

Comparison of Metabolic Pathways between Cancer Cells and Stromal Cells in Colorectal Carcinomas: a Metabolic Survival Role for Tumor-Associated Stroma

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Abstract

Understanding tumor metabolism is important for the development of anticancer therapies. Immunohistochemical evaluation of colorectal adenocarcinomas showed that cancer cells share common enzyme/transporter activities suggestive of an anaerobic metabolism [high lactate dehydrogenase 5 (LDH5)/hypoxia-inducible factor α s (HIF α s)] with high ability for glucose absorption and lactate extrusion [high glucose transporter 1 (GLUT1)/monocarboxylate transporter (MCT1)]. The tumor-associated fibroblasts expressed proteins involved in lactate absorption (high MCT1/MCT2), lactate oxidation (high LDH1 and low HIF α s/LDH5), and reduced glucose absorption (low GLUT1). The expression profile of the tumor-associated endothelium indicated aerobic metabolism (high LDH1 and low HIF α s/LDH5), high glucose absorption (high GLUT1), and resistance to lactate intake (lack of MCT1). It is suggested that the newly formed stroma and vasculature express complementary metabolic pathways, buffering and recycling products of anaerobic metabolism to sustain cancer cell survival. Tumors survive and grow because they are capable of organizing the regional fibroblasts and endothelial cells into a harmoniously collaborating metabolic domain. (Cancer Res 2006; 66(2): 632-7)

Introduction

Glycolysis, the transformation of glucose to pyruvate, is a key step for the acquisition of ATP in all mammalian cells, including cancer tissues. Glucose transporters are commonly overexpressed in human malignancies enhancing glucose influx in the proliferating cancer cells (1). In well-oxygenated normal cells, pyruvate enters the mitochondria where, by the enzymic activity of pyruvate dehydrogenase, it is transformed to acetyl-CoA, the substrate for ATP production through the Krebs cycle (2). Suboptimal oxygen availability switches on cellular metabolism to anaerobic pathways for ATP production, which occurs through pyruvate transformation to lactic acid via the catalytic activity of lactate dehydrogenase 5 (LDH5 isoenzyme; ref. 3). Certainly, the hypoxic tumor environment favors the intensification of anaerobic metabolic pathways in cancer cells. Nevertheless, for reasons rather unclear, cancer cells have an inherent tendency to turn to anaerobic glycolysis even in the presence of high oxygen tension, a phenomenon first described by Otto Warburg (Nobel Prize 1931; ref. 4). Thompson et al. showed

in a recent study that activation of a single oncogene, Akt, is sufficient to stimulate aerobic glycolysis in tumors (5), whereas Akt is known to activate, under hypoxic conditions, hypoxia-inducible factor-1 α (HIF-1 α) as well (6), and this in turn up-regulates enzymes involved in anaerobic glycolysis and glucose intrusion (7). Akt also accelerates lipid formation, and this is an important pathway for membrane synthesis essential for cell growth (8).

In a previous study, we noted that pyruvate dehydrogenase (PDH), the enzyme responsible for catalysis of the above reaction, is repressed in lung cancer cells, apparently switching to anaerobic glycolysis (9). In contrast, tumor-associated fibroblasts maintained a rather strong PDH expression whereas reduced PDH-kinase1 (PDK1) activity, indicating an intense aerobic metabolism. On the other hand, HIF-1 α and its downstream regulated protein LDH5 are overexpressed in human malignancies but, again, only in the cancerous component. LDH5 is down-regulated in the tumor-associated stroma, which often overexpresses the LDH1 isoenzyme, favoring oxidation of lactic acid back to pyruvate (10, 11). These observations bring forward a new concept where tumor cells and their tumor-associated stroma and vessels should not be merely seen as a unified morphologic domain (12, 13) but also as functional domain with a genuine metabolic cooperation between the anaerobic (cancer cells) and aerobic compartments (newly formed fibroblasts and vessels).

Whether this well-organized structural and functional domain is, indeed, a key feature safeguarding cancer cell survival and growth is a hypothesis that needs further consideration, all the more when metabolic manipulation of tumors may lead to new therapeutic approaches (14). The newly formed tumor-associated stroma, loose and edematous as it is, was thought as being an essential, although complementary, phenomenon of tumor growth facilitating tumor cell invasion in parallel with endothelial cell migration (12, 13). This process of new stroma formation, occurring during the neoplastic growth, was called stromatogenesis (or, rather unorthodoxically, stromagenesis) as distinct to reactive fibrosis that was generally thought to oppose tumor cell infiltration (15). Furthermore, the newly formed stroma may be a major buffer of acidity through the absorption of lactic acid released by cancer cells and recycling it back to pyruvate. The study of monocarboxylate transporters, pumps responsible for the influx and efflux of lactic acid and pyruvate from cells, together with the expression profile of enzymes (PDH, PDK1, LDH1, and LDH5) and pumps (glucose transporter 1, GLUT1) involved in glucose metabolism would provide further evidence on the validity of the above hypothesis (16).

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Materials and Methods

Formalin-fixed, paraffin-embedded tissues from 70 colorectal adenocarcinomas and 20 normal colonic samples were retrieved from the archives of

the Department of Pathology, Democritus University of Thrace, Alexandroupolis, Greece. Histologic diagnosis, grade of differentiation, depth of muscle invasion, and lymph node status were assessed on H&E-stained sections. Serial sections were cut at 3 μ m and mounted on slides for immunohistochemistry. Table 1 reports the primary antibodies used for detecting the expression of the various proteins involved in glucose metabolism and transport through the cellular membranes.

Details of the immunohistochemical techniques employed have been reported in previous studies (9–11, 15). Briefly, sections were deparaffinized and peroxidase was quenched with methanol and 3% H₂O₂ for 15 minutes. Thereafter, slides were placed in antigen unmasking buffer, pH 6.0 (code: TAR001, ILEM, Italy), and microwaving followed (3 \times 4 minutes). The primary antibodies were applied overnight at room temperature. Following washing with TBS, sections were incubated with a secondary antibody (Kwik Biotinylated Secondary, 0.69A Shandon-Upshaw, Pittsburgh, PA) for 15 minutes and washed in TBS. Kwik Streptavidin peroxidase reagent (0.39A Shandon-Upshaw) was applied for 15 minutes, and sections were again washed in TBS. For the MCT22-A, the EnVision Detection kit (DAKO, Glostrup, Denmark) was used to improve staining results. The color was developed by 15-minute incubation with 3,3'-diaminobenzidine solution, and sections were weakly counterstained with hematoxylin.

Normal immunoglobulin G was substituted for the primary antibody as negative controls at the same concentration with the primary antibodies. For positive controls, appropriate tissue sections were used: (a) skeletal muscle, adult brain, and small intestine reported to bear specific affinity for monocarboxylate transporters (MCT); (b) normal lung for PDH, LDH1, and PDK1 (9, 10); and (c) hypoxic rat renal tissue for HIFs and LDH5 obtained after ligation of the renal artery.

The expression patterns of the various antigens under investigation were studied in cancer cells, tumor-associated fibroblasts, and tumor-associated vessels with reference to normal tissues on a conference microscope by two pathologists. Vascular density was assessed using the anti-CD31 monoclonal antibody as previously reported (11). Statistical values were calculated by Fisher's exact two-tailed *t* test, using the GraphPad Prism 4.0 package.

Results

PDH was expressed strongly in the cytoplasm of all normal colonic mucosae: 20 samples (Fig. 1B, a). It was also expressed in tumor-associated fibroblasts (60-100% of the optical fields

examined; Fig. 1B, b) in 63 of 70 (90%) of the cancer cases in the series. Similarly, PDH was detected in a large percentage of tumor-associated vessels (median, 30%; range, 20-50%), after comparison with anti-CD31 staining. In contrast, a suppression of the PDH expression in cancer cells was found in 60% of colorectal adenocarcinomas (Fig. 1B, b); in 42 of 70 (60%) cases, PDH expression was strong in <50% of cancer cells (range, 0-50%; median, 30%), whereas an extensive staining ranging from 60% to 80% of cancer cells was noted in the remaining 30 of 70 (40%) cases. This finding, compared with a 100% expression of normal intestinal tissues or to a 90% expression of tumor-associated fibroblasts, makes the difference highly statistically significant ($P < 0.0001$).

Staining for PDK1 (an enzyme inhibiting PDH activity) was usually seen, although slight, in the normal colon (Fig. 1B, c), but this was absent from the tumor-associated fibroblasts in all 70 cancer cases analyzed (Fig. 1B, d). The expression of PDK1 in tumor-associated vessels was sporadic, as recognized with anti-CD31 immunostaining, and occurred in <10% (range, 0-10%; median, 3%) of vessels. Cancer cells, however, did show a strong cytoplasmic PDK1 activity in 70% of colorectal tumors studied, regardless of the PDH reactivity (Fig. 1B, d). Strong PDK1 expression in 60% to 90% of cancer cells was noted in 49 of 70 (70%) cases, whereas the absence of PDK1 reactivity was rather infrequent, noted in 4 of 70 (6%) cases. Again, this finding was statistically significant compared with the complete (100%) absence of PDK1 expression in tumor-associated fibroblasts ($P < 0.0001$).

LDH1 is an LDH isoenzyme with markedly reduced ability to transform pyruvate to lactate compared with LDH5, favoring oxidation of lactate back to pyruvate. The pattern of staining for these enzymes was both cytoplasmic and nuclear. LDH1 was expressed strongly and consistently in the normal colon (Fig. 1B, e) but only in 35 (50%) of the 70 colorectal carcinomas examined; these showed a strong LDH1 expression in 60% to 90% of cancer cells, the remaining 35 cases showing a reduced percentage of cellular expression (30-50%). The newly formed tumor-associated fibroblasts and vessels expressed LDH1 extensively (>60-100% of

Table 1. Details of the antibodies, dilutions, and antigen retrieval methods used in this study

Primary antibody	Dilution/incubation time	Antigen retrieval	Specificity	Source
A-213226	1 μ g/mL (overnight)	MW	PDH*	Molecular Probes, Eugene, OR
A-21325	1 μ g/mL (overnight)	MW	PDH [†]	Molecular Probes
C-20	1 μ g/mL (overnight)	MW	PDK1	Santa Cruz Biotechnology, Santa Cruz, CA
ESEE 122	1:20 (overnight)	MW	HIF1 α	Oxford University
EP 190b	Neat (overnight)	MW	HIF2 α	Oxford University
ab9001	25 μ g/mL (75 min [‡])	MW	LDH1	Abcam, Cambridge, UK
ab9002	25 μ g/mL (75 min [‡])	MW	LDH5	Abcam
sc-14916	10 μ g/mL (overnight)	MW	MCT1	Santa Cruz Biotechnology
sc-14926	10 μ g/mL (overnight)	MW	MCT2	Santa Cruz Biotechnology
MCT-22A	5 μ g/mL (overnight)	MW	MCT2	Alpha Diagnostics, San Antonio, TX
sc-14934	10 μ g/mL (overnight)	MW	MCT4	Santa Cruz Biotechnology
Ab652	10 μ g/mL (75 min [‡])	MW	GLUT1	Abcam
JC70 (CD31)	1:20 (30 min [‡])	Protease XXIV	Endothelium	DAKO

Abbreviation: MW, microwave heating.

*It recognizes the E2/E3bp PDH subunit.

[†] It recognizes the E2 PDH subunit.

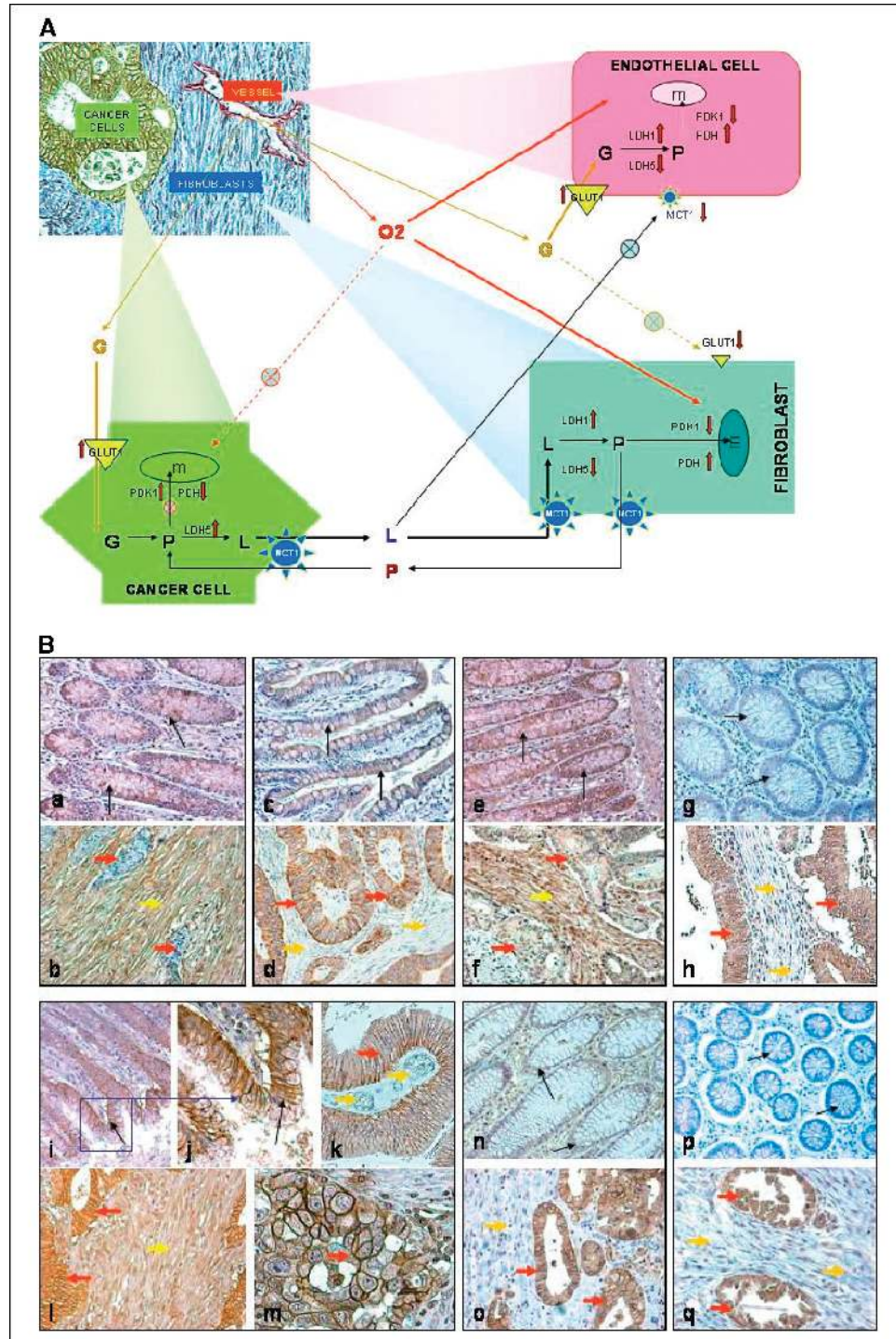
[‡] At room temperature.

optical fields) in 37 of 70 (53%) cases (Fig. 1B, f), the remaining showing a less extensive reactivity. Blood vessels, in particular, showed an invariably strong LDH1 expression regarding 10% to 50% (median, 30%) of the CD31-positive vasculature.

LDH5 was not expressed in normal colonic mucosae (Fig. 1B, g) or tumor-associated fibroblasts and vessels (Fig. 1B, h), although a focal stromal cell reactivity was noted in a small number of cancer cases (18 of 75, 24%). LDH5 was up-regulated in both cytoplasm and nuclei (of >50% of cancer cells) in 54 of 70 (77%) of the colorectal tumors investigated (Fig. 1B, h). Apparently, expression

was significantly higher in cancer cells compared with normal colonic mucosa or the adjacent tumor-associated fibroblasts ($P < 0.0001$).

MCT1 was noted consistently in cancer cells of all (70 of 70, 100%) colorectal adenocarcinomas studied (Fig. 1B, k-m). The expression was both membranous and cytoplasmic and was invariably strong (Fig. 1B, k and m). The percentage of cancer cells stained positively ranged from 50% to 100% (median, 80%). MCT1 was also expressed strongly in the tumor-associated fibroblasts in all cases examined (Fig. 1B, l) but not in the



tumor-associated vessels (Fig. 1B, k). Normal colonic tissues expressed cytoplasmic and membranous MCT1 in both surface and glandular cells (Fig. 1B, i); characteristically, membranous reactivity was basal and lateral (Fig. 1B, j). In contrast to the intratumoral endothelium, the vascular network of the normal colon and rectum consistently expressed MCT1. However, the normal submucosa and the muscular layer were devoid of reaction. MCT2 did not show a membranous pattern of staining in cancer cells after immunostaining, but it did show a strong cytoplasmic expression in both cancer cells and tumor-associated fibroblasts. MCT4 was only weakly expressed in the tumor environment.

Normal colon (Fig. 1B, n) and tumor-associated fibroblasts did not show GLUT1 expression in any of the samples examined (Fig. 1B, o). In contrast, cancer cells and tumor-associated vessels exhibited a strong GLUT1 expression. Membranous overexpression of GLUT1 was noted in 80% (56 of 70) of carcinomas analyzed (Fig. 1B, o), with an extent of staining ranging from 10% to 80% of cells (median, 30%). This membrane expression of GLUT1 by cancer cells was significantly higher than the 0% detection rate noted in normal intestinal mucosa and tumor-associated fibroblasts ($P < 0.0001$). In contrast to tumor-associated fibroblasts, the newly formed endothelial cells expressed GLUT1 well above the levels exhibited by mature colon vessels. Compared with the anti-CD31 staining, a median of 20% of tumor vessels (range, 10-40%) reacted with GLUT1.

Overexpression of HIF1 α , HIF2 α , or both (scored as strong cytoplasmic and/or nuclear expression in >50% of cancer cells per sample) was a common event in cancer cells, occurring in 48% (34 of 70), 43% (30 of 70), and 60% (42 of 70) of cases, respectively (Fig. 1B, q). HIF localization was both cytoplasmic and nuclear, consistent with HIF synthesis in the cytoplasm and subsequent translocation in the nuclei. By contrast, extensive HIF expression was detected in the adjacent stroma of no >12 of 70 (17%) colorectal carcinomas in the series (Fig. 1B, q), whereas it was totally absent from the normal colon (Fig. 1B, p). HIFs were significantly overexpressed in cancer cells compared with normal mucosal cells (lack of expression in 20 of 20 samples examined) or to tumor-associated fibroblasts and vessels ($P <$

0.0001). Tumor-associated vessels showed some HIF reactivity in 18 of 70 (25%) cases studied, but in these, reactivity was <5% (range, 0-5%; median, 3%) of the total CD31-positive vasculature.

Discussion

In this study, we examined the expression of six major proteins involved in cellular aerobic and anaerobic metabolism, focusing on differences between normal tissues, cancer cells and tumor-associated stroma. PDH was strongly expressed in tumor-associated stroma and vessels, whereas suppression of PDH expression was found in cancer cells in the majority of colorectal adenocarcinomas. In contrast, PDK1 (an enzyme inhibiting PDH activity) was absent from the tumor-associated fibroblasts and vessels but was strongly expressed in cancer cells in the majority of colorectal tumors studied. These findings suggest that the normal colon and the fibroblasts of the newly formed tumor-associated stroma, having high PDH and low PDK1 activity, are dependent on aerobic metabolism. Cancer cells, on the other hand, by lacking PDH expression while up-regulating PDK1, are likely to have a blocked or, at least, suppressed aerobic metabolic ability. The benefits of suppressing PDH in hypoxic conditions are that this would reduce oxygen use and help cell survival and also reduce generation of toxic free radicals that occurs when the Krebs cycle runs under low-oxygen conditions.

LDH1, the LDH isoenzyme favoring oxidation of lactate back to pyruvate, was expressed consistently in the normal colon and in half of the cancer cases analyzed, showing also an extensive localization in the tumor-associated fibroblasts and vessels. By contrast, LDH5 was up-regulated predominantly in cancer cells but not in the tumor-associated stroma. These patterns of LDH1/LDH5 staining in normal and malignant colorectal tissues match well with those of PDH/PDK1 expression. Tumor-associated fibroblasts and vessels, together with the normal colon, by having low LDH5/PDK1 expression and high PDH/LDH1 reactivity, favor aerobic glycolysis with metabolism proceeding to pyruvate and then mitochondrial pathways. Cancer cells, exhibiting a widespread

Figure 1. A, the proposed model of tumor metabolism. *Step 1*, glucose and pyruvate reach the tumor-associated fibroblasts through the tumor-associated vasculature followed by absorption of glucose by GLUT1, which is expressed strongly (\uparrow) in the cellular membranes of cancer cells (*o*). Glucose absorption by stromal fibroblasts is much lower (\otimes reduced uptake), as suggested by the lack of GLUT1 expression (\downarrow low levels; *B, o*). In contrast, endothelial cells are enriched with GLUT1, which enables them to absorb glucose directly from the blood. *Step 2*, in cancer cells, glucose is first transformed into pyruvate and subsequently to lactate through the intense activity of LDH5 (*B, h*). Pyruvate is unlikely to be used by cancer cell mitochondria (*B, m*) intensively, as PDH is suppressed (*B, b*) and PDK1 (inhibiting PDH activity) is overexpressed (*B, d*). Cancer cells may, therefore, have minimal requirements for oxygen so that oxygen use is reduced (\otimes). *Step 3*, the high concentrations of lactate in the cancer cell cytoplasm is rapidly extruded to the extracellular matrix through the intense activity of the MCT1 membrane monocarboxylate pump (*B, k-m*). This protects cancer cells from acidic death and results in acidification of the extracellular matrix. Lactate can diffuse into the circulation through blood vessels. *Step 4*, the high expression of MCT1 in stromal fibroblasts (*B, l*) results, under low pH matrix conditions, in intense absorption of lactate that is eventually used as a fuel to acquire energy after its oxidation back to pyruvate. This is suggested by the lack of LDH5 (*B, h*) and HIF (*B, q*) expression in stromal fibroblasts, the presence of LDH1 (*B, f*), the impressive lack of PDK1 (*B, d*), and the overexpression of PDH (*B, b*). Aerobic metabolism is, therefore, the main source of energy acquired by fibroblasts; thus, the oxygen diffused from the tumor-associated vessels is used mainly by the stroma and not by cancer cells. Blood vessels also share an enzymic/transported profile characteristic of aerobic metabolism, but unlike fibroblasts, endothelial cells do not express MCT1 (*B, k*), presumably reducing absorption of lactate and protecting themselves from acidic death. *Step 5*, excess pyruvate production within fibroblasts creates a gradient between cytoplasm and extracellular matrix, with MCT1 exporting pyruvate that can be used subsequently by cancer cells as a fuel, ending again in lactate production. *B*, PDH expression is strongly positive in normal intestinal mucosa (*a*; black arrows) and in tumor-associated stroma (*b*; yellow arrows), but it is absent from cancer cells (*b*; red arrows). PDK1 is weakly expressed in the normal mucosa (*c*; black arrows); it is absent from the tumor-associated stroma (*d*; red arrows), but it is strongly expressed in the cytoplasm of colorectal cancer cells (*d*; red arrows). LDH1 is strongly expressed in normal intestinal mucosa (*e*; black arrows) and in the tumor-associated stroma (*e*; yellow arrows), but its expression is variable in cancer cells (weak expression exhibited in *e*; red arrows). Lack of LDH5 expression is seen in normal intestinal mucosa (*g*; black arrows). Strong LDH5 expression is evident in cancer cells (red arrows) but not in the adjacent tumor-associated fibroblasts (*h*; yellow arrows). Strong basal and lateral membranous MCT1 expression is noted in normal intestinal mucosa (*i* and *j*; black arrows). MCT1 shows a strong membranous and cytoplasmic reactivity in colorectal tumor cells (*k* and *m*; red arrows). Strong expression is also noted in tumor-associated fibroblasts (*l*; yellow arrows). Intratumoral vessels were unreactive to MCT1 antibody (*k*; yellow arrows). GLUT1 is strongly expressed by cancer cells (*o*; red arrows); a weak expression is noted in colorectal mucosal cells (*n*; black arrows), but it is absent from the tumor-associated fibroblasts (*o*; yellow arrows). HIF1 α is not detected in the normal intestinal mucosa (*p*; black arrows), but it is strongly expressed in colorectal cancer cells (*q*; red arrows); HIF1 α is by and large absent from the tumor-associated fibroblasts (*q*; yellow arrows). Magnification, $\times 100$ (*i* and *l*) and $\times 200$ (*a-h, j, k*, and *m-q*).

LDH5/PDK1 reactivity and reduced PDH repression, are directed to anaerobic glycolysis for energy, favoring the transformation of pyruvate to lactate.

MCT1 is a protein participating in the bidirectional transmembrane exchange of lactic acid and pyruvate (16). The direction of transportation depends upon the pH gradient between intracellular and extracellular environment (16–19). Admittedly, the immunohistochemical patterns of MCT1 indicated here are in direct contrast with those reported by Lambert et al. (20), where MCT1 was suppressed in colorectal cancer tissues. In our study, a distinct clear and strong membranous expression of the pump was noted consistently in cancer cells of all colorectal adenocarcinomas but not in the adjacent stroma or the normal colonic mucosa, leaving no doubt for the specificity of the staining. Any discrepancy between Lambert's study and ours should be sought in the specificity of the antibodies used. It is notable that Lambert et al. failed to detect any membranous reactivity with the antibody used, which is in discordance with the cell surface localization of MCTs and their pump function. Although studies on MCT1 expression in human malignancies are rare, overexpression of MCT1 in brain tumors has been also reported (21) and, in our experience, MCT1 up-regulation is a feature in a variety of human malignancies.⁴

The strong membranous expression of MCT1 by cancer cells suggest a role in the efflux of lactate from the tumor cells to the tumor-supporting stroma. As anaerobic glycolysis is likely to be low in stromal fibroblasts, lactate concentration is lower in fibroblasts than in the extracellular matrix, which is enriched by lactate released from the cancer cells. The MCT1 expression in stromal fibroblasts favors the absorption of lactate from the acidic extracellular matrix; this is because of the concentration gradient from cancer cells to extracellular matrix and then stromal cells. Ultimately, lactate may be used as a fuel by the tumor-associated fibroblasts and vessels. Following lactate oxidation, the pyruvate formed can be used either as a fuel by the fibroblasts themselves or can be extruded into the matrix by MCT1 for subsequent absorption and consumption by cancer cells. Tumor-associated vessels, however, although rich in LDH1, do not express MCT1 and this may reflect a protective endothelial mechanism to prevent lactate absorption and vascular destruction from acidosis.

MCT2 showed a strong cytoplasmic expression in both cancer cells and tumor-associated fibroblasts. Whether this pattern of expression represents a predominantly organelle localization of MCT2 (e.g., mitochondrial) is not known, although the high affinity of MCT2 for pyruvate and the experimental evidence of the presence MCT2 in mitochondrial membranes (16) is pointing towards a role of MCT2 in the mitochondrial import of pyruvate following lactic acid oxidation. In fact, the Krebs cycle can run under quite low oxygen concentrations; this may be an adaptive response to use the lower pyruvate levels to maintain residual Krebs function and ATP generation at least. With regard MCT4, this was only weakly expressed in the tumor environment, suggesting a minimal role in the metabolic intratumoral communication.

Glucose, the main energy source for cancer cells and tumor-associated stroma, reaches the tumor environment through diffusion from the peritumoral and intratumoral vasculature. The expression levels of GLUT1, a key pump for the absorption of glucose by cells, are expected to define the rate of glucose influx

into the cells. In the present study, normal colon did not show GLUT1 expression, but this presumably reflects the limits of immunohistochemical technique to detect membranous GLUT1 protein at the baseline concentrations present in the normal tissues or use of other GLUTs. Similarly, the tumor-associated fibroblasts were uniformly negative for GLUT1, suggesting a low level activity. In contrast, cancer cells and tumor-associated vessels exhibited a strong GLUT1 expression. The irregularly, and often poorly, vascularized intratumoral environment indicates that glucose disposition is rather limited and abnormally distributed within tumors. The lack of expression of GLUT1 in tumor-associated fibroblasts probably reflects the specific up-regulation of GLUT1 by various oncogenes. The activation of lactate oxidation by the stromal fibroblasts would use lactate for energy and spare glucose for cancer cells.

In contrast to tumor-associated fibroblasts, the newly formed endothelial cells expressed GLUT1 well above the levels exhibited by mature colon vessels, suggesting active uptake of glucose from the blood stream, ready to be used aerobically for energy production. The oxygen, diffused through the tumor-associated vasculature, seems to be necessary for the survival of intratumoral endothelium and stroma but is unlikely to have a major contribution to energy production for cancer cells, as it is indicated by the low PDH, high PDK1, high LDH5, and high GLUT1 cancer cell reactivity.

Overexpression of HIF1 α , HIF2 α , or both was a tumor-specific phenomenon concerning more than half of colorectal carcinomas examined. However, HIF expression was confined in cancer cells and was only occasionally seen in the stroma. These patterns of HIF staining are in accordance with those of LDH5 and GLUT1 reactivity, as transcription of their corresponding genes is under the immediate control of HIFs (7). Lactate and glucose metabolites inhibit prolyl-hydroxylases, thus contributing to further up-regulation of HIF in tumors compared with tumor-associated stroma (22, 23).

From the above immunohistochemical findings, it becomes apparent that a contribution to surviving the adverse microenvironment in colorectal adenocarcinomas may derive from well-organized metabolic domains composed of the invading tumor cells and the newly formed tumor-associated fibroblasts and tumor-associated endothelium. These heterogeneous cellular elements work together, with opposite qualities. The cancer cells, sharing in common enzyme/transporter activity, are dependent upon anaerobic metabolism with a high ability for glucose absorption and lactate extrusion. The tumor-associated fibroblasts exhibit patterns that are involved in aerobic metabolism, lactate absorption, and lactate oxidation. Finally, the tumor-associated endothelium express enzymes/transporters involved in aerobic metabolism with high capacity for lactate oxidation, high ability for glucose absorption, and resistance to lactate intrusion.

Putting this information together, a model of tumor metabolism may be reached, as indicated and explained in Fig. 1. This is, of course, based only upon immunohistochemical findings. Clearly, direct investigation of the biochemical fluxes is needed both *in vivo* and in tumor and stromal lines. Nevertheless, it provides a basis for further study. The distinctly different but complementary metabolic function of tumor cells, tumor-associated fibroblasts, and vessels, shows a complex spatial organization compatible with synergistic support to each other. This tumor domain may be insufficient to support tumor growth and survival if it was not to use a stromal "sump" to control pH and removal of toxic metabolites.

⁴ Unpublished data.

Indeed, many investigators stressed the unique molecular profile of tumor-associated fibroblasts (24–26). These are, in essence (together with the extracellular matrix), the main component of stromatogenesis (i.e., the process of new stroma formation occurring concurrently with tumor cell invasion and angiogenesis; refs. 12, 13, 15). The newly formed stroma not only provides tumor cells with a substratum suitable for tumor growth and expansion but also offers them a metabolic support essential for cancer cell survival and growth. Targeting the function of enzymes involved in this fine metabolic interplay between cancer and stromal cells might give rise to a new area

for therapeutic research. Already, from the area of diabetes research, there are inhibitors of PDH kinase, which could be assessed clinically in cancer.

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