells and to suppress the growth of fibroblasts has yielded poor results in neurofibroma. Tumour specimens from 17 patients were processed for cell culture and grown at 37°C with 5% CO_2 and 100% humidity. Key modifications in limiting fibroblast proliferation included Dulbecco modified medium devoid of 10% foetal calf serum at the initial stage of the cell culture. Following 60% confluence, the unattached cells (Schwann cells) were preferentially detached. The presence of Schwann cells and the absence of fibroblast were confirmed through the staining with S-100 protein and vimentin primary antibodies. Light microscopy demonstrated the typical spindle-shaped cells. Thus, the researchers describe an easy and efficient method of obtaining Schwann cells from NF1 tissues. These pure cultures of Schwann cells are useful tools for the study of the pathogenesis of NF1 *in vitro*.

Key words: NF1, cell culture, Schwann cells, fibroblast, foetal calf serum, antibosies

INTRODUCTION

Neurofibromatosis type 1 (NF1)-Von Recklinghausen disease is the most frequent single-gene disorder that affects the nervous system. NF1 is inherited in an autosomal dominant manner with an estimated incidence of about 1 in 3000 (Wu *et al*., 2006). The *NF1* gene is mapped to chromosome 17, (17p21). Its product neurofibromin reduces cell proliferation (Wu *et al*., 2006). Neurofibromas are heterogeneous and complex benign tumours consisting mostly of Schwann cells and fibroblasts and other cell types such as axons, perineurial cells, mast cells, pericytes, endothelial cells, smooth muscle cells, collagen and cells with intermediate features (Peltonen *et al*., 1988; Korf, 1999).

Schwann cells are reported to constitute the major cell type (40-80%) in neurofibroma as defined by positive staining for the S-100 protein (Peltonen *et al*., 1988). Majority of the previous researches conducted using primary Schwann cells has been largely restricted to the use of either Schwann cells from animal origin or commercially procured Schwann cell lines. Furthermore, these cell lines derived from animal source do not often represent all the distinct characteristic of the parent cell from which it is derived from. Therefore, studies using this cell lines can be misleading and thus would lead to inaccurate and wrong inferences.

The apparent unavailability of a standardised and validated *in vitro* culture of NF1 derived Schwann cells has greatly hampered the progress of studies into the pathophysiology of NF1. Therefore, only a little progress has so far been achieved in unravelling the tumourigenesis of NF1 derived Schwann cells particularly as it relates to the NF1 genotype within the Schwann cells. This lack of a standardised and validated Schwann cell cultures from neurofibroma has been attributed to a

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number of factors among which are that NF1 are relatively uncommon tumours (1/3000 birth) and therefore, it is not easy to readily obtain samples and even those samples obtained do not survive in cell cultures due to fungal contamination. Importantly, fibroblasts often and quite readily overgrow, overwhelm and contaminate the cell culture (Nair *et al*., 2007).

Thus, the researchers carry out this study in order to establish primary Schwann cell cultures from neurofibromas which are devoid of fibroblasts so that the cellular pathophysiology of these Schwann cells can be studied in more details.

MATERIALS AND METHODS

After obtaining informed consent from patients due to undergo surgery at the Royal Liverpool and Broad Green Hospitals UK and with ethical approval, the resected tissues are placed in a container and transported on ice. Subsequently, the tumour specimens were wash with sterile Phosphate-Buffered Saline solution (PBS) with pH 7.6 in a petri dish. Tumour specimen were minced into 2 mm^3 pieces and incubated with 0.1% trypsin (Sigma) and 0.05% collagenase type 1 (Sigma) at 37°C in Dulbecco Modified Eagle Meduim (DMEM) with penecillin and streptomycin and 10% foetal calf serum overnight in order to aid dissociation.

Specimens were examined the following day for evidence of dissociation. Once the dissociation is evident, the specimens were transfer to centrifuge tubes and centrifuged. The supernatant is discarded and the cell pellets were washed twice with PBS. This procedure was repeated 3 times. Finally, the dissociated cells and the explants were resuspended in Dulbecco Modified Eagle Meduim (DMEM) with penecillin and streptomycin. Important step employed in order to limit or control fibroblast proliferation included using Dulbecco modified medium devoid of 10% foetal calf serum at the initial stage of the cell culture.

Cultures were grown at 37°C with 5% CO_2 and 100% humidity. The lack of foetal calf serum tends to limit the growth of adherens cells (Fibroblast-like cells) and thus the floating or the cells in suspension (Schwann cell-like cells) were not overwhelmed. Cultures were constantly monitored for growth and contamination by the aid of an inverted light microscope. Following 60% confluence, the unattached cells (Schwann cells) were pipette out of the culture flask and transfer on to a fresh culture flask containing DMEM with 10% foetal calf serum. This preferential pipetting was repeated for all the culture plates.

RESULTS AND DISCUSSION

Fourteen out of the seventeen NF1 culture were successfully cultured. The presence of Schwann cells and the absence of fibroblast were confirmed through the staining with S-100 (DAKO) and Vimentin antibodies. Light microscopy demonstrated the typical bipolar spindle-shaped cells (Fig. 1).

The level of contamination particularly by fungi was minimal and most common amongst cultures that were between 7-8th weeks old. Most cultures remain viable for up to 3-6 weeks. Thus, it seems likely the higher the level of passage and age of the culture the more likely for contamination by fungus and fibroblast over growth to occur. Similar observation has been reported by other researchers (Nair *et al*., 2007).

At the initial stage of the cultures, the researchers observed different subsets of cells which we thought may represent the de-differentiated Schwann cells and young fibroblastic cells. This observation has been also been reported (Nair *et al*., 2007).

However, sooner rather than later, the cell culture was populated by the bipolar spindle shape cells (Fig. 2) in all the preferentially pipetted cells. In other to characterise the bipolar spindled shape cells, the S-100 protein (DAKO) was utilised and the cells were positively stained. Thus, confirming that the bipolar cells

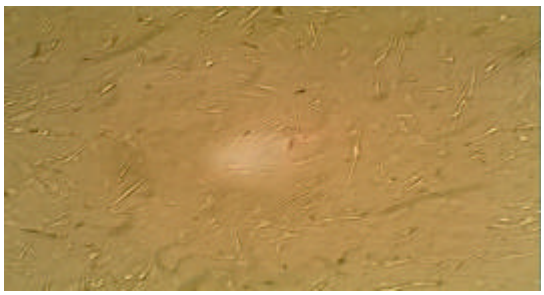

Fig. 1: Schwann cells and fibroblasts in cell suspension of Neurofibroma x100

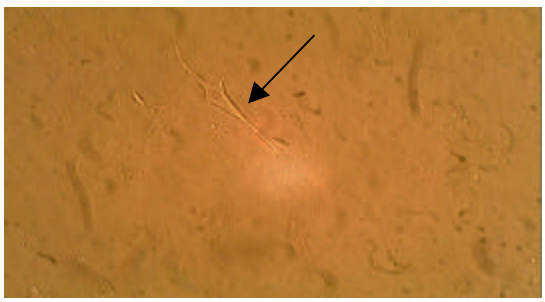

Fig. 2: Typical bipolar spindle-shaped cell (arrow) x100

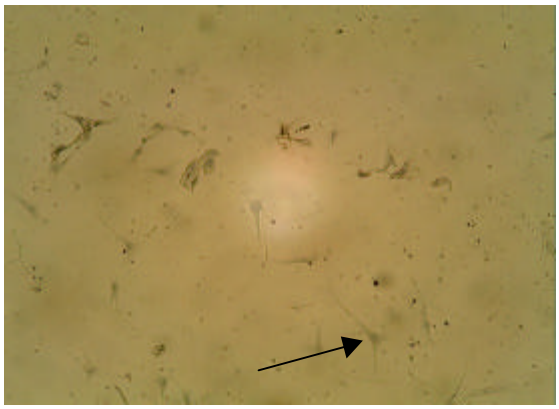

Fig. 3: S-100 nuclei staining in spindle shaped cell x100

are derivatives of Schwann cells (Fig. 3). To the best of the knowledge only few studies have been carried out in order to establish primary Schwann cell cultures from Neurofibromatosis type 1. Many of these attempts were hampered and overwhelmed with contaminations by either fibroblast or fungi or both.

The most successful study in the recent past is that conducted by Nair *et al.* (2007) in their attempt to culture primary Schwann cells from vestibular schwannomas. However, Armati *et al.* (1990) were reported to have demonstrated a selective method that favours the growth of Schwann cells from normal tissues and not from neoplastic tissues. Here, the researchers demonstrate a simple and efficient method of establishing a primary Schwann cell culture from Neurofibromatosis type-1 tumours.

The cell phenotype characterization with the calcium soluble binding protein S-100 of the bipolar cells suggests that they are of Schwann cell origin. This positive staining with S-100 protein may suggest that the Schwann cells derived from neurofibroma retain certain phenotypic characteristic with their normal counterparts. Importantly, we observed that the immunoreactivity of the Schwann cells to the S-100 protein was predominantly nuclei.

This observation is consistent with the reports of Nair *et al.* (2007) in which it was observed that even though the S-100 immunoreactivity was localised to the cytoplasm primarily, some nuclear reactivity was also noted. The significance of this nuclei staining is not fully understood. However, Flatmark *et al.* (2003) have demonstrated significant nuclear localisation in tumours and this nuclear localisation has been correlated strongly with tumor stage particularly when compared with cytoplasmic staining.

Some researchers have reported that the time required for tumour digestion is between 1-3 h (Milner *et al.*, 1997; Nair *et al.*, 2007). In this study the researchers observed that it takes much longer time to digest NF1 tumours with collagenase and trypsin and infact even after overnight digestion of the tissues with collagenase and trypsin complete dissociation of the tumour was not fully achieved.

This may be due to the fact that unlike in vestibular schwannoma that is entirely made up of Schwann cell, neurofibroma are predominatly made up of Schwann cells, fibroblasts and perineurial cells. Thus, it seems likely that a much longer time is required in order to completely dissociate the fibrous tissues from the Schwann cells. This may in part explain why overnight digestion with collagenase and trypsin may indeed be necessary in order to completely dissociate a neurofibroma tissue into its primary constituents.

Furthermore, the perineurial fibroblast of the neurofibroma which is made up alternate layers of flatten polygonal cells that are rich in collagen and fibroblast (Armati *et al.*, 1990) may also contribute to the longer time required for the digestion of neurofibroma tissue as compare with tumours such as Schwannoma that are devoid of perineurial fibroblast.

Additionally, Bunge *et al.* (1989) have suggested that the perineurial cells usually assume a fibroblastic characteristics when they are lack intrafascicular constituents and thus reverse to thier previous morphology similar to that when the neural structures are regenerating.

CONCLUSION

Finally, the researchers have described a simple and efficient method for obtaining an essentially primary Schwann cell cutures from neurofibroma devoid of fibroblast.

These cultures have shown to retain certain vital *in vivo* characteristics with their normal counter parts such as positive staining with S-100 protein. Thus, the researchers strongly suggest that these cultures would be useful tools in the study of the pathophysiology of neurofibromatosis type 1 and the subsequent treatment regimen for neurofibromatosis type 1.

ACKNOWLEDGEMENTS

The researchers wish to acknowledge the assistance rendered by the staff members of the Pancreatic Research

group for the use of the Primary Tissue Culture Laboratory the Department of Surgery, School of Cancer Studies, University of Liverpool. The researchers are also grateful to Niger state Government of Nigeria for funding of this study.

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