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Expression Profiling of the Ephrin (*EFN*) and Eph Receptor (*EPH*) Family of Genes in Atherosclerosis-related Human Cells

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Ephrin B1 and its cognate receptor, Eph receptor B2, key regulators of embryogenesis, are expressed in human atherosclerotic plaque and inhibit adult human monocyte chemotaxis. Few data exist, however, regarding the gene expression profiles of the ephrin (*EFN*) and Eph receptor (*EPH*) family of genes in atherosclerosis-related human cells. Gene expression profiles were determined of all 21 members of this gene family in atherosclerosis-related cells by reverse transcription–polymerase chain reaction analysis. The following 17 members were detected in adult human peripheral blood monocytes: *EFNA1* and *EFNA3* – *EFNA5* (coding for ephrins A1 and A3 – A5); *EPHA1, EPHA2, EPHA4* – *EPHA6* and *EPHA8* (coding for Eph receptors A1, A2, A4 – A6 and A8); *EFNB1* and *EFNB2* (coding for ephrins B1 and B2); and *EPHB1* – *EPHB4* and *EPHB6* (coding for Eph receptors B1 – B4 and B6). THP-1 monocytic cells, Jurkat T cells and adult arterial endothelial cells also expressed multiple *EFN* and *EPH* genes. These results indicate that a wide variety of ephrins and Eph receptors might affect monocyte chemotaxis, contributing to the development of atherosclerosis. Their pathological significance requires further study.

KEY WORDS: Atherosclerosis; Inflammation; Cell migration; Ephrin; Eph receptor; Gene expression profile

Introduction

In the development of atherosclerosis, monocytes transmigrate through the endothelium and differentiate into macrophages.[1,2] It was previously demonstrated that ephrin B1 cell signalling peptide and its cognate receptor, ephrin receptor B2 (EphB2), which are key regulators of embryogenesis and morphogenesis,[3,4] are expressed in atherosclerotic lesions, and that both ephrin B1 and EphB2 inhibit monocyte chemotaxis.[5] There are few data, however, on the gene expression profile of the ephrin (*EFN*) and Eph receptor (*EPH*) family of genes in atherosclerosis-related human cells. The present study, therefore, analysed the expression of all 21 members of the *EFN* and *EPH* gene family in adult human monocytes and related cells.

Materials and methods

This study was performed in accordance with the International Code of Medical Ethics of the World Medical Association (Declaration of Helsinki).

CELL PURIFICATION AND CULTURE

Mononuclear cells from venous blood of healthy adult volunteers were prepared using Lymphoprep™ (Axis-Shield, Oslo, Norway). Monocytes were enriched by counter-flow centrifugal elutriation (R5E elutriation system; Hitachi Koki, Ibaraki, Japan) as described previously.[5] THP-1 monocytic cells and Jurkat T cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a 5% carbon dioxide atmosphere. Adult human coronary artery endothelial cells (HCAEC) were obtained from the Applied Cell Biology Research Institute (Kirkland, WA, USA) and maintained in CSC medium (Applied Cell Biology Research Institute) at 37 °C in a 5% carbon dioxide atmosphere. For experiments with HCAEC cells up to the third passage were used.

RNA ISOLATION AND RT–PCR TEMPLATE PREPARATION

Total RNA was isolated from the cells using Isogen reagent (Nippon Gene, Tokyo, Japan),[5–8] and was cleared of genomic DNA by the use of genomic DNA wipe-out buffer from the QuantiTect™ Reverse Transcription Kit (Qiagen, Hilden, Germany). Reverse transcription–polymerase chain reaction (RT–PCR) was used in the present study instead of a microarray[9] because of its specificity. Forward and reverse primers were designed for particular exons within each gene using human genomic DNA as the common positive control template. The exon–intron structures of all human *EFN* and *EPH* genes were identified through the Map Viewer Web site (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi). The primer sets used in this study are shown in Table 1. Ex Taq™ polymerase (TaKaRa, Tokyo, Japan) was used for PCR and the reaction mixture was assembled to a total volume of 10 µl as follows: 6.65 µl water, 1.0 µl 10 × Ex Taq™ buffer, 0.8 µl dNTP mixture (comprising 2.5 mM of each nucleotide), 1.0 µl forward and reverse primers (5 µM of each primer), 0.5 µl template and 0.05 µl Ex Taq™ polymerase. The PCR was carried out with pre-heating (94 °C for 2 min) and 30 or 35 cycles of amplification (94 °C for 20 s, 55 °C or 60 °C for 30 s and 72 °C for 40 s). DNA-cleared RNA without reverse transcription and human genomic DNA (50 nM; Clontech, Palo Alto, CA, USA) were used as negative and positive control templates, respectively. For all cell types, PCR was repeated three to five times and representative data are shown.

Results

VALIDATION OF RT–PCR CONDITIONS

Each primer set amplified a single PCR product from genomic DNA (Fig. 1, lane 3 in each column) and no product from the DNA-cleared RNA (Fig. 1, lane 2 in each column). Thus, the RT–PCR products were specific to the target genes and were derived from the synthesized cDNA. When expression of the genes coding for ephrin B1 (*EFNB1*) and EphB2 (*EPHB2*) were examined in human monocytes, THP-1 cells and HCAEC (Fig. 1), expression was consistent with our previous data obtained by RT–PCR using different primers and an immunofluorescence technique.[5] The present RT–PCR method was, therefore, reliable for analysis of the

TABLE 1:
Primer sets, annealing temperatures (temp.) and expected amplified fragment sizes for reverse transcription–polymerase chain reaction analysis of genes encoding ephrin cell signalling peptides and their cognate ephrin receptors

Gene encoding	Gene name	Accession No.	Exon No.	Primers (forward/reverse: 5′ → 3′)	Temp. (°C)	Fragment size (base pairs)
Ephrin A1	*EFNA1*	NM_004428	2	ACACCATACATGTGCAGCTG/ACAGTATGTACTGCTCCATG	55	99
Ephrin A2	*EFNA2*	NM_001405	4	CGAGACCCTGTACGAGGCTC/GCTGCTACACGAGTTATTGC	55	58
Ephrin A3	*EFNA3*	NM_004952	2	TACGTGCTGTACATGGTGAG/AGAGAGAAGGCGCTGTAGC	55	146
Ephrin A4	*EFNA4*	NM_005227	2	CAACGATTACCTAGACATTGTC/GTAGTAGTAAGTCTCTCCAG	55	247
Ephrin A5	*EFNA5*	NM_001962	2	GTGACTACCATATTGATGTCTG/GACGGAGTCCTCATAGTGAG	55	71
Ephrin receptor A1	*EPHA1*	NM_005232	6	AGGATGTCAGATACAGTGTG/TGACATGCACTGCAGGTGTG	55	135
Ephrin receptor A2	*EPHA2*	NM_004431	5	TGTCTACAGCGTCACCTGCG/ATGCTGACACTGGCAGTACG	65	221
Ephrin receptor A3	*EPHA3*	NM_005233	5	ACGAGACCTCAGTTATCCTG/AGAAGGTCTGTCACTGTCAC	65	190
Ephrin receptor A4	*EPHA4*	NM_004438	7	TGAGCGAAGCTATCGTATAG/CTCACTGAAGTCTCCATAGC	55	127
Ephrin receptor A5	*EPHA5*	NM_004439	3	TACAGAGGTCAGAGATGTAG/AGACAGCCAAGTGTCGTAC	55	140
Ephrin receptor A6	*EPHA6*	XM_114973	5	AGAGTGCTGAAGAGCGTGAC/ATATCCTGTACTGCAGATGC	55	95
Ephrin receptor A7	*EPHA7*	NM_004440	3	ACAGACTATGACACTGGCAG/CTCTGCACTGCTGACACATG	65	330
Ephrin receptor A8	*EPHA8*	NM_020526	11	AGTTCACCATCATGCAGCTG/AAGTCAGACACCTTGCAGAC	65	145
Ephrin B1	*EFNB1*	NM_004429	5	GTCCTACTACTGAAGCTACG/CTCTTGGACGATGTAGACAG	55	222
Ephrin B2	*EFNB2*	NM_004093	5	GCATCATCTTCATCGTCATC/GCTGACCTTCTCGTAGTGAG	65	221
Ephrin B3	*EFNB3*	NM_001406	2	ATGTGCTGTACCCTCAGATC/ATGATGTAGTAATCGTGGTGCG	65	271
Ephrin receptor B1	*EPHB1*	NM_004441	3	AGAAGTCAGTGGCTACGATG/TGCAGTCTCTCACAGTGAAG	65	161
Ephrin receptor B2	*EPHB2*	NM_004442	6	GCAGTGTCCATCATGCATC/AGTACTGCAGCTCATAGTCC	65	109
Ephrin receptor B3	*EPHB3*	NM_004443	5	ACCTCACTGATCCTCGAGTG/GTTGTCATCACAGCGTGAGC	65	129
Ephrin receptor B4	*EPHB4*	NM_004444	11	GAGCTGTGTGGCAATCAAG/ACTCTGTGAGAATCATGACG	55	161
Ephrin receptor B6	*EPHB6*	NM_004445	9	CTGAGAGCCGAGTGTTAGTG/TGACATTGATGGCTGCAGC	65	123

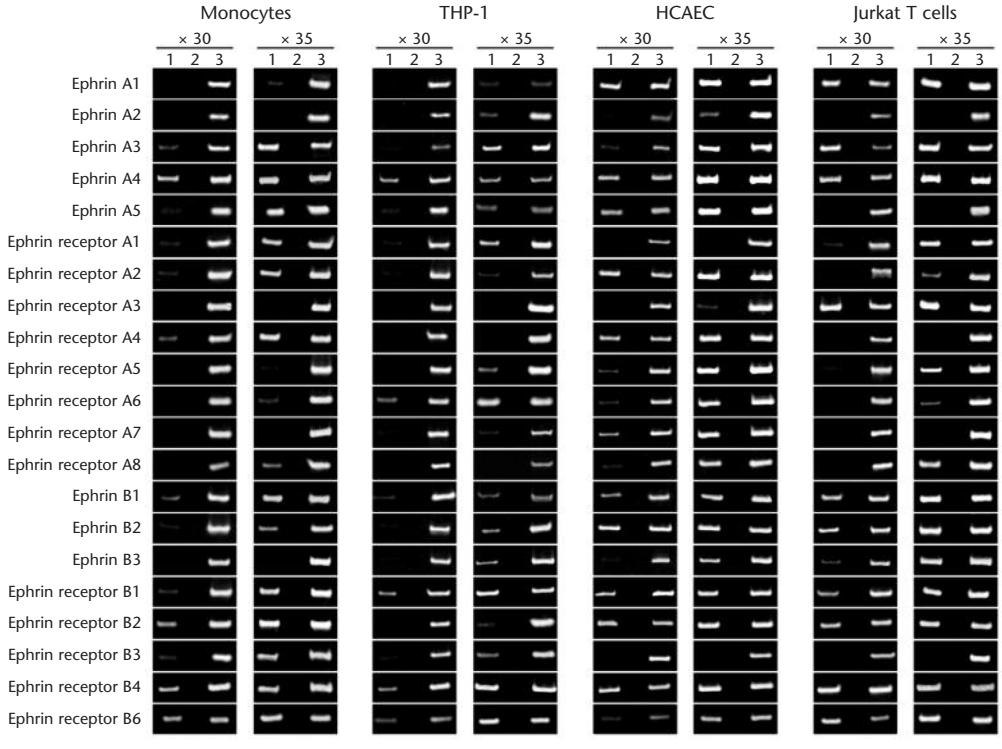

FIGURE 1: Expression profiling of genes encoding ephrin cell signalling peptides and their cognate ephrin receptors in adult human peripheral blood monocytes, THP-1 cells, adult human coronary artery endothelial cells (HCAEC) and Jurkat T cells by reverse transcription–polymerase chain reaction (RT–PCR). The templates used were: lanes 1, cDNA; lanes 2, DNA-cleared RNA; lanes 3, genomic DNA and the PCR was carried out for 30 (× 30) or 35 (× 35) cycles using the primer sets listed in Table 1

expression of the human *EFN* and *EPH* family of genes.

EFN AND *EPH* EXPRESSION IN MONOCYTES AND THP-1 CELLS

In adult human peripheral blood monocytes, multiple *EFN* and *EPH* genes of both the A and the B subclasses were detected. All *EFN* and *EPH* genes were detected except those coding for ephrin A2, EphA3 and EphA7 and ephrin B3 (Fig. 1, monocytes, × 35). Strong signals were observed for the genes coding for ephrin A4 and EphB2, EphB4 and EphB6 (Fig. 1, monocytes, × 30). In human monocytic THP-1 cells, all *EFN* and *EPH* genes were found except those coding for EphA3, EphA4 and EphA8 (Fig. 1, THP-1, × 35) and robust signals were obtained for the genes coding for ephrin A4, EphA6, EphB1, EphB4 and EphB6 (Fig. 1, THP-1, × 30). The expression patterns of the *EFN* and *EPH* genes in adult human monocytes and THP-1 cells showed similarities, though with some disparities which might have been due to immortalizing processes occurring in THP-1 cells.[10]

EFN AND *EPH* EXPRESSION IN HCAEC AND JURKAT T CELLS

Multiple members of the A and B subclasses of *EFN* and *EPH* genes were also detected in HCAEC and Jurkat T cells. In HCAEC, all members except the genes coding for EphA1

and EphB3 were found (Fig. 1, endothelial cells, × 35) and strong signals were detected for the genes coding for ephrins A1, A4 and A5, EphA2 and EphA4, ephrins B1 and B2, and EphB1, EphB2 and EphB4 (Fig. 1, endothelial cells, × 30). In Jurkat T cells, all members except the genes coding for ephrins A2 and A5, EphA4, EphA7 and EphB3 were detected (Fig. 1, Jurkat, × 35) and robust bands were obtained for the genes coding for ephrins A1, A3 and A4, EphA3, ephrins B1 and B2, EphB1, EphB2, EphB4 and EphB6 (Fig. 1, Jurkat, × 30). The pattern of redundant expression of *EFN* and *EPH* genes in HCAEC and Jurkat T cells was consistent with previous reports.[11,12]

Discussion

Ephrins are divided into two subclasses according to the way in which they are bound to the cell membrane: those of subclass A (ephrins A1 – A5) are attached to the plasma membrane by a glycosylphosphatidylinositol anchor, whereas those of subclass B (ephrins B1 – B3) have a single transmembrane domain.[3,4] Ephrins of subclasses A and B interact primarily with Eph receptors of subclasses A (EphA1 – EphA8) and B (EphB1 – EphB4 and EphB6), respectively. Characteristically, ephrins and Eph receptors can mediate bidirectional signalling: classical forward signalling by Eph receptors via their intrinsic tyrosine kinase activity and reverse signalling by ephrins of subclass B via their conserved cytoplasmic domain.[3] Despite intensive study, the significance of ephrins and Eph receptors in adults is still unclear.

We previously reported that ephrin B1 and EphB2 were expressed in both dilated and stenotic lesions associated with atherosclerosis.[5] In the inflammatory process, monocytes adhere to endothelial cells during transmigration[13] and to T lymphocytes as antigen-presenting macrophages.[14] Through these cell-to-cell interactions, ephrins and Eph receptors on monocytes/macrophages can bind to their counterparts on other types of cell or to other monocytes/macrophages. Ephrin B1 and reverse signalling by EphB2 inhibit monocyte chemotaxis.[5] Several ephrins of both subclasses A and B can inhibit the chemotaxis of Jurkat T cells[12] and ephrin B1 promotes endothelial cell migration.[15]

These findings suggest that a wide variety of ephrins and Eph receptors might modulate the chemokine-conditioned transmigration/chemotaxis of monocytes.[9] The ephrin/Eph receptor system might provide clues about the regulatory mechanisms of monocytes/macrophages and the mechanisms underlying other macrophage-related inflammatory diseases in adults,[16 – 20] and requires further study.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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