Phospholipid Remodeling and Eicosanoid Signaling in Colon Cancer Cells

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Phospholipid remodeling and eicosanoid synthesis are central to lipid-based inflammatory reactions. Studies have revealed that membrane phospholipid remodeling by fatty acids through deacylation/reacylation reactions increases the risk of colorectal cancers (CRC) by allowing the cells to produce excess inflammatory eicosanoids, such as prostaglandins, thromboxanes and leukotrienes. Over the years, efforts have been made to understand the lipid remodeling pathways and to design anti-cancer drugs targeting the enzymes of eicosanoid biosynthesis. Here, we discuss the recent progress in phospholipid remodeling and eicosanoid biosynthesis in CRC.

Keywords: Colon cancer, Arachidonic acid, Lipid remodeling, Cyclooxygenase, Lipooxygenase, Prostaglandins, Leukotrienes

Introduction

Colorectal cancer (CRC) is prevalent in both developed and developing countries. Epidemiological studies indicate that the consumption of high-fat and high-calorie diets could be linked to CRC. In addition, genetic pre-disposition, such as Lynch syndrome (hereditary non-polyposis colorectal cancer) has also been implicated in inducing this deadly disease¹ ². In the U.S., however, the overall incidence of colon cancer is on the decline and it has been predicted that this disease will be reduced further, if healthier life-styles are followed and anti-cancer medications are made available to the general population³. While the mortality rates for colorectal and other cancers are decreasing in the U.S., for a large population in the developing countries they are on the rise. It is anticipated that the incidence of cancers, including colorectal, breast, lung, and prostate cancer will increase 60% by 2030 in those countries⁴. The incidences and survival rates of CRC in India are, however, low compared with those in other Asian countries, and this can probably be attributed to the consumption of a diet richer in vegetables and fruits⁵.

CRC Genes

Adenocarcinomas are the most common form of CRC. They are the cancerous tumors of the epithelial tissue and are differentiated from adenomas or polyps². The two subtypes of adenocarcinomas are the signet-ring-cell and mucinous-cell carcinomas. The signet-ring-cell carcinoma is considered to be an aggressive form of CRC and can be life threatening⁶. The mucinous adenocarcinoma is also considered an aggressive form of cancer and this may be partially due to its ability to spread rapidly⁷. As far as locations are concerned, the majority of colonic tumors are formed in the distal part of the colon⁸. The deficient-in-mismatch-repair (dMMR) colonic tumors preferentially localize in the proximal section⁹. Although the actual reason for this preferential
localization is not well-understood, it is hypothesized that this could be due to the presence of typical mismatch-repair (MMR) characteristics, such as chromosomal instability, loss of heterozygosity and Wnt/β-catenin signaling defects in colorectal cancers.

β-Catenin is a transcriptional co-activator that interacts with Wnt/β signaling pathway to regulate embryonic development and cellular homeostasis. The phenomenon of DNA methylation of CpG islands (CpG island is a GC-rich region of the genome) is common in sporadic MSI colonic tumors with CpG island methylation phenotype (CIMP). Abnormal DNA methylation of CpG islands affects the function and expression of tumor-suppressor genes. Interestingly, CIMP is not common in Lynch syndrome and it is more frequently localized in the proximal region of the colon, where it is linked to serine/threonine-protein kinase B-Raf (BRAF). CpG island methylation is an epigenetic phenomenon and is involved in the modulation of gene transcription in normal cells. However, in neoplasia hypermethylation, CpG islands induce unregulated transcription and alters the chromatin structure. Thus, it appears that MSI, CIMP and BRAF mutation and epigenetic events, although interlinked, do not belong to a consolidated pathway, which makes the entire process incredibly complex. More research is needed to provide understanding of this process.

### Fatty acid metabolism and CRC

Another important aspect of CRC is elevated fatty acid metabolism and eicosanoid biosynthesis. It has been demonstrated that the levels of stearic acid (C18:0), arachidonic acid (C20:4) and decosahexanoic acid (C22:6) increase significantly in CRC patients, although the study has a small sample, but results have been interesting and suggested that fatty acids play important roles in CRC. Furthermore, polymorphisms in fatty acid metabolic genes have been linked to CRC. In 2003, we reviewed the importance of arachidonic acid (AA) in CRCs. Since then, hundreds of papers have been published underscoring the involvement of fatty acids, diets and CRC. It is hypothesized that while long-chain ω-6 polyunsaturated fatty acids (PUFA; AA for example) are potential inducers of CRCs, ω-3 PUFA suppresses tumorigenesis by interacting with protein kinase CβII signaling pathways.

However, a recent epidemiological study suggests that the relationship between ω-6 and ω-3 fatty acid levels and CRC induction/prevention may depend on gender, locations of tumors (proximal or distal colon), and time of the follow-up study. The study has found that ω-3 PUFA could be linked with the formation of tumors in the distal colon in both men and women in the U.S. However, more follow-up studies are required to confirm this interesting
finding. A large-scale genome-wide study in East Asian population has identified new sets of CRC-linked genetic loci, including fatty acid-metabolism loci, indicating that fatty-acid metabolism could be key to CRC induction. Therefore, it is conceivable that the association between ω-6 and ω-3 PUFA is more complex than was thought previously and that it may work in concert with several other gene products and pathways of CRC described above.

**Phospholipid remodeling and eicosanoid synthesis**

Fatty acid metabolism and the synthesis of downstream inflammatory molecules are regulated in part by phospholipid remodeling reactions. In this reaction, newly acquired fatty acids are incorporated into phospholipids (PLs) or lysophospholipids (LPLs) of plasma membranes to generate a phosphoglyceride with a new acyl chain at the SN2 position of the glycerol (Fig. 1, compartment A). The enzymes, such as phospholipase A2 (PLA2) and acyltransferases (ATs) are important for carrying out the remodeling reaction. Lysophosphatidylcholine acyltransferase 1 (LPCAT1), the enzyme that transacylates lysophosphatidylcholine (LPC) into phosphatidylcholine (PC) is overexpressed significantly in CRCs, indicating a direct correlation between the transacylation reaction and tumorigenesis/malignancy. It is shown that the rate of AA incorporation in various PLs and its simultaneous release from arachidonoyl-PL increases significantly in activated human T cells.

AA is also distributed in various cellular PLs and this redistribution is proposed as being associated with CoA-dependent and -independent transacylation reactions. The transfer of AA from arachidonoyl-PL to other PLs, especially phosphatidylethanolamine has been observed in activated T cells, which could be connected with the cell division and proliferation. There are numerous reports in the literature that suggest the possible participation of PUFA and PUFA metabolic enzymes in the initiation and progression of CRC, and many of these studies indicate the direct involvement of PL remodeling reactions.

It can be assumed that PLA2 serves as a key regulator of PL remodeling. PLA2 cleaves fatty acid (usually AA) from the SN2 position of a PL which then functions as a precursor for various types of eicosanoids (illustrated in Fig. 1, compartment B). Based on the substrate preferences, co-factor requirements and genetic/structural uniqueness, PLA2 families can be divided into three major classes -

(i) small secreted PLA2 (sPLA2-Ca2+-dependent),
(ii) large cytosolic PLA2 (cPLA2-Ca2+-dependent),
and (iii) Ca2+-independent intracellular PLA2 (iPLA2) and platelet activating factor (PAF) secreted by...
PLA2 (PLA2G7)\textsuperscript{30}. sPLA2, cPLA2, and PAF-PLA2 are shown to be associated with CRC. For example, sPLA2 (PLA2G10), which preferably catalyzes phosphatidylcholine (PC) plays an important role in forming tumors in the colon by releasing various small-molecule lipid mediators\textsuperscript{31}. It is demonstrated earlier that AA released from arachidonoyl-PC by PLA2G10 undergoes cyclization by cyclooxygenase (COX) and lipoxygenase (LOX) pathways to produce small, inflammatory lipid molecules\textsuperscript{32}. Likewise, genetic polymorphisms of group 4A cPLA2\textsubscript{α}s result in phenotypic features similar to those seen in patients with FAP\textsuperscript{33}. Thus, cPLA2\textsubscript{α} may act as a modulator of the disease process. The deletion of PLA2 locus (plag2g4) in APC\textsuperscript{Min} mice is shown to reduce the formation of new tumors, when compared with APC\textsuperscript{Min/cPLA2\textsubscript{α}} mices\textsuperscript{34}.

The activation of colonic carcinoma cells HT29 by epidermal growth factor (EGF) upregulates cPLA2\textsubscript{α} activity and produces a significant increase in cell proliferation, indicating that this enzyme is responsible for the growth and proliferation of HT29 cells\textsuperscript{35}. It is also demonstrated that cPLA2\textsubscript{α}-mediated HT29 proliferation and the concomitant increase in prostaglandin E2 (PGE2) production involves PKA and PKB/Akt pathways\textsuperscript{35}. Similar to groups 4 and 10 PLA2\textsubscript{S}, PAF-PLA2 (PLA2G7) is also linked with CRC. Based on cellular and genetic studies, it is observed that the deletion of the plag2g7 gene reduces intestinal polyps through a mechanism that involves β-arrestin, Akt phosphatase, and intrinsic apoptotic pathway\textsuperscript{36}. In contrast to these three groups of PLA2\textsubscript{S}, the role of iPLA2 in CRC is still not clear and further studies are required to elucidate its role in the initiation and progression of colon cancers\textsuperscript{37}.

It has already been discussed above that PL remodeling by deacylation/reatylation reactions serves as a regulator of eicosanoid biosynthesis. AA or other PUFAs released from PLs (or PL-like molecules) by the action of a specific PLA2 serve as precursors of COX, LOX and cytochrome P450 enzymes to produce eicosanoids and other bioactive lipid mediators like hydroxyeicosatetraenoic acids (HETEs) and hydroperoxyeicosatetraenoic acids (HPETEs) (Fig. 1, compartment B). In addition to genetic predisposition, frequent inflammation in the colon is considered as a risk factor of CRC. Molecules like prostaglandins G2 (PGG2), H2 (PGH2), E2 (PGE2), D2 (PGD2) and F2\textalpha (PGF2\textalpha), prostaglandin I2 (PGI2) and thromboxane A2 (TXA2) are the products of two separate COX enzymes, i.e. COX-1 and -2 (Fig. 1B).

While COX-1 is expressed constitutively, COX-2 is an inducible enzyme. The expression of COX-2 increases in inflammatory, mitogenic and cancerous cells\textsuperscript{38}. PGs are secreted by the cells and act through autocrine and paracrine mechanisms via G-protein-coupled prostaglandin receptors (EPs). Eight membrane receptors have been identified so far, including EP1, EP2, EP3, and EP4 for PGE2, DP for PGD2, FP for PGF2, IP for PGI2, and TP for thromboxane A2. Reports suggest that expression and function of eicosanoid receptors are linked to CRC and that they play roles in polyp formation in the colon\textsuperscript{39}. COX-2 expression increases dramatically in CRC and acts through PKC and Ras-signaling pathway\textsuperscript{40,41}.

Using genetic knockout mice, it is demonstrated that COX-2 (but not COX-1) induces colorectal tumors and that suppressing its activity either genetically or chemically reduces tumor formation\textsuperscript{42}. The growth and motility of colon cancer cells is also reported to be dependent upon PGE2 production, one of the products of the COX-2 enzyme\textsuperscript{43}. PGE2 exerts its effect by activating phosphatidylinositol 3 kinase/protein kinase B (PI3K/PKB) pathway. Another product of the COX-1/COX-2 pathway is TXA2 and it has been observed that TXA2 can restore the migration of colonic carcinoma cells that have been inhibited by COX-2 inhibitors\textsuperscript{44}.

AA is also metabolized by 5-lipoxygenase (5-LOX) enzyme to synthesize leukotrienes (LTs), and this reaction is facilitated by 5-LOX activating factor or FLAP\textsuperscript{45}. There are several different types of LTs, which include leukotrienes A4 (LTA4), B4 (LTB4), C4 (LTC4), D4 (LTD4) and E4 (LTE4). LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4} are collectively known as cysteinyl leukotrienes (CysLTs) because of the presence of cysteine residues in the molecule. LTE\textsubscript{4} is the most stable of all of the CysLTs. Because LTA4 and LTB4 contain no cysteine, they are not considered as CysLTs\textsuperscript{46}. In addition to 5-LOX, there are also 12-LOX and 15-LOX enzymes, which facilitate the synthesis of 15-, 12- and 8-hydroperoxyeicosatetraenoic acids (15-, 12-, and 8-HPETEs). These short-lived, bioactive lipid mediators are quickly converted to LTs by dehydration reactions\textsuperscript{45}. 

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Interestingly, the 15-LOX activity declines in CRC, resulting in a reduction of apoptosis\(^\text{47}\). Several G-protein coupled receptor (GPCR) families of receptors function as LT or CysLT receptors that include CysLT1R, CysLT2R, BLT1, and BLT2. LOX activities increase several fold during the initiation and progression of colorectal and other cancers\(^\text{30}\). Increased expression of CysLT1R has been observed in cultured colon cancer cell lines, and the changes in the CysLT1R/CystLT2R ratio are linked to CRC\(^\text{45}\). Once the CysLT1R is activated by LTD4, CRC-linked proteins such as COX-2, β-Catenin and Bcl-2 are stimulated. In addition, CysLT1R activation also involves the participation of PI3K, glycogen synthase kinase 3β (GSK-3β), cAMP-response-binding protein (CREB), p90 ribosomal S6 kinase and PI3K-Rac pathways. These signaling molecules/pathways are associated with the survival, proliferation and migration of cancer cells\(^\text{45,49}\).

### Drugs targeting the remodeling/eicosanoid pathway

Aspirin (acetylsalicylic acid) is a common analgesic and anti-inflammatory drug. This non-steroidal anti-inflammatory drug (NSAID) was reported to use treat patients suffering from glycosuria and diabetes mellitus\(^\text{50}\). It has been found that aspirin is effective in reducing the pain of pancreatic and colonic cancer\(^\text{51}\) and studies conducted over the past forty years have indicated that NSAIDs, such as aspirin, sulindac, ibuprofen, celecoxib and indomethacin minimize the incidence of CRC by 40\%\(^\text{52}\). Effects of aspirin and other NSAIDs on risk and survival were conducted and it is observed that a daily intake of aspirin (75 mg) lowers the incidence of CRC in high-risk subjects. However, NSAID intake prior to the diagnosis of the disease has failed to yield any significant results\(^\text{53}\).

As far as the molecular mechanisms are concerned, aspirin and other NSAIDs can act through COX-dependent and-independent pathways and influence various signaling pathways, such as TGF-β-induced apoptotic pathways, sphingosine phosphatase and kinase-mediated angiogenesis, and cytokines/platelet-derived growth-factor-mediated cells\(^\text{54}\). The COX-independent actions of NSAIDs involve the NFkB family of molecules, which includes p50 and RelA (p65) heterodimer, I-kappaB, Wnt/B catenin signaling pathway, β-catenin destruction complex, tumor-promoting genes and proteins and cell cycle complex to mention a few.

Aspirin stimulates the cell cycle checkpoint proteins, allowing the cells to upregulate tumor suppression proteins\(^\text{54}\).

Similar to COX enzymes, the deactivation of 5-LOX activity also blocks cell proliferation by inducing apoptosis. The 5-LOX activity is associated with polyp formation and may direct myeloid-derived suppressor cells to the polyp site\(^\text{55}\). The inhibitors of LOX pathways are useful in treating asthma and arthritis. Curcumin, meclofenamate, auranofin, baicalein, caffeic acid, esculetin, L-655, 238, lycopodiene, NDGA, MK-886 and ziluton target LOX enzymes and have been shown to be effective in treating various cancers\(^\text{56}\). Anti-LOX drugs, such as pranlukast, zafirlukast and montelukast are also available and are useful for treating asthma patients. These drugs are CysT1R antagonists and are effective in reducing colonic tumor growth in experimental nude mice model\(^\text{55}\). Cyst LT1R antagonists such as ZM 198 and ZM 615 decrease the growth of colon cancer cells in culture. It is noted that the combined effects of LTR1 antagonists and COX-2 inhibitors are more effective in blocking cancer cell proliferation and inducing apoptosis\(^\text{56}\).

### Future studies

Future studies should be focused on identifying common genes and proteins that connect the remodeling network with other pathways. In this context, the recent development of genome-wide screening (GWS) and metabolomic study should yield conclusive results. In addition, the screening of small molecules and the development of non-toxic therapies need to be considered. Currently, there are many new drugs to treat CRCs, but they are not designed to target the COX/LOX pathways. It will be interesting to test, if NSAID molecules can be coupled to these new drugs to make cancer therapy more effective. For example, in the past, attempts were made to design nitric oxide (NO)-donating NSAIDs (NO-NSAID), which showed promising results in reducing the growth of cancer cells\(^\text{57}\).

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