ELSEVIER

Biochimica et Biophysica Acta 1316 (1996) 203–209

BBA Biochimica et Biophysica Acta

Coordinate expression of nuclear and mitochondrial genes involved in energy production in carcinoma and oncocytoma

Abdelaziz Heddi [a,b], Hélène Faure-Vigny [c], Douglas C. Wallace [a,*], Georges Stepien [c]

[a] Department of Genetics and Molecular Medicine, Emory University School of Medicine, 1462 Clifton Road, Room 446, Atlanta, GA 30322, USA
[b] Laboratoire de Biologie Appliquée, UA-INRA 227, INSA, 20 av. Albert Einstein, F-69621 Villeurbanne, France
[c] Centre de Génétique Moléculaire et Cellulaire, CNRS-UMR 106, 43 bd. du 11 Novembre 1918, F-69622 Villeurbanne, France

Received 19 February 1996; accepted 4 April 1996

Abstract

The expression of mitochondrial and nuclear genes involved in ATP production was examined in renal carcinomas, renal oncocytomas, and a salivary oncocytoma. Renal carcinomas were found to have a reduced mitochondrial DNA (mtDNA) content while oncocytomas had increased mtDNA contents. This parallels morphological changes in mitochondrial number in these tumours. In the carcinomas, mtDNA transcripts were decreased 5- to 10-fold relative to control kidneys, suggesting that mitochondrial transcript levels depend on the mtDNA content. In renal oncocytomas, mtDNA transcripts were slightly reduced in spite of a high mtDNA content. However, in the salivary gland oncocytoma, mtDNA transcripts were increased more than 10-fold in parallel with a 10-fold increase in mtDNA content. The expression of the nuclear DNA oxidative phosphorylation genes, ATPsynβ and ANT2, was reduced up to 4-fold in renal carcinoma. In contrast, the levels of these two nuclear gene transcripts were induced about 4-fold in renal oncocytoma and up to 30-fold in salivary gland oncocytoma. Moreover, the ANT2 precursors were observed to change in oncocytomas. These data suggest a coordinated regulation of nuclear and mitochondrial gene expression in renal carcinomas and the specific induction of nuclear OXPHOS gene expression in oncocytomas.

Keywords: Carcinoma; Oncocytoma; mitochondrial DNA; Gene expression; Oxidative phosphorylation

1. Introduction

A variety of tumour types have been reported to have an altered energy metabolism. Many tumours show an increase in glycolysis, while sustaining an oxidative metabolism. This appears to result from the association of hexokinase with the mitochondria [1], thus channelling mitochondrial ATP to the cytosol driving glycolysis. In many solid tumours, the altered metabolism is associated with reductions in mitochondria and mtDNA levels [2,3] and changes in mitochondrial size and shape [2] and membrane potential [4,5]. In SV40-transformed diploid fibroblasts as well as HeLa and EBV-transformed lymphoblasts, the reduction in cellular mtDNA levels is associated with a coordinate induction in nDNA and mtDNA OXPHOS transcripts and induction of the ANT isoforms ANT1 and ANT2 [3]. This could reflect a compensatory mechanism for responding to a reduced energy metabolism in these transformed cells. Oncocytomas stand in marked contrast to most other solid tumours in that they accumulate large numbers of hypertrophied mitochondria occasionally containing granular deposits. This unique feature of oncocytomas has led to the cytological designation of 'oxyphil tumour cell' [6–8]. The mechanism for the extraordinary proliferation of mitochondria in oncocytoma cells is unknown. However, it is possible that it reflects an induction of mitochondrial biogenesis.

Mitochondria contain their own genome which encodes thirteen polypeptides involved in OXPHOS [ND1 to 6 and ND4L, COI to COIII, ATP6 and 8, and cytochrome *b* (cyt *b*)], as well as the 12S and 16S ribosomal RNAs and

Abbreviations: OXPHOS, oxidative phosphorylation; ATPsynβ, β subunit of the ATP synthase; ANT, adenine nucleotide translocator; GAPDH, glyceraldehyde phosphate dehydrogenase; kb, kilobase(s); bp, base pair(s).

* Corresponding author. Fax: +1 (404) 7273949.

0925-4439/96/$15.00 Copyright © 1996 Elsevier Science B.V. All rights reserved.
PII S0925-4439(96)00026-9

transfer RNAs required for mitochondrial protein synthesis [9]. The other mitochondrial proteins are encoded in the nucleus, translated in the cytoplasm, and imported into the mitochondria [10,11]. The approximately 75 nDNA OXPHOS polypeptides, including the catalytic β subunit of the ATPsynthase (ATPsynβ) [12,13] which make up the five mitochondrial complexes (I through V), are assembled with the mtDNA polypeptides within the mitochondrion. The mitochondrial protein that exchanges ATP and ADP across mitochondrial internal membrane, the adenine nucleotide translocator (ANT) [14], is also nuclear encoded and has three isoforms: ANT1, ANT2 and ANT3. ANT1 is primarily expressed in heart and skeletal muscle, ANT2 appears to facilitate ATP-ADP equilibration between the mitochondria and cytosol in anaerobic cells, and ANT3 is expressed in all tissues [15–21].

To investigate the physiology of the oxyphil oncocytomas, we have examined the mRNA levels for mtDNA, and nDNA OXPHOS genes and compared these to renal carcinomas. The mitochondrial ND2, ND5/6 (complex I) and cyt *b* (complex II) transcripts were quantified in control kidney and salivary gland, in two renal tumours (carcinoma and oncocytoma) and in a salivary gland oncocytoma. The RNA levels of the OXPHOS ANT2 and ATPsynβ genes as well as of the glycolytic GAPDH gene were also determined in these tissues. The nuclear and mitochondrial transcript patterns were observed to differ in the various tissues, indicating that changes in energy metabolism are complex in these tumour tissues.

2. Materials and methods

2.1. Autopsy and biopsy tissues

Total RNAs were isolated from Kidney and salivary gland oncocytomas, Kidney carcinomas and their matched controls. ONC_2 and ONC_3 samples and their matched controls (Table 1) were provided and histologically examined by Dr. Peter A. McCue MD (Thomas Jefferson University Hospital, Surgical Pathology, Philadelphia). The rest of the samples were provided with their respective clinical descriptions (Table 1) by Drs. Jackson Gates, MD; William Hardman, MD; Emma Diaz, MD; and Marian Pennington, MD (Department of Pathology at Emory University School of Medicine, Atlanta). All biopsy samples used for RNA extraction were frozen immediately in liquid Nitrogen after the surgical excision, except the post mortem ONC_2, ONC_3 and their matched controls (C_4) which were collected within 4 h to prevent RNA degradation. The clinical and histological descriptions are listed on Table 1.

2.2. Nuclear and mitochondrial probes

The ANT2 probe was a 1200 bp *Xho*I/*Hin*dIII restriction fragment obtained from pSKHANT21 which was derived from subcloning the hp21 cDNA (Dr R. Baserga, Temple University Medical School, Philadelphia, PA) into pBluescript SK- (Stratagene). The ATPsynβ probe was a 950 bp EcoRI restriction fragment of a cDNA encompassing the C-terminal two-thirds of the coding sequence [12]. The GAPDH and 18S rDNA probes were full length cDNAs subcloned into pBluescript SK- (Stratagene).

The specific mtDNA ND2, ND5 + ND6 and cyt *b* probes were generated by PCR amplification from 5 ng of HeLa DNA using primers listed in Table 2. The PCR conditions involved 35 cycles of 1 min at 93°C, 1 min at the hybridization temperature (Table 2) and 1 min at 72°C.

2.3. RNA isolation and Northern blot analysis

Total cellular RNA was isolated by the guanidinium isothiocyanate procedure [22] from 0.2 to 0.5 g of tissue pulverized in liquid nitrogen (Ultra-Turrax T25). RNA samples (10 to 15 μg) were denatured by incubating at 55°C for 20 min in 1 M deionized glyoxal, 50% (v/v) dimethyl sulfoxide (DMSO), 10 mM NaH_2PO_4 (pH 7.0), and electrophoresed in 1.3% agarose (Ultrapure, BRL) gels containing 10 mM NaH_2PO_4 (pH 7.0). Gels were blotted overnight onto nylon membranes (Hybond-N, Amersham)

Table 1
Clinical and histological properties of carcinoma and oncocytoma biopsies

Tumour type	Matched control	Age and sex	Histological description
CAR1	C1	71, M	Renal cell carcinoma, clear cell type, grade II, without capsular or vascular invasion.
CAR2	C2	38, M	Renal cell carcinoma, grade II, clear cell type. with focal venous invasion.
CAR3	no matched control	62, F	Renal cell carcinoma, clear and granular cell types, nuclear grade II with extension into peripheric fat.
ONC1	C3	54, M	Renal oncocytoma, eosinophilic granular cytoplasm, enlarged and hyperchromatic nuclei in several areas.
ONC2	C4	71, F	Renal oncocytoma, hypertrophied cells packed with mitochondria.
ONC3	C4	71, F	Same as ONC_2. duplicated sample from a different autopsy region.
ONC4	C5	68, M	Oxyphilic adenoma, superficial parotid mass.

Table 2
Oligonucleotide pairs used for polymerase chain reaction amplification of mtDNA probes in Northern analysis

Primer pairs	mtDNA region	T_H (°C)	mtDNA genes
5′-GCACCCCTCTGACATCC 3′	4831–5917	51	ND2
5′-ccacaagcttCGGTCGGCGAACATCAGTGG 3′			
5′-ccaagcttGGGGATTGTGCGGTGTGTG 3′	13172–14606	53	ND5 + ND6
5′-CTTCTCCTATTTATGGGGGT 3′			
5′-ccaagcttCAACTACAAGAACACCAATG 3′	14728–15865	53	cyt *b*
5′-ccaagcttCAATTAGGGAGATAGTTGG 3′			

Sequence homology with human mtDNA [9] is indicated in capital letters.

in 20 × SSC (3 M NaCl, 0.3 M Na citrate, pH 7.0) and the blots were baked at 80°C for 2 hours. The RNA blots were prehybridized for 6 to 16 hours at 55°C (Hybaid oven) in 5 × SSPE (3.6 M NaCl, 100 mM Na_2PO_4, 20 mM EDTA, pH 7.0), 5 × Denhardt, 50% formamide, 0.2% SDS and 0.05% (w/v) of sheared and denatured salmon sperm DNA. Hybridization was performed overnight at 42°C in the same buffer following addition of 6×10^6 cpm of ^{32}P-dCTP labelled probe [23] plus 100 mg/ml sheared denatured salmon sperm DNA. Blots were washed three times (10 min) in 1 × SSC and 0.1% SDS at 55°C and autoradiographs were obtained by exposing Amersham hyperfilms with intensifying screens at −80°C for 5 h to 4 days. Autoradiographic exposure intensities were analysed and quantified using a video camera and the Minichromax (Nautil interfaces) and Biolab A (CNRS-HEVEA) programs. The amount of RNA blotted for each sample was normalized by hybridization of the Northern blots with human 18S rDNA probes. Synthetic control transcripts were loaded on gels destined for ANT hybridization to control for the specificity of hybridization. Synthetic RNAs for ANT1 and ANT2 were transcribed using T7 polymerase from pTZHANT11 [15] and pSKHANT21. T3 polymerase was used for the full length ANT3 from pKSFHANT3 [21]. Little if any cross-hybridization between the ANT1 probe and the two other isoform transcripts occurred under our experimental conditions.

2.4. DNA purification and Southern blot analysis

Total cellular DNA was extracted from 0.1 to 0.2 g of tissue pulverized in liquid nitrogen. The samples were digested overnight with proteinase K (12 μg/ml) at 50°C in STE (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 2 mM EDTA and 0.6% SDS) and then with RNAse A (5 μg/ml) for 60 min at 37°C. The proteins were removed by organic extraction followed by precipitation with 1.5 M NaCl and centrifugation for 15 min at 1000 × *g*. 1–5 μg of DNA was digested with 15 units of *Bam*HI, *Sst*I or *Eco*RI; the fragments were separated on a 0.8% agarose gel, and transferred onto nylon membranes (Hybond-N, Amersham). Filters were prehybridized and hybridized as described above, first with the 18S DNA probe, exposed to X-ray film, rinsed twice for 30 min at 90°C in 1 × SSC + 0.1% SDS, then hybridized a second time with a total mitochondrial DNA probe linearized with *Bam*HI, and exposed to X-ray film. The band intensities were compared by densitometric analysis.

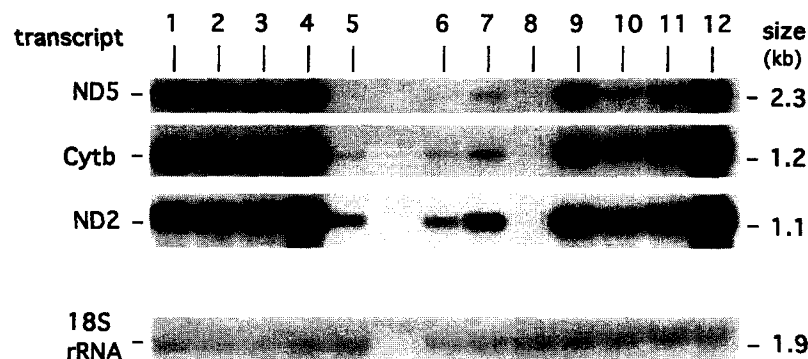

Fig. 1. MtDNA transcript analysis for controls, carcinomas and oncocytomas. Northern blot autoradiographs hybridized with mtDNA and 18S rDNA probes. The transcripts analysed are indicated on the left and their apparent sizes on the right. Lanes 1 to 5: C1 to C5 controls; lane 6, 7 and 8 were CAR1 to CAR3 carcinomas, respectively; lanes 9 to 12 were ONC1 to ONC4 oncocytomas, respectively (see experimental procedures). One of three blots sequentially hybridized to the four probes is presented. The blot was exposed for 12 h except for the rDNA probes (5 h).

3. Results

3.1. Mitochondrial gene expression

Quantitation of the transcript levels from the mitochondrial ND2, ND5/6 and cyt *b* genes in control kidney, renal oncocytoma, renal carcinoma, control salivary gland and salivary gland oncocytoma revealed different patterns of expression (Figs. 1 and 2). Data for kidney RNAs are the average from 2 or 3 different patients and the standard error is indicated (Fig. 2). For the salivary gland control and oncocytoma, only one sample was available.

Comparison of the mitochondrial OXPHOS gene mRNA levels from two types of renal tumours to those from control kidneys revealed that all tumour mRNA levels were decreased. The reductions were most marked for the renal carcinoma which ranged from 5-fold for ND2 to 10-fold for ND5. For the renal oncocytomas, the ND2 and ND5 mRNAs were only reduced 1.6- to 1.8-fold. In contrast, a comparison of the mRNA levels of the salivary gland oncocytoma versus control revealed a 14-fold increase for the cyt *b* transcript and an 18-fold increase for the ND2 transcript.

3.2. Quantitation of mtDNA in DNA fractions from controls and tumour biopsies

The relative amounts of mtDNA in controls and tumours were determined by the ratio of the hybridization bands detected by the mtDNA probe versus a nuclear 18S rDNA probe in genomic blots (Fig. 3). The densitometric analysis of the autoradiographs showed that the mtDNA/18S rDNA ratio is 3-fold higher in renal oncocytoma than in control kidney but is 5-fold lower in carcinoma (Table 3). In salivary gland oncocytoma, the mtDNA/18rDNA ratio is 10-fold higher than in control.

Table 3
Quantitation of mtDNA versus nDNA of control and tumours

	mtDNA	nDNA	mt/nDNA
Control kidney	11.2	6.5	1.7
Kidney oncocytoma	91.1	16.9	5.4
Kidney carcinoma	7.8	22.3	0.4
Control salivary gland	6.0	38.4	0.2
Salivary gland oncocytoma	32.9	15.9	2.1

The values were determined by quantitative scanning densitometry of the Southern blots (see Fig. 3). Data have been expressed in arbitrary units (mt = mitochondrial; n = nuclear) and based on one individual of each tumour used in this study.

The mtRNA levels were adjusted for the mtDNA/nDNA ratios to give a relative mitochondrial transcript ratios (data from Figs. 2 and 3 and Table 3). This revealed that relative to the template levels, the mitochondrial mRNA transcript levels in carcinoma were comparable to control kidney. The renal oncocytoma transcript to template ratio was 5-fold lower than in control kidney, while this ratio in the salivary gland oncocytoma was comparable to the control salivary gland.

3.3. Nuclear OXPHOS gene expression

The expression of the nuclear OXPHOS genes ATP-synβ, ANT2, and the glycolytic GAPDH gene were also

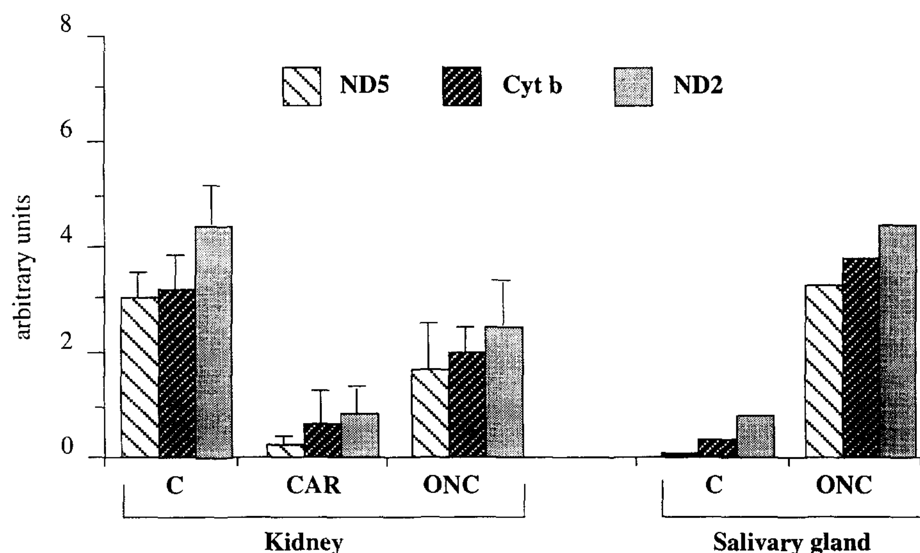

Fig. 2. Quantitation of the mtDNA transcripts from controls, carcinomas and oncocytomas. The values were determined by scanning densitometry of the Northern blots. C: control; CAR: carcinoma; ONC: oncocytoma. Data are expressed in arbitrary units normalized to the relative intensity of the 18S nuclear rRNA band (see Fig. 1). The values for the kidney are the average from at least three different biopsies, with the bars indicating the standard deviation. The salivary gland values are from a single sample.

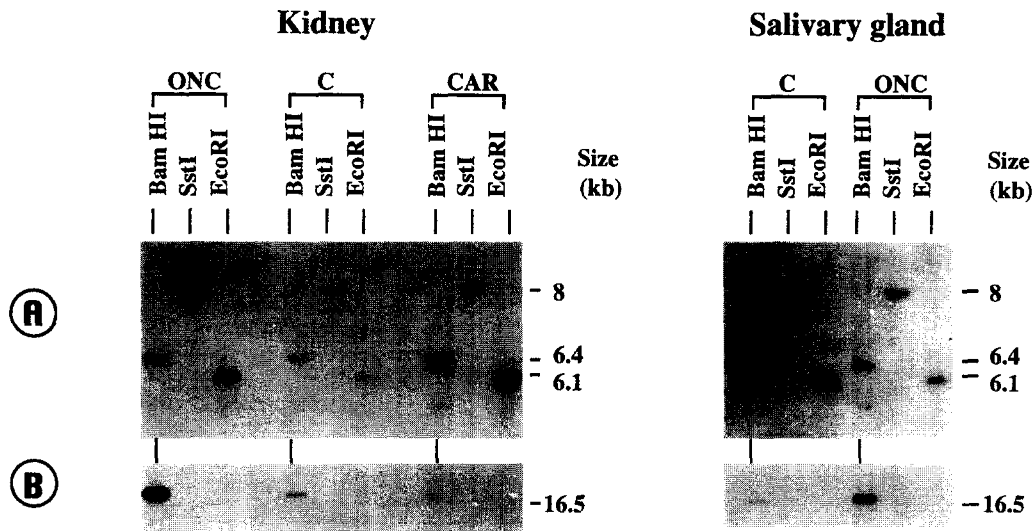

Fig. 3. MtDNA and nDNA analysis of controls, carcinomas and oncocytomas. Southern blot autoradiographs involving 1 to 5 μg of total cellular DNA digested with *Bam*HI, *Sst*I and *Eco*RI. C: control; CAR: carcinoma; ONC: oncocytoma. The same filter was hybridized with a 18S rRNA nuclear probe (panel A), then washed and hybridized again with a total mtDNA probe (panel B). The intensities of the *Bam*HI 16.5 kb mtDNA band and the 6.4 kb 18S rDNA band were quantitated (see Table 2).

analysed in these samples (Fig. 4). The mRNA levels were normalized to the nuclear encoded 18S rRNA (Fig. 5). The specificity of ANT2 cDNA hybridization was confirmed by including in vitro transcripts from the three isoforms ANT1, ANT2 and ANT3 cDNAs on the blots (see Section 2).

A 4-fold decrease of the ATPsynβ and ANT2 transcript levels was observed in renal carcinoma as compared to control kidney. In contrast, the levels of these transcripts were increased 4-fold in the renal oncocytoma and up to 30-fold in the salivary gland oncocytoma. The GAPDH mRNA level was increased in all tumours, ranging from about 1.6-fold in renal oncocytoma up to 20-fold in salivary gland oncocytoma.

Paradoxically, the level of the ANT2 precursor which was detected in the autoradiographs (Fig. 4) did not parallel the mature transcript levels. The mature ANT2 mRNA versus ANT2 precursor mRNA ratio is similar in control

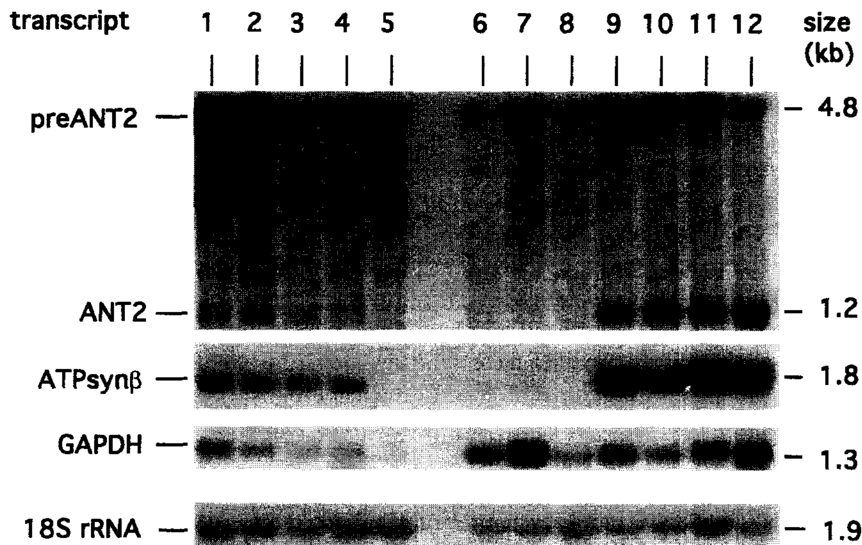

Fig. 4. Nuclear OXPHOS gene transcripts from controls, carcinomas and oncocytomas. Northern blot autoradiographs hybridized with the nuclear ANT2, ATPsynβ, GAPDH and 18S rDNA probes. The probes used are listed on the left and their apparent transcript sizes on the right. preANT2: ANT2 unprocessed transcript. Lanes 1 to 5: C1 to C5 controls; lane 6, 7 and 8 were CAR1 to CAR3 carcinomas, respectively; lanes 9 to 12 were ONC1 to ONC4 oncocytomas, respectively (see experimental procedures). One of the three blots sequentially hybridized with the four probes is presented. The blot was exposed for 12 h except for the rDNA probes (5 h).

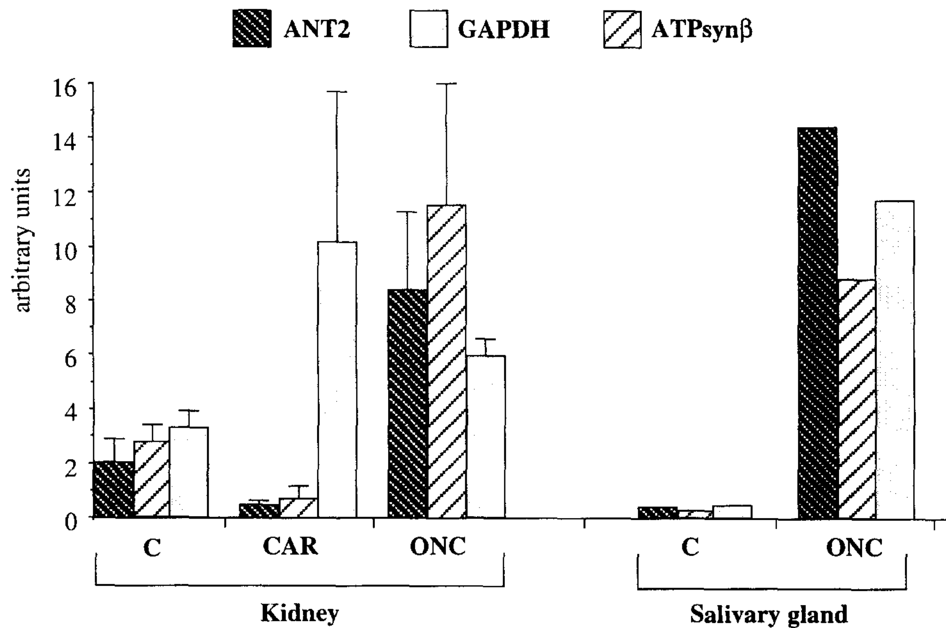

Fig. 5. Quantitation of nuclear OXPHOS gene transcripts from controls, carcinomas and oncocytomas. The values were determined by quantitative scanning densitometry of the Northern blots. C: control; CAR: adenocarcinoma; ONC: oncocytoma. Data are expressed in arbitrary units normalized to the relative intensity of the 18S nuclear rRNA band (see Fig. 4). The values for the kidney are the average of at least three different biopsies with the standard deviations shown. The salivary gland data was from a single determination.

kidney, control salivary glands and renal carcinoma. However, this ratio was increased 4-fold in renal oncocytoma and 30-fold in salivary gland oncocytoma relative to the corresponding control tissues.

4. Discussion

Analysis of the mitochondrial and nuclear OXPHOS gene transcripts in renal carcinomas, renal and salivary gland oncocytomas relative to controls revealed that the mtDNA ND2, ND5 and cyt *b* transcripts were substantially decreased in the renal carcinomas, only slightly reduced in renal oncocytomas, but increased in salivary gland oncocytoma. Furthermore, changes in the expression of the nuclear OXPHOS genes for ATPsynβ and ANT2, parallelled the changes in mtDNA OXPHOS gene expression.

These changes in mtDNA transcript levels were associated with changes in the relative levels of tumour cell and control mtDNAs. The renal carcinomas were found to have a 4-fold reduction in mtDNA number as compared to control kidney, whereas the renal oncocytoma cells had a 3-fold increase in their mitochondrial DNA content. Similarly, the salivary gland oncocytoma had a 10-fold increase in mtDNA content. The mtRNA/mtDNA ratios in both the renal carcinoma and salivary gland oncocytoma were comparable to control ratios. However, the renal oncocytoma ratio was significantly less than controls, suggesting a change in mtDNA transcription or mitochondrial mRNA stability.

The nuclear OXPHOS gene transcript levels parallelled the mtDNA content as well. The renal carcinomas showed a substantial reduction in ATPsynβ and ANT2 transcript levels, but an increase of the glycolytic GAPDH transcript. This is consistent with the reduced levels of renal carcinoma mtDNAs and the well-documented reduction in mitochondrial content in most solid tumours [2]. By contrast, the ATPsynβ and ANT2 mRNA levels were elevated in the renal and salivary gland oncocytomas in parallel with their increased mtDNA content and proliferation of abnormal mitochondria [24–27]. A high ANT3 (ANT constitutive isoform) transcript level corroborates this high mitochondrial biogenesis (Stepien, unpublished observation).

The induction of OXPHOS genes has previously been observed in cultured cells immortalized by viruses or cellular oncogenes [28,3], as consequences of mtDNA damage in ischemic hearts [29], and also in presence of a high percentage of mutated mtDNA in tissues of patients with mitochondrial myopathies [30,31]. This is similar to the induction seen in the oncocytomas and suggests that nuclear regulatory factors such as those binding the OXBOX and REBOX motifs [32–34] could play a role in altering the OXPHOS gene expression.

The reduction in mitochondrial transcript levels in the renal carcinoma indicates that other factors also could play an important role in the cancer cell energy metabolism. One of these factors could be the difference in the rate of division between mitochondrial and nuclear systems. Another could be a change in the regulation of the glycolytic and oxidative energy genes. The high level of the GAPDH

expression suggests that the cell metabolism have switched to a glycolytic status. This metabolic switch might be associated with a decrease in nuclear ATPsynβ transcription in carcinoma.

Since the proposed role of the ANT2 isoform is to transport glycolytic ATP from the cytoplasm into the mitochondrial matrix [21] during times of OXPHOS deficiency, the high ANT2 transcript levels in oncocytomas would be consistent with nonfunctional mitochondria. ANT2 induction was also observed in Kearns-Sayre syndrome tissues (liver, kidney, brain) which exhibit 65% to 86% of deleted mitochondria [31]. Whereas most of ANT2 RNA is in the precursor (unspliced) form in renal carcinoma, more than 50% is in the mature form in oncocytoma, suggesting a higher requirement for cytosolic ATP to maintain mitochondrial proliferation in oncocytomas.

The discovery of clear changes in OXPHOS gene expression in solid tumours provides the opportunity to study the molecular mechanisms involved in energetic processes of cancer cells. Further, molecular investigations in nuclear OXPHOS gene promoters should lead to identify *cis* and *trans* elements regulating gene expression during the neoplastic transformation.

Acknowledgements

This work was supported by grants from the Association pour la Recherche sur le Cancer (ARC) and the Institut Electricité Santé awarded to G. S., and NIH grants NS21328, NS30164, HL45572 and DK45215 awarded to DCW. We thank T. Horton for technical assistance.

References

[1] Bustamante, E., Morris, H.P. and Pedersen, P.L. (1981) J. Biol. Chem. 256, 8699–8704.
[2] Pedersen, P.L. (1978) Prog. Exp. Tumor Res. 22, 190–274.
[3] Torroni, A., Stepien, G., Hodge, J.A. and Wallace, D.C. (1990) J. Biol. Chem. 265, 20589–20593.
[4] Johnson, L.V., Walsh, M.L., Bockus, B.J. and Chen, L.B. (1981) J. Cell. Biol. 88, 526–535.
[5] Chen, L.B. (1988) Annu. Rev. Cell. Biol. 4, 155–181.
[6] Tandler, B., Hutter, R.V.P. and Erlandson, R.A. (1970) Lab. Invest. 23, 567.
[7] Ross Hunter, V., Pauly, D.F. and Wolkowicz, P.E. (1990) Human Pathology 21, 437–442.
[8] Freddo, T.F. and Leibowitz, H.M. (1991) Cornea. 10, 175–182.
[9] Anderson, S., Bankier, A.T., Barrel, B.G., De Bruijn, M.L.J., Coulson, D.R., Droin, J., Eperson, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Stadenk, R. and Young, I.G. (1981) Nature 290, 457–465.
[10] Tzagoloff, A. and Myers, A.M. (1986) Ann. Rev. Biochem. 55, 249–288.
[11] Chomyn, A. and Attardi, G. (1992) In: Molecular Mechanisms in Bioenergetics, L. Ernster (ed.), pp. 496–500, Elsevier Science, Amsterdam.
[12] Wallace, D.C., Ye, J., Neckelmann, N., Singh, G., Webster, K.A. and Greeberg, B.D. (1987) Curr. Genet. 12, 81–90.
[13] Neckelmann, N., Warner, C.K., Chung, A., Kudoh, J., Minoshima, S., Fukayama, R., Maekawa, M., Shimizu, Y., Shimizu, N., Liu, J.D. and Wallace, D.C. (1989) Genomics 5, 829–843.
[14] Klingenberg, M. (1981) Nature 290, 449–454.
[15] Neckelmann, N., Li, K., Wade, R., Shuster, R. and Wallace, D.C. (1987) Proc. Natl. Acad. Sci. USA 84, 7580–7584.
[16] Battini, R., Ferrari, S., Kaczmarek, L., Calabretta, B., Chen, S. and Baserga, R. (1987) J. Biol. Chem. 262, 4355–4359.
[17] Houldworth, J. and Attardi, G. (1988) Proc. Natl. Acad. Sci. USA 85, 377–381.
[18] Li, K., Hodge, J.A. and Wallace, D.C. (1990) J. Biol. Chem. 265, 20585–20588.
[19] Cozens, A.L., Runswick, M.J. and Walker, J.E. (1989) J. Mol. Biol. 206, 261–280.
[20] Ku, D., Kagan, J., Chen, S., Chang, C., Baserga, R. and Wurzel, J. (1990) J. Biol. Chem. 265, 16060–16063.
[21] Stepien, G., Torroni, A., Chung, A., Hodge, J.A. and Wallace, D.C. (1992) J. Biol. Chem. 267, 14592–14597.
[22] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1987) Current Protocols in Molecular Biology, Greene Publishing Associates/Wiley-Interscience, New York.
[23] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[24] Goebel, H.H., Schulz, F. and Rama, B. (1980) Acta Neurochir. 51, 195–201.
[25] Kobayashi, H., Hosino, M. and Tauchi, H. (1972) Nagoya J. Med. Sci. 34, 25–32.
[26] Krech, R., Zerban, H. and Bannasch, P. (1981) Eur. J. Cell. Biol. 25, 331–339.
[27] Ordoñez, N.G., Ro, J.Y. and Ayala, A.G. (1992) Am. J. Surg. Pathol. 16, 1007–1012.
[28] Glaichenhaus, N., Léopold, P. and Cuzin, F. (1986) EMBO J. 5, 1261–1265.
[29] Corral-Debrinski, M., Stepien, G., Shoffner, J.M., Lott, M.T., Kanter, K. and Wallace, D.C. (1991) J. Am. Med. Ass. 266, 1812–1816.
[30] Heddi, A., Lestienne, P., Wallace, D.C. and Stepien, G. (1993) J. Biol. Chem. 268, 12156–12153.
[31] Heddi, A., Lestienne, P., Wallace, D.C. and Stepien, G. (1994) Biochim. Biophys. Acta 1226, 206–212.
[32] Li, K., Warner, C.K., Hodge, J.A., Minoshima, S., Kudoh, J., Fukuyama, R., Maekawa, M., Shimizu, Y., Shimizu, N. and Wallace, D.C. (1989) J. Biol. Chem. 264, 13998–14004.
[33] Chung, A.B., Stepien, G., Haraguchi, Y., Li, K. and Wallace, D.C. (1992) J. Biol. Chem. 267, 21154–21161.
[34] Haraguchi, Y., Chung, A.B., Neill, S. and Wallace, D.C. (1994) J. Biol. Chem. 269, 9330–9334.