The Key Components of Schwann Cell-like Differentiation Medium

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Abstract

Schwann cell-like cells differentiated from adipose-derived stem cells may have important role in peripheral nerve regeneration. In this poster we present our study on the individual effects of growth factors in Schwann cell-like differentiation medium. There were six groups in the study. In the control group, we supplemented the rat adipose-derived stem cells with normal cell culture medium. In group I, we fed the cells with Schwann cell-like differentiation medium (normal cell culture medium supplemented with platelet-derived growth factor, basic fibroblast growth factor, forskolin, and glial growth factor). In other groups, we removed the components of the medium one at a time from the differentiation medium so that group II lacked glial growth factor, group III lacked forskolin, group IV lacked basic fibroblast growth factor, and group V lacked platelet-derived growth factor. We examined the expression of the Schwann cell specific genes with qRT-PCR and immunofluorescence staining in each group. Groups III and IV, lacking forskolin and basic fibroblast growth factor respectively, had the highest expression levels of integrin-β4, and p75. Group I showed a 3.2 fold increase in the expression of S100, but the expressions of integrin-β4 and p75 were significantly lower compared to groups III and IV. Group II [glial growth factor (-)] did not express significant levels of Schwann cell specific genes. The gene expression profile in group IV most closely resembled Schwann cells. Immunofluorescence staining results were parallel with qRT-PCR results. In conclusion, our data suggests that glial growth factor is a key component of Schwann cell-like differentiation medium.

Background

The peripheral nervous system has a limited capacity to heal after injury. A successful regeneration relies on a multitude of adjusted intrinsic and extrinsic factors such as Schwann cells (SCs), macrophages, fibroblasts and endothelial cells. After nerve injury, SCs become activated, assume a more primitive phenotype, and stimulate axonal growth by up regulating growth-related genes and by secretion of neurotrophins.

Since SCs has an essential role in the peripheral nerve regeneration the distance and speed of regeneration can be improved by SCs transplantation to the site of nerve injury. Despite the encouraging results obtained in preclinical studies, the main obstacles in the clinical translation of SC therapy are limited donor tissue availability, donor site morbidity, and long culture times. Recent research on peripheral nerve healing is actively focused on finding alternative sources for SCs; mesenchymal stem cells (MSCs) have therefore been investigated for their potential to develop a SC phenotype.

Adipose tissue is a rich source of MSCs. Adipose-derived mesenchymal stem cells (ASCs) can be differentiated into SC-like cells via co-culture with SCs or more commonly with a mixture of growth factors (GF) (**Table.1**).

Table 1. The growth factors used for the Schwann cell differentiation of ASCs.	
Platelet derived growth factor (PDGF)	5 ng/ml
Basic fibroblast growth factor (bFGF)	10 ng/ml
Forskolin	14 µM
Glial growth factor (GGF; Heregulin)	252 ng/ml

Aim

In this study, our aim is to examine the individual effects of GFs in ASCs-SC differentiation medium by observing the changes in gene expression patterns of ASCs in response to the removal of the components (one at a time) from the differentiation medium.

Methods

i. Isolation and characterization of ASCs

We harvested ASCs from the inguinal fat pads of female Lewis rats by enzymatic digestion (**Figure 1**) and analyzed the immunophenotype of ASCs with flow cytometry. Futhermore, we differentiated ASCs into adipogenic, osteogenic and chodrogenic lineages to verify their multipotency.

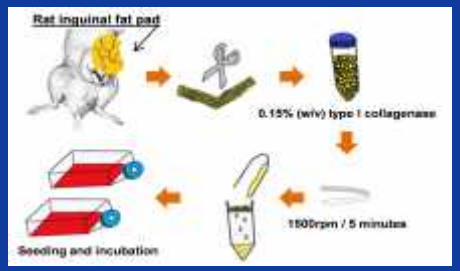

Figure 1.The harvest steps of ASCs

ii. SC-like differentiation of ASCs

We divided ASCs in culture flasks into 6 groups. In the control group, we supplemented the cells with cell growth medium. In all the other groups, initially we treated the cells with 1 mM β-mercaptoethanol (BME) for 24 hours and 35 ng/ml all-trans-retinoic acid (RA) for 72 hours. In group I, we added SC differentiation medium cell cultures. In other groups, we removed the components of the SC differentiation medium one at a time from the medium (**Table 2**). We fed the cells in all groups with corresponding differentiation medium for two weeks. We evaluated SC differentiation by immunofluorescence staining for SC specific proteins and real time qRT-PCR.

Table 2. Study groups.				
Groups	**PDGF**	**bFGF**	**Forskolin**	**GGF**
Group I	+	+	+	+
Group II	+	+	+	-
Group III	+	+	-	+
Group IV	+	-	+	+
Group V	-	+	+	+
Control	-	-	-	-

Results

i. Isolation and characterization of ASCs

ASCs expressed MSC markers CD90, and CD44 but lacked endothelial cell marker CD31 and leukocyte marker CD45 on their surface (**Figure 2**). Additionally, ASCs differentiated into adipogenic, osteogenic, and chondrogenic lineages as a proof of their multipotency (**Figure 3**).

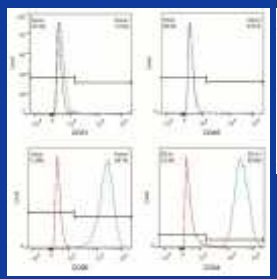

Figure 2. Flowcytometric analysis revealed that ASCs expressed MSC markers (CD90 and CD44) but they were negative for endothelial cell marker (CD31) and leucocyte marker (CD45).
Figure 3. ASCs were differentiated into osteogenic (A), adipogenic (B) and chondrogenic (C) lineages to demonstrate their multipotency. Microbars, 100 µm (A); 50 µm (B, C).

ii. IF staining and qRT-PCR for SC specific proteins

S100 protein was positive in all the groups, but the staining in groups I, III, IV and V was stronger than in other groups (**Figure 4A**). The weakest staining for S100 was in group II, which lacked GGF. Overall, the IF staining pattern that was most compatible with SC phenotype was observed in groups IV and V. The gene expression profile in groups III and IV were most compatible with a SC genetic profile **(Figure 4B)**.

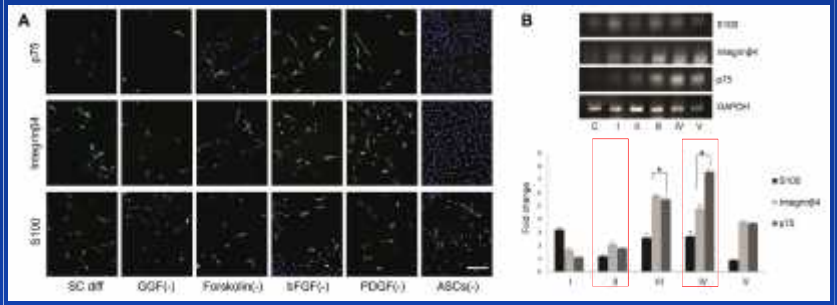

Figure 4. (A) The results of IF staining **(B)** The results of qRT-PCR.

Conclusion

Glial growth factor is a key component of Schwann cell-like differentiation medium. The omission of GF from SC differentiation medium decreases the SC differentiation potential of ASCs significantly.