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Original Paper

Expression of the Aromatic L-Amino Acid Decarboxylase mRNA in Human Tumour Cell Lines of Neuroendocrine and Neuroectodermal Origin

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Neuroendocrine differentiation of lung tumours is characterised by the expression of several neuroendocrine markers and is confined mostly to specific histological subtypes, i.e. small cell carcinomas and carcinoids. One of the markers seen in neuroendocrine tumours, high activity of the aromatic L-amino acid decarboxylase (AADC), is helpful in distinguishing the classic and variant small cell lung tumour subtypes. Here, we have analysed the expression and quantified the level of mRNA coding for AADC in human tumour cell lines by use of the reverse transcription and polymerase chain reaction (RT–PCR). High amounts of mRNA were detected in classic small cell lung carcinomas and a neuroblastoma cell line. Other cell lines (melanomas, non-small cell lung carcinomas and osteosarcoma) also showed AADC expression, but the levels were 2–3 orders lower. Also, the tissue-specific (neuronal versus liver-specific) mRNA type has been estimated. Small cell lung carcinomas, neuroblastoma and melanoma expressed messenger RNA specific for neuronal tissues. Importantly, the non-small cell lung carcinoma cell lines expressed either liver-specific (non-neuronal) mRNA (cell line A549) or predominantly the neuronal (cell line NCI-H520) AADC message. These data indicate that a range of tumour cell lines transcribe the *AADC* gene and that two distinct types of AADC mRNA which reflect the embryonal (neuronal or non-neuronal) origin of the tumour may be produced in non-small cell lung cancer cells. © **1997 Elsevier Science Ltd.**

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INTRODUCTION

AMONG LUNG cancers, classic small cell lung carcinomas (SCLC) and a subset of non-small cell lung tumours (NSCLC) express neuroendocrine markers. Previous studies have shown that dopa decarboxylase, chromogranin A (mRNA and immunoreactivity) and synaptophysin in lung tumour cell lines and specimens are markers for neuroendocrine differentiation [1–3]. High dopa decarboxylase activities have been found in classic SCLC that distinguished them from variant types [1]. Other proteins, more or less specific for the neuroendocrine phenotype, are often found in lung cancer [4, 5]. The most common lung tumours with neuroendocrine differentiation are classic SCLC and carcinoids. However, a subset of NSCLC (about 10–15%) also display neuroendocrine features, including aromatic L-amino acid decarboxylase (AADC) activity. It is believed that the neuroendocrine tumours arise from cells dispersed in the bronchial epithelium which are similar to cells distributed in some other organs and sharing neuroendocrine features.

AADC (EC 4.1.1.28, dopa decarboxylase) catalyses the formation of dopamine from L-dopa as a substrate. The enzyme is also able to convert 5-hydroxytryptophan to serotonin, as well as to decarboxylate several other amino acids, such as tyrosine, tryptophan and phenylalanine, although with lower efficiency, to the corresponding amines [6]. AADC is expressed in both the central and the peripheral nervous systems in neurons producing dopamine and serotonin

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and in the chromaffin cells of the adrenal medulla. The enzyme is also expressed in neuroendocrine cells distributed in several organs such as lung and intestine (amine precursor uptake and decarboxylation, APUD system) and also in the liver and kidney [6].

The human *AADC* gene is present as a single copy and its coding region is distributed over 15 exons in the genome [7]. The cDNA has been cloned [8] and shown to code for a protein 480 amino acids long. Rat cDNA that codes for a protein product very similar to the human enzyme has also been cloned [9]. It has been demonstrated that the mRNA transcribed in neuronal and neuroendocrine tissues differs from the non-neuronal type (expressed in liver and kidney) by the untranslated 5′-end [10–12]. This difference is due to the alternative promoter usage followed by alternative splicing [10–13]. In both rat and human genomic DNA, the non-neuronal promoter is located upstream and the neuronal promoter downstream, closer to the second exon which is common to both tissue systems. In rat tissues, the splicing acceptor in neural cells is located five bases downstream from that in the non-neuronal tissue [10, 11]. In humans, the different non-neuronal or the neuronal first exons are spliced to a common acceptor site [12]. Thus, human AADC mRNAs have an identical second exon in both groups of tissues.

Recently, a hepatocyte nuclear factor-1 (HNF-1) has been demonstrated to bind to a motif in the rat non-neuronal AADC promoter [14]. The core of this sequence is a short A/T-rich stretch located −49 bp to −35 bp relative to the transcriptional start which is completely homologous to a motif found in a human gene. HNF-1 cDNA, when transfected into AADC non-expressing cells, stimulated transcription from the cotransfected promoter/reporter construct [14]. However, the factors participating in transcription of the neuronal type of AADC are unknown.

Here, we present data demonstrating the expression of AADC mRNA in several types of human tumour cells. Using the quantitative reverse transcriptase–polymerase chain reaction (RT–PCR), even very low levels of the messenger RNA were detected in a range of human tumour cell lines. Using this approach, AADC was found in tumour cell types which have been shown previously to be negative when the dopa decarboxylase activity or Northern blot signals were examined. We also show that, while all SCLC cell lines analysed express a neuronal-type message, NSCLC lines may differ and express either non-neuronal or neuronal forms of AADC mRNA.

MATERIALS AND METHODS

Cell lines

The human tumour cell lines used in this study are listed in Table 1. The lines were purchased from American Type Culture Collection (ATCC) except some melanoma cell lines (Hbl, Scl, IgR3, Dau, Dor, Beu, and LND1) which have been described previously [15] and were generously provided by Dr G. Ghanem (Brussels). All lung cancer cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin and streptomycin. Melanoma and neuroblastoma cell lines were maintained in Eagle's minimum essential medium supplemented with non-essential amino acids, 1 mM sodium pyruvate, 10% fetal bovine serum, 2 mM L-glutamine, penicillin and streptomycin. Other lines were cultured in media recommended by ATCC.

Table 1. *Relative levels of AADC mRNA in human tumour cell lines*

Cell line		Relative AADC mRNA levels
NCI-H69	SCLC, classic type	6720
NCI-H146	SCLC, classic type	10 000
NCI-H209	SCLC, classic type	4517
NCI-H345	SCLC, classic type	11 554
NCI-H378	SCLC, classic type	7084
NCI-H82	SCLC, variant type	233
A 549	NSCLC (adenocarcinoma)	51.4
NCI-H520	NSCLC (squamous carcinoma)	10.4
SK-MES-1	NSCLC (squamous carcinoma)	2.1
SK-N-SH	neuroblastoma	4093
SaOS-2	osteosarcoma	15.4
U2-OS	osteosarcoma	3.8
RPMI 7951	melanoma	16.1
A 375	melanoma	4.5
SK-MEL-2	melanoma	5.3
Dau	melanoma	1.3
Hbl	melanoma	2.1

AADC mRNA levels are shown relative to levels found in cell line H146. In the following cell lines, very low or undetectable levels of AADC mRNA were noted: NSCLC cell lines A427, NCI-H460, NCI-H661, NCI-H1299, Calu-1; NCI-H446 (SCLC); melanoma cell lines HT144, SK-MEL-1, SK-MEL-5, SK-MEL-28, SK-MEL-31, Malme 3M, IgR3, LND1, Beu, Dor, SCl; neuroepithelioma cell line SK-N-MC; colon carcinoma SW480, HeLa, and 293 cells. The experiment was carried out twice with similar results.

AADC mRNA detection by RT–PCR

Part of the *AADC* cDNA has been recovered from NCI-H375, a small cell lung carcinoma cell line. Briefly, total RNA was reverse transcribed by a reverse transcriptase (Gibco-BRL, Gaithersburg, Maryland, U.S.A.) using the oligonucleotide: 5′..CCGGAATTCACTTTGTTGGAACCCTTTAGC (P1 in Figure 1, complementary to sequences in exons 13 and 14) as a primer and the cDNA was directly amplified by using a sense primer: 5′..CCGGAATTCATCCCCTCAGATGGCAACTTC (P2 in Figure 1, derived from sequences in exon 6) and the antisense primer used for reverse transcription (above). The primer design is based on the human cDNA sequence published by Ichinose and associates [8]. *Eco*RI sites were introduced into primers to facilitate subsequent cloning. The PCR mix was amplified in 30 cycles, consisting of 1 min at 94°C, 1 min 52°C and 2 min at 72°C for each cycle by using AmliTaq DNA polymerase (Perkin-Elmer, Foster City, California, U.S.A.). The 0.64 kb PCR product was gel purified, cleaved by *Eco*RI, cloned into

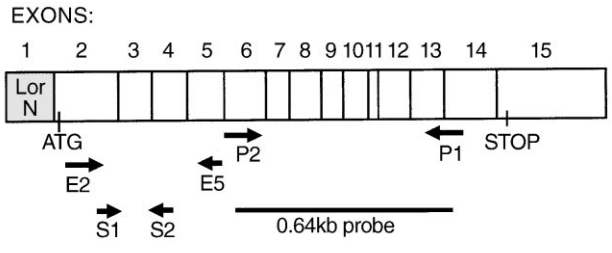

Figure 1. Summary of the structure of the AADC mRNA. Alternative first exon (L or N, shaded), start and stop codons, primers and hybridisation probe used in this study are shown. The primers were used for reverse transcription, PCR and sequencing as indicated in the text.

pGEM7ZF+ (Promega, Madison, Wisconsin, U.S.A.) and verified by sequencing (fmol DNA Sequencing System, Promega). The sequence was identical to the published human cDNA sequence [8].

Cytoplasmic RNAs were isolated from cell lines by a modification of the described method [16], the integrity checked on denatured agarose gels and 2 μg of RNA were added to the RT reaction (final volume 20 μl). To check the quality of RNAs further, all RT mixes were tested by PCR with primers specific for human glyceraldehyde-3-phosphate dehydrogenase (5′..CGTCTTCACCACCATGGAGAA and 5′..TCTTACTCCTTGGAGGCCATG) and were found positive (not shown). RT and PCR were carried out as described above and the products were electrophoresed in 1.5% agarose and, in order to detect only specific bands on the gel, the gel was blotted on Nylon N+ (Amersham) and hybridised with the *AADC* probe (*Eco*RI-cleaved 0.64 kb insert labelled with the oligo-priming method). This probe spans several exons on the *AADC* cDNA sequence as is shown in Figure 1. Filters were then washed for 30 min in high stringency conditions (0.1×SSPE/0.1%SDS) at 65°C and autoradiographed.

Quantitative RT–PCR

To quantitate the levels of AADC mRNA in cell lines, reverse transcription was performed as described above and a kinetic PCR assay [17] was carried out as follows. Samples were amplified for an increasing number of cycles and an aliquot of each sample was slot blotted onto Nylon N+. The membrane was hybridised with the 0.64 kb *AADC* probe (Figure 1), washed at high stringency and autoradiographed. The small rectangles of the membrane-containing samples were cut off and counted in a liquid scintillator. A reference sample (RNA from NCI-H146 cell line) was processed through RT and PCR with each set of samples. In Table 1, the values are given as relative with the value for the cell line NCI-H146 set as 10 000.

Analysis of 5′-ends of mRNA by PCR

An approach similar to that employed by Ichinose and associates [12] was used to ascertain the type of mRNA produced. Cytoplasmic RNA from cell lines displaying appreciable AADC mRNA levels were reverse transcribed with random hexaprimers (Pharmacia, Uppsala, Sweden) and reverse transcriptase (SuperScript II, Gibco BRL). 2 μl of the RT mix were amplified for 35 cycles (40 s 94°C, 1 min 54°C and 1 min 72°C each) with a mix of two sense primers, 5′..TTCTGTGCCTCTTAACTGTCACTG as neuronal (N) and 5′..CCTGTAAGGAATTCGAATTTCCAGCA as non-neuronal (L) primer, and an antisense primer (5′..TCCCTCAATGCCTTCCATGTAGTT, primer A). The primers are shown in Figure 2. The PCR products specific for the neuronal and non-neuronal mRNA were identified on 4% NuSieve 3:1 gels as 177 bp and 133 bp bands, respectively. The identity of the products was further confirmed by *Eco*RI cleavage.

Identification of AADC mRNA variants containing or lacking exon 3

AADC cDNA synthesised by a reverse transcription with a gene-specific antisense primer (above, primer P1 in Figure 1) was also subjected to PCR to determine forms of mRNA that contain or lack exon 3. The same primers as described

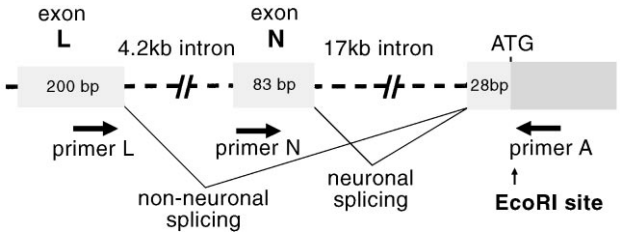

Figure 2. Schematic view of the two alternative first exons and common second exon of the human *AADC* gene (a). After transcription and splicing, mRNAs with two different 5′-ends are produced in neuronal or non-neuronal tissues. The positions of neuron-specific (N), non-neuronal (L) and exon 2 primer (A) used in this study are indicated. mRNA sequences are shown as shaded boxes.

[18] were used (primers E2 and E5 in Figure 1, 5′..CCCAAGCTTCACCTCTGACAGAGCCCAG and 5′..GCCACCAGCTTCTCCATGA, respectively) and the PCR products were sequenced with nested primers flanking exon 3 to verify the splicing junctions and exon 3 sequences (5′..GACATCATCAACGACGT as S1 and 5′..TCCATCATCACAGTCTC as S2, shown in Figure 1).

RESULTS

Expression of AADC mRNA

When analysing expression of the *AADC* gene, in many samples, RT–PCR products often gave more bands on agarose gels (not shown). Therefore, Southern blotting was carried out to identify the specific products. Figure 3 shows a Southern blot of PCR products amplified from reverse transcribed RNAs and hybridised with the *AADC* probe. Strong signals are seen in cell lines of classic SCLC NCI-H69, NCI-H209, NCI-H146, NCI-H345, NCI-H378 and a neuroblastoma line SK-N-SH. A variant SCLC, NCI-H82, surprisingly shows AADC expression as well. This line has been reported to have only negligible dopa decarboxylase activity [1]. Another variant SCLC line (NCI-H446) essentially lacks AADC mRNA as does the neuroepithelioma line SK-N-MC. In addition to the main prominent hybridising signal, additional bands of higher and lower sizes could be seen in samples with high expression levels (NCI-H146, NCI-H378, NCI-H345, SK-N-SH). The probe hybridised to these additional bands with the same intensity as to the main 0.64 kb band, indicating that *AADC* sequences were detected. All bands are products of amplification of cDNA because no bands were seen when no reverse transcription occurs (reverse transcriptase omitted in the RT reaction, not shown). Thus, presumably, more mRNA variants resulting from alternative splicing in the region between exons 6 and 13 are present in some cell lines with high AADC mRNA levels. Among NSCLC, A549 and, less prominently, NCI-H520 hybridised with the specific probe (Figure 3). Very weak or no signals were seen in several other NSCLC cell lines. The AADC message was also detected in two osteosarcoma cell lines SaOS-2 and U2-OS and in melanoma cell line RPMI 7951. Several other melanoma lines displayed low signals or were negative (Figure 3).

Quantificative measurements of the AADC mRNA expression levels

The quantitative kinetic PCR method can be used to estimate the levels of the specific message in cellular RNA [17].

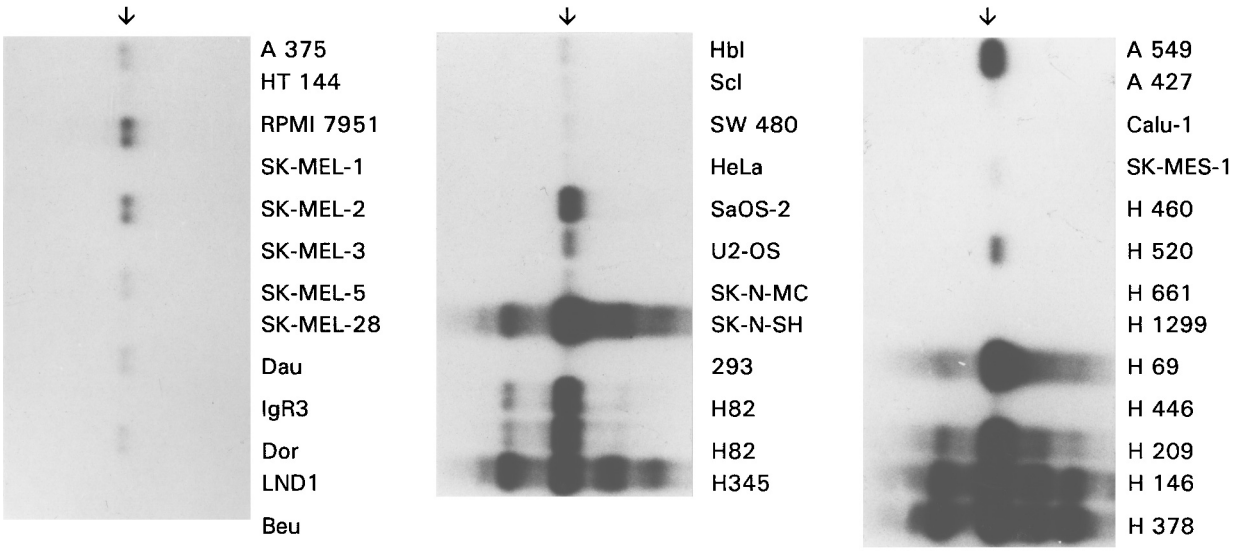

Figure 3. Southern blot of PCR products obtained by amplification of reverse transcription reactions with AADC-specific primers and resolved on the 1.5% agarose gels. Blots were hybridised to a 0.64 kb *AADC* cDNA fragment. Arrow indicates the predominant hydridising signal (0.64 kb long). The description of cell lines is in Table 1 footnote. One of two experiments that were carried out with two different RNA preparations is shown. The results of these experiments were the same. Hybridisation, washing, autoradiography and photographs were made simultaneously under identical conditions for the whole set of samples.

PCR products were processed as described in Materials and Methods and the log of counts was plotted against the number of PCR cycles. As an example of these experiments, Figure 4 shows that this relationship is linear in several cell lines. Similar results were obtained with other cell lines studied; only lines that showed appreciable signals in the previous experiment (Figure 3) were analysed in the quantitative assay. The regression curves plotted from cpm data obtained from all samples were parallel or nearly parallel (Figure 4), indicating that the difference in the amount of PCR product and hence the difference in the initial number of molecules can be estimated between any two samples processed together in the same set. Even very low levels, 3–4 orders below the high-level samples, could be quantitated.

Table 1 summarises the levels of AADC mRNA in the cell lines analysed by the quantitative method. The values in Table 1 do not strictly correlate with the intensity of the signals shown in Figure 3 in several samples. As the signals in Figure 3 are only qualitative, the values estimated by kinetic PCR (Table 1) reflect mRNA levels more accurately. The levels found in several classic SCLC and neuroblastoma SK-N-SH are extremely high when compared to all other samples analysed. Intermediate levels were seen in cell lines NCI-H82 (varian SCLC) and A549 (NSCLC) and lower levels were observed in other cell lines listed (Table 1).

PCR analysis of 5′-ends of AADC mRNAs

Figure 2 schematically shows the 5′-end of the human *AADC* gene. Two alternative first exons L and N are transcribed from alternative promoters in non-neuronal (liver-specific) and neuronal tissues, respectively [12]. To determine the type of mRNA (neuronal versus non-neuronal) expressed in tumour cell lines having readily observable messenger levels, we used primers specific for both alternative first neuron-specific or non-neuronal exons and co-amplified the cDNAs with both these primers and the 3′-primer complementary to a sequence in the second exon, common to both forms of messages. In Figure 2, the position of primers used in this experiment is indicated. As expected, all SCLC, neuroblastoma and melanoma cell lines revealed a neuronal type of messenger RNA, as recognised by the presence of a neuronal PCR product 177 bp long (Figure 5). However, A549, a lung adenocarcinoma cell line, expressed exclusively non-neuronal mRNA as indicated by the appearance of a

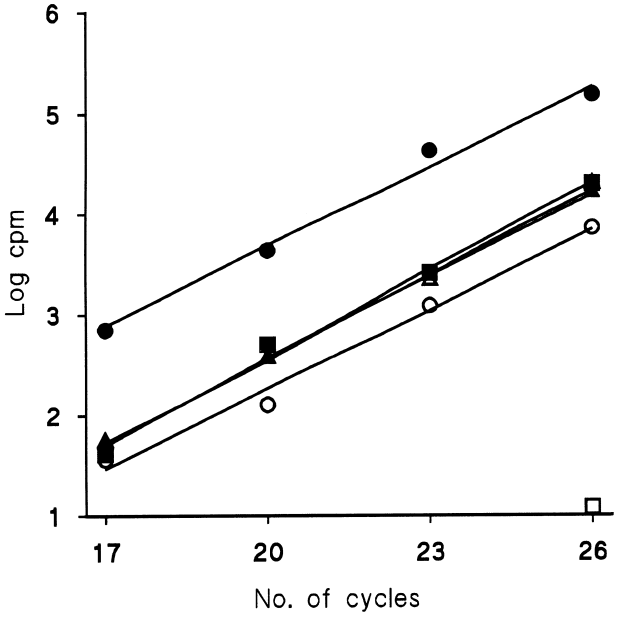

Figure 4. An example of the PCR-kinetic method to estimate the AADC messenger RNA levels in cell lines. Samples were processed through reverse transcription, PCR, slot blotting, hybridisation and counting as described in Materials and Methods. Tumour cell lines shown: ●, NCI-H146, RT mixture 100-times diluted; ■, NCI-H146, 1000-times diluted; ▲, A549, 10-times diluted; ○, NCI-H520; △, NCI-H82, 10-times diluted; □, NCI-H1299 (negative control).

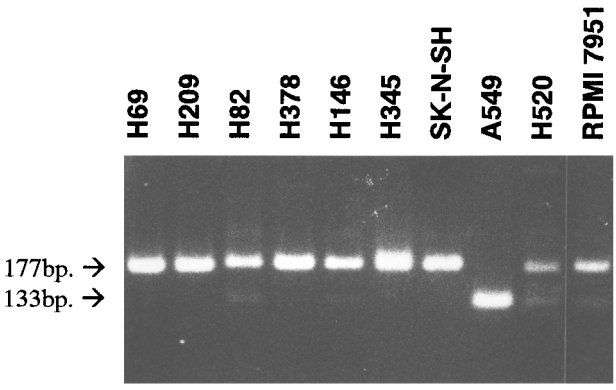

Figure 5. Different types of AADC mRNA expressed in human tumour cell lines. The PCR product 177 bp long indicates the presence of neuronal type AADC mRNA and the 133 bp product is characteristic for non-neuronal type mRNA. Reverse transcription followed by PCR was performed with primers indicated in Figure 2 and Materials and Methods.

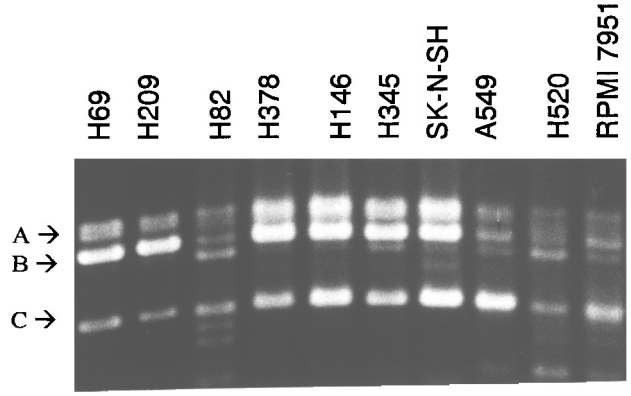

Figure 6. AADC mRNA isoforms in tumour cell lines. PCR products which indicate AADC mRNAs containing (band B) or lacking (band C) exon 3 sequences in human tumour cell lines. Band A contains exon 3 and additional sequences (see Results).

133 bp product. Squamous cell carcinoma NCI-H520 showed a predominantly neuronal product and a weak non-neuronal mRNA-specific band (Figure 5). Three other cell lines, NCI-H82, NCI-H146 and RPMI 7951, also revealed minor liver-specific bands. To confirm the specificity of the PCR products, the amplified DNAs were cleaved by *Eco*RI (the cleavage site resides in the AADC first exon cDNA sequence [8]). The cleavage yielded the expected sizes of the fragments (data not shown). Thus, the PCR products were authentic fragments amplified in the 5′-region of the AADC mRNA. Also, separate amplifications with common complementary primer and either a liver- or neuron-specific primer gave results similar to those seen in the co-amplifications described above (not shown).

Splicing variants of AADC mRNA

In normal tissues, the AADC mRNA consists of two variant forms that differ in the presence or absence of exon 3 [18, 19] and codes for proteins 480 or 442 amino acids long, respectively. Functional biochemical studies have shown that the shorter protein is enzymatically inactive [18]. Here, we have also analysed the presence of these two forms of AADC mRNA in several tumour cell lines. Figure 6 shows the three principal amplified products which were synthesised by PCR from primers as described [18] (primers E2 and E5, Figure 1). Band B, 574 bp long, corresponds to the full-length message that contains exon 3 (Figure 6). A 471 bp fragment (band C, Figure 6) indicates the presence of the mRNA lacking exon 3. The authenticity of these fragments was confirmed by purifying the bands from the gel and sequencing by the use of nested primers (primers S1 and S2 in Figure 1, sequencing data not shown). Band A in Figure 6 consists of at least two PCR products approximately 610–630 bp long and indicates that other variant forms of AADC mRNA may exist that contain additional sequences (an internal oligonucleotide probe hybridised equally to all three bands, 471 bp, 574 bp and 610–630 bp, not shown). Since the exon 3/exon 4 boundary is preserved (and the exon 2/exon 3 boundary is not) in this 610–630 bp band (not shown), exon 3 presumably contains additional 5′ sequences in these splicing variants. The relative abundancies of the splicing variants that contain or lack exon 3 differ among the cell lines. For example, NSCLC tumour cells (A549) show a much higher proportion of the smaller (inactive) form than all classic types of SCLC (Figure 6). Also, a low ratio of band B (representing the message for the active enzyme) to other bands is seen in lines NCI-H82 and NCI-H520. This may indicate that even though the total level of AADC mRNA is relatively high, the final protein product can be mostly inactive. Thus, as in normal tissues [18], the two main AADC mRNA splicing variants also exist in tumour cells.

DISCUSSION

Neuroendocrine differentiation in human lung tumours, mostly detected by biochemistry or immunocytochemistry, is associated with the presence of protein markers that are indicative of the neural origin. SCLC is a tumour characterised by the presence of several neuroendocrine markers like chromogranin A or L-amino acid decarboxylase (AADC), the enzyme necessary for the synthesis of catecholamines. Classic subtypes of SCLC tumours were reported to contain very high levels of AADC enzymatic activity [1, 20]. It has been reported in numerous studies that a variant subclass of SCLC tumours has more aggressive growth and poorer response to chemotherapy. Variant tumour cell lines do not express this marker, as measured by the activity of cell extracts to decarboxylate L-dopa [1] and the variant tumour phenotype frequently correlates with high c-myc expression levels [21].

In this study, it is shown that AADC messenger RNA is expressed in a range of human tumour cell lines (normal tissues known to express AADC were not analysed here). We used a sensitive RT–PCR method to detect and quantitate the mRNA levels. The highest expression was found in SCLC lines and a neuroblastoma cell line SK-N-SH. All these cell lines also expressed a chromogranin A message when analysed by Northern blotting (data not shown), a finding consistent with the previously reported correlation between dopa decarboxylase activity and chromogranin mRNA in both SCLC and NSCLC [2]. The NCI-H82 SCLC cell line classified as a variant type and reported to have only negligible dopa decarboxylase activity [1] showed, however, an appreciable AADC mRNA expression level (Table 1). Similarly, A549 lung adenocarcinoma and NCI-H520 squamous lung carcinoma cell lines with reported extremely low or

undetectable dopa decarboxylase enzymatic activities [1, 22] or undetectable AADC signals on Northern blots [23] showed detectable AADC messenger RNA levels (Table 1). This may be explained by the extreme sensitivity of the RT–PCR assay that detects even minute quantities of mRNA. Furthermore, since only the isoform containing the third exon is enzymatically active [18], the low ratio of mRNA coding for active and inactive enzyme as shown in Figure 6 for the NCI-H82 cell line could then explain, provided that both mRNA isotypes are equally efficient in translation, the discrepancy between the reported low catalytic activity and relatively high AADC mRNA level described here. As previously reported, melanomas and sarcomas did not have any detectable AADC enzymatic activity. Here, clearly detectable mRNA for AADC were found in one of two sarcoma cell lines and in the minority of melanoma lines tested.

A small subset of NSCLC tumours also exhibit neuroendocrine features (reviewed in Ref. [24]). We showed here that A549 lung adenocarcinoma cells express a type of AADC mRNA specific for non-neuroendocrine tissues such as liver and kidney (Figure 5). Another NSCLC line, NCI-H520, revealed predominantly the neuronal form of message and also showed a minor non-neuronal-specific band (Figure 5). These two NSLC cell lines did not express another neuroendocrine marker, chromogranin A (not shown). However, analysis of the 5′-end of AADC described here suggests a different embryonal origin of the parent tumours. Furthermore, osteosarcoma cell line SaOS-2 expressed non-neuronal RNA as expected, since it is of non-neuroectodermal (mesenchymal) origin (data not shown). RPMI 7951 melanoma cells, derived from the neural crest, expressed predominantly the neuronal form (Figure 5). The presented initial data thus suggest that analysis of the 5′-end of the AADC mRNA, if this is expressed in a sufficient amount in the tumour tissue, might be helpful in determining the neuroectodermal and neuroendocrine origin of the tumour. A common ancestor multipotent cell can exist for neural crest-derived tissues—melanocytes, cells of the peripheral nervous system and adrenal medulla [25]—and it is therefore not surprising that some melanomas express AADC, although at low levels.

For the liver-specific transcription of the *AADC* gene, hepatocyte nuclear factor 1 seems to be essential [14]. However, it is not known what transcription factors and mechanisms underlie the mammalian neuronal type *AADC* gene transcription. Human and rat *AADC* 5′-flanking sequences have been cloned and both neuronal and non-neuronal promoters identified [7, 11, 12, 26]. A 560 bp region, upstream relative to the translational start site without intron 1 sequences, directed reporter gene expression in neuroblastoma cells but not in several non-neuronal tumour cell lines; the construct with most of the first intron sequences was inactive [26]. Interestingly, when the human neuronal promoter has been removed from the *AADC* gene context, reporter gene expression was also observed in cells that do not express endogenous AADC [27], suggesting that additional sequences outside the promoter are required to achieve tissue-specific transcription. It has also been suggested that the upstream non-neuronal promoter may inhibit transcription from the neuronal promoter [14, 27]. Studies with *AADC* promoter transgenes similarly showed that other regions of the endogenous *AADC* gene are presumably involved in neuronal expression because the neuronal promoter also directed expression of the reporter transgene in non-neuronal tissues [28]. Recently, the presence of neuronal and liver-specific mRNA coding for AADC has also been confirmed by *in situ* hybridisation in rat tissues [29].

In summary, the analysis of AADC mRNA in human tumour cell lines, as described in this paper, shows that even a low amount of mRNA can be detected in cells derived from various tumour types. The usefulness of this analysis, for example, as an aid in the detection of residual tumour cells in circulation after therapy, needs to be evaluated in further studies. Also, the analysis of tumour samples and correlation of the expression of one of the two AADC mRNA types with the presence of other neuroendocrine markers, such as synaptophysin or chromogranin A, are needed to evaluate further the significance of expression of the particular type of message. Although transcriptional regulation of the *AADC* gene in neuronal tissues is not understood, the finding that NSCLC may express different types of AADC mRNA (neuronal and non-neuronal) points to the relevance of ascertaining the embryonal origin of the cell from which the particular lung tumour arises and suggests that tumours expressing non-neuronal mRNA might not be of neuroendocrine origin.

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