ONCOLOGY

Comparative Analysis of Family 1 Cytochrome P-450 mRNA Expression in Human Intestinal Adenocarcinoma and Intact Portion of the Intestine

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The expression of mRNA of proteins involved in the transformations of cytostatics (cytochrome P-450 1A1 and 1B1 isoforms) and genes encoding proteins participating in their regulation (Ah receptor, AHRR and ARNT) in intestinal tumors and intact portions of the intestine were studied. The expression of cytochrome P-450 1A1 increased in poorly differentiated tumors in comparison with its expression in intact portions of the intestine (tumor/intact tissue=1.65). The expression of cytochrome P-450 1B1 was higher in well-differentiated tumors (tumor/intact tissue=1.62). The possibility of practical use of high expression of cytochrome P-450 isoforms in tumors in comparison with intact intestinal tissue is discussed.

Key Words: *cytochrome P-450; intestinal adenocarcinoma; cytostatics*

The majority of cytostatics used for the treatment of tumors are substrates of cytochrome P-450 isoforms. In some cases oxidation of the cytostatic by cytochrome P-450 leads to its activation, and this fact indicates that this compound is a pro-drug (cyclophosphamide, doxorubicin, thiotepa, *etc.* [10]), in others the initial drug dosage form is an active antitumor factor, while oxidation by cytochrome P-450 deactivates the drug (for example, docetaxel, tamoxifen, *etc.* [2]). Hence, depending on the antitumor drug, expression of cytochrome P-450 isoforms in tumors can be a positive (in therapy with pro-drugs) or a negative factor (in therapy with "direct effect" cytostatics). Hence, the level of cytochrome P-450 isoforms expression can be a factor largely determining tumor sensitivity to drugs, and treatment strategy should be planned with consideration for the data about this expression. Results of studies of the levels of cytochrome P-450 isoforms expression in tumors are ambiguous. It was shown on experimental animal tumors that the level of cytochrome P-450 isoforms expression in them was as a rule lower than in intact homologous tissue. The expression of cytochrome P-450 isoforms in human tumors differs depending on the organ in which the tumor develops and tumor type. Family 1 cytochrome P-450 isoforms, specifically 1A1 and 1B1, are particularly interesting. Regulation of the expression of these isoforms is associated with functioning of Ah receptors, ARNT and AHRR proteins; Ah receptor and ARNT protein are positive, while AHRR protein is negative regulators. Cytochromes 1A1 and 1B1 are not expressed in cells without Ah receptor expression. Transfection of Ah receptor gene stimulates constitutive expression of cytochrome P-450 isoforms 1A1 and 1B1 [12]. Compounds activated by family 1 cytochrome P-450 isoforms are used for antitumor therapy at present

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and new compounds are developed. Among these drugs are flavone [8] and benzothiazole [1,3,13] derivatives. On the other hand, there are drugs inactivated by family 1 isoforms (ellipticine, tamoxifen [10]).

We compared the expression of mRNA for cytochromes 1A1 and 1B1 and Ah receptor, ARNT, and AHRR genes involved in their regulation in highly prevalent human intestinal tumors and in intact portions of the intestine.

MATERIALS AND METHODS

Specimens of intestinal tumors and intact intestinal tissue were obtained during surgery and were directly frozen in liquid nitrogen. Frozen tissue was fragmented and treated with TRIzol (Gibco BRL Life Technologies) according to the instruction for isolation of mRNA. The concentration of total RNA was evaluated by absorption at λ=260 nm. Reverse transcription (RT) reaction with 6-nucleotide sequence (random primer) and with synthetic hexanucleotides was carried out for obtaining cDNA. The reaction was carried out with 4 μg total RNA at 42°C for 1 h and exposed for 5 sec at 94°C for inactivation of the enzyme. The resultant cDNA was used in PCR. Reaction with water instead of RNA served as the control in all experiments. The protocol of PCR for all studied genes was as follows: 40 sec denaturation at 94°C, 10 sec annealing of primers at 60°C, 10 sec synthesis of the product at 72°C, and 2 min exposure at 72°C after the cycles. The number of cycles varied from 25 to 30 depending on the studied gene. In the control, RNA was used instead of cDNA. The following enzymes were used in RT-PCR: reverse transcriptase, MuLV, TaqSE DNA polymerase (Sibenzyme). Primers and hexanucleotides were synthesized by Helicon Company.

The content of cDNA for amplification of specific genes was equalized by the amount of β-actin mRNA. The RT and amplification reactions were carried out on a Tertsik device. The sequences of primers and sizes of the resultant amplified fragments are presented in Table 1. The RT-PCR products were separated by electrophoresis at 150 V in 2% agarose gel prepared on TBE buffer. Electrophoregrams were densitometried. The gels were analyzed using Scion Image 4.0.2 software.

RESULTS

All studied genes were expressed in normal tissue and tumors irrespective of the histological diagnosis. According to histological analysis, the studied tumors were well, moderately, and poorly differentiated adenocarcinomas. Table 2 presents mRNA levels of 1A1 and 1B1 and of Ah receptor, ARNT and AHRR, evaluated in intestinal tumors, in proportion to their levels in intact parts of the intestine of the same patient. The mean level of the expression of regulatory protein (Ah receptor, ARNT, AHRR) in the tumor irrespective of its histological diagnosis and in intact portions of the intestine was approximately the same, except the expression of AHRR in poorly differentiated tumors, in which the expression of this gene was somewhat lower than in intact portions of the intestine. The expression of 1A1 cytochrome P-450 in poorly differentiated tumors was higher than in moderately differentiated adenocarcinomas, while in moderately differentiated adenocarcinomas, in turn, higher than in well-differentiated tumors. The expression of cytochrome P-450 1B1 mRNA in tumors exhibited an op-

TABLE 1. Primers Used in the Study

	Nucleotide sequence	Size, b. p.	Number of cycles
CYPIA1	5'-TAGACACTGATCTGGCTGCAG-3'	146	35
	5'-GGGAAGGCTCCATCAGCATC-3'		
CYPIBI	5'-AACGTCATGAGTGCCGTGTGT-3'	360	30
	5'-GGCCGGTACGTTCTCCAAATC-3'		
β-Actin	5'-GTGGGGCGCCCCAGGCACCA-3'	572	22
	5'-TCCTTAATGTCACGCACGATTTC-3'		
AHR	5'-ACTTAACGGATGAAATCCTGACG-3'	689	32
	5'-TCCATTCTCAAACTTGTTTAAAA-3'		
ARNT	5'-CGGAACAAGATGACAGCCTAC-3'	225	30
	5'-ACAGAAAGCCATCTGCTGCC-3'		
AHRR	5'-AGACTCCAGGACCCACAA-3'	406	30
	5'-CAGCGTCGGACCACACA-3		

TABLE 2. Proportion of the Content of mRNA of Cytochrome P-450 1A1, 1B1, Ah Receptor, ARNT, and AHRR in Intestinal Tumors and in Intact Portions of the Intestine

No, sex	1A1	1B1	AHR	AHRR	ARNT
Well-differentiated adenocarcinomas					
024 F	1.17	1.86	0.68	1.46	1.16
014 F	0.74	1.12	0.61	0.49	1.54
016 M	1.45	1.74	0.86	1.20	1.65
003 M	1.76	1.68	1.53	0.53	0.94
Mean	1.30±0.43	1.62±0.33*	0.91±0.42	0.92±0.49	1.32±0.33
Moderately differentiated adenocarcinomas					
020 M	1.35	1.18	1.21	1.24	1.03
006 F	0.88	0.98	1.23	0.88	1.13
007 M	1.98	1.12	0.96	0.64	0.81
008 F	1.43	0.90	0.98	0.41	0.90
009 M	1.58	0.79	1.01	0.94	1.00
012 F	1.05	1.52	1.57	0.74	1.14
013 M	1.67	2.19	1.55	1.26	0.89
0.15 F	2.06	1.75	0.78	1.26	1.12
Mean	1.50±0.37	1.38±0.48	1.16±0.26	0.92±0.31	0.98±0.12
Poorly differentiated adenocarcinomas					
023 F	1.59	0.90	1.22	0.63	0.92
021 F	1.73	0.81	0.89	0.74	1.37
019 M	1.85	1.11	1.26	1.15	0.52
005 F	1.72	0.92	0.90	0.66	0.79
010 M	1.74	0.85	0.79	1.14	1.12
017 M	1.24	1.23	1.21	1.24	0.87
Mean	1.65±0.22*	0.98±0.16	1.05±0.21	0.74±0.28	0.94±0.29

Note. *$p<0.05$ compared to normal tissue.

posite trend to expression of cytochrome P-450 isoform 1A1: its level was higher in well-differentiated tumors than in moderately differentiated ones and higher in moderately differentiated ones than in poorly differentiated tumors. No relationship between the level of expression of cytochrome P-450 isoforms and Ah receptor, ARNT, and AHRR proteins was detected. This does not contradict the notions on the mechanisms regulating the expression of cytochrome P-450 family 1 isoforms, because it was previously shown that the level of constitutive expression of its isoforms depends not only on the level of expression of the known regulatory genes, but also on other heretofore unknown factors. Previous studies of the expression of cytochrome P-450 isoforms in various human tumors showed that the level of expression depended on the tumor histogenesis. The expression of some isoforms was lower in some tumor types in comparison with their expression in normal tissue. For example, the expression of 2A6 minor isoform was increased in hepatomas [11]. Relationship between the expression of 1B1 isoform and the level of tumor differentiation was demonstrated for mammary tumors: expression of 1B1 was associated with poor tumor differentiation [4].

Reports about the levels of cytochrome P-450 isoform expression in intestinal tumors are scanty. An increase of family 1 isoform expression in human intestinal tumors was detected by P. Gibson *et al.* [7]. Other authors, evaluating the levels of 23 isoforms of cytochrome P-450 in intestinal tumors by the immunohistochemical method [9], showed higher expression of some isoforms, including 1B1, in the tumors, but did not analyze the relationship between expression and histological diagnosis. High expression of 1A1 and 1B1 in the tumors suggests the creation of drugs activated by these isoforms. Family 1 enzymes are inducible and their level sharply increases in response to such substrates as polycyclic aromatic carbohydrates or chlorinated dioxines. It was shown on LS180 human intestinal

carcinoma cell culture that 1B1 was an inducible enzyme in response to classical cytochrome P-450 inductors 2,3,7,8-tetrachlorodibenzo-p-dioxin or benz(a)pyrene [5]. Hence, the volume of active preparation, forming from the pro-drug activated by 1A1 or 1B1, can be amplified by injection of the inductor. However, clinical use of "classical" inductors is impossible because of their high toxicity. A recent publication presents data on the synthesis of a new type of family 1 inductors; this compound (triazole derivative) cause no toxic effect characteristic of the "classical" inductors [6]. The use of inductors can amplify the antitumor effect of the cytostatic if this compound is activated by an inducible enzyme, while the effect of a direct-effect compound can be attenuated.

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