ONCOLOGY

Expression of mRNA for Several Enzymes of Xenobiotic Detoxification in Normal and Spontaneously Transformed Mesothelial Cells and Mesothelioma Cells of Rats

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Expression of mRNA for the *mdr1* gene, cytochrome P450 isoforms 1A1 and 1B1, Ah receptor, and ARNT protein regulating the concentration of cytochrome P450 mRNA was compared in normal and spontaneously transformed mesothelial cells and mesothelioma cells from rats. Expression of cytochrome P450 1A1 and 1B1 mRNA decreased in transformed mesothelial and mesothelioma cells compared to normal mesothelial cells. mRNA for the *mdr1* gene was undetected in normal mesothelial cells. Expression of mRNA for the Ah receptor and ARNT protein did not differ in cultured cells.

Key Words: *cytochrome P450; P-glycoprotein; Ah receptor; tumor transformation*

Cytochrome P450 isoforms (CYP) and P-glycoprotein (*mdr1* gene product) are proteins regulating intracellular xenobiotic concentration. The effects of these proteins are mediated by various mechanisms. P-glycoprotein operates as a transmembrane pump and eliminates xenobiotics from the cell. CYP oxidizes xenobiotics. Oxidation products interact with hydrophilic compounds (*e.g.*, glutathione) and are eliminated from the organism. Oxidation of xenobiotics sometimes results in activation of substances determining cell dysfunction, toxicity, and carcinogenic transformation. Expression of the *mdr1* gene increases [4], while expression of CYP isoforms decreases in tumors [5]. These changes determine drug resistance of tumors. 1A1 and 1B1 are the major isoforms of CYP involved in oxidation of various compounds, including polycyclic aromatic carbohydrates. Expression of these isoforms is induced by several substances. The Ah receptor and ARNT play a major role in the induction and are responsible for signal transduction in the nucleus. Cultured mesothelial cells easily undergo spontaneous transformation and gain malignant activity (growth in semisolid agar, supermonolayer growth). Previous studies showed that CYP 1A1 is expressed in normal mesothelial cells [2]. We studied expression of mRNA for CYP 1A1 and 1B1, regulatory proteins Ah receptor and ARNT, and *mdr1* gene in cells at various stages of tumor transformation. Experiments were performed with normal (6th culture passage) and spontaneously transformed mesothelial cells (30th culture passage) and mesothelioma cells from rats.

MATERIALS AND METHODS

The methods for culturing of normal mesothelial cells and asbestos-induced mesothelioma cells from rats were described previously [1]. The isolated parietal pleura was treated with collagenase to obtain the culture of mesothelial cells. Disaggregated cells were washed and put in a 96-well plate with

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Ham F12 medium (Gibco) and 10% fetal serum (HyClone). After attaining confluence the cells from wells containing only polygonal cells (without fibroblasts) were taken for further experiments with cell division.

Mesotheliomas in Wistar rats were induced by intraperitoneal treatment with asbestos. The culture of mesothelioma cells was obtained by the method described for the culture of normal mesothelial cells. The cells expressed cytokeratins and vimentin. Therefore, they belonged to cells of mesothelial type.

Experiments were performed with cultures of normal mesothelial cells (passages 4-7 and 30) and mesothelioma cells (passages 4-7). Induction was initiated by addition of benz(a)anthracene (BA, 5 μg/ml) dissolved in acetone. The time of exposure was 1 day.

mRNA was isolated using TRIzol reagent (Gibco BRL Life Technologies). Total RNA concentration was determined by optical density at 260 nm. The reverse transcription reaction with six-reading frames of a random nucleotide sequence (random primer) was conducted to obtain cDNA. To perform a comparative study of mRNA expression, the concentration of cDNA for gene amplification was leveled by the concentration of β-actin mRNA. Table 1 shows nucleotide sequence of primers for specific genes.

RESULTS

In the early passages, mesothelial cells looked like polygonal epithelial structures and formed individual islets of regular shape (Fig. 1). Confluence was attained after 15-20 days (subculturing 1:3). Further culturing was accompanied by the appearance of fibroblast-like cells with long processes that intersected each other in a dense monolayer. These cells prevailed in the follow-up period. Starting from passages 22-24, mesothelial cells formed focuses of superconfluent growth (Fig. 2, *a*) and gained the ability to grow in semisolid agar. These characteristics are typical of transformed cells. Cell polymorphism was observed in the culture of mesothelioma cells. Apart from epithelial cells, we revealed long and elongated cells. They had long processes that crawled over other cells and formed the multilayer structure. Cell nuclei differed in size a were hyperchromic. Many nuclei were large and had irregular shape. We often found multinuclear cells (Fig. 2, *b*).

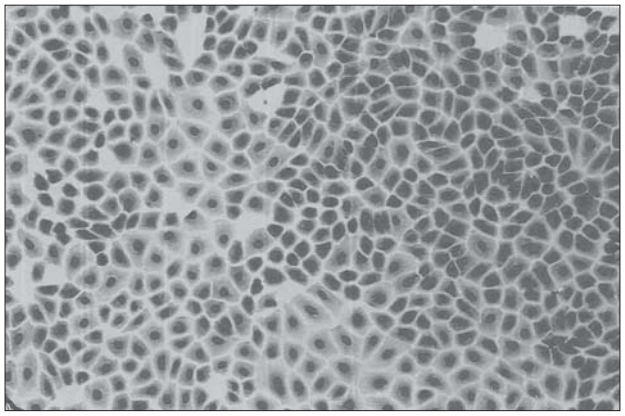

Fig. 1. Cultured mesothelial cells of the 6th passage. Hematoxylin staining, ×128.

We compared expression of genes for xenobiotic resistance in cultures at various stages of tumor transformation. *CYP 1A1* mRNA was expressed only in early-passage mesothelial cells (normal mesothelium, Fig. 3). Addition of BA was followed

TABLE 1. Nucleotide Sequence of PCR Primers

Gene	Nucleotide sequence of primers	Amplicon size
β-Actin	Sense 5'-TGCAGAAGGAGATTACTGCC-3'	211
	Antisense 5'-GCAGCTCAGTAACAGTCCG-3'	
CYP1A1	Sense 5'-CCATGACCAGGAACTATGGG-3'	341
	Antisense 5'-TCTGGTGAGCATCCAGGACA-3'	
CYP1B1	Sense 5'-ACCGCAAACTTCAGCAACTTC-3'	427
	Antisense 5'-GTGTTGGCAGTGGTGGCATG-3'	
AHR	Sense 5'-TCCATGTAGCAGTGCCAGG-3'	212
	Antisense 5'ATATCAGGAAGAGGCTGGGC-3'	
ARNT	Sense 5'-GTCTCCCTCCCAGATGATGA-3'	218
	Antisense 5'-AAGAGCTCCTGTGGCTGGTA-3'	
mdr1	Sense 5'-CCCATCATTGCAATAGCAGG-3'	167
	Antisense 5'-GTTCAAACTTCTGCTCCTGA-3'	

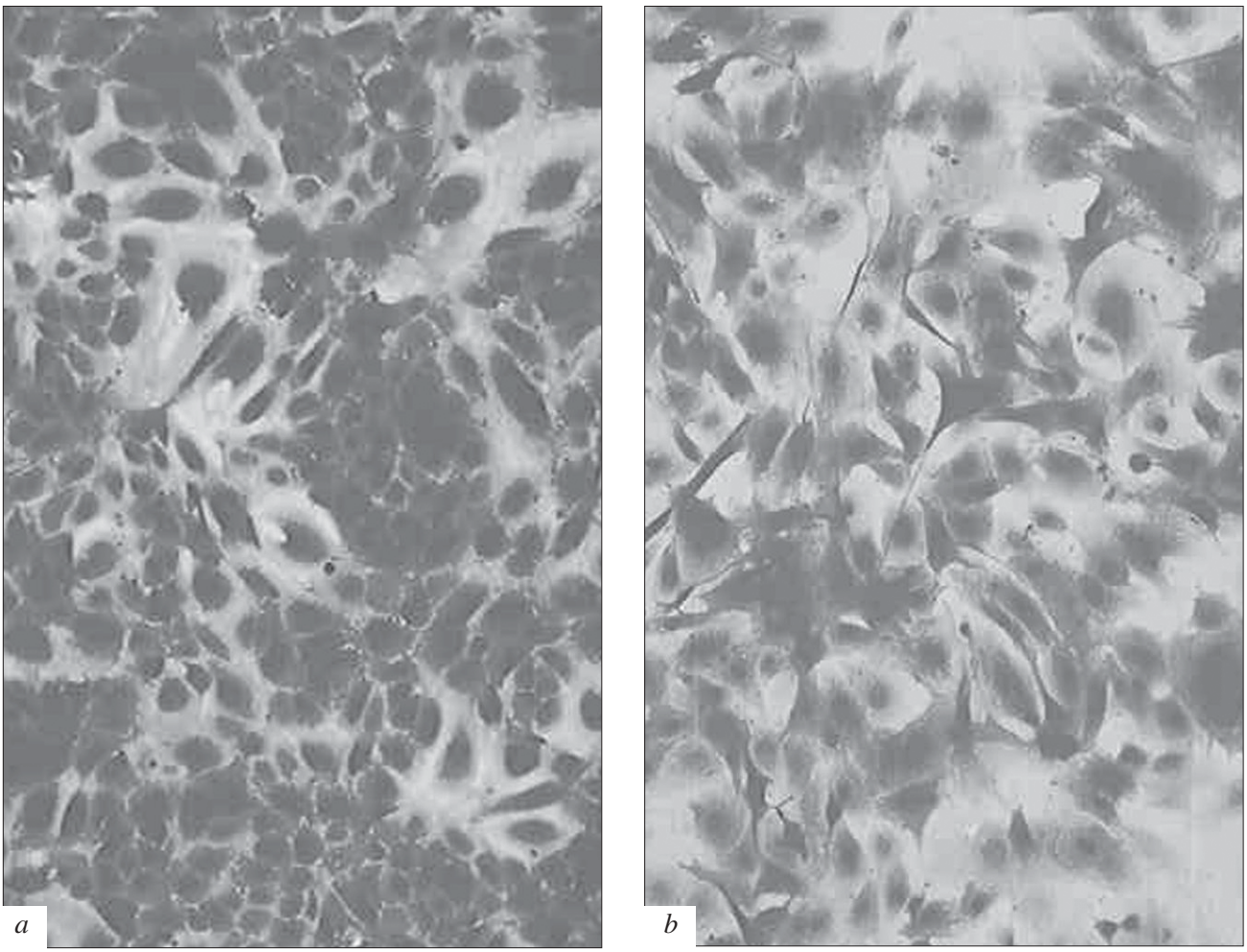

Fig. 2. Cultured mesothelial (*a*) and mesothelioma cells (*b*) of the 30th passage. Hematoxylin staining, ×320.

by weak induction. Expression of *CYP 1A1* mRNA was not detected in mesothelioma cells and mesothelial cells of the 30th passage. These cells had signs of transformation. It should be emphasized that we took into account BA-treated and untreated cells.

CYP 1B1 mRNA was expressed in the test cells. The degree of expression decreased in the following order: early-passage mesothelial cells>mesothelial cells of the 30th passage>mesothelioma cells. BA induced *CYP 1B1* mRNA in all cultures.

Expression of mRNA for proteins involved in transduction of the induction signal (Ah receptor and ARNT) practically did not differ in the test cells. A negative correlation was found between expression of mRNA for the *mdr1* gene and CYP 1A1 in transformed cells and mesothelioma cells. This correlation was not revealed in early-passage mesothelial cells. BA had no effect on mRNA for the *mdr1* gene, which is consistent with published data that Ah receptor does not regulate expression of this gene [3].

Animal studies showed that carcinogen treatment decreased the concentration of CYP isoforms in experimental tumors [5]. In the presence of this carcinogen, transformed cells with low ability to activate xenobiotics (*i.e.*, decreased level of CYP) survived better than normal and transformed cells exhibiting high-intensity formation of active metabolites. Therefore, the decrease in CYP concentration in tumor cells probably results from selection by survival in a carcinogen-containing medium.

Experiments with spontaneously transformed mesothelial cells in the absence of carcinogen showed that the decrease in CYP isoforms is associated with transformational events, but not with better survival in a carcinogen-containing medium.

Our experiments showed that CYP expression in tumors decreases at the stage of cell transformation. Expression of P450 isoforms 1A1 and 1B1 is regulated by different mechanisms. mRNA for *CYP 1A1* is present only in early-passage mesothelial cells. mRNA for *CYP 1B1* is expressed in various cells. Expression of *CYP 1B1* mRNA tends to de-

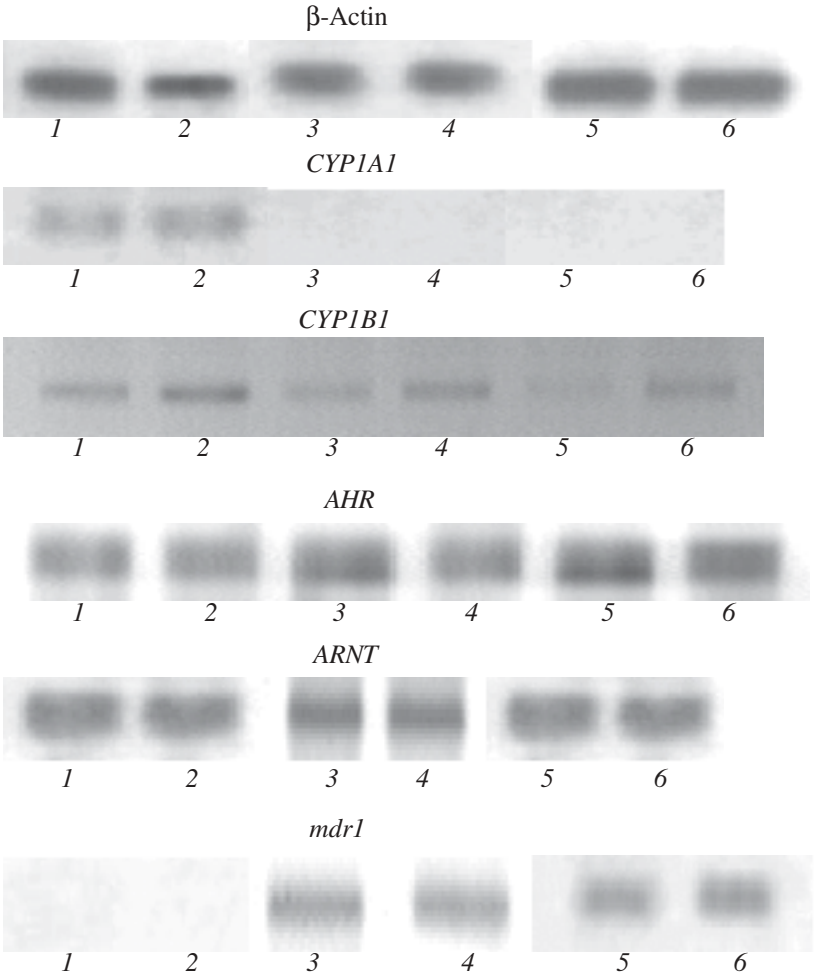

Fig. 3. Constitutive and benz(a)anthracene-induced (BA) expression of genes for *β-actin*, *CYP 1A1*, *CYP 1B1*, *AHR*, *ARNT*, and *mdr1* in mesothelial cells (passages 7 and 30) and mesothelioma cells. Mesothelium (7th passage, *1*); mesothelium (7th passage)+BA (*2*); mesothelium (30th passage, *3*); mesothelium (30th passage)+BA (*4*); mesothelioma (*5*); mesothelioma+BA (*6*).

crease in malignant cells. Expression of mRNA for the Ah receptor and ARNT does not differ in the test cells, which indicates that the absence of CYP 1A1 mRNA in transformed cells of the mesothelium and mesothelioma is not related to the impairment of signal transduction at the level of these components. The mechanism underlying these changes remains unclear.

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