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Gingival and dermal fibroblasts: Their similarities and differences revealed from gene expression

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Gene expression profiles in normal human gingival and dermal fibroblasts were investigated using DNA microarrays. Their fundamental characteristics were almost identical, but 5% of their genes were uniquely expressed. These results help us to choose an optimal cell source for effective fibroblast-based cell therapy that is dependent on differential gene expression profiles.

[**Key words:** Fibroblast; Gingival tissue; Anti-aging; Microarray; Antioxidant]

Fibroblasts are widely used for regenerative medicine in clinics, such as gingival (1) or facial skin treatment (2). In fact, fibroblasts are considered to be a mixture of various types of cells of "spindle shape" and as such there are no clearly defined biomarkers of fibroblasts. Gingival and dermal fibroblasts are similar in their morphology and function. However, it is considered that cultured cells retain the original characteristics of the tissue of origin and therefore may induce differential therapeutic effects. For example, gingival wounds are known to heal relatively quickly with less scar formation compared with skin wounds, which may imply that gingival fibroblasts have a higher capability for regeneration in cell-based therapies (3). The reason for this phenomenon may be partly due to characteristic differences between gingival and dermal fibroblasts including the expression of migration stimulating factor (4) and matrix formation (5) but these differences remain largely unknown. Recently, the characteristics of dermal fibroblasts have been reported to be different depending on the skin source, such as face, trunk and palmoplantar skin (6). Although the expression of fibronectin and its alternative splice variants are known to be different between trunk and oral mucosal fibroblasts, there is still no detailed report on the functional differences between gingival and dermal fibroblasts (7). In this study, we investigated differential gene expression in normal gingival and dermal fibroblasts using DNA microarray to investigate the difference between the vague fibroblast-type cells from different tissue origin to achieve higher therapeutic effect in cell therapy.

This study conformed to the tenets of the Declaration of Helsinki. Dermal and oral tissues were obtained from healthy volunteers (8 cases of facial skin from the postauricular crease: 5 females, 3 males, average age 48, and 8 cases of oral mucosa from the posterior vestibule: 6 females, 2 males, average age 43) whose informed consent was obtained according to a protocol approved by the ethics committee of Nagoya University Hospital. After enzymatic digestion, dermal and mucosal fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum at 37°C in the presence of 5% CO_2 for about 4 weeks as reported previously (8). Total mRNAs were extracted from cells between passages 4–5 by Trizol reagent (Invitrogen, Carlsbad, CA, USA) and were applied to Human Focus Arrays (Affymetrix, Santa Clara, CA, USA) for microarray analysis according to the manufacturer's protocol (http://www.affymetrix.com/support/technical/manuals.affx). The gene expression data were analyzed by Arrayassist (Stratagene, La Jolla, CA, USA).

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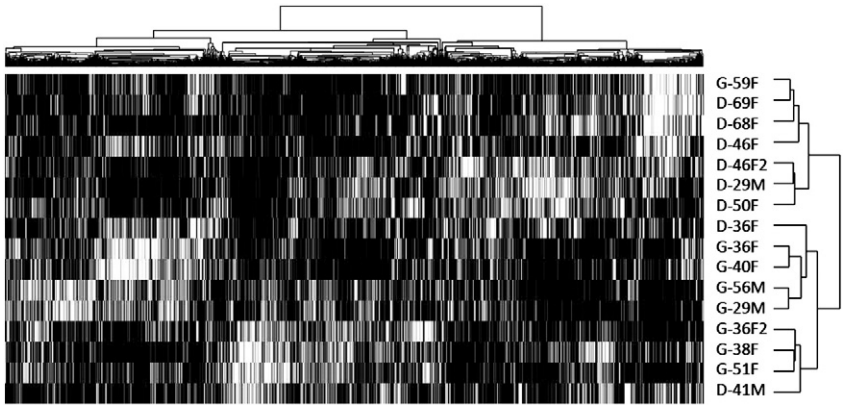

FIG. 1. Hierarchical clustering of 5284 genes comparing gingival and dermal fibroblasts (8 clinical samples were collected for each tissue origin). Samples are designated by tissue origin (G; gingival/D; dermal) followed by ages and sex (M; male/F; female). Rows, samples; columns, genes. White, highly expressed in dermal fibroblasts (>0.8); Black, highly expressed in gingival fibroblasts (−1.0), intermediate colors represents the gradient between white and black.

Briefly, 8500 probes on the array, normalization and scaling (MAS5), flag-positive gene selection, unpaired *t*-test, and CV selection (<20) resulted in 5284 genes to analyze (GEO accession number: GSE22029). GO (Gene ontology) analysis was performed using the software default settings to find the gene group related to the same category of biological function by searching common key terms that were reported for each gene.

From comparison of expression profiles of 5284 genes in dermal and gingival fibroblasts, only 5% (278 genes) showed a significant difference in the levels of expression after noise reduction. These results and the GO analysis indicated that the fundamental characteristics of both dermal and gingival fibroblasts were almost identical. On the other hand, among 278 genes, 164 and 114 genes were uniquely expressed in gingival and dermal fibroblasts, respectively. Interestingly, the number and fold differences of those uniquely expressed genes were more evident in gingival fibroblasts. Hierarchical clustering of total genes showed that the difference between gingival and dermal fibroblasts was larger than the difference between ages or gender of patients (Fig. 1). This indicates that gingival and dermal fibroblasts retain most of their functions in common, although there were some genes that specifically characterized each fibroblast type. Table 1 indicates representative GO hierarchies, which showed significant fold differences. Major differences were observed in extracellular matrix-related genes, oxidoreductase activity-related genes, cytokine activity-related genes and growth factor-related genes.

TABLE 1. Gene list from microarray analysis of gingival fibroblasts and dermal fibroblasts.

GO hierarchy	Gene title	Probe set ID	*p*-value	Tissue specificity	Fold change
Aging	T-box 2	40560_at	0.002	gingival	2.68
Odioreductase activity	hydroxysteroid (17-beta) dehydrogenase 2	204818_at	0.000	gingival	15.69
	dehydrogenase/reductase (SDR family) member 3	202481_at	0.000	gingival	5.45
	aldo-keto reductase family 1, member B10 (aldose reductase)	206561_s_at	0.002	gingival	4.31
	superoxide dismutase 3, extracellular	205236_x_at	0.004	gingival	3.97
	lectin, galactoside-binding, soluble, 3 binding protein	200923_at	0.000	gingival	3.38
	cytochrome P450, family 26, subfamily B, polypeptide 1	219825_at	0.010	dermal	6.69
	prostaglandin-endoperoxide synthase 1	215813_s_at	0.003	dermal	3.12
Antioxidant activity	superoxide dismutase 3, extracellular	205236_x_at	0.004	gingival	3.97
	prostaglandin-endoperoxide synthase 1	215813_s_at	0.003	dermal	3.12
Extracellular matrix	glypican 3	209220_at	0.001	gingival	13.29
	collagen, type IV, alpha 1	211980_at	0.000	gingival	4.24
	superoxide dismutase 3, extracellular	205236_x_at	0.004	gingival	3.97
	lectin, galactoside-binding, soluble, 3 binding protein	200923_at	0.000	gingival	3.38
	matrix metallopeptidase 12 (macrophage elastase)	204580_at	0.003	dermal	25.54
	tenascin C (hexabrachion)	201645_at	0.006	dermal	6.56
	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	217428_s_at	0.005	dermal	3.86
Cytokine activity	chemokine (C-X-C motif) ligand 14	218002_s_at	0.009	gingival	8.20
	platelet-derived growth factor alpha polypeptide	205463_s_at	0.001	gingival	6.21
	tumor necrosis factor (ligand) superfamily, member 10	202688_at	0.007	gingival	5.81
	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	203666_at	0.007	gingival	4.01
	midkine (neurite growth-promoting factor 2)	209035_at	0.002	gingival	3.07
	cytochrome P450, family 26, subfamily B, polypeptide 1	219825_at	0.010	dermal	6.69
Growth factor activity	insulin-like growth factor 2 (somatomedin A)	202409_at	0.003	gingival	21.88
	platelet-derived growth factor alpha polypeptide	205463_s_at	0.001	gingival	6.21
	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	203666_at	0.007	gingival	4.01
	jagged 1 (Alagille syndrome)	216268_s_at	0.004	gingival	3.33
	placental growth factor, vascular endothelial growth factor-related protein	209652_s_at	0.007	gingival	3.09
	midkine (neurite growth-promoting factor 2)	209035_at	0.002	gingival	3.07
Insulin related	insulin-like growth factor 2 (somatomedin A) /// insulin–insulin-like growth factor	202409_at	0.003	gingival	21.88
	insulin-like growth factor binding protein 4	201508_at	0.010	gingival	2.00
	insulin-like growth factor binding protein 6	203851_at	0.010	gingival	2.42
Angiogenesis related	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	204642_at	0.002	gingival	3.61
	endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 7	220816_at	0.005	dermal	4.56