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Characterization of the Cell Membrane During Cancer Transformation

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1. Introduction

Colorectal cancer is one of the most common cancers diagnosed worldwide. The development of colorectal cancer, like many types of cancer is a multistage process that involves many different pathways. In particular, deregulation of cell-cell communication plays an important role. Moreover, cell-cell comunication is indispensable for the maintenance of homeostasis in a multicellular organism. *Gap*-type junctions are one of the most common and perhaps most interesting, mediators of intercellular communications. Digestive tract gap junctions are also important and are flanked by various cell types within each layer of the wall. The composition and organisation of gap junction channel subunits plays a critical role in determining the properties of these channels, including conductance properties and pH sensitivity. Structurally, gap junctions are composed of transmembrane proteins which form structures called connexons, with a single connexon consistings of six peripherally arranged subunits of integral membrane proteins known as connexins. Correspondingly, normal human epithelial cells in the colon have been found to express the connexins, Cx32 and Cx43. Moreover, in our previous studies Cx26 expression was detected in normal colon epithelium as well as in colorectal cancer tissues (Contreras et al., 2002, Cascio, 2005, van Zeijl et al., 2007).

A number of biological and chemical substances affect the function of gap junctions. For example junctions can be inhibited following the phosphorylation of connexin proteins or following exposure to agents that disrupt the accumulation of connexin or mediate local damage to cellular membranes. The function of membrane channels also require the presence of particular species of lipid in the surrounding membrane. Locke and Harris were the first to identify endogenous phospholipids tightly associated with connexin channels and these results suggested that specific phospholipids are associated with different connexin isoforms to form connexin-specific regulatory networks and/or structural interactions with lipid membranes. Ongoing studies of connexin channel function and cell biology to characterize lipid-protein interactions and membrane biophysics are providing valuable insight into these processes (Locke & Harris, 2009).

Phenomena associated with changes in cell membranes are suspected to play an important role during the cancer transformation. At physiological pH, the cell membrane surface is

negatively charged, which is determined based on the number of negative and positive charge carriers present (i.e., phosphates, carboxyl and amino groups of proteins and phospholipids). Furthermore, electrical properties of a membrane are determined by acid-base and complex formation equilibria at the membrane and in response to surrounding medium components. For example, membrane components including – proteins, phospholipids, and free fatty acids contribute to this equilibria. Correspondingly, we hypothesis that the electrical charge of tumor cells can indirectly represent changes that have occurred during cell transformation and may indicate tumor cell status.

2. The cell membrane

Biological membranes are essential boundaries within a living cell. The cell membranes separate the interior of the cell from its microenvironment and also participate in intercellular communication.

2.1 Electric properties of cell membranes

For a biological membrane, its electrical charge and difference in potential between the membrane and surrounding solution are key properties. Cell membrane charge has been found to increase during tumorigenesis and decrease during necrosis (Dołowy, 1984). Correspondingly, investigations of factors that influence membrane electric charge during cancer transformation have been performed. These factors include determining pH, acidic (C_{TA}) and basic (C_{TB}) functional group concentrations and their average association constants with hydrogen (K_{AH}) or hydroxyl (K_{BOH}) ions (Dobrzyńska et al., 2006).
The electrical properties of a membrane are determined by acid-base and complex formation equilibria. Both membrane and surrounding medium components contribute to this equilibria, with the former including proteins, phospholipids and fatty acids (Gennis, 1989; Tien, 1974). As a result, we hypothesise that the electrical charge of tumor cells can be indirectly estimated from changes detected in tumor cells that are concomitant with their transformation during tumorigenesis.

2.1.1 Surface charge density cell membrane

Surface charge density dependence on pH of normal and tumor large intestine cell membrane are similarly shaped (Fig. 1). For example, an increase in positive surface charge density is observed at low pH values until a plateau is reached. Conversely, at high pH values, the proportion of negative charges present increases until it reaches a plateau. Overall, an increase in negative charge at low pH values as well as in positive charge at high pH is observed in human large intestine tumor cells compared to unaffected cells (Szachowicz-Petelska et al., 2002).

2.1.2 Theory

The dependence of surface charge density of a cell membrane on pH of electrolyte solution can be described according to four equilibria factors. Two equilibria concern negative groups and involve sodium and hydrogen ions, and two other equilibria refer to positive groups and involve hydroxide and chloride ions. These factors can then be expressed as follows written in the form:

$$A^- + H^+ \Leftrightarrow AH \quad (1)$$

$$A^- + Na^+ \Leftrightarrow ANa \quad (2)$$

$$B^+ + OH^- \Leftrightarrow BOH \quad (3)$$

$$B^+ + Cl^- \Leftrightarrow BCl \quad (4)$$

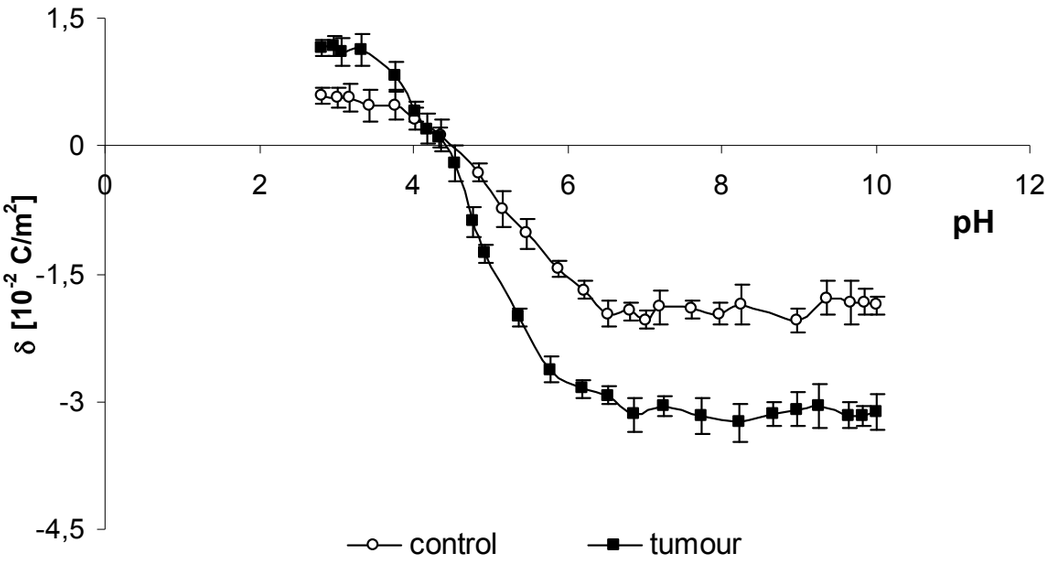

Fig. 1. The dependence of surface charge density on pH for normal and colorectal cancer cell membranes from several patients.

An association constant of the H^+ Na^+, OH^- and Cl^- ions with functional groups can be expressed according to the following equations:

$$K_{AH} = \frac{a_{AH}}{a_{A^-} \cdot a_{H^+}} \quad (5)$$

$$K_{ANa} = \frac{a_{ANa}}{a_{A^-} \cdot a_{Na^+}} \quad (6)$$

$$K_{BOH} = \frac{a_{BOH}}{a_{B^+} \cdot a_{OH^-}} \quad (7)$$

$$K_{BCl} = \frac{a_{BCl}}{a_{B^+} \cdot a_{Cl^-}} \quad (8)$$

Here:

K_{AH}, K_{ANa}, K_{BOH} and K_{BCl} represent association constants,

a_{A^-}, a_{AH}, a_{ANa}, a_{B^+}, a_{BOH} and a_{BCl} represent surface concentrations, that are present on the membrane surface,

and a_{H^+}, a_{Na^+}, a_{OH^-} and a_{Cl^-} represent corresponding concentrations in solution.

Surface charge density (δ) is expressed as follows:

$$\delta = (a_{B^+} - a_{A^-}) \cdot F \tag{9}$$

where F=96487 [C/mol] - Faraday constant.
And functional group concentration balances can be expressed as follows:

$$C_{TA} = a_{A^-} + a_{AH} + a_{ANa} \tag{10}$$

$$C_{TB} = a_{B^+} + a_{BOH} + a_{BCl} \tag{11}$$

where C_{TA} and C_{TB} represent the total surface concentrations functional groups.
Elimination of a_{A^-}, a_{AH}, a_{B^+}, and a_{BOH} values from above equations yields the following formula:

$$\frac{\delta}{F} = \frac{C_{TB} \cdot a_{H^+}}{a_{H^+}(1 + K_{BCl} \cdot a_{Cl^-}) + K_{BOH} \cdot K_w} - \frac{C_{TA}}{K_{AH} \cdot a_{H^+} + K_{ANa} \cdot a_{Na^+} + 1} \tag{12}$$

It is difficult to carry out the regression function of Eqn. (12) to determine the C_{TA}, C_{TB}, K_{AH} and K_{BOH} constants.
Simplifying to one fraction and making transformations described in this work (Dobrzyńska et al., 2006), we can receive the equation of a straight line for high and low ion concentration H^+, from which C_{TA}, C_{TB}, K_{AH} and K_{BOH} values can be established.
The coefficients could be determined using linear regression and C_{TA}, C_{TB}, K_{AH} and K_{BOH} values could be calculated. However, in determining each values, there are points that would need to be considered in the regression, both for high and low H^+ concentration ranges.

2.1.3 Parameters characterizing the cell membrane

In this study C_{TA}, C_{TB} and K_{BOH} values for a cell membrane were found to be affected by cancer cell transformation, and were higher than the same parameters assayed in unmodified cells (Figs. 2-4). Meanwhile K_{AH} was found to decrease in cancer cells versus normal cells (Fig. 3).
In normal cells, the aminophospholipids such as phoshatidylserine (PS) and phosphatidylethanolamine (PE) are asymmetrically distributed across the plasma membrane e.g., they primarily localize to the cell's inner membrane leaflet (Stafford & Thorpe, 2011; Marconescu & Thorpe, 2008). This membrane lipid asymmetry is maintained by a group of P-type ATPases known as aminophospholipid translocases (APTLs). These APTLs catalyze the active transport of PS and PE from the external side to the internal side of the leaflet of the plasma membrane (Devaux, 1992). The distribution of PS, a component of the skeleton, has been shown to undergo changes, which could cause an increase in the proportion of negatively charged groups present at high pH values. As a result, anionic phospholipids present on tumor vessels could potentially represent tumor-specific markers for targeting and imaging (Ran et al., 2002).
Hypoxia/reoxygenation and acidity-induced exposure of anionic phospholipids, most likely phosphatidylserine and phosphatidylethanolamine (Zhao et al., 1998; Ran et al., 2002). According to previous studies both hypoxia and acidity can exist in a tumor. In particular,

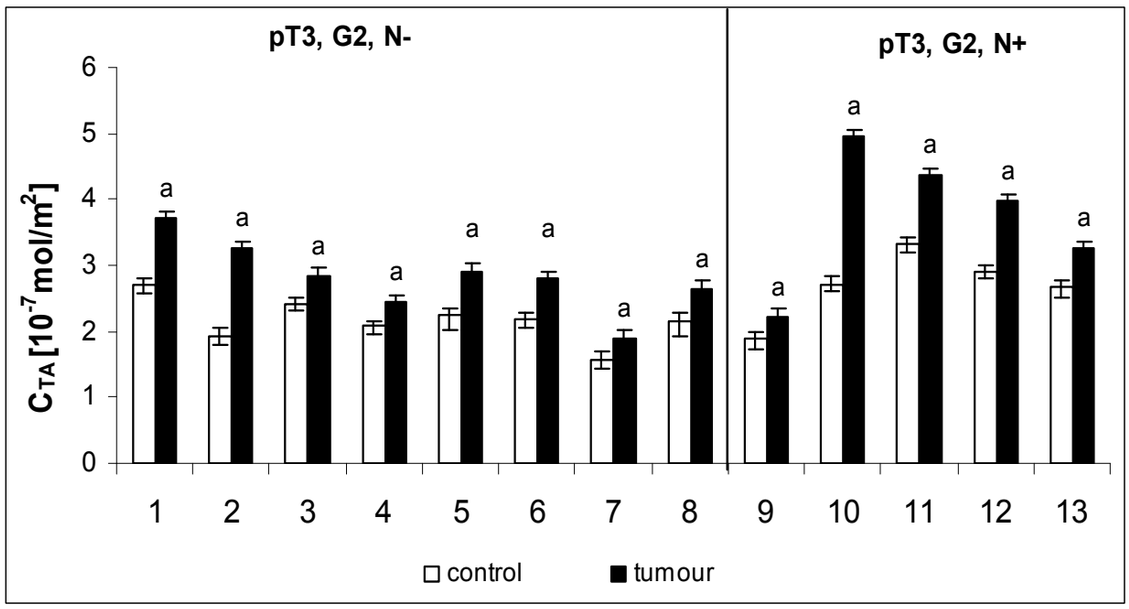

Fig. 2. The concentration of acidic functional groups present on pT3 stage, G2 grade human colorectal cancer cell membranes associated with metastasis (N+) and not associated with metastasis (N-). [a] $p<0.05$, compared with control.

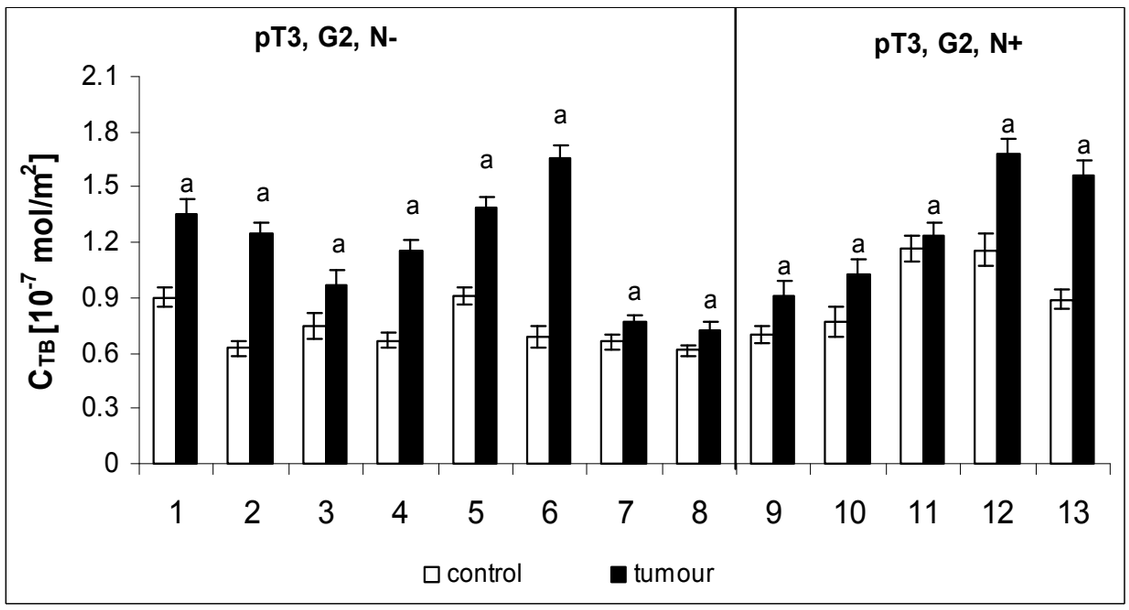

Fig. 3. The concentration of basic functional groups present on pT3 stage, G2 grade human colorectal cancer cell membranes associated with metastasis (N+) and not associated with metastasis (N-). [a] $p<0.05$, compared with control.

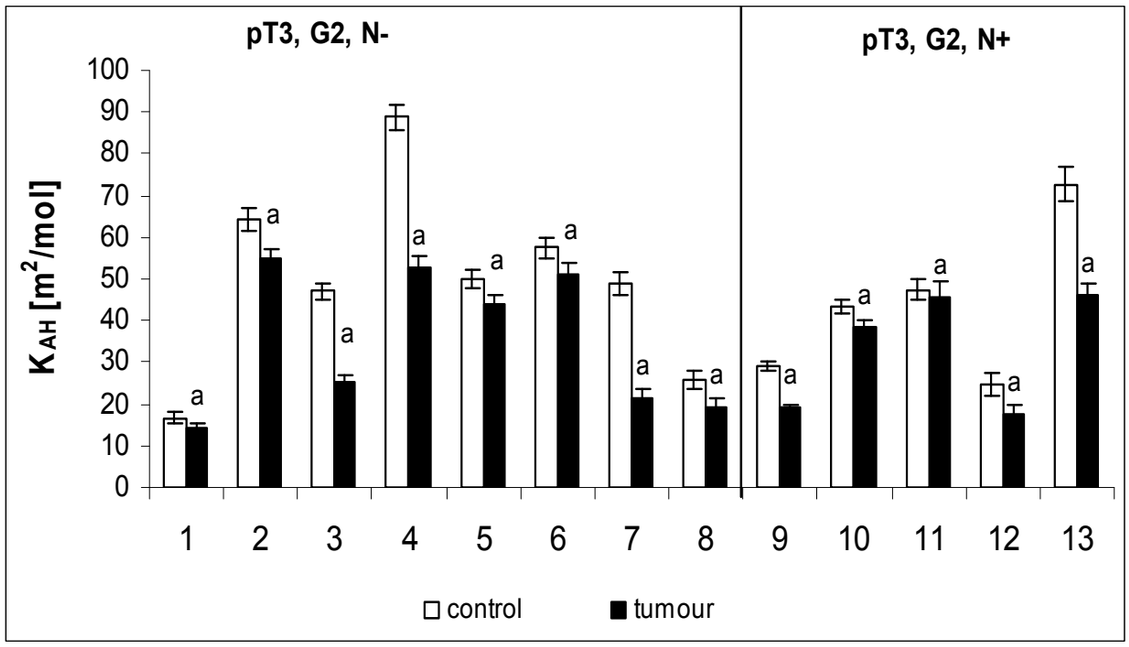

Fig. 4. The average association constant for hydrogen ions associated with pT3 stage, G2 grade human colorectal cancer cell membranes associated with metastasis (N+) and not associated with metastasis (N-). [a] $p<0.05$, compared with control.

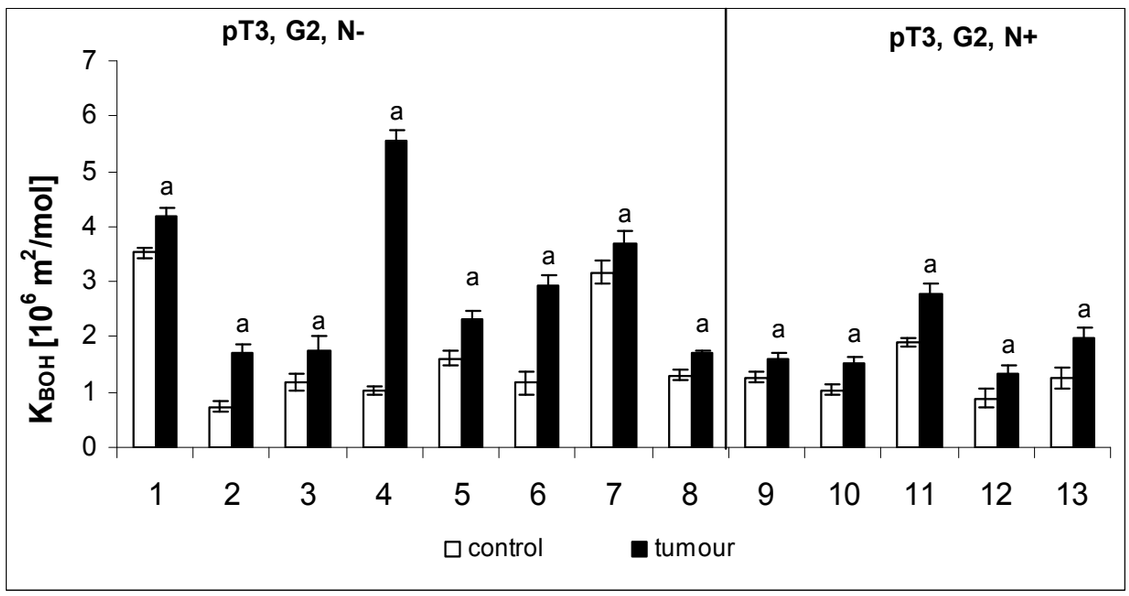

Fig. 5. The average association constant for hydroxyl ions associated with pT3 stage, G2 grade human colorectal cancer cell membranes associated with metastasis (N+) and not associated with metastasis (N-). [a] $p<0.05$, compared with control.

hypoxia represents an important cellular stressor that can trigger a survival program by which cells attempt to adapt to a new environment. Typically, these adaptations will largely affect cell metabolism and/or stimulation of oxygen delivery (Bos et al., 2004).
Cell membrane charge is also affected by sialic acid present in glycolipids and glycoproteins. It has previously been hypothesized that sialic acid influences the concentrations of acid and basic groups present on the cell surface as well as association constants of positively and negatively charged groups during cancer transformation. An increase in the content of sialic acid in glycolipids and glycoproteins has been confirmed in, and increased sialic acid content has been found to provoke an increase in the surface concentration of acid groups (Erbil et al., 1986; Narayanan, 1994; Wang, 2005).

2.2 The compounds present in the cell membranes of human colorectal cancer

Neoplasms produce and secrete agents at trace levels inside of cells. These agents can include carcinogenic antigens, hormones, metabolites, growth factors, enzymes and cytokines (Skrzydlewska et al., 2005; Koda et al., 2004). In malignant cells, the ultrastructural architecture of the cell membrane is altered, partially as a result of changes in the quantities of membrane components present. Correspondingly, the transport of agents through the cell membrane is affected, thereby altering the biological properties of a cell. In many cases, expression levels of proteins, phospholipids and free unsaturated fatty acids are also affected due to enzyme disorders associated with biosynthesis processes that are altered. It is hypothesized that quantitation of the changes in the levels of phospholipids and structural proteins at the cell surface can reflect the extent of disintegration and impairment of genomic functioning that has occurred as a result of mutations associated with malignant transformation (Baldassarre et al., 2004; Tsunada et al., 2003).
Changes in membrane composition have the potential to affect cell growth and interactions between cells (including cells of the immune system), as well as the function of proteins and other components present at the cell membrane. For example, the immune system depends on interactions between different cell types for its function and these interactions are mediated by the membrane composition of the cells involved (Yaqoob, 2003). Moreover, immune cell activation (e.g., cell proliferation, phagocytosis) and tumor growth (malignancy) are processes associated with an increased rate of *de novo* synthesis and turnover of membrane phospholipids (Field & Schley, 2004).

2.2.1 Changes in the phospholipids composition of human colorectal cancer cell membranes

Phospholipids are an integral part of a cell membrane and determine its structure. Accordingly, different biological conditions are associated with differences in membrane phospholipids composition particularly during cancer transformation (Dobrzyńska et al., 2005; Szachowicz-Petelska et al., 2007).
For example, most cases of colorectal cancer involve an increase in the concentration of all phospholipid types at the cell membrane, including: phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (Table 1).
Previous studies have shown that an increase in the concentration of phospholipids in the cell membrane is associated with human colon cancer cells (Dueck et al., 1996) and murine mammary tumor cells (Monteggia et al., 2000). Moreover, this increase has been proposed to be the result of enhanced cell membrane synthesis related to accelerated neoplasm cell replication (Ruiz-Cabello & Cohen, 1992). Furthermore, the mechanisms involved can vary

Patient no	Type of phospholipid	Phospholipid content detected (mg/g tissue)	
		Control	Tumor
1.	PI	0.010 ± 0.002	0.225 ± 0.020[a]
	PS	0.016 ± 0.003	0.100 ± 0.010[a]
	PE	0.550 ± 0.010	0.890 ± 0.030[a]
	PC	0.675 ± 0.011	1.100 ± 0.061[a]
2.	PI	0.012 ± 0.003	0.239 ± 0.040[a]
	PS	0.028 ± 0.002	0.151 ± 0.022[a]
	PE	0.510 ± 0.020	0.740 ± 0.081[a]
	PC	0.116 ± 0.010	1.237 ± 0.099[a]
3.	PI	0.074 ± 0.008	0.081 ± 0.007
	PS	0.086 ± 0.006	0.131 ± 0.010[a]
	PE	0.494 ± 0.021	0.902 ± 0.051[a]
	PC	0.648 ± 0.024	1.240 ± 0.085[a]
4.	PI	0.087 ± 0.009	0.248 ± 0.020[a]
	PS	0.097 ± 0.007	0.097 ± 0.006
	PE	0.901 ± 0.050	0.932 ± 0.050
	PC	1.139 ± 0.061	1.245 ± 0.089[a]
5.	PI	0.064 ± 0.005	0.109 ± 0.010[a]
	PS	0.086 ± 0.004	0.114 ± 0.015[a]
	PE	0.498 ± 0.012	0.768 ± 0.080[a]
	PC	0.677 ± 0.018	1.054 ± 0.095[a]
6.	PI	0.020 ± 0.002	0.056 ± 0.006[a]
	PS	0.024 ± 0.002	0.096 ± 0.009[a]
	PE	0.432 ± 0.012	0.951 ± 0.092[a]
	PC	0.707 ± 0.019	1.368 ± 0.101[a]
7.	PI	0.009 ± 0.001	0.030 ± 0.015[a]
	PS	0.010 ± 0.011	0.021 ± 0.010
	PE	0.419 ± 0.023	0.828 ± 0.052[a]
	PC	0.675 ± 0.034	1.182 ± 0.065[a]
8.	PI	0.036 ± 0.012	0.136 ± 0.016[a]
	PS	0.042 ± 0.015	0.103 ± 0.050[a]
	PE	0.468 ± 0.028	0.895 ± 0.039[a]
	PC	0.686 ± 0.039	1.287 ± 0.070[a]

Table 1. The phospholipid content of pT3 stage, G2 grade human colorectal cancer cell membranes not associated with metastasis (N-). [a] $p<0.05$, compared with control.

depending on the cell type, cell growth phase and malignancy status. For example, the greatest changes in the content of PC and PE have been observed in the G_1 phase of the cell cycle, during which activity of the enzymes controlling biosynthesis, catabolism and metabolism of phospholipids is maximal (Jackowski et al., 1996; Jackowski et al., 1994). As shown in Table 1 the PC content detected in normal mucosa in lesions of colorectal cancer cells and in other cancer cells was found to be higher than that of other phospholipids. These observations are consistent with the results of previous studies.

Patient no	Type of phospholipid	Phospholipid content detected (mg/g tissue)	
		Control	Control
9.	PI	0.044 ± 0.002	0.225 ± 0.011[a]
	PS	0.043 ± 0.002	0.145 ± 0.009[a]
	PE	0.642 ± 0.011	0.909 ± 0.019[a]
	PC	0.783 ± 0.012	1.406 ± 0.025[a]
10.	PI	0.150 ± 0.009	0.160 ± 0.008
	PS	0.055 ± 0.003	0.055 ± 0.003
	PE	0.540 ± 0.012	0.545 ± 0.013
	PC	0.925 ± 0.022	1.555 ± 0.028[a]
11.	PI	0.044 ± 0.002	0.144 ± 0.009[a]
	PS	0.025 ± 0.001	0.075 ± 0.005[a]
	PE	0.381 ± 0.018	0.396 ± 0.011
	PC	0.475 ± 0.020	1.281 ± 0.016[a]
12.	PI	0.018 ± 0.001	0.113 ± 0.008[a]
	PS	0.031 ± 0.003	0.110 ± 0.007[a]
	PE	0.551 ± 0.018	0.592 ± 0.013[a]
	PC	0.698 ± 0.021	0.933 ± 0.026[a]
13.	PI	0.026 ± 0.003	0.039 ± 0.003[a]
	PS	0.013 ± 0.001	0.026 ± 0.002[a]
	PE	0.433 ± 0.019	0.546 ± 0.014[a]
	PC	0.770 ± 0.030	1.240 ± 0.019[a]

Table 2. The phospholipid content of pT3 stage, G2 grade human colorectal cancer cell membranes associated with metastasis (N+). [a] $p<0.05$, compared with control.

Differences in membrane phospholipid content can also affect the potential for metastasis (Podo, 1999; Dobrzyńska et al., 2005). For example, malignant neoplasm cells associated with a greater number of metastases were characterized by a higher PC/PE ratio than malignant neoplasm cells with fewer metastases (Table 2).

2.2.2 Changes in the membrane free unsaturated fatty acid composition of human colorectal cancer cells

Free fatty acids are present in cell membranes, with the former present at low levels and the latter having a strong influence on the structure, properties and functions of the cell membrane. Polyunsaturated free fatty acids (PUFAs) also participate in the normal functioning of a cell, particularly by contributing to intracellular cell signaling. In addition, PUFAs represent nutritional components of a human diet and can indirectly affect tumorigenesis. For example, long-chain n-3 fatty acids have been shown to alter co-stimulatory molecules and activation markers, as well as calcium signaling and protein kinase C translocation at the cell membrane of immune cells (Hughes & Pinder, 2000). Similarly, the incorporation of n-3 fatty acids in the membrane of other cell types has been shown to alter membrane permeability, membrane fluidity and hormone and growth factor

binding (Hashimoto et al., 1999; Lund et al., 1999). In colorectal cancer cells, reduced levels of PUFAs have been detected in the membrane, concomitant with increased levels of arachidonic and oleic acids, and lower levels of linoleic and α-linolenic acids (Table 3) (Szachowicz-Petelska et al., 2002, 2007).

Moreover, decreased levels of linoleic and α-linolenic acids have been detected in the plasma and erythrocytes of colorectal cancer patients. These changes are probably due to metabolic alterations caused by the illness and not necessarily by malnutrition (Baro et al., 1998). In addition, two clinical investigations have reported a significant increase in plasma and tissue concentrations of arachidonic acid (AA) in colorectal cancer patients compared with control patients (Neoptolemos et al., 1991; Hendrickse et al., 1994). This increase may be related to an enhancement of lipid peroxidation, which is a feature of rapidly growing cells (Skrzydlewska et al., 2001, 2005). Alternatively, increased AA levels could be due to elevated desaturase activity involving linoleic acid (LA) and α-linolenic acid (ALA), possibly leading to increased formation of prostaglandins and other lipoxygenase products (Dommels et al., 2002).

Other classes of unsaturated fatty acids include the palmitoleic (n-7) and oleic (n-9) family, both of which can be produced by most cells in humans and, thus, are not essential (Pandian et al., 1999). Levels of oleic acids have been found to be increased in colon cancer cells (Table 3). Furthermore, a significant elevation in the concentration of oleic acid has been detected in the plasma of colorectal cancer patients (Baro et al., 1998). Correspondingly, an almost statistically significant increase in the intake of oleic acid was found in another study of high-risk subjects for colorectal cancer (Schloss et al., 1997). These results may be due to changes in oleic acid metabolism as part of the pathogenic process. It has also been shown that human colon tumor growth is promoted by oleic acid (Calder et al., 1998) via mechanisms of increased fatty acid oxidation and a disturbance of membrane enzymes (Suziki et al., 1997).

Work by Rakheja et al., demonstrated that an overall reduction in free unsaturated fatty acids was associated with cancer cell membranes, while another recent report detected an elevated proportion of saturated versus unsaturated total fatty acids in colonic adenocarcinoma (Rakheja et al., 2005). In the latter case, the increase in saturated total fatty acids was attributed to elevated levels of the enzyme fatty acid synthase (Rashid et al., 1997). Furthermore, saturated fatty acids may be targeted to lipid raft microdomains, which are rich in cholesterol, sphingolipids and phospholipids with saturated fatty acid side chains (Swinnen et al., 2003; Rakheja et al., 2005). Recently, an increased intake of dietary n-3 fatty acids has been shown to decrease levels of sphingomyelin, cholesterol and caveolin-1 collectively, suggesting that n-3 fatty acids can modulate the composition of lipid rafts (Martin et al., 2005). Moreover, polyunsaturated fatty acids have been proposed to play a role in cancer therapy and to perturb membrane lipids rafts, thereby affecting cell functions (Hardman, 2004; Ma et al., 2004).

Under pathological conditions, such as hypoxia/reoxygenation, byproducts of AA that are generated can reduce gap junction-mediated coupling (Martinez & Saez, 2000). Dommels et. al., demonstrated that short-term incubation with LA, α-ALA or AA did not influence gap junctional intercellular communication (GJIC), yet long-term incubation with LA and α-ALA did inhibit GJIC of colon cells. Although the exact mechanisms mediating the inhibition of GJIC remain unclear, it is hypothesized that the associated cytotoxicity releated to the disruption of gap junctions is mediated by lipid peroxidation products. This hypothesis is supported by the observation that incubation with PUFAs, such as AA, can completely abolish GJIC (Dommels et. al., 2002).

Patient no	Type of fatty acid	Fatty acid content detected (mg/g tissue)	
		Control	Control
1.	18:2n-6	0.059 ± 0.005	0.014 ± 0.002[a]
	18:3n-3	0.045 ± 0.002	0.032 ± 0.005[a]
	16:1	0.032 ± 0.009	0.027 ± 0.007
	20:4n-6	0.036 ± 0.008	0.050 ± 0.010
2.	18:2n-6	0.028 ± 0.005	0.014 ± 0.005[a]
	18:3n-3	0.086 ± 0.010	0.071 ± 0.011
	16:1	0.021 ± 0.005	0.028 ± 0.005
	20:4n-6	0.064 ± 0.007	0.071 ± 0.008
3.	18:2n-6	0.033 ± 0.006	0.011 ± 0.003[a]
	18:3n-3	0.055 ± 0.005	0.039 ± 0.008[a]
	16:1	0.022 ± 0.003	0.022 ± 0.004
	20:4n-6	0.044 ± 0.009	0.061 ± 0.007[a]
4.	18:2n-6	0.022 ± 0.004	0.003 ± 0.001[a]
	18:3n-3	0.034 ± 0.006	0.028 ± 0.005
	16:1	0.016 ± 0.003	0.016 ± 0.003
	20:4n-6	0.028 ± 0.005	0.031 ± 0.006
5.	18:2n-6	0.014 ± 0.004	0.007 ± 0.001[a]
	18:3n-3	0.034 ± 0.006	0.017 ± 0.003[a]
	16:1	0.010 ± 0.002	0.014 ± 0.002
	20:4n-6	0.027 ± 0.004	0.041 ± 0.006[a]
	18:1	0.058 ± 0.007	0.075 ± 0.008[a]
6.	18:2n-6	0.016 ± 0.004	0.003 ± 0.001[a]
	18:3n-3	0.024 ± 0.005	0.019 ± 0.004
	16:1	0.005 ± 0.001	0.008 ± 0.001[a]
	20:4n-6	0.024 ± 0.004	0.035 ± 0.005[a]
	18:1	0.011 ± 0.002	0.027 ± 0.004[a]
7.	18:2n-6	0.009 ± 0.002	0.002 ± 0.001[a]
	18:3n-3	0.019 ± 0.004	0.009 ± 0.002[a]
	16:1	0.005 ± 0.001	0.005 ± 0.001
	20:4n-6	0.015 ± 0.003	0.026 ± 0.005[a]
	18:1	0.009 ± 0.002	0.019 ± 0.004[a]
8.	18:2n-6	0.057 ± 0.008	0.007 ± 0.001[a]
	18:3n-3	0.071 ± 0,009	0.036 ± 0.005[a]
	16:1	0.028 ± 0.005	0.043 ± 0.004[a]
	20:4n-6	0.064 ± 0.007	0.071 ± 0.007
	18:1	0.019 ± 0.004	0.056 ± 0.006[a]

18:2n-6, linoleic acid;18:3n-3, α-linolenic acid;16:1, palmitoleic acid;20:4n-6, arachidonic acid;18:1, oleic acid.

Table 3. PUFA content of pT3 stage, G2 grade human colorectal cancer cells not associated with metastasis (N-). [a] $p<0.05$, compared with control.

2.2.3 Changes in membrane proteins of human colorectal cancer cells

Currently, membrane proteins are classified into five groups according to their putative functions. These include: 1) receptor proteins associated with various extracellular ligands such as growth factors and hormones, 2) channel proteins that mediate the transportation of ions and small molecules across the membrane, 3) various enzyme proteins such as phospholipases and phosphatases, 4) regulatory proteins associated with functional proteins such as p21 and 5) cellular adhesion proteins such as cell - CAMs. In the latter case, most CAMs belong to one of four protein families: immunoglobulin (Ig), superfamily (IgSF), integrins, cadherins or selectins.

Structural changes in membrane proteins are associated with changes in the electrical potential of tumor cell membranes. These changes also correspond with altered biological properties exhibited by tumor cells. For example, a decrease in levels of E-cadherin expression in colorectal cancer cells has been shown to affect the diversification of cells in a tumor as well as the probability that tumor cells will contribute to distant metastasis.

While characterization of membrane proteins of tumor cells has made progress and provided valuable insight into the role of the cell membrane in tumorigenesis, additional studies are still needed to elucidate tumor-specific mechanisms associated with these changes (Kojima, 1993).

3. Conclusions

A higher proportion of phospholipids present in cell membranes results in a larger number of functional groups present at the cell surface and these can include: amino, carboxy and phosphate functional groups. Correspondingly, in acidic medium (e.g., a low pH), the charge associated with the phospholipid population at the cell surface is mainly determined by the amino groups present. In contrast, carboxy and phosphate groups present in a basic medium (e.g., a high pH) are key. For large intestine cell membranes, the main component of the outer layer is PC and at higher concentrations, PC can provoke an increase in both C_{TA} and C_{TB} values. In addition, when cells undergo transformation the association constant of negatively charged groups present (e.g., K_{AH}) decreases while the association constant of positively charged groups (e.g., K_{BOH}) increases.

Anionic phospholipids associated with tumor vessels also potentially represent markers for tumor vessel targeting and imaging (Ran et al., 2002). In addition, alterations in the distribution of PS, a component of the skeleton, can cause an increase in C_{TA} values.

Therefore, an evaluation of the membrane status of tumor cells may be an important consideration in future studies of tumor biology.

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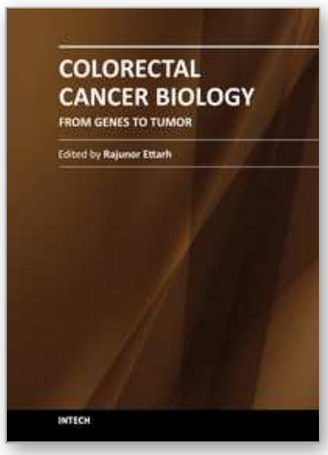

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Colorectal cancer is a common disease, affecting millions worldwide and represents a global health problem. Effective therapeutic solutions and control measures for the disease will come from the collective research efforts of clinicians and scientists worldwide. This book presents the current status of the strides being made to understand the fundamental scientific basis of colorectal cancer. It provides contributions from scientists, clinicians and investigators from 20 different countries. The four sections of this volume examine the evidence and data in relation to genes and various polymorphisms, tumor microenvironment and infections associated with colorectal cancer. An increasingly better appreciation of the complex inter-connected basic biology of colorectal cancer will translate into effective measures for management and treatment of the disease. Research scientists and investigators as well as clinicians searching for a good understanding of the disease will find this book useful.

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