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Human hair follicles as a peripheral source of tyrosine hydroxylase and aromatic l-amino acid decarboxylase mRNA

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Abstract

Total RNA from human hair follicles was reverse transcribed and amplified using primers specific for aromatic l-amino acid decarboxylase (AADC) and tyrosine hydroxylase (TH). Agarose electrophoresis of the amplified products showed reverse transcription polymerase chain reaction (RT-PCR) products of the expected size for both TH and AADC. Sequencing showed that the amplified products matched the known sequences of TH and AADC. This study identifies hair follicles as a convenient, uninvase, source of the mRNA for TH and AADC. Analysis of these mRNA's may be useful in the diagnosis and investigation of conditions resulting from qualitative changes in the genes that code for these enzymes. © 1997 Elsevier Science Ireland Ltd.

Keywords: Tyrosine hydroxylase; Aromatic l-amino acid decarboxylase; Hair follicle; Keratinocyte

Inherited defects affecting tyrosine hydroxylase (TH) [5,6] and aromatic l-amino acid decarboxylase (AADC) [4,7] have recently been described. Establishing the molecular cause of these defects is complicated by the lack of an easily obtainable source of tissue which contains the TH and AADC mRNA or protein. Detection of mutations has therefore relied on sequence analysis of genomic DNA [5,6] which is laborious and does not allow detection of different mRNA species in cases where multiple splicing exists. We recently demonstrated the presence of TH and AADC mRNA in human keratinocytes [2]. These cells can be cultured in the laboratory and could provide a useful source of mRNA for reverse transcription polymerase chain reaction (RT-PCR), however, the methods for collection of skin tissue and the culture of keratinocytes are not standard practice in most laboratories. Human hair roots contain cuticle keratinocytes which are derived from the follicle bulb cells during the development of

human hair [8]. These hair follicle keratinocytes are reported to be similar to the keratinocytes in skin epidermis. Furthermore, hairs are intimately associated with multiple structures which respond to adrenergic input [12]. We therefore examined human hair follicles to determine whether they might provide a source of TH or AADC mRNA that could be more easily obtained in a clinical setting.

We first tested whether total RNA extracted from hair roots contained detectable levels of TH and AADC mRNA. Hair roots (approximately 10) were collected from the scalp and placed immediately in 1 ml of ice-cold RNA extraction solution (guanidinium/isothiocyanate/phenol/chloroform) [13] and stored at –20°C. On thawing, the tubes were left at 4°C for 30 min and then centrifuged for 15 min and the aqueous phase removed. Total RNA was precipitated from the aqueous phase with isopropanol [13] and resuspended in 30 µl H₂O. The purified total RNAs (8 µl) were checked for integrity by separation in a 1.5% agarose gel and visualization using ethidium bromide staining.

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Fig. 1. Ethidium bromide-stained 2% agarose gel showing RT-PCR products from human hair roots. Lanes 1 and 5, DNA size standard, ready-load 1 kb DNA ladder from Life Technologies (Gaithersburg, MD, USA); Lanes 2 and 3, AADC product (expected size 597 bp) from two sources of human hair; Lanes 6 and 7, TH product (expected size 391 bp) from two sources of human hair; Lane 4, negative control.

RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (8 μ l in a 20 μ l reaction mix). For TH, the reverse transcription primer was complementary to a sequence in exon 10 (5'-GCGTGGA-CAGCTTCTCAATTTC-3', bp 1124–1103 in [3]; Acc# X05290). For AADC, the reverse transcription primer was complementary to a sequence in exon 14 (5'-GTGACATGGAACCAAGTGG, bp 1406–1388 in [11]; Acc# M88700). After denaturation (94°C for 5 min), annealing was performed at 56°C for 30 min. RT proceeded at 41°C for 1 h and was terminated by heating at 95°C for 5 min.

For both TH and AADC, two rounds of PCR were performed. The upstream primers for TH and AADC were specific for sequences in the respective 3rd exons (TH: 5'-GTACTTCGTGCGCCTCGAGGTG, bp 406–427; AADC: 5'-CCATTGGCTGCATCGGCTTCTC, bp 379–400) and were used in conjunction with the downstream primers described above. After initial denaturation and hot start PCR using 10 μ l of the RT mix in a 50 μ l PCR reaction, 40 cycles of denaturation for 30 s at 96°C; annealing for 40 s at 55°C; elongation for 50 s at 72°C were performed. PCR was concluded with 10 min at 72°C and final incubation at 4°C. A second round of PCR was carried out using 2 μ l of the first PCR products as templates in a final mix of 50 μ l. The same upstream primers were used but nested downstream primers (for TH located in exon 7, 5'-CCAGCAAAGCAAAGGCCTCC, bp 868–849 in TH1; for AADC located in exon 8, 5'-TCCACTC-CATTCAGAA- GGTGC, bp 964–944) were substituted for the previous primers. The same PCR program and cycle number were employed. The amplified products were analyzed on a 2% agarose gel using 8 μ l of the 50 μ l reaction and visualized using ethidium bromide staining. RT-PCR products of the expected size for both TH (391 bp) and AADC (597 bp) were detected (Fig. 1) suggesting that hair roots might be a convenient source of mRNA.

In order to confirm that the amplified RT-PCR products represent bona fide TH and AADC mRNA, we determined the sequence of the amplified products. The DNA sequence was determined by the dideoxynucleotide chain termination method [10]. PCR products were incubated at 37°C for 15 min with exonuclease to degrade excess PCR primers and shrimp alkaline phosphatase to degrade the remaining dNTPs and the reactions terminated by heating at 80°C for 15 min. After adding specific sequencing primers (TH, 5'-TGTCAGAGCTGG-ACAAGTGT, bp 621–602; AADC, 5'-TCACACAG-GCCGCTATCATG, bp 611–592), double-stranded DNA sequencing was performed using sequenase version 2.0 (United States Biochemical) following the manufacturer's protocol. The PCR products were denatured by heating 2–3 min at 100°C and cooled for 5 min in an ice-water bath. [$\Delta\alpha^{35}$]dATP was added to the PCR product/primer cocktail and the extension reaction allowed to proceed for 5 min at room temperature. The termination mix was added and the sequencing reaction allowed to proceed at 37°C for 10 min. Sequencing products were separated using a 6% acrylamide-7 M urea sequencing gel. Exposure time of the autoradiograph was 48 h at room temperature. This analysis revealed that the amplified products matched the known sequences of TH and AADC, respectively (Fig. 2).

As the result of recent studies, we are becoming increasingly aware of inherited defects in the enzymes responsible for synthesis and catabolism of the catecholamines and serotonin [1,4,5,7]. Here, we are concerned with the TH and AADC genes. As a first step in understanding the molecular basis for altered levels of metabolites and changes in enzyme activity, it is appropriate to determine whether there are changes in the coding sequences of the

Fig. 2. Double-stranded sequencing results from TH (A) and AADC (B), matches the published sequences (664–677 for TH and 654–667 for AADC).

suspect genes that might alter enzymatic function. This can be done by sequencing genomic DNA from patients or more readily by sequence analysis of the relevant mRNA. Direct analysis of the genomic sequence is tedious since both genes contain 15 or more exons, some of which are less than 30 bp in length. Analysis of the mRNA is preferred since the entire coding sequence is present and the mRNAs are only ~2 kb in length. However, analysis of these genes via the mRNA has been difficult due to the lack of an easily accessible source of tissue containing the message. Catecholamines serve as precursors to melanin and since hair is pigmented by melanin and hair follicles are intimately associated with sweat glands, arterioles, and erector pili muscles, all of which respond to adrenergic input [12], we reasoned that hair follicles might be a potential source of mRNA for genes in this pathway. We show here that hair follicles, obtained by the fairly uninvase method of simple pulling of scalp hair, contain mRNA for both TH and AADC in sufficient quantities to be analyzed by RT-PCR. It has recently been demonstrated that keratinocytes contain the enzymes necessary to synthesize and catabolize catecholamines [9]. These cells are intimately associated with the hair root and they contain the mRNA for TH and AADC [2]. It is, therefore, possible that keratinocytes are recovered by this method and provide the source of mRNA; however, since the final sample obtained is crude, we do not know the nature of the various cells recovered nor do we know yet which cell types actually contain the TH and AADC mRNAs.

Whatever the cellular origin of the TH and AADC mRNA, hair roots, provide a convenient source of mRNA which may prove useful in diagnosing conditions resulting from qualitative changes in the TH and AADC. It is possible that there may also be expression of other catecholamine related genes in follicles (e.g. dopamine β -hydroxylase, monoamine oxidase, catechol-*O*-methyltransferase, adrenergic receptors). If so, analysis may permit changes in the mRNA sequence to be determined.

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