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Research Article

Micro-array chip analysis of carbonyl-metabolising enzymes in normal, immortalised and malignant human oral keratinocytes

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Abstract. Enzymes involved in various protective and metabolic processes of carbonyl compounds were analysed utilising a micro-array method in a three-stage in vitro model for oral carcinogenesis involving cultured normal, immortalised and malignant human oral keratinocytes. A complete transcript profiling of identified carbonyl-metabolising enzymes belonging to the ADH, ALDH, SDR and AKR families is presented. Expression of 17 transcripts was detected in normal, 14 in immortalized and 19 in malignant keratinocytes of a total of 12,500 genes spotted on the micro-array chip. For the detected transcripts, about half were changed by cell transformation, and for the various enzyme families, differences in expression patterns were observed. The detected AKR transcripts displayed a conserved pattern of expression, indicating a requirement for the keratinocyte phenotype, while most of the detected SDRs displayed changed expression at the various stages of malignancy. The importance of multiple experiments in using a micro-array technique for reliable results is underlined and, finally, the strength of the method in detecting co-expressed enzymes in metabolic pathways is exemplified by the detection of the formaldehyde-scavenging pathway enzymes and the polyol pathway enzymes.

Key words. Alcohol dehydrogenase; aldehyde dehydrogenase; carbonyl-metabolising enzymes; micro-array chip.

Carbonyl-metabolising enzymes are involved in a variety of detoxification steps and metabolic pathways. Carbonyl compounds, in particular aldehydes, show chemical reactivity as electrophiles that interact with nucleophilic centres in proteins or nucleic acids. Although several effects of carbonyl metabolites are beneficial, many are deleterious, including cytotoxicity, mutagenicity and carcinogenesis. These compounds originate from endogenous or exogenous sources and as defence, several carbonyl-metabolising enzymes have evolved. These enzymes can be divided into four protein families: zinc-containing alcohol dehydrogenases [ADHs; members of the medium-chain dehydrogenase/reductase (MDR) superfamily], aldehyde dehydrogenases (ALDHs), short-chain dehydrogenases/reductases (SDRs) and aldo-keto reductases (AKRs) [1–6]. The protein families are further divided into different enzymes, groups/classes and isoenzymes.

The mammalian ADHs are regarded as general detoxifiers of alcohols and aldehydes and are today classified into six classes, of which five have been identified in humans [2, 7]. ADH3, or glutathione-dependent formaldehyde dehydrogenase, is the ancestral form of the ADHs that has been detected in all genomes characterised and

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has been assigned a specific role in the oxidation of the probable human carcinogen formaldehyde [8, 9]. Sixteen ALDHs have been identified in human that are grouped into nine gene families [3]. The ALDH1A1 and ALDH2 enzymes are connected to the ADH enzymes in that they metabolise aldehyde intermediates in the oxidative pathway of ethanol metabolism. The SDR superfamily is the largest group with approximately 2000 genes annotated in the data banks. However, the numbers identified in humans are limited and a large number of these SDR enzymes have been identified in specific metabolic pathways, several of which are associated with metabolic disorders [10]. The AKR family consists of the aldose and aldehyde reductases and has recently been enlarged to include several dihydrodiol dehydrogenases [6].

Most of these enzymes are expressed in highly regulated manners and a majority have liver-specific expression while others are ubiquitously expressed or specific for other tissues. For several of the enzymes, tissue expression patterns have been determined by classical techniques including Northern and Western blot analyses [11, 12]. Today, expression patterns for these enzymes can be correlated in various cellular contexts with powerful array-based methods. These techniques enable the simultaneous analysis of expression patterns of thousands of genes.

As portal of entry, the oral mucosa is a documented target for toxic compounds, e.g., various electrophiles, and a capacity for biotransformation might be expected for carbonyl compounds such as aldehydes. This fact implies the need for detailed characterisation of the enzymatic defence in oral keratinocytes and in vitro model systems. Development of serum-free culture conditions have permitted the establishment of replicative cultures of normal keratinocytes from human oral mucosa [13]. These conditions are also applicable to the non-malignant, oral SV40 T antigen-immortalised keratinocyte line SVpgC2a, and the malignant, oral squamous carcinoma cell line SqCC/Y1 [14, 15]. Recent characterisation of these three cell lines demonstrated keratin expression patterns similar to normal tissue, oral dysplasia, and well-differentiated oral squamous cell carcinoma [16]. Furthermore, the normal and transformed keratinocyte cell lines model the step-wise development of oral cancer, reflecting the acquisition of immortality [14], loss of p53 tumour suppressor function and eventually gaining a tumorigenic phenotype [13, 15]. Notably, the use of one standardised culture condition provides an identical environment and exogenous influence on gene expression, allowing for a comparison of transcripts of carbonyl-metabolising enzymes in the various cell types.

Recent studies indicated a significant ability of human oral mucosal cells for detoxification of aldehydes (especially formaldehyde [9]). Moreover, retained ADH3 activity in normal keratinocytes, SVpgC2a and SqCC/Y1, indicated that the capacity for formaldehyde metabolism may be preserved during cell transformation. In this study, we extend the expression analysis to the set of carbonyl-metabolising enzymes identified in humans using a micro-array-based method demonstrating that several of the detected transcripts display expression changes associated with cell transformation.

Materials and methods

Processing of cell cultures

For processing of primary cell cultures, human buccal tissue was selected (approved by the ethical committee at Karolinska Institutet). Primary keratinocyte lines were derived following incubation of tissue with 0.17% trypsin in phosphate-buffered saline (PBS) at 4 °C for 18–24 h, and the subsequent seeding of keratinocyte aggregates and single cells at 5×10^3 cells/cm^2 onto fibronectin/collagen-coated dishes in serum-free EMHA (epithelial medium with high levels of amino acids). The immortal cell line SVpgC2a, derived by transfection and stable integration of the SV40 T antigen into buccal keratinocytes, and the buccal carcinoma cell line SqCC/Y1 were also cultured in serum-free EMA. The normal cell types were used in passages 1–5, the SVpgC2a line in passages 59–64, and the SqCC/Y1 line in passages 115–120 [13–15].

Preparation of labelled cRNA and array hybridisation

Methods for cRNA preparation and the following hybridisation as well as data analysis were provided by the manufacturer (Affymetrix). Briefly, total RNA was prepared with RNeasy (Qiagen) from 3×10^6 cells of each type. Double-stranded cDNA was synthesised from 25 µg total RNA using a cDNA synthesis kit (SuperScript Choice system; GibcoBRL Life Technologies). Labelled cRNA was in vitro transcribed with 1.5 µg cDNA as template incorporating biotinylated CTP and UTP (Enzo RNA Transcript Labeling Kit; Enzo Diagnostics). The cRNA was purified with RNeasy affinity columns (Qiagen) and subsequently fragmented in 40 mM Tris-acetate, pH 8.1, 100 mM potassium acetate, 30 mM magnesium acetate at 94 °C for 35 min. Triplicates of 10 µg of cRNA from three separate RNA isolations and subsequent cRNA preparations from each cell line were hybridised to oligonucleotide arrays HuFL6800 (one experiment) or HG_U95A (two experiments) at 45 °C for 16 h according to the manufacturer's protocol (Affymetrix). Arrays were washed under stringent conditions, stained with streptavidine-phycoerythrin (Molecular Probes), washed again and subsequently scanned at 570 nm using a Hewlett Packard Gene Array scanner. Obtained data were analysed with GeneChip3.1 software (Affymetrix). The detection of gene expression is based on the absolute call

(Pos/Neg Ratio, Positive Fraction and Log Average Ratio) and the Average Difference which directly correlates with the expression level (for detailed information see GeneChip3.1 Expression Analysis Algorithm Tutorial; Affymetrix). For the comparison analysis, a global scaling was used where the target intensity was set to 250 for each chip. If a gene was detected as present in two out of the three hybridisation experiments, the gene transcript was considered as present in the cell line (present/absent are indicated as +/– in tables 1–5). For those genes that were only represented on the HG_U95A chip, only those transcripts that were detected in both hybridisation experiments were considered as present. Present genes were further divided into three groups depending on expression levels: low expression (Average Difference < 100) $+^{i}$; moderate expression (100 < Average Difference < 500) $+^{ii}$; high expression (Average Difference > 500) $+^{iii}$. Gene expression was considered increased/decreased if two of the three hybridisation experiments yielded a significant twofold change (+/–) or more among the samples with normal keratinocytes as reference. This limit is in agreement with other mammalian studies [17].

Results

Micro-array chips with oligonucleotides corresponding to 6800 (HuFL6800) and 12,500 (HG_U95A) human genes were hybridised with labelled cRNA prepared from normal, immortalised (SVpgC2a) and malignant (SqCC/Y1) human oral keratinocytes to determine transcript expression levels of carbonyl-metabolising enzymes. Clearly, single hybridisations of this kind are not sufficient for reliable results. This uncertainty was overcome by multiple experiments using independent RNA preparations. The three separate hybridisations were compared to normal keratinocytes within the same hybridisation experiment. The total number of expressed genes in the three cell lines was 3894, 4666 and 4210, respectively. The carbonyl-metabolising enzymes were analysed according to four protein families: ADH, ALDH, SDR and AKR. Of a total of 57 genes from these families present on the chip, 17 were expressed in normal keratinocytes, 14 in SVpgC2a and 19 in SqCC/Y1. Expression of 34 genes was not detected in any cell type (a few recently cloned genes coding for carbonyl-metabolising enzymes were not represented on the chip). The results are summarised in tables 1–5 and discussed in more detail below.

Transcripts of the ancestral form of ADH, i.e. ADH3, were detected in all the investigated cell types (table 1). Transcripts for esterase D, the enzyme metabolising the second step in formaldehyde oxidation was also present in all cell types. The other forms of human ADH transcripts were not detectable in any cell type. The expression of the related sorbitol dehydrogenase was detected in all three cell types, while the expression of quinone reductase (ζ-crystallin type) was decreased in the malignant cells.

The cytosolic ALDH1A1 (ALDH1) was not detected in any cell type, while the mitochondrial ALDH2 and an ALDH1 type, ALDH1A3, transcripts were present in normal and malignant keratinocytes, and ALDH3A1 (ALDH3) transcript was only present in the malignant keratinocytes (table 2). ALDH4A1 (ALDH4) and the more recently identified ALDHs, ALDH7A1 (ATQ1) and

Table 1. Expression of ADHs in normal and transformed oral keratinocytes.

Protein	Accession No.	Normal oral keratinocytes		SVpgC2a (fold change)		SqCC/Y1 (fold change)	
		present/ absent	score* (n/3)	present/ absent	score * (n/3)	present/ absent	score * (n/3)
ADH1A	M12963	–	0	–	0	–	0
ADH1B	X03350	–	1	–	0	–	0
ADH1C	M12272	–	0	–	0	–	0
ADH2	X56411	–	0	–	0	–	0
ADH3	M81118	$+^{ii}$	3	$+^{ii}$	3	$+^{ii}$	3
ADH4	X76342	–	0	–	0	–	0
ADH5	M68895	–	0	–	0	–	0
CRYZ (quinone reductase, ζ-crystallin)	L13278	$+^{ii}$	3	$+^{ii}$	3	– (–5.0)	0
SDH (sorbitol dehydrogenase)	L29254	$+^{iii}$	3	$+^{ii}$	3	$+^{ii}$	3
Esterase D[+]	AF112219	$+^{iii}$	3	$+^{ii}$	3	$+^{iii}$	3

Transcripts present/absent are indicated as +/–. Present genes are divided into three groups depending on expression levels: moderate expression, $+^{ii}$; high expression, $+^{iii}$. The indicated fold change in expression level is given with the expression level in normal oral keratinocytes as a base.

* Score indicates in how many of the three hybridisation experiments the gene was detected as present.

[+] Esterase D is not a member of the ADH family but is included because of it's involvement in formaldehyde oxidation together with ADH3.

Table 2. Expression of ALDHs in normal and transformed oral keratinocytes.

Protein	Accession No.	Normal oral keratinocytes		SVpgC2a (fold change)		SqCC/Y1 (fold change)	
		present/ absent	score* (n/3)	present/ absent	score* (n/3)	present/ absent	score* (n/3)
ALDH1A1 (ALDH1)	K03000	–	0	–	0	–	0
ALDH1A2 (RALDH2)+	AB015228	–	1	–	1	–	0
ALDH1A3 (ALDH6)	U07919	+ ii	3	–	1	+ iii	3
ALDH1B1 (ALDH5)	M63967	–	0	–	0	–	0
ALDH1L1 (FTDH)+	AF052732	–	0	–	0	–	0
ALDH2 (ALDH2)	X05409	+ ii	3	–	1	+ ii	2
ALDH3A1 (ALDH3)	M74542	–	0	–	0	+ i	2
ALDH3A2 (ALDH10)	U46689	+ ii	3	+ ii	2	+ ii	2
ALDH3B1 (ALDH7)	U10868	–	0	–	0	–	0
ALDH3B2 (ALDH8)	U37519	–	0	–	0	–	0
ALDH4A1 (ALDH4)	U24266	+ iii	3	+ ii	2	+ ii (–2.2)	3
ALDH5A1 (SSDH)	AL031230	–	0	–	0	–	1
ALDH6A1 (MMSDH)	M93405	–	0	–	1	–	0
ALDH7A1 (ATQ1)	S74728	+ iii	2	+ ii	2	+ ii	2
ALDH9A1 (ALDH9)	U34252	+ ii	3	+ ii	3	+ ii	2

Transcripts present/absent are indicated as +/–. Present genes are divided into three groups depending on expression levels: low expression, + i; moderate expression, + ii; high expression, + iii. The indicated fold change in expression level is given with the expression level in normal oral keratinocytes as base. Old nomenclature given within parentheses.
* Score indicates in how many of the three hybridisation experiments the gene was detected as present.
+ The gene was not represented on the HuFL6800 chip.

ALDH9A1 (ALDH9), were monitored in all three cell types.

The SDR family is a diverged group with several specific functions, and ERAB was the only form detected in all cell types (table 3). 2,4-Dienoyl-CoA reductase, 17-β-hydroxysteroid dehydrogenase type 2 where quinoid dihydropterin reductase transcripts were detected in normal keratinocytes but were down-regulated in the transformed cells. Hydroxysteroid 17-β-dehydrogenase 4 was present in both SVpgC2a and SqCC/Y1, while 3-β-hydroxybuturate dehydrogenase and galactose-4-epimerase were only present in SqCC/Y1. For the majority of the genes in this family, no transcripts could be detected under these experimental conditions. However, for the expressed transcripts, almost all displayed changed expression in the cells representing various stages of cell transformation.

The thoroughly investigated aldehyde reductase (AKR1A1), aldose reductase (AKR1B1) and dihydrodiol dehydrogenase (AKR1C1) were expressed in all three cell types, as was aflatoxin aldehyde reductase (AKR7A2) (table 4). AKR1C3 was detected in the SVpgC2a cells only. Four out of five detected transcripts were not changed by cell transformation.

Transcript levels of the housekeeping genes for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-actin were determined in all three cell types as controls (table 5).

Discussion

Transformation of carbonyl compounds into less toxic compounds or into metabolites that are essential for cell metabolism is a prerequisite for all living species. Several protein superfamilies have evolved for this purpose, e.g. ADH, ALDH, SDR and AKR. Individual proteins within these families have been investigated to a certain extent by Northern blot and/or with other classical techniques. However, today, expression levels can be determined simultaneously for multiple genes using micro-array chip-based technology, including comparison of transcripts in different cell types.

Within the ADH family, a member of the MDR superfamily, only ADH3/glutathione-dependent formaldehyde dehydrogenase was detected in the oral keratinocytes, reflecting normality and different stages of cell transformation. This finding is consistent with an earlier study on formaldehyde-metabolising activities in these cell types and the fact that ADH3 is present in all, tissues and species investigated so far [8, 9, 11]. The second enzyme in the glutathione-dependent formaldehyde metabolising pathway, esterase D/S-formylglutathione hydrolase (not a strict member of the carbonyl-metabolising enzyme group) was also expressed in the three different keratinocytes, indicating that these two enzymes are co-expressed and coupled in the detoxification of formaldehyde. Therefore, these cell lines represent true functional models for in vitro studies of formaldehyde metabolism in human oral epithelium. The observation of co-expres-

Table 3. Expression of SDRs in normal and transformed oral keratinocytes.

Protein	Accession No.	Normal oral keratinocytes		SVpgC2a (fold change)		SqCC/Y1 (fold change)	
		present/ absent	score* (n/3)	present/ absent	score* (n/3)	present/ absent	score* (n/3)
BDH (3-β-hydroxybutyrate dehydrogenase)	M93107	–	1	–	1	+ [ii]	2
CBR1 (carbonyl reductase 1)	AB003151	ND‡	1	ND‡	1	ND‡	1
CBR3 (carbonyl reductase 3)+	AB004854	–	0	–	0	–	0
DECR1 (2,4-dienoyl CoA reductase 1)	U78302	+ [i]	2	–	0	+ [i]	2
ERAB (L-3-hydroxyacyl CoA dehydrogenase)+	AB035555	+ [ii]	2	+ [iii]	2	+ [iii]	2
FABGL (member of the short-chain ADH family)	D82061	–	1	–	0	+ [ii]	2
FVT1 (follicular lymphoma variant translocation 1)	X63657	–	1	–	0	–	1
GALE (galactose-4-epimerase)	L41668	–	1	–	0	+ [ii]	2
Hep27 (short-chain alcohol dehydrogenase member)+	U31875	–	0	–	0	–	0
HSD3B1 (hydroxy-delta 5 steroid dehydrogenase)	M38180	–	0	–	1	–	0
HSD3B2 (hydroxy-delta 5 steroid dehydrogenase 2)	M77144	–	0	–	0	–	0
HSD11B1 (hydroxysteroid 11-β dehydrogenase 1)	M76665	–	1	–	0	–	0
HSD11B2 (hydroxysteroid 11-β dehydrogenase 2)	U26726	–	0	–	0	–	0
HSD17B1 (hydroxysteroid 17-β dehydrogenase 1)	M84472	–	0	–	0	–	0
HSD17B2 (hydroxysteroid 17-β dehydrogenase 2)	L40802	+ [ii]	2	– (–3.0)	0	–	0
HSD17B3 (hydroxysteroid 17-β dehydrogenase 3)	U05659	–	0	–	0	–	0
HSD17B4 (hydroxysteroid 17-β dehydrogenase 4)	X87176	–	1	+ [ii]	2	+ [ii]	2
HPGD (15-hydroxy-prostaglandin dehydrogenase)	L76465	–	0	–	0	–	0
retSDR1 (Short-chain dehydrogenase 1)+	AF061741	–	0	–	0	–	0
RDH (3-α hydroxysteroid dehydrogenase)+	U89281	–	0	–	0	–	0
RDH4 (microsomal retinol dehydrogenase)+	AF057034	–	0	–	0	–	0
RDH5 (9-cis-11-cis retinol dehydrogenase 5)	AF037062	–	0	–	0	–	0
SPR (Sepiapterin reductase)+	M76231	–	0	–	0	–	1
QDPR (quinoid dihydropterin reductase)	M16447	+ [ii]	2	–	1	–	1

Transcripts present/absent are indicated as +/–. Present genes are divided into three groups depending on expression levels: low expression: + [i]; moderate expression, + [ii]; high expression, + [iii]. The indicated fold change in expression level is given with the expression level in normal oral keratinocytes as base.

* Score indicates in how many of the three hybridisation experiments the gene was detected as present.

+ The gene was not represented on the HuFL6800 chip.

‡ CBR1 was not represented on the HG_U95A chip.

sion of metabolically coupled enzymes is further visualised with aldose reductase and sorbitol dehydrogenase, enzymes forming the polyol pathway. These enzymes are essential in metabolising excess glucose that cannot be metabolised through ordinary glycolysis. ADH3 and sorbitol dehydrogenase are the only classical zinc-containing ADHs that are expressed in keratinocytes. ADH4 is the only ADH that is solely extrahepatically expressed with high expression levels in epithelial cells [18]. However, ADH4 transcripts were neither detected in normal nor in transformed keratinocytes. This finding is in line with a previous study where ADH3 but not ADH4 activ-

Table 4. Expression of AKRs in normal and transformed oral keratinocytes.

Protein	Accession No.	Normal oral keratinocytes		SVpgC2a (fold change)		SqCC/Y1 (fold change)	
		present/ absent	score* (n/3)	present/ absent	score* (n/3)	present/ absent	score* (n/3)
AKR1A1 (aldehyde reductase)	J04794	+iii	3	+iii	3	+iii	3
AKR1B1 (aldose reductase)	X15414	+iii	3	+iii	3	+iii	3
AKR1C1 (dihydrodiol dehydrogenase 1)	U05861	+iii	3	+iii	3	+ii	2
AKR1C3 (dihydrodiol dehydrogenase 3)	D17793	–	1	+i	2	–	1
AKR1C4 (dihydrodiol dehydrogenase 4)	M33375	–	1	–	0	–	0
AKR1D1 (4-3-ketosteroid 5-β reductase)	Z28339	–	0	–	0	–	0
AKR7A2 (aflatoxin aldehyde reductase)+	AF026947	+ii	2	+iii	2	+iii	2
AKR7A3 (aflatoxin B 1-aldehyde reductase)+	AF040639	–	0	–	0	–	0
AKR1B10 (aldose reductase)+	U37100	–	0	–	0	–	0

Transcripts present/absent are indicated as +/–. Present genes are divided into three groups depending on expression levels: low expression: +i; moderate expression, +ii; high expression, +iii.
* Score indicates in how many of the three hybridisation experiments the gene was detected as present.
+ The gene was not represented on the HuFL6800 chip.

Table 5. Expression of housekeeping genes detected in normal and transformed oral keratinocytes.

Protein	Accession No.	Normal oral keratinocytes		SVpgC2a (fold change)		SqCC/Y1 (fold change)	
		present/ absent	score* (n/3)	present/ absent	score* (n/3)	present/ absent	score* (n/3)
GAPDH	M33197	+iii	3	+iii	3	+iii	3
β-actin	X00351	+iii	3	+iii	3	+iii	3

Transcripts present/absent are indicated as +/–. Present genes are divided into three groups depending on expression levels: high expression, +iii.
* Score indicates in how many of the three hybridisation experiments the gene was detected as present.

ity was monitored in the three cell lines [9]. Activities for both ADH3 and ADH4 have previously been detected in oral epithelia [19], but during extended culture and transformation of oral keratinocytes, ADH3 expression is retained and ADH4 expression is lost. The loss of expression of certain phase I enzymes is a commonly observed feature for in vitro-cultured cells [20].

Today, the ALDH family consists of 16 different enzymes in the human. Several of these have been characterised in recent years but for many, a specific function remains to be revealed. The classical types, the cytosolic ALDH (ALDH1A1) and the mitochondrial ALDH (ALDH2) are often referred to as low-K_m ALDHs together with the recently isolated ALDH1B1 (old name: ALDH5). ALDH2 was detected in normal keratinocytes and in the transformed cell line, supporting the expression of low-K_m ALDHs in many cell types [12]. The low-K_m ALDHs participate in alcohol metabolism but none of the main alcohol-oxidising enzymes were detected in the keratinocyte cell lines. These enzymes are usually coupled and co-expressed. ALDH1A3 (old names: ALDH6/RALAH3) has been postulated to participate in retinoid metabolism in parallel with ALDH1A1 and ALDH1A2 (retinal dehydrogenase 2). The function for ALDH1A3 may be as a back-up enzyme in retinoid metabolism. Apart from ALDH1A3, no true retinoid-metabolising enzyme was identified. Together, the lack or low expression of retinol/retinal active ADH/ALDH expression addresses the question about retinoic acid metabolism in keratinocytes. ALDH3A2 and ALDH9A1 were expressed in all three keratinocyte types, indicating that the expressions of transcripts for these enzymes are retained during

cell transformation. This is an observation that correlates with the detection of ALDH9A1 in all cell types [3].

The largest group of carbonyl-metabolising enzymes is the SDR family. This group harbours several retinoid-metabolising enzymes, but none of these were detected in the keratinocytes. The only SDR detected in all cell lines was ERAB, while a number of SDR enzymes were variably detected in one or two of the cell lines (cf. table 3). The fact that most SDRs participate in specific metabolic pathways, not present in keratinocytes, may explain the low number of SDRs detected in these cells. Notably, neither FVT1 nor Hep27 were expressed in any cell types, proteins that have been implicated in carcinogenesis [5]. However, of the detected SDRs, all except ERAB showed changes in expression levels at various stages of cell transformation, indicating potential involvement in the multi-step process of carcinogenesis.

For a long time, the AKR group was the smallest family of carbonyl-metabolising enzymes but several recent additions have enlarged it significantly. The thoroughly investigated AKR1A1 and AKR1B1 were expressed in all three types of keratinocyte. Again, the importance of these functions are visualised in accordance with the variety of identified metabolic pathways for these enzymes [5, 6]. However, results from disruption of the aldose reductase gene (*Akr1b1*) in mice show that the gene product is not essential per se [21]. The detection of AKR1C1 and AKR7A3 also underlines essential roles for these enzymes in cellular metabolism.

With the application of a micro-array chip technique, the expression of a large number of genes can be determined simultaneously. The methodology demands that multiple experiments are performed, preferably with batch independent chips and RNA preparations. This is the first time that a complete investigation of expression of carbonyl-metabolising enzyme transcripts in human oral keratinocytes has been performed. Of the investigated enzymes, relatively few were associated to the keratinocyte phenotype, a feature particularly highlighted by the SDR family. In the various cells, several of the detected transcripts were similarly expressed indicating their essentiality for keratinocyte biology and that in several aspects, the transformed cell lines may be regarded as experimental models for normality. However, for the transcripts with monitored expression changes, including quinone reductase, ALDH1A3, ALDH2, ALDH3A1, hydroxysteroid 17-β dehydrogenase 2, hydroxysteroid 17-β dehydrogenase 4, quinoid dihydropterin reductase and AKR1C3, a potential involvement of the respective enzymes in malignant transformation is indicated. Finally, the detection of both ADH3 and sorbitol dehydrogenase with esterase D and AKR1B1, respectively, illustrates co-expression of enzymes in metabolic pathways.

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