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Phosphoglycerate mutase, 2,3-bisphosphoglycerate phosphatase and enolase activity and isoenzymes in lung, colon and liver carcinomas

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Summary We have compared the levels of phosphoglycerate mutase, 2,3-bisphosphoglycerate phosphatase and enolase activities and the distribution of their isoenzymes in normal colon, liver and lung tissues, and in colon, liver and lung adenocarcinoma, lung squamous cell carcinoma and lung carcinoid. All tumours presented higher phosphoglycerate mutase and enolase activities and lower 2,3-bisphosphoglycerate phosphatase activity than the normal tissues. No changes were observed in the phosphoglycerate mutase isoenzyme patterns analysed by cellulose acetate electrophoresis. All specimens contained mainly type BB isoenzyme, traces of type MB isoenzyme and no type MM isoenzyme. However, the tumours had decreased levels of 2,3-bisphosphoglycerate mutase and 2,3-bisphosphoglycerate mutase–phosphoglycerate mutase hybrid enzyme. Determined by agarose gel electrophoresis, αα-enolase was the isoenzyme predominant in normal lung, colon and liver tissue, although αγ- and γγ-enolase were also present in all tissues. In colon, liver and non-endocrine lung tumours, the proportions of αγ- and γγ-enolase decreased. In contrast, in carcinoid tumours of the lung, the proportions of these isoenzymes increased.

Keywords: 2,3-bisphosphoglycerate mutase; 2,3-bisphosphoglycerate phosphatase; enolase; phosphoglycerate mutase; isoenzyme; lung, colon and liver adenocarcinoma; lung squamous cell carcinoma; lung carcinoid

Phosphoglycerate mutase (D-phosphoglycerate 2,3-phosphomutase, EC 5.4.2.1, PGM) and enolase (2-phospho-D-glycerate hydrolyase, EC 4.2.1.11) are glycolytic enzymes that catalyse consecutive reversible reactions connecting the two ATP-generating reactions in the glycolytic pathway. PGM catalyses the conversion of 3-phosphoglycerate, product of the first ATP-generating reaction, into 2-phosphoglycerate in the presence of the cofactor 2,3-bisphosphoglycerate. Enolase catalyses the conversion of 2-phosphoglycerate into phosphoenolpyruvate, substrate of the second ATP-generating reaction. In addition to the main mutase activity, PGM possesses collateral 2,3-bisphosphoglycerate synthase or 2,3-bisphosphoglycerate mutase activity (BPGM: 1,3-bisphosphoglycerate + 3-phosphoglycerate → 3-phosphoglycerate + 2,3-bisphosphoglycerate) and 2,3-bisphosphoglycerate phosphatase activity (BPGP: 2,3-bisphosphoglycerate → 3-phosphoglycerate + Pi), which is stimulated by 2-phosphoglycolate (for reviews, see Fothergill-Gilmore and Watson, 1989; Wold, 1971).

In mammalian tissues, there are three isoenzymes of PGM, which result from the homodimeric and the heterodimeric combinations of two different subunits coded by separate genes and designated M (muscle) and B (brain). In early fetal life, type BB-PGM is the only form present. During myogenesis, the isoenzyme phenotype undergoes transition, type BB-PGM being replaced by the MM-form, through the MB isoenzyme. In skeletal muscle, there is an almost complete transition from the BB- to the MM-PGM, but in heart muscle complete transition does not occur (Omenn and Cheung, 1974; Omenn and Hermodson, 1975; Adamson, 1976; Edwards and Hopkinson, 1977; Mezquita and Carreras, 1981; Mezquita et al, 1981). During sperm cell differentiation, a switch from type BB-PGM to type MM-PGM also occurs (Fundele et al, 1987). Therefore, in adult mammals, mature sperm cells and skeletal muscle contain almost exclusively type MM-PGM, whereas type BB-PGM is found in most other tissues. Only in heart are the three PGM isoenzymes present in substantial amounts (Omenn and Cheung, 1974; Omenn and Hermodson, 1975; Rosa et al, 1975; Adamson, 1976; Edwards and Hopkinson, 1977; Mezquita and Carreras, 1981; Carreras et al, 1981; Mezquita et al, 1981; Bartrons and Carreras, 1982; Prehu et al, 1984; Pons et al, 1985*a*,*b*; Fundele et al, 1987).

In addition to PGM isoenzymes, in mammalian tissues there are three other enzyme proteins that have PGM-, BPGM- and 2-phosphoglycolate-stimulated BPGP activities, with a PGM–BPGP and a PGM–BPGM activity ratios lower than those of PGM isoenzymes. One of these enzymes is the 2,3-bisphosphoglycerate synthase-phosphatase or 2,3-bisphosphoglycerate mutase (EC 5.4.2.4), which is a homodimer of a subunit that possesses great homology with PGM subunits (Sasaki et al, 1975; Kappel and Hass, 1976; Sasaki et al, 1976; Narita et al, 1979). The other two enzyme proteins are heterodimers resulting from the combination of a BPGM subunit with a PGM subunit either type M or type B (Rosa et al, 1984; Pons et al, 1985*a*). The BPGM homodimer is particularly abundant in erythrocytes. The BPGM-type M PGM hybrid is present in the tissues that express type M PGM subunit (skeletal muscle and heart), and the BPGM-type B PGM hybrid is present in the tissues that express type B PGM subunit. Moreover, in mammalian tissues, there are two other enzyme forms that possess only BPGP activity not stimulated by 2-phosphoglycolate (2-phosphoglycolate non-stimulated BPGP) and seem to be monomeric (Carreras et al, 1981; Pons et al, 1985*b*).

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Table 1 Levels of PGM and BPGP activities in human lung, colon and liver normal tissues and tumours

Tissue	Tumour	Case no.	Normal tissue					Tumour				
			PGM		BPGP		PGM/BPGP	PGM		BPGP		PGM/BPGP
			U g^{-1}	U mg^{-1}	mU g^{-1}	mU mg^{-1}		U g^{-1}	U mg^{-1}	mU g^{-1}	mU mg^{-1}	
Lung	Adenocarcinoma	1	1.5	0.05	600	18.5	3	4.2	0.14	340	11.0	12
		2	1.8	0.06	170	5.9	11	5.4	0.19	125	3.5	43
		3	2.1	0.08	495	19.4	4	4.0	0.14	220	7.5	18
		4	2.7	0.08	185	5.9	15	9.1	0.29	100	3.2	91
		5	3.0	0.07	150	3.5	20	7.5	0.2	80	2.5	93
		6	3.8	0.1	145	4.7	26	5.1	0.16	70	1.7	72
		7	5.7	0.2	190	8.1	30	10.2	0.4	125	4.9	81
		Mean ± s.e.m	2.9 ± 0.5	0.09 ± 0.01	276 ± 71	9.4 ± 2.5	15.6 ± 3.9	6.5 ± 0.9	0.2 ± 0.03	151 ± 36	4.9 ± 1.2	59 ± 12
	Squamous cell carcinoma	1	2.4	0.06	586	21.2	4	4.5	0.16	495	14.2	9
		2	2.4	0.06	596	16.5	4	4.8	0.2	340	13.9	14
		3	1.5	0.06	295	12.2	5	4.8	0.16	167	5.7	28
		4	4.5	0.2	115	5.1	39	11.1	0.5	100	4.9	111
		Mean ± s.e.m.	2.7 ± 0.6	0.09 ± 0.03	398 ± 117	13 ± 3.4	13.6 ± 8.6	6.3 ± 1.6	0.2 ± 0.08	275 ± 88	9.6 ± 2.5	41 ± 23
	Carcinoid	1	7.5	0.26	298	10.5	25	13.8	0.46	210	7.0	65
		2	6.6	0.13	186	3.8	35	20.4	0.4	147	3.1	138
		Mean ± s.e.m.	7.0 ± 0.45	0.1 ± 0.06	112 ± 56	7.1 ± 3.3	30 ± 5	17.1 ± 3.3	0.4 ± 0.03	178 ± 31	5.0 ± 1.9	101 ± 36
Colon	Adenocarcinoma	1	8.8	0.22	1521	38.1	5	20.1	0.64	225	7.2	89
		2	8.7	0.4	676	36.3	13	18.3	0.49	596	5.8	31
		3	7.2	0.25	465	16.3	15	14.5	0.35	235	5.7	62
		4	6.6	0.25	1190	44.6	5	10.8	0.33	340	10.4	32
		5	6.3	0.21	676	23.6	9	15.9	0.6	615	22.2	26
		6	7.5	0.2	676	18.7	11	12.6	0.26	440	9.1	28
		7	7.8	0.3	1593	54.1	5	24.6	1.1	597	25.8	41
		8	7.2	0.4	549	31.0	12	11.4	0.6	348	17.6	33
		9	7.8	0.37	771	36.7	10	14.4	0.5	996	36.1	14
		10	8.4	0.4	1047	49.8	8	12.0	0.66	897	49.0	13
		Mean ± s.e.m.	7.6 ± 0.2	0.29 ± 0.02	916 ± 126	35.0 ± 4.0	9 ± 1	15.4 ± 1.3	0.55 ± 0.07	528 ± 83	18.8 ± 4.6	37 ± 7
Liver	Adenocarcinoma	1	6.9	0.46	215	10.0	32	8.1	0.36	170	7.6	47
		2	13.5	0.29	335	7.1	40	18.0	0.47	111	5.2	162
		3	15.6	0.27	200	6.0	78	20.7	0.6	175	5.5	118
		4	12.0	0.31	225	5.8	53	22.2	0.6	140	3.8	158
		5	15.3	0.4	200	5.3	77	19.3	0.8	110	3.0	175
		6	12.0	0.33	155	4.2	76	15.9	0.6	90	3.4	176
		Mean ± s.e.m.	12.5 ± 1.2	0.34 ± 0.02	221 ± 24	6.4 ± 0.8	59 ± 8	17.3 ± 2.0	0.57 ± 0.06	132 ± 14	4.7 ± 0.6	139 ± 20

The activity is expressed as units per g wet tissue and as units per mg extracted protein. The comparisons for the U mg^{-1} of protein data are as follows: PGM activity: normal lung vs normal liver; normal lung vs normal colon; normal liver vs normal colon, $P < 0.001$. Lung adenocarcinoma vs normal tissue, $P < 0.01$; lung squamous cell carcinoma vs normal tissue, $P < 0.04$; colon adenocarcinoma vs normal tissue, $P < 0.002$; hepatocarcinoma vs normal tissue, $P < 0.009$. BPGP activity: normal colon vs normal colon vs normal liver, $P < 0.001$. Lung adenocarcinoma vs normal tissue, $P < 0.01$; colon adenocarcinoma vs normal tissue, $P < 0.002$; hepatocarcinoma vs normal tissue, $P < 0.003$. All other comparisons are not significant.

Enolase molecules are dimers composed of three distinct subunits coded by separate genes and designated α (liver), β (muscle) and γ (brain) (for a review, see Day, 1982). The αα isoenzyme exists in most mammalian tissues. ββ and αβ enolase are found predominantly in skeletal and heart muscle. γγ and αγ enolases are present mainly in nervous tissue and in tissues with neuroendocrine cells. They have frequently been designated as neuron-specific enolase. The βγ hybrid has not been found, probably because the β and the γ enolase subunits are not expressed in the same tissue (for reviews, see Kato et al, 1983*a*; Taylor et al, 1983; Haimoto et al, 1985; Royds et al, 1985; Schmechel, 1985; Marangos and Schmechel, 1987).

The present study was undertaken to determine the distribution of total PGM, BPGP and enolase activities and isoenzymes in lung, colon and liver carcinoma as a first step to studying the expression of these isoenzymes in neoplastic cells. Only one report has been published about PGM isoenzymes in brain tumours (Omenn and Cheung, 1974; Omenn and Hermodson, 1975) and, to our knowledge, no data exist on other tumours. Numerous studies have been published on the distribution of enolase isoenzymes in human tumours (for reviews, see Taylor et al, 1983; Royds et al, 1985; Schmechel, 1985; Gerbitz et al, 1986; Marangos and Schmechel, 1987; Kaiser et al, 1989). However, most data have been obtained by immunohistochemical and immunoassay techniques. To our knowledge, only two reports have been published on the distribution of enolase isoenzyme proteins in lung tumours (Pahlman et al, 1986; Batandier et al, 1987), and no data exist on the distribution of enolase isoenzymes in colon and liver tumours.

MATERIALS AND METHODS

Materials

Enzymes, substrates, cofactors and biochemicals were purchased from either Boehringer (Mannheim, Germany) or Sigma (St Louis, MO, USA). β-Mercaptoethanol was from Merck (Darmstadt,

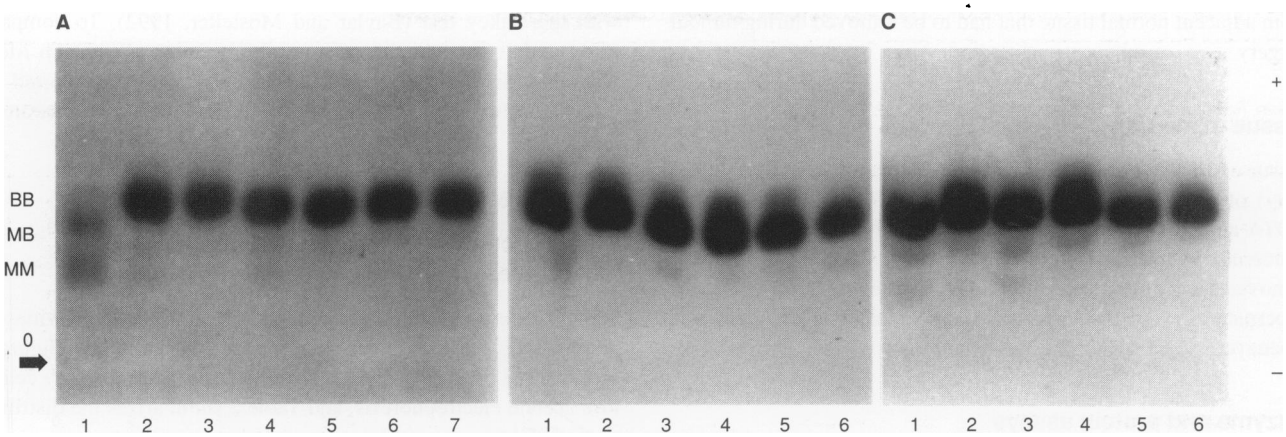

Figure 1: Electrophoretograms of PGM isoenzymes in extracts of human normal tissues and tumours. (**A**) Lung: lane 1, heart extract; lanes 3 and 5, squamous cell carcinoma; lane 7, adenocarcinoma; lanes 2, 4 and 6, normal tissues. (**B**) Colon: lanes 2, 4 and 6, adenocarcinoma; lanes 1, 3 and 5, normal tissues. (**C**) Liver: lanes 2, 4 and 6, adenocarcinoma; lanes 1, 3 and 5, normal tissues

Table 2 PGM isoenzymes in lung, colon and liver normal tissues and tumours

			Normal tissue			Tumour		
Tissue	**Tumour**	**Case no.**	**MM**	**MB**	**BB**	**MM**	**MB**	**BB**
Lung	Adenocarcinoma	1	0	0	100	0	0	100
		2	0	8	92	0	8	92
		3	0	0	100	0	0	100
		4	0	0	100	0	0	100
		5	0	3	97	0	0	100
		6	0	3	97	0	8	92
		7	0	0	100	0	0	100
	Squamous cell carcinoma	1	0	4	96	0	1	99
		2	0	4	96	0	0	100
		3	0	4	96	0	2	98
		4	0	0	100	0	0	100
	Carcinoid	1	0	0	100	0	9	91
		2	0	0	100	0	0	100
Colon	Adenocarcinoma	1	0	0	100	0	0	100
		2	0	2	98	0	0	100
		3	0	0	100	1	1	99
		4	0	1	99	0	0	100
		5	3	6	91	0	2	98
		6	3	7	90	0	3	97
		7	0	6	94	0	0	100
		8	0	0	100	0	0	100
		9	0	4	96	0	3	97
		10	0	6	94	0	3	97
Liver	Adenocarcinoma	1	0	1	99	0	0	100
		2		ND			ND	
		3	0	2	98	0	1	99
		4	0	3	97	0	2	98
		5	0	4	96	0	2	98
		6	0	3	97	0	0	100

The results are expressed as a percentage of the total activity on electrophoresis. ND, not determined.

Germany) and bovine serum albumin was from Calbiochem (La Jolla, CA, USA). Other chemicals were reagent grade. Agar noble was obtained from Difco Laboratories (Detroit, MI, USA). Cellulose acetate strips were from Helena Laboratories (Beaumont, TX, USA) and agarose gels were from Ciba-Corning (Palo Alto, CA, USA).

Tissue samples

Tumour samples were obtained from surgical resection specimens: seven lung adenocarcinomas, five lung squamous cell carcinomas, two carcinoid tumours of the lung, ten colon adenocarcinomas and six hepatocarcinomas. Samples of normal tissue were obtained

from adjacent normal tissue that had to be removed during tumour surgery.

Tissue extraction

Tissue extracts were prepared by homogenization in three volumes (w/v) of cold 20 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 1 mM β-mercaptoethanol with a Polytron homogenizer (Lucerne, Switzerland) (position 5, 20 s). Cellular debris was removed by centrifugation at 4°C for 30 min at 12 500 ***g***, and the supernatants were used for the assay of enzyme activities and isoenzymes.

Enzyme and protein assays

PGM activity was measured spectrophotometrically at 30°C by coupling the formation of 2-phosphoglycerate from 3-phosphoglycerate with the enolase, pyruvate kinase and lactate dehydrogenase catalysed reactions (Beutler and Stratton, 1975), as previously described (Durany and Carreras, 1996). BPGP activity was assayed by measuring the appearance of inorganic phosphate from 2,3-bisphosphoglycerate (Joyce and Grisolia, 1958). The reaction mixture was contained in a total volume of 1 ml: 50 mM triethanolamine, pH 7.6, 1 mM 2,3-bisphosphoglycerate, 2.5 mM 2-phosphoglycolate (as activator) and the sample tested. After incubation at 37°C, the reaction was stopped by the addition of 0.25 ml of 25% trichloroacetic acid. After centrifugation, the inorganic phosphate was estimated in the supernatant by the method of Itaya and Ui (1966). Enolase activity was measured spectrophotometrically at 30°C by coupling the formation of phosphoenolpyruvate with the pyruvate kinase and lactate dehydrogenase catalysed reactions (Bergmeyer, 1983), as previously described (Joseph et al, 1996). Enzyme activities were expressed as U g^{-1} wet tissue and as U mg^{-1} protein (1 Unit = 1 μmol substrate converted per min). Protein was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

Isoenzyme analysis

The methods described previously were used to evaluate PGM isoenzymes by cellulose acetate electrophoresis (Durany and Carreras, 1996) and enolase isoenzymes by agarose gel electrophoresis (Joseph et al, 1996).

Separation of BPGP from PGM and BPGM

PGM isoenzymes, BPGM and the BPGM-PGM hybrids were separated from 2-phosphoglycolate non-stimulated BPGP by highly resolutive gel filtration fast liquid chromatography (FPLC system and Superdex 75 HR column from Pharmacia LKB Biotechnology, Sweden). The column was equilibrated with extraction buffer. A sample (0.2 ml) of the tissue extract was injected, the column was eluted at a flow rate of 0.1 ml min^{-1} and 500-μl fractions were collected.

Statistical analysis

A one-way analysis of variance with repeated measures was employed for statistical evaluation and used to compare enzyme activities among different tissues. When a significant *P*-value was obtained ($P < 0.05$), the difference between means was located with the Tukey test (Baylar and Mosteller, 1992). To compare PGM activity in tumour and control tissues, the Wilcoxon *t*-test was used (Baylar and Mosteller, 1992). Values are reported as means ± s.e.m. Data were analysed by InStat statistical software.

RESULTS

Distribution of PGM and BPGP activities and isoenzymes

Table 1 summarizes the levels of total PGM and BPGP activities in normal lung, colon and liver tissues, and in their tumours. Figure 1 shows some of the PGM isoenzyme patterns determined by cellulose acetate electrophoresis, and Table 2 summarizes the distribution of PGM isoenzymes in normal and tumour tissues.

As shown, lung tissue presents a lower PGM content than liver and colon tissues ($P < 0.001$), which have similar PGM concentration. Normal lung, liver and colon tissues contain almost exclusively BB-PGM with traces of MB-PGM isoenzyme. Colon is the tissue with the highest BPGP activity ($P < 0.001$), and no significative difference is observed between the levels of this activity in lung and liver. The PGM–BPGP activity ratio in colon and in lung is not significantly different. In contrast, liver possesses a higher PGM–BPGP activity ratio. These results indicate that lung, colon and liver tissues differ in their PGM content and in the concentration of some of the other enzymes that also possess BPGP activity (BPGM, BPGM–PGM hybrid and 2-phosphoglycolate non-stimulated BPGP).

It has been shown that 2-phosphoglycolate non-stimulated BPGP has a lower molecular weight than PGM, BPGM and BPGM–PGM hybrids, and that they can be separated by gel filtration chromatography (Carreras et al, 1981; Pons et al, 1985*b*). Therefore, we compared the PGM–BPGP activity ratio in liver and lung extracts before and after gel filtration FPLC. It was found that neither the enzyme activities nor the PGM–BPGP activity ratio changed significantly after chromatography. There was correlation between the levels of the enzyme activities in the crude extracts and in the peaks containing the PGM, the BPGM and the BPGM–PGM hybrid isolated by gel filtration chromatography. The peak from liver extract was the peak with the highest PGM activity. It was concluded that the contribution of the 2-phosphoglycolate non-stimulated BPGP enzyme to the total BPGP activity of the tissues is almost negligible, and that they differ in the concentration of PGM, BPGM and BPGM–PGM hybrid.

As summarized in Table 1, all tumours present higher PGM activity levels than the corresponding normal tissues. However, no changes are observed in the PGM isoenzyme patterns. As the normal tissues, tumours contain mainly type BB-PGM, with only traces of the MB-PGM form (Figure 1 and Table 2). In contrast to PGM activity, the levels of BPGP activity in all tumours are lower than in normal tissues. Correspondingly, the PGM–BPGP activity ratios in tumours are higher than in control tissues.

The fact that tumours present opposite changes in the levels of PGM activity and of BPGP activity suggests that tumoral tissues change both the concentration of PGM and the concentration of the other enzymes that also have BPGP activity. Extracts of liver carcinoma and of lung carcinoid were filtered through a FPLC column in order to separate the 2-phosphoglycolate non-stimulated BPGP. The total enzyme activities and the PGM–BPGP activity ratio of the extracts were similar before and after chromatography. This shows that, as in normal tissues, the contribution of 2-phosphogly-

Table 3 Levels of enolase activity in human lung, colon, and liver normal tissues and tumours

			Normal tissue		Tumour	
Tissue	**Tumour**	**Case no.**	**U g^{-1}**	**U mg^{-1}**	**U g^{-1}**	**U mg^{-1}**
Lung	Adenocarcinoma	1	6	0.1	17	0.3
		2	5	0.07	21	0.3
		3	3	0.1	20	0.5
		4	2	0.08	26	0.86
		5	5	0.12	17	0.47
		6	7	0.15	19	0.48
		7	4	0.12	11	0.28
		Mean ± s.e.m.	4.5 ± 0.6	0.10 ± 0.01	18.7 ± 1.7	0.45 ± 0.07
	Squamous cell Carcinoma	1	15	0.2	23	0.2
		2	9	0.1	24	0.5
		3	3	0.06	16	0.3
		4	8	0.12	33	0.61
		5	4	0.17	7	0.36
		Mean ± s.e.m.	7.9 ± 2.1	0.13 ± 0.02	20.6 ± 4.3	0.39 ± 0.07
	Carcinoid	1	3	0.1	5	0.23
		2	4	0.07	11	0.19
		Mean ± s.e.m.	3.5 ± 0.5	0.085 ± 0.01	8.0 ± 3.0	0.21 ± 0.02
Colon	Adenocarcinoma	1	6.1	0.1	19.6	0.4
		2	7.0	0.1	8.3	0.1
		3	5.4	0.1	14.5	0.2
		4	5.2	0.09	14.3	0.2
		5	5.1	0.2	17.8	0.4
		6	7.6	0.2	8.7	0.1
		7	6.9	0.1	12.9	0.3
		8	4.5	0.2	11.2	0.3
		9	7.4	0.1	18.2	0.4
		10	8.5	0.1	11.6	0.2
		Mean ± s.e.m.	6.4 ± 0.4	0.13 ± 0.015	13.7 ± 1.2	0.26 ± 0.04
Liver	Hepatocarcinoma	1	9	0.8	8	0.5
		2	14	0.2	22	0.4
		3	15	0.2	21	0.6
		4	11	0.2	12	0.3
		5	17	0.4	20	0.8
		6	18	0.3	20	0.7
		Mean ± s.e.m.	14 ± 1.4	0.35 ± 0.1	17.2 ± 2.3	0.56 ± 0.07

The activity is expressed as units per g wet tissue and as units per mg extracted protein. The comparisons for the U mg^{-1} of protein data are as follows: normal lung vs normal liver, $P<0.001$; normal colon vs normal liver, $P<0.01$; lung adenocarcinoma vs normal tissue, $P < 0.05$, colon adenocarcinoma vs normal tissue, $P < 0.05$. All other comparisons are not significant.

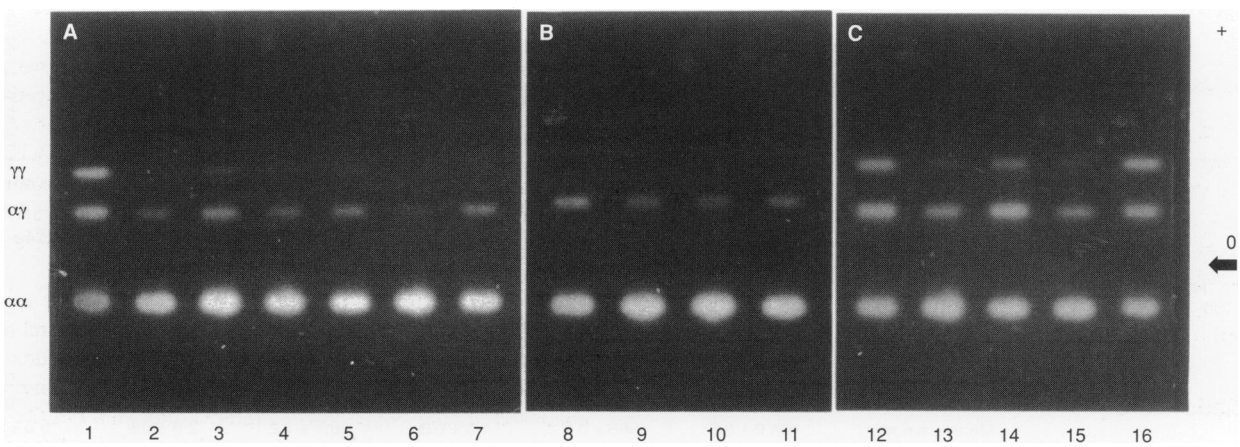

Figure 2 Electrophoretograms of enolase isoenzymes in extracts of human lung normal tissue and tumours. (**A**) Adenocarcinoma: lanes 2, 4 and 6, tumours; lanes 3, 5 and 7, normal tissues. (**B**) Squamous cell carcinoma: lanes 8 and 10, tumours; lanes 9 and 11, normal tissues. (**C**) Carcinoid: lanes 12 and 14, tumours; lanes 13 and 15, normal tissues. Lanes 1 and 16, human brain extracts (cortex)

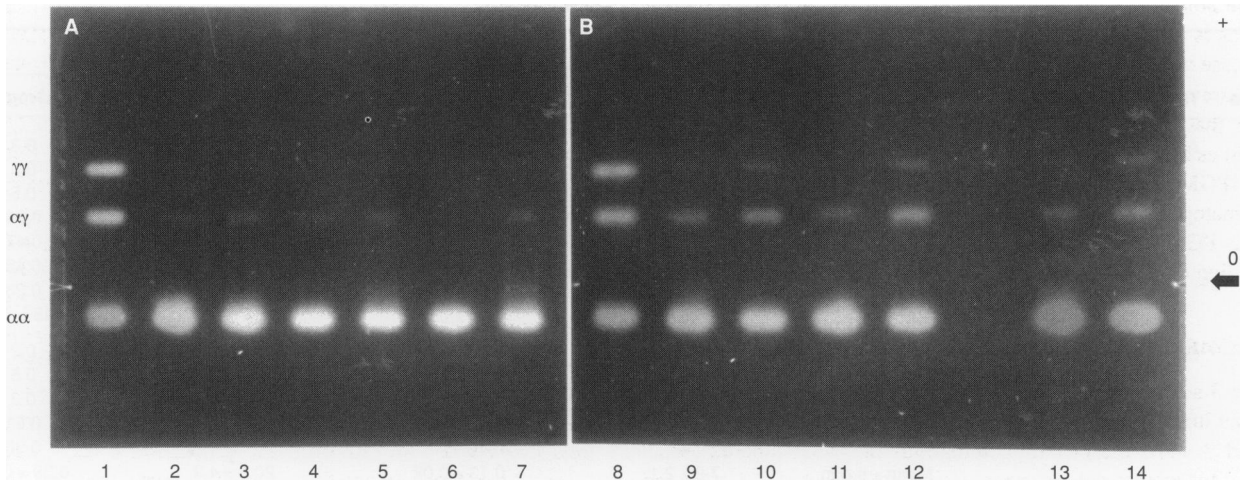

Figure 3: Electrophoretograms of enolase isoenzymes in extracts of human liver and colon normal tissues and tumours. (**A**) Liver: lanes 2, 4 and 6, hepatocarcinomas; lanes 3, 5 and 7, normal tissues. (**B**) Colon: lanes 9, 11 and 13, adenocarcinomas; lanes 10, 12 and 14, normal tissues. Lanes 1 and 8 human brain extracts (cortex)

Table 4 Enolase isoenzymes in human lung, colon and liver normal tissues and tumours

			Normal tissue			Tumour		
Tissue	**Tumour**	**Case No.**	αα[a]	αγ[b]	γγ[c]	αα	αγ	γγ
Lung	Adenocarcinoma	1	77	20	3	94	4	2
		2	62	31	7	85	13	2
		3	73	23	4	76	22	2
		4	73	23	4	85	13	2
		5	77	19	4	84	14	2
		6	76	21	3	96	4	0
		7	76	22	2	78	20	2
		Mean ± s.e.m.	73.4 ± 2.0	22.7 ± 1.5	3.8 ± 0.6	85.4 ± 2.8	12.8 ± 2.6	1.7 ± 0.3
	Squamous cell carcinoma	1	74	22	4	84	15	1
		2	90	10	0	92	8	0
		3	81	17	2	85	13	2
		4	84	14	2	70	28	2
		5	78	20	2	88	12	0
		Mean ± s.e.m.	81.4 ± 2.7	16.6 ± 2.1	2.0 ± 0.6	83.8 ± 3.7	15.2 ± 3.4	1.0 ± 0.4
	Carcinoid	1	70	26	4	44	32	24
		2	70	25	5	50	37	13
		Mean ± s.e.m.	70 ± 0.0	25.5 ± 0.5	4.5 ± 0.5	47 ± 3	34.5 ± 2.5	18.5 ± 5.5
Colon	Adenocarcinoma	1	77	19	4	86	12	2
		2	70	24	6	87	10	3
		3	70	21	9	89	9	2
		4	74	20	6	84	14	2
		5	75	19	6	90	8	2
		6	79	15	6	94	4	2
		7	69	25	6	79	19	2
		8	89	8	3	82	15	3
		9	84	12	4	86	11	3
		10	77	18	5	81	16	3
		Mean ± s.e.m.	76.4 ± 2.0	18.1 ± 1.6	5.5 ± 0.5	85.8 ± 1.4	11.8 ± 1.4	2.4 ± 0.2
Liver	Hepatocarcinoma	1	96	3	1	93	6	1
		2	93	6	1	94	4	2
		3	92	6	2	100	0	0
		4	95	5	0	96	3	1
		5	95	5	0	97	3	0
		6	94	4	2	100	0	0
		Mean ± s.e.m.	94.2 ± 0.6	4.8 ± 0.5	1.0 ± 0.3	96.7 ± 1.2	2.6 ± 0.9	0.6 ± 0.3

The results are expressed as a percentage of the total enolase activity on electrophoresis. The comparisons are as follows: [a]Lung vs liver, $P < 0.001$; colon vs liver, $P < 0.001$. [b]Lung vs liver, $P < 0.001$; colon vs liver, $P<0.001$. [c]Lung vs colon, $P < 0.01$; lung vs liver, $P < 0.05$; colon vs liver, $P < 0.001$. All other comparisons are not significant.

colate non-stimulated BPGP to the total BPGP activity in tumour tissues is not relevant. In addition, these results indicate that the decrease of the total BPGP activity found in tumours is caused by a decrease of the BPGM and of the BPGM–PGM hybrid. It has to be noted that there was a correlation between the assay of the enzyme activities in the crude extracts and in the peaks containing the PGM, the BPGM and the BPGM–PGM hybrid isolated by gel filtration chromatography. Those peaks corresponding to tumour tissues had higher PGM activity and lower BPGP activity than the peaks corresponding to normal tissues.

Distribution of enolase activity and isoenzymes

Table 3 summarizes the levels of total enolase activity in normal human lung, colon and liver tissues, and in their tumours. Figures 2 and 3 show the electrophoretograms of some tumours determined by agarose gel electrophoresis, and Table 4 summarizes the data obtained.

As shown, liver tissue possesses a higher enolase content than lung ($P < 0.001$) and colon ($P < 0.01$) tissues, which have similar enolase concentration. All tumours have higher enolase activity than the corresponding normal tissues, although only in lung and colon adenocarcinoma are the differences observed statistically significant.

The normal tissues possess all the three enolase isoenzymes, although the proportion of αα-enolase is much larger than the proportions of αγ-and γγ-enolase. Normal lung and colon tissues present enolase isoenzymes in similar proportions. Liver tissue has lower proportions of enolase isoenzymes containing the γ subunit (αγ- and γγ-enolase). Five of seven lung adenocarcinomas and four of five squamous cell carcinomas of the lung show a lower content of αγ- and γγ-enolase than the corresponding normal tissue. In contrast, one squamous cell carcinoma and two carcinoid tumours of the lung present higher proportions of the enolase isoenzymes containing the γ subunit than the normal tissues. Colon and liver carcinomas have lower proportions of αγ- and γγ-enolase than normal tissues, although in hepatocarcinomas the decrease in the proportion of the enolase isoenzymes containing the γ subunit is less evident than in colon adenocarcinomas.

DISCUSSION

Our results show that, in normal human lung, colon and liver tissue, the PGM and the enolase activities are much higher than the BPGP activity, which is in agreement with the different functions of these enzymes. PGM and enolase are enzymes of the main glycolytic pathway, whereas the BPGP activity participates in the 2,3-bisphosphoglycerate bypass (or Rapoport–Luebering shunt), a collateral deviation of glycolysis (Rapoport, 1968).

The data presented on the distribution of PGM isoenzymes in normal human lung, colon and liver agree with preliminary results (Durany and Carreras, 1996) and confirm that in all these tissues type BB-PGM is the predominant PGM isoenzyme. They contain traces of MB-PGM and no detectable MM-PGM. Our results also show that in these tissues most of the total BPGP activity is caused by PGM, BPGM and BPGM-PGM hybrid. The contribution of 2-phosphoglycolate non-stimulated BPGP is very low. Similar results were obtained in other tissues of pig and cat (Carreras et al, 1981; Pons et al, 1985*b*).

Our data on the distribution of enolase isoenzymes in tissues are in agreement with the results of others. It has been shown by immunoassay that the three enolase subunits are present in normal human lung, colon and liver, although the proportion of α subunit is much larger than the proportions of β and γ subunits (Schmechel et al, 1978; Marangos et al, 1979, 1980; Kato et al, 1983*a*, *b*; Taylor et al, 1983; Fujita et al, 1987; Marangos and Schmechel, 1987). Hullin et al (1980) found that γγ-enolase determined by ion-exchange chromatography represented 0.7%, 1.3% and 0.04% of the total enolase activity in extracts of lung, large intestine and liver respectively. Batandier et al (1987) showed by electrophoretic analysis that αα-enolase was the predominant isoenzyme in human lung. αγ-Enolase represented a low proportion (5.75 ± 0.25%) of the total enolase activity and γγ-enolase was not detectable. The results presented here confirm that the γ-enolase subunit is expressed in normal lung, colon and liver, and show that, in lung and in colon tissues, αγ-enolase represents an appreciable proportion (21 ± 5.1% and 18.1 ± 1.6% respectively) of the total enolase activity. In liver, the proportion of αγ-enolase is lower (4.8 ± 0.5%) than in the two other tissues.

In all lung, colon and liver tumours, we found an increase in PGM and enolase activities and a decrease in 2-phosphoglycolate-stimulated BPGP activity. In contrast, in brain tumours a decrease of the enolase and PGM activities was observed (Joseph et al, 1996; N Durany and J Carreras, unpublished results).

In the tumours studied, we have not detected changes in the distribution of PGM isoenzymes, but we have observed some alterations in the enolase isoenzyme patterns. In colon and liver carcinomas, and in most adenocarcinomas and squamous cell carcinomas of the lung, we found a decrease in enolase isoenzymes containing type γ subunit (αγ- and γγ-enolase). In contrast, in two carcinoid tumours and in one squamous cell carcinoma of the lung, we observed an increase in these enolase isoenzymes. In addition, we have deduced that the decrease of the 2-phosphoglycolate-stimulated BPGP activity observed in all tumours reflects a decrease of the BPGM and the BPGM–PGM hybrid enzyme levels.

Our results on enolase isoenzymes in tumours can be compared with data from other authors but, to our knowledge, no previous data exist on PGM isoenzymes in lung, colon and liver tumours. By enzyme immunoassay, it has been shown that the α-enolase subunit is abundant and that the β-enolase subunit is present at low levels in most lung tumours (Nakajima et al, 1985; Fujita et al, 1987). The presence of the γ-enolase subunit in lung neuroendocrine tumours has been well documented by immunohistochemistry, but its presence in non-neuroendocrine tumours is controversial (for reviews, see Marangos and Schmechel, 1987; Kaiser et al, 1989). Pahlman et al (1986) found using ion-exchange chromatography that non-small-cell carcinoma cell lines had measurable quantities of neuron-specific enolase, although its levels were 10- to 100-fold lower than those found in small-cell carcinoma cell lines. Batandier et al (1987) found by electrophoresis αγ-enolase in significantly higher proportion in lung neuroendocrine carcinomas than in non-neuroendocrine tumours. γγ-Enolase was present at high levels in lung neuroendocrine carcinomas and consistently absent in non-neuroendocrine tumours. In disagreement with these authors, we have detected γγ-enolase in non-neuroendocrine lung tumours, although at lower levels than in normal lung tissues and lung carcinoids. The discrepancy is probably owing to a higher sensitivity of the method of detection used by us.

By immunoassay, the γ-enolase subunit was detected in gastric and gut carcinoids (Tapia et al, 1981; Simpson et al, 1984; Nash et al, 1986), and in a cell line from a neuroendocrine tumour of colon (Reeve et al, 1986). In contrast, no γ-enolase staining was found in

colon adenocarcinomas (Simpson et al, 1984; Vinores et al, 1984; Fukuda et al, 1989), and low levels of reactivity were detected in a cell line from a colon carcinoma (Reeve et al, 1986). As indicated above, we have detected αγ- and γγ-enolase in colon adenocarcinomas, although at a lower proportion than in normal tissue.

By immunohistochemistry, γ-enolase was found to be negative in hepatocellular carcinoma (Vinores et al, 1984; Fujita et al, 1987), although it was present in one of two cases of fibrolamellar carcinoma with neurosecretory granules (Fujita et al, 1987). Our results indicate that, although at a very low proportion, αγ- and γγ-enolase isoenzymes can be detected in liver carcinomas.

In conclusion, we have found that in hepatocarcinoma, colon and lung adenocarcinoma, lung squamous cell carcinoma and carcinoid tumour of the lung, the expression of PGM subunits does not suffer any qualitative change. In tumour tissues, the total PGM activity increases but the relative expression of type B and type M PGM subunits does not vary. As in the normal tissues, type B subunit is predominant. The total enolase activity also increases in all tumours studied by us but, in contrast to PGM, the distribution of enolase isoenzymes in tumour tissues presents some alterations. In two carcinoid tumours and in one squamous cell carcinoma of the lung, the proportion of enolase isoenzymes containing type γ subunit was found to be increased. In all other tumours, it was found to be decreased. The changes in the proportions of enolase isoenzymes in tumours could reflect a change in the proportions of the different cell types present in the tissues. But a change in the expression of α- and γ-enolase subunits cannot be excluded, since it has been shown that cells undergoing mitosis activate the expression of the α-enolase subunit. It has been reported that α-enolase is expressed at relatively high levels in all actively proliferating human cell lines but at very low levels in normal resting peripheral blood lymphocytes, in which its synthesis is induced upon mitogenic stimulation (Giallongo et al, 1986*a*, *b*). Moreover, it has been shown that expression of the genes encoding four glycolytic enzymes, including enolase, is specifically stimulated in quiescent rat fibroblast by either epidermal growth factor or serum (Matrisian et al, 1985).

From the diagnostic point of view, we conclude that PGM and enolase isoenzymes are not good markers for the tumours studied by us. Only in carcinoid tumours of the lung can the increase in neuron-specific enolase have clinical applications, as already indicated by others (Royds et al, 1985; Schemechel, 1985; Gerbitz et al, 1986; Marangos and Schmechel, 1987; Kaiser et al, 1989).

ABBREVIATIONS

BPGM, 2,3-bisphosphoglycerate mutase; BPGP, 2,3-bisphosphoglycerate phosphatase; PGM, phosphoglycerate mutase.

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