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The roles of sPLA2-IIA (Pla2g2a) in cancer of the small and large intestine

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TABLE OF CONTENTS

1. ABSTRACT

The mouse secretory phospholipase A2 group IIA ($sPLA_2$-IIA) gene *Pla2g2a* has been identified as a susceptibility gene for cancer of the small and large intestine. Interestingly, unlike most previously identified tumor susceptibility genes, *Pla2g2a* does not behave like a classical oncogene or tumor suppressor gene. Hence, identification of its biological functions in tumor development may shed new light on general mechanisms that modulate colon cancer risk. So far, $sPLA_2$-IIA has been proposed to play a role in anti-bacterial defense, inflammation and eicosanoid generation, in clearance of apoptotic cells, and in the Wnt signaling pathway. More recently, comparison of RNA expression profiles of colon from *Pla2g2a*-transgenic to *Pla2g2a*-deficient mice confirmed and even extended $sPLA_2$-IIA's diverse biological effects. In this review we aim to summarize current knowledge about the various links of $sPLA_2$-IIA to cancer of the gastro-intestinal tract, and propose several models to illustrate its putative biological effects on tumor development.

2. INTRODUCTION

Genetic predisposition to sporadic cancer is determined by multiple genetic factors due to germline polymorphisms in tumor susceptibility genes, genes that are 'somehow' responsible for variation in cancer risk among the population (1,2). Inheritance of cancer risk remains largely unnoticed due to genetic heterogeneity of the human population, the plethora of tumor susceptibility genes, and incomplete penetrance of their effects. Familial cancer syndromes form an exception, as they are characterized by germline variation in a single cancer susceptibility gene with high penetrance towards the cancer phenotype, and therefore tend to behave as a monogenic trait. A number of these high-penetrance tumor susceptibility genes have been pinpointed onto the genome by linkage analysis studies and were eventually identified, like the *APC* tumor suppressor gene in the case of familial adenomatous polyposis (FAP) which predisposes to colorectal cancer (CRC)(3-5). Functional analysis of these high-penetrance susceptibility genes reveals valuable information about molecular pathways through which they stimulate tumor development, pathways that often are of general relevance to the development of non-familial, sporadic cancers. In the case of *APC*, such research revealed a direct link between the Wnt-signaling pathway and colon tumorigenesis (6-8). Hence, classical forward genetics research of cancer risk reveals genes that can be used as 'anchorpoints' to identify and investigate new molecular pathways that modulate tumor development.

2.1. *Pla2g2a* is a susceptibility gene for intestinal cancer

Identification of intermediate-penetrance and low-penetrance tumor susceptibility genes from the human population is hampered by its genetic heterogeneity. Mouse models of human cancer have proven to be a useful alternative to overcome this problem (9-14). $Apc^{Min/+}$mice carry a germline mutation in one of their *Apc* alleles and develop tumors throughout their intestinal tract, thereby resembling human FAP (15,16). On the genetic background of inbred strain C57BL/6 (B6), $Apc^{Min/+}$mice develop numerous (sometimes >100) tumors in their small and large intestines. However, when crossed back to several other inbred strains of mice because of genetic modifiers, this high tumor number dropped significantly (17). Linkage analysis using segregating crosses between inbred strains B6 and AKR revealed a locus on mouse chromosome 4 that was named *Mom1*, the modifier of $Apc^{Min/+}$-induced neoplasia-1 (18). One of the genes located within this genomic segment was the secretory phospholipase A_2 group IIA ($sPLA_2$-IIA) gene, *Pla2g2a*, which was proposed as the candidate gene for the *Mom1*-locus based on: 1) Presence of a polymorphism in *Pla2g2a*, causing one allele to produce a truncated $sPLA_2$-IIA protein, whereas the other allele is fully functional (19); 2) The strain distribution pattern, showing that inbred strains susceptible to intestinal cancer inherited the truncated form of $sPLA_2$-IIA (C57BL/6, BTBR, 129Sv, A/J, P/J), whereas all non-susceptible strains (AKR, SWR, MA, CAST, DBA, Balb/c, C3H) inherited the functional *Pla2g2a* allele; and 3) localization of its expression to Paneth cells in the crypts of the small intestine (20). Final proof that *Pla2g2a* is indeed a susceptibility gene for cancer of the small and large intestine was obtained when the functional AKR-derived *Pla2g2a* allele was introduced as a transgene onto the B6-$Apc^{Min/+}$ background, and was shown to strongly reduce tumor multiplicity (21,22).

2.2. $sPLA_2$-IIA, an unexpected suspect

High-penetrance cancer susceptibility genes that were identified in familial cancer syndromes like FAP (*APC*), hereditary non-polyposis colorectal cancer (*MLH1*, *MSH2*, *MSH6*), familial breast cancer (*BRCA1*, *BRCA2*), retinoblastoma (*RB*), and Li-Fraumeni syndrome (P53) represent germline alterations in tumor suppressor genes that are also frequently mutated in sporadic cancer (3). The main effect of *Pla2g2a* on mouse intestinal cancer risk is also relatively large. Among the loci that were suspected to influence $Apc^{Min/+}$-induced intestinal cancer risk, *Mom1* exhibited by far the largest effects and therefore has been described as a 'major' factor (20). Similarly, a study using genome-wide linkage analysis to investigate susceptibility to carcinogen-induced tumors of the small intestine also revealed only one locus with a significant effect, susceptibility to small intestinal cancer-1 (*ssic1*), which co-localized with the *Mom1* region (23). Compared to the numerous tumor susceptibility genes for colon, lung, and skin cancer whose main effects are partly or completely masked by their involvement in epistatic genetic interactions (10,24-27), the main effect of *Pla2g2a* on intestinal cancer susceptibility is quite impressive. Moreover, the fact that the human *PLA2G2A* gene is located on chromosome 1p35-1p36 (28), a genomic region that is frequently deleted in CRC and has been correlated with poor prognosis and metastasis (29-34), raised the expectation that *PLA2G2A* might be a tumor suppressor gene that is frequently mutated in human sporadic cancer. However, careful analysis of the *PLA2G2A* gene in many human CRC cell lines and colon tumor samples revealed little to no evidence that it acquired any somatic mutations (35-38). Also, from a functional point of view, *Pla2g2a* is unlikely to act as a classical tumor suppressor gene. In general, mutations in oncogenes and tumor suppressor genes must exhibit cell-autonomous effects to drive clonal expansion of cells in which these mutations occurred. $sPLA_2$-IIA is a *secretory* protein that is much more likely to have paracrine effects than autocrine cell-autonomous effects. In other words, incidental somatic mutations in *Pla2g2a* are unlikely to trigger tumorigenesis, while germline variation that affects its function in all cells may dramatically alter the (micro)environment in which cancer initiation and progression occurs. Hence, $sPLA_2$-IIA defines a promising 'anchorpoint' to identify and investigate new (oncogene- and tumor suppressor gene-independent) molecular pathways that modulate tumorigenesis in the mammalian intestine.

2.3. Aim of this review

Biochemical studies of phospholipase A_2 (PLA_2) enzymes have been performed for over 40 years, and have been published in more than 15,000 papers. Yet, despite this wealth of information the exact mechanism(s) by which $sPLA_2$-IIA modulates cancer risk remains unclear. In this review, we aim to summarize some of the biological

mechanisms in which $sPLA_2$-IIA is involved, and to propose several models to indicate the putative effects of $sPLA_2$-IIA in development of cancer of the gastro-intestinal tract. We will propose that the overall context of specific gene expression (cell and tissue type, developmental stage, presence or absence of inflammatory stimulants such as flora, signaling from neighboring cells, expression of co-factors, type and stage of disease, etc.) significantly contribute to whether a molecule such as $sPLA_2$-IIA acts anti- or pro-inflammatory, anti- or pro-apoptotic, or anti- or pro-oncogenic. In the gastro-intestinal (GI) tract, there are many examples of factors that may act in either a protective or damaging fashion in specific stages of disease pathogenesis. In addition to $sPLA_2$-IIA, cytosolic PLA_2 ($cPLA_2$), various peroxisome proliferator-activated receptors (PPARs), transforming growth factor–beta (TGF-beta) and even cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE_2) act in this manner. Understanding the complexity of gene action is important as we discuss the activities of $sPLA_2$-IIA and controversies surrounding its role in cancer.

3. $sPLA_2$-IIA FUNCTION

The first studies of mammalian PLA_2 started around 1967, and investigated the 'pancreatic PLA_2' enzyme that was isolated from the pancreatic juice of rats, dogs, pigs and humans. Around 1986, the first non-pancreatic mammalian PLA_2 enzyme was discovered, which was isolated from platelets and from the synovial fluid of rheumatoid arthritis. By now, the superfamily of PLA_2 genes consists of at least 34 members and includes five distinct types of enzymes (families), which have been classified into 15 groups and many subgroups in a systematic manner (39). At present, 'pancreatic PLA_2' is referred to as PLA_2 group IB, and 'platelet PLA_2' or 'synovial fluid PLA_2' as group IIA-PLA_2, gIIA-PLA_2, PLA2G2A or $sPLA_2$-IIA . We will refer to it as $sPLA_2$-IIA. The one aspect that all PLA_2 enzymes have in common is their enzymatic phospholipase activity, *i.e.,* the ability to catalyze hydrolysis at the *sn-2* position of membrane glycerophospholipids resulting in the release of free fatty acids and lysophospholipids. The main characteristics that discriminate them into distinct PLA_2 families are their calcium requirement for optimal enzymatic activity, sequence homology of the catalytic site, and intra- or extra-cellular mode of action. Secretory PLA_2-IIA belongs to a family of more than 10 mammalian secretory PLA_2s ($sPLA_2$) that require mM amounts of Ca^{2+}. Group IV PLA_2 enzymes belong to the family of cytosolic PLA_2s ($cPLA_2$) that require μM amounts of Ca^{2+}, whereas the family of group VI PLA_2s or Ca^{2+}-independent PLA_2s ($iPLA_2$) require no calcium for their enzymatic activity. Moreover, there are the families of group VII and group VIII platelet-activating factor acetylhydrolases (PAF-AH) and the group XV lysosomal PLA_2. For an extensive overview of the characteristics of the different PLA_2 families we refer to a review by Kudo and Murakami (40). Here, we focus on $sPLA_2$-IIA and briefly mention group IV $cPLA_2$ ($cPLA_2$-IV, $cPLA_2$-IV alpha), which we will refer to hereafter as $cPLA_2$.

3.1. Biochemical properties and biological activities of $sPLA_2$-IIA

3.1.1. Enzymatic Activities

Secretory PLA_2 group IIA belongs to the subfamily of group II $sPLA_2$s, comprising PLA_2 group IIA, IIC, IID, IIE, IIF, and PLA_2 group V. All of these enzymes are encoded by a cluster of highly homologous genes located within a ~250kb genomic segment on human chromosome 1p36, and on its homologous region on mouse distal chromosome 4. They have similar molecular weights, ranging from 14-16 kD. One important feature that discriminates $sPLA_2$-IIA from other group II PLA_2 family members is its highly cationic nature, which allows it to bind tightly to anionic heparanoids such as heparin and heparan sulphate proteoglycans (HPSGs). As such, a large proportion of $sPLA_2$-IIA protein will stick to cell surface molecules, as has been observed for recombinant PLA_2 transfected into HEK293 cells (40). When bound to glycerophosphatidylinositol-anchored HSPGs such as glypicans, $sPLA_2$-IIA is transferred to punctate compartments containing caveolin (41). Enzymatically, the cationic nature of the $sPLA_2$-IIA protein (pI > 10.5) causes it to be much more effective in hydrolyzing fatty acids from negatively charged lipid membranes containing anionic phospholipids like phosphatidylserine (PS) than in releasing fatty acids from uncharged lipid membranes (42,43). The outer leaflets of unperturbed mammalian cells are characterized by neutral lipid composition enriched in phosphatidylcholines, sphingomyelin and cholesterol, thus unperturbed cells are poor substrates for extracellular $sPLA_2$-IIA and thereby prevent indiscriminate hydrolysis of healthy cells (42). Consistent with this idea, healthy mammalian cells are highly resistant to exogenous $sPLA_2$-IIA, requiring very high concentration of the protein to elicit arachidonic acid release (41). Perturbation of cell membranes by phospholipid scramblase that alters membrane symmetry increases $sPLA_2$-IIA activity and arachidonic acid release (44). Secretory PLA_2-IIA does not exhibit a strict selectivity for the types of fatty acids that are being released, but arachidonic acid is certainly one of its major hydrolytic products. Arachidonic acid can be processed by cyclooxygenases (COX's) and lipoxygenases (LOX's) to form eicosanoids including prostaglandins and leukotrienes. As a group, eicosanoids are important mediators of inflammation (45). Besides their enzymatic activity, $sPLA_2$-IIA and several other $sPLA_2$s can also function as ligands for PLA_2-binding proteins, of which the M-type PLA_2 receptor has been best characterized (46,47).

3.1.2. $sPLA_2$-IIA Expression Patterns

Secretory PLA_2-IIA has been extensively studied in human tissues where its activity is associated with inflammation, host defense against bacteria, blood coagulation and atherosclerosis (48). Human $sPLA_2$-IIA is expressed in prostate epithelial cells, coronary vascular smooth muscle cells, kidney uriniferous tubular epithelium, respiratory epithelial cells, pulmonary arterial smooth muscle cells, placenta, hepatocytes, stomach, small and large intestine, spleen, thymus, tonsil, parotid and lacrine glands, cartilage and bone marrow, seminal plasma, tears, platelets, neutrophils, eosinophils, mast cells, macrophages,

and liver Kupfer cells (40,49-53). In contrast, the range of $sPLA_2$-IIA tissue expression in mice is far more limited. Mouse $sPLA_2$-IIA has been shown to be strongly expressed in intestine, very weakly expressed in prostate, and moderately expressed in mouse skin (54-57). Expression in other mouse tissues and cells such as mast cells and macrophages has been controversial (58), but the preponderance of evidence is that murine $sPLA_2$-IIA is expressed in both mast cells and macrophages, and probably other cells as well.

3.1.3. Inducers and downstream effectors of $sPLA_2$-IIA

Factors that stimulate $sPLA_2$-IIA include bacterial lipopolysaccarides (LPS), interleukin (IL)-1beta, IL-6, tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, phorbol esters, and cyclic AMP (cAMP) elevating agents (40,59). The $sPLA_2$-IIA promoter has TATA and CAAT boxes and binding sites for AP-1, C/EBPs, CREB, NF-kappaB, STAT, PPAR-gamma, LXR/RXR heterodimers and NFATs (60-63). In some contexts $sPLA_2$-IIA activation is dependent upon prior activation by $cPLA_2$ and 12/15-LOX (discussed below). Factors that inhibit $sPLA_2$-IIA include TGF-beta, IL-10, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and insulin growth factor (IGF) (40,59). Some of the molecules and pathways that act downstream of $sPLA_2$-IIA include ERK1/2 (64), inducible nitric oxide synthase (iNOS) in RAW264.7 macrophages (65), EGF, based on its ability to increase activation of EGF receptor (EGFR) in A431 cells (54,55), MUC16 in human conjunctive epithelium (66), and in human microvascular cells $sPLA_2$-IIA induces NF-kappaB, intercellular adhesion molecule (ICAM)-1, IL-8, epithelial derived neutrophil activating peptide (ENA)-78/CXCL5 and growth regulated protein Gro-alpha/CXCL1 (67).

3.1.4. $sPLA_2$-IIA's enhancing factor and phospholipase functions lie in separate domains

Secretory PLA_2-IIA binds to the 180 kDa M-type receptor, a cell surface protein that may be involved in important physiological functions that are mediated by the binding of PLA_2's and other ligands (46,47,68). The M-type receptor is expressed in the colon (47), thus these receptors may have some importance in $sPLA_2$-IIA's functions in intestinal tumorigenesis. Accordingly, there are reports that some functions of $sPLA_2$-IIA do not rely on its hydrolytic activity (69), and that even arachidonic acid release can result solely from binding of $sPLA_2$-IIA to its M-type cell surface receptor (70). Catalytically-inactive $sPLA_2$-IIA mutants which were unable to cause the release of arachidonic acid from cytokine-primed mast cells still retained the ability to enhance COX-2 expression (52). Furthermore, the ability of $sPLA_2$-IIA to enhance EGFR activation in A431 cells is located in a separate amino terminal domain from the distal phospholipase domain (71,72), although it is notable that other $sPLA_2$'s can inhibit EGF induced receptor activation in A431 cells (73). Other reported $sPLA_2$-IIA activities that are likely to be independent of its enzymatic activity are the induction of iNOS in murine macrophages (65) and the release of cytokines from human monocytes, lung macrophages and eosinophils (74-77).

3.1.5. Interactions between $sPLA_2$-IIA and $cPLA_2$

Cytosolic PLA_2 is a 85 kDa serine esterase that is widely expressed in mammals, except in lymphocytes (43,60). There is substantial evidence that $sPLA_2$-IIA and $cPLA_2$ interact in complex pathways that result in arachidonic acid release, COX-2 activation, and eicosanoid production but the context (species, tissue and cell type, presence of requisite cofactors, nature of stimulatory agent, and disease state) appears to govern the existence and direction of these interactions. Genetic knockouts of these genes and PLA_2-specific inhibitors have provided crucial evidence regarding the role of specific PLA_2 proteins in different cell types in mice, and potentially inform about their function in human tissues. For instance, although both $sPLA_2$ and $cPLA_2$ enzymes have been implicated in arachidonic acid release for eicosanoid generation, studies using $cPLA_2$-IValpha knockout mice showed that this particular PLA_2 enzyme is crucial for the release of arachidonic acid for prostaglandin and leukotriene production (78-81). Nevertheless, hydrogen peroxide-induced arachidonic acid release by murine mesangial cells is mediated by crosstalk between $sPLA_2$-IIA and $cPLA_2$, where $sPLA_2$-IIA activates $cPLA_2$ causing the release of arachidonic acid (82). Secretory PLA_2 also activates $cPLA_2$ in mouse mast cells (70), and in human 1321N1 astrocytoma cells (83). In the other direction, $sPLA_2$-IIA is under the control of $cPLA_2$ and 12/15-LOX in rat fibroblastic 3Y1 cells (84) and in mouse $P388D_1$ macrophages. However, $sPLA_2$-IIA and $cPLA_2$ expression are not per definition always connected. For example, $cPLA_2$ but not $sPLA_2$-IIA induces COX-2 and IL-8 in response to EGF and other stimulants in human A549 lung epithelial cells, a response that is mediated by PPAR-gamma (85). Cytosolic PLA_2 seems to be solely responsible for the induction of CD36 by IL-13 in human monocytes, a pathway also mediated by PPAR-gamma (86). Cytosolic PLA_2 has also been identified to be responsible for the inhibition of macrophage clearance of apoptotic cells (87). In the GI tract, there are similarities and differences in the actions of $sPLA_2$-IIA and $cPLA_2$. Secretory PLA_2-IIA appears to play a dominant role in gastric cells in terms of prostaglandin production, wound repair, defense against pathogens, and protection against gastric cancer progression (49,88,89). In the small intestine $sPLA_2$-IIA is expressed by Paneth cells from where it is secreted into the crypt lumen, allowing $sPLA_2$-IIA to play a bactericidal role that is unavailable to $cPLA_2$ (90,91).

3.1.6. Interactions between $sPLA_2$-IIA and PPARs

As discussed before, the (inter)actions of $sPLA_2$-IIA and $cPLA_2$ can result in generation of fatty acids and eicosanoids, including leukotriene B4 (LTB_4) and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) which may function as ligands for peroxisome proliferator-activated receptors (PPARs) (92-96). PPARs protect the GI tract from inflammation and neoplasia. Mutations in the PPAR-gamma gene have been identified in human colorectal cancers (97), and the intestinal-specific ablation of PPAR-gamma was shown to enhance tumorigenesis in *Apc*$^{Min/+}$ mice (98). Ligands of PPAR-gamma promote growth arrest and apoptosis in human colon cancer cells (99,100). Similar ligands protect against experimentally-induced colitis in

rodents (101,102), and PPAR-gamma ligands have proven their therapeutic value in treatment of human inflammatory bowel disease (IBD) (103,104), a finding that is consistent with the reduction in PPAR-gamma expression observed in IBD patients (105). PPAR-alpha ligands slow the growth of human colon cancer cells, a phenotype associated with the inhibition of COX-2 (106). PPAR-alpha ligands also significantly reduce intestinal tumor multiplicity in *Apc*$^{Min/+}$ mice (107). As mentioned previously, sPLA$_2$-IIA produces a number of downstream effectors that can bind and activate PPARs. In turn, the sPLA$_2$-IIA promoter contains a PPAR response element (PPRE), thus there is reason to believe that PPARs and sPLA$_2$-IIA interact in signaling pathways (108). For example, TNF-alpha induces sPLA$_2$-IIA in mesangial cells, via an autocrine loop involving sPLA$_2$-IIA and PPAR-alpha activation (109). PPAR-alpha also controls Paneth cell differentiation, and thus indirectly controls sPLA$_2$-IIA activity, via interaction with the Hedgehog signaling pathway (110). We observed that sPLA$_s$-IIA regulates the expression of several genes that are targets of PPARs. Secretory PLA$_2$-IIA upregulates *Dscr1*, a tumor suppressor gene that is induced by PPAR-gamma where it is involved in a regulatory loop with nuclear factor of activated T cells (NFAT) molecules. In turn, NFATs can bind the sPLA$_2$-IIA promoter and regulate its transcription (111,112).

3.1.7. sPLA$_2$-IIA functional complexity and experimental challenges

Despite apparently straightforward results obtained from defined 'simple' models whereby COX's and PGE$_2$ are "pro-inflammatory" and "oncogenic" and PPARs such as PPAR-gamma are "anti-inflammatory" and "anti-oncogenic", the signaling pathways that connect sPLA$_2$-IIA to cPLA$_2$, COXs, LOXs, and PPARs may constitute complex feedback, cell-type specific mechanisms that are difficult to interpret. Depending on the cell and tissue type, PPAR-gamma can either repress or induce expression of COX-2, in cooperation with sPLA$_2$-IIA or cPLA$_2$. In rat vascular smooth muscle cells PPAR-gamma synergized with IL-1beta to induce COX-2, several prostaglandins (PGE$_2$, PGD$_2$, PGI$_2$) and sPLA$_2$-IIA (113). PPAR-gamma agonists induce COX-2 and PGE$_2$ in carcinogen-treated human epidermoid carcinoma KB cells (114) and both n-3 and n-6 polyunsaturated fatty acids (PUFAs) induce COX-2 and PGE$_2$ expression via PPAR-gamma activation in human keratinocytes (115). It has even been proposed that overexpression of COX-2 in breast cancer is a favorable prognostic marker because of its co-expression with PPAR-gamma (116). In contrast, in the TNBS model of IBD in rats, rosiglitazone, a PPAR-gamma agonist, ameliorated chronic inflammation, a phenotype associated with upregulation of PGE$_2$ but the down-regulation of COX-2 (117). PPAR-gamma also cooperates with cPLA$_2$ in promotion of mitogenesis in human liver cancer cells (118) and attenuates colonic inflammation in the intestinal epithelia via down-regulation of the Toll-like receptor-4 (TLR4) and COX-2 (119). Finally, in macrophages, statins, which can activate sPLA$_2$-IIA, activate PPAR-gamma through an ERK 1/2 and p38 MAPK-dependent, COX-2 dependent pathway (120), and all of these molecules are known downstream effectors of sPLA$_2$-IIA as well. In addition, we note that there are many reports that PPAR-gamma can also inhibit COX-2 and PGE$_2$ but in their entirety the literature supports a very complex relationship between PPARs, PLA$_2$ and their downstream mediators.

Finally, we note the deficiencies in some previous studies of sPLA$_2$-IIA and other sPLA$_2$s that have employed enzymatic assays, antibodies, antisense RNAs and pharmacological inhibitors to evaluate the presence and function of sPLA$_2$-IIA in human and mouse tissues and disease pathologies. Prior to the late 1990's, many of these methodologies mistakenly detected and targeted multiple sPLA$_2$ family members (or even cPLA$_2$ enzymes). Given the high homology between PLA$_2$s, these errors are not surprising. In some cases, it was later determined that the reported expression of sPLA$_2$-IIA in a certain tissue actually was sPLA$_2$-V or that the source of a particular prostaglandin was actually cPLA$_2$. This problem is also true for supposed sPLA$_2$-IIA-specific chemical inhibitors. In more recent years, with the use of genetic mouse models, the role of sPLA$_2$-IIA in specific pathologies has begun to become clearer although significant questions remain.

3.2. sPLA$_2$-IIA has potent bactericidal activity

Host-bacterial mutualism in the gastrointestinal tract is critical to human health (121,122). When homeostastic processes that govern host-bacterial interactions breakdown, our normal bacterial flora can cause chronic inflammation that significantly elevates the risk for human gastric and colorectal cancers (123-125). In several mouse models of inflammatory intestinal cancer (such as the IL-10 and TGF-beta1 knockout mice) transfer of animals to germ-free conditions substantially or completely eliminated the incidence of inflammation and cancer (126-128). Thus, it is proposed that dysregulation of host responses to normal bacterial flora underlie susceptibility to some inflammatory GI cancers. In addition, infectious bacteria such as *Streptococcus bovis* also promote colon carcinogenesis in humans and rodent models (129,130). The management of normal enteric and infectious bacterial flora by sPLA$_2$-IIA in prevention of inflammatory disease in both mice and humans is well-documented. Secretory PLA$_2$-IIA is stored in secretory granules of platelets, neutrophils, mast cells, gastric cells, goblet cells and Paneth cells, and is also expressed by macrophages (22,131-137). All these types of cells have a common function in the defense against microbacteria. Moreover, high levels of sPLA$_2$-IIA have been found in human tears (138). The positively charged residues on the surface of the sPLA$_2$-IIA protein allow it to penetrate through the negatively charged cell wall of Gram-positive bacteria, where its enzymatic activity is responsible for membrane phospholipid degradation and ultimately bacterial killing (42,139-141). Overexpression of human sPLA$_2$-IIA in transgenic mice results in decreased mortality in experimental *Staphylococcus aureus* infection and improved clearance of bacteria from organs and body fluids (142), and provides protection against *Bacillus anthracis* infection (143). Hence, enzymatic activity of sPLA$_2$-IIA forms a first line of defense against Gram-positive bacteria

such as *Bacillus*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Clostridium* (144,145).

Gram-negative bacteria are coated with LPS. Secretory PLA_2-IIA exhibits only weak direct bactericidal activity against Gram-negative bacteria like *Escherichia coli* (146). Nevertheless, upon experimental *E. coli* infection, mice transgenic for $sPLA_2$-IIA showed lower rates of mortality and less bacterial growth in tissues than their $sPLA_2$-deficient B6 littermates (147), indicating that $sPLA_2$-IIA does play a role in defense against Gram-negative bacteria. In humans, chronic gastritis caused by the Gram-negative bacterium *Helicobacter pylori* is a strong risk factor for gastric cancer (148) where *H. pylori* has been shown to activate beta-catenin (149). Human $sPLA_2$-IIA is bactericidal against *H. pylori in vitro* (150) but *H. pylori*'s effect is modeled *in vivo* in the stomach of mice via the use of the related species *H. felis*. Infection of C57BL/6 mice with *H. felis* causes severe gastritis that eventually results in neoplastic development (151). A key early event in this gastritis is the breakdown of the epithelial phospholipid barrier (152). In several *H. felis* studies, comparison of inbred mouse strains that expressed either a wildtype or mutant allele of $sPLA_2$-IIA indicated that expression of $sPLA_2$-IIA provided resistance to both inflammation and neoplastic development (153). The protective role of $sPLA_2$-IIA was further demonstrated by another study in which the expression of a human transgene in C57BL/6 mice prevented infection with *H. felis* (154). In another study, Balb/c mice that express a wildtype $sPLA_2$-IIA gene were shown to sharply upregulate $sPLA_2$-IIA in the stomach upon challenge with *H. felis*, resulting in rapid resolution of the inflammation (155). These effects of $sPLA_2$-IIA on Gram-negative pathogens are probably due to receptor-mediated activity rather than to direct bactericidal activity. Knockout mice that lack the M-type PLA_2 receptor *Pla2r1* were demonstrated to be more resistant to LPS-induced endotoxic shock than their wild-type littermates (156). Hence, $sPLA_2$-IIA and the PLA2R1 receptor are likely involved in the innate immune response against Gram-negative bacteria. Finally, in another study, C57BL/6-*Apc*$^{Min/+}$ mice ($sPLA_2$-IIA-negative) developed three-fold more colon tumors after infection with the naturally occurring Gram-negative bacterium *Citrobacter rodentium* (157). This study confirmed the oncogenic role of bacterial flora in colon carcinogenesis and emphasized the action of bactericidal agents such as $sPLA_2$-IIA in management of gut flora to protect against tumorigenesis.

3.3. $sPLA_2$-IIA is involved in inflammation

Since the identification of $sPLA_2$-IIA from synovial fluid of patients with rheumatoid arthritis (158), high levels of $sPLA_2$-IIA have been detected in many inflammatory, autoimmune, and allergic disease like acute pancreatitis, septic shock, adult respiratory distress syndrome, Crohn's disease, ulcerative colitis, bronchial asthma, and allergic rhinitis (159). Serum levels of $sPLA_2$-IIA can serve as an index of disease activity in rheumatoid arthritis (160). Moreover, expression of $sPLA_2$-IIA by inflammatory cells is induced by pro-inflammatory stimuli like LPS, TNF-alpha, IL-1beta, IL-6, and IFN-gamma, and strongly repressed by anti-inflammatory glucocorticoids (40,161), indicating that expression of $sPLA_2$-IIA is strongly associated with, and regulated by inflammatory responses.

3.3.1. $sPLA_2$-IIA, a pro-inflammatory factor

As already mentioned, the enzymatic activity of PLA_2 enzymes results in the generation of bioactive molecules such as lysophospholipids and free fatty acids, of which the release of arachidonic acid is of particular interest because it is the first and rate-limiting step in the generation of eicosanoids. Free arachidonic acid is metabolized by COXs and LOXs into various prostaglandins and leukotrienes, respectively, bioactive molecules that have a wide range of effects, including modulation of inflammatory responses (45,162). Although $cPLA_2$ enzymes have been implicated in the release of arachidonic acid specifically for eicosanoid generation (78-81), there is functional coupling between $sPLA_2$s and $cPLA_2$. Secretory PLA_2-IIA and $sPLA_2$-V have been demonstrated to enhance eicosanoid generation, *e.g.,* by stimulating expression of inducible COX-2 in conjunction with other stimuli (44,163-166). COX-2 (also known as prostaglandin H synthase-2) converts arachidonic acid to PGH_2, the immediate substrate for a variety of prostaglandin and thromboxane synthases (45). In the GI tract, COX-2 is best known for its promotion of inflammation, proliferation of cancer cells and resistance to apoptosis (167-171). Although the mechanisms through which $sPLA_2$s stimulate COX-2 induction have not been resolved completely, $sPLA_2$-mediated generation of lysophosphatidylcholine (lyso-PC) may play a role in this process (172). In addition, lyso-PC has been demonstrated to be a chemoattractant for monocytes, suggesting that $sPLA_2$s mediate the influx of inflammatory cells during inflammation (173). Moreover, $sPLA_2$-IIA and several other $sPLA_2$s can also function as ligands for PLA_2-binding proteins like the M-type PLA_2 receptor PLA2R1 (46,47). This receptor is expressed by various inflammatory cells like neutrophils and macrophages. Stimulation of the PLA2R1 by $sPLA_2$s induces activation of signal transduction pathways involving p38 MAPK, ERK1/2, phosphatidylinositol 3-kinase (PI3K) and Akt, and results in the induction of iNOS and the production of various cytokines that mediate inflammatory responses against bacterial infections (64,65,75,77).

Secretory PLA_2-IIA is implicated in the development of inflammatory bowel disease (IBD) in both humans and rodents. In humans, its mRNA and protein expression is upregulated in both ulcerative colitis and Crohn's Disease, with an increase in expression detected in the colonic mucosa, in the intestinal submucosa in mast cells and macrophages, and in the serum of IBD patients (137,174-178). In rodents, inhibitors of $sPLA_2$-IIA protect rats from TNBS-induced colitis (179); mouse $sPLA_2$-IIA is upregulated in the colonic mucosa following treatment with azoxymethane and dextran sulfate sodium (DSS) (180) and $sPLA_2$-IIA is a candidate for a quantitative trait locus (QTL) for experimental IBD (181). All together, these data indicate that $sPLA_2$-IIA modulates inflammatory responses by attracting inflammatory cells and by stimulating the

production of various mediators of inflammation, both through its enzymatic and its receptor-mediated actions.

3.3.2. $sPLA_2$-IIA, an anti-inflammatory factor

As we have discussed, $sPLA_2$-IIA is implicated in inflammatory, presumably damage-inducing processes, but can it also play a protective function? There is direct and indirect evidence that this may be the case, and we will argue that this protective role may underlie its resistance to intestinal cancer. First, in mice we can directly compare the phenotypes of inbred strains that are naturally mutant or wildtype for the *Pla2g2a* allele. Several studies of gastric inflammation employing mice that expressed a wildtype allele of *Pla2g2a*, either as an endogenous copy or a transgene, clearly demonstrated that $sPLA_2$-IIA^+ mice were resistant to bacterially-induced gastric inflammation in comparison with C57BL/6 mice that expressed a mutant copy of *Pla2g2a*. Notably, in the DSS model of experimental IBD, Melgar and colleagues treated C57BL/6 and Balb/c mice ($sPLA_2$-IIA wildtype) with DSS and observed that the C57BL/6 mice progressed to chronic inflammation while the Balb/c mice significantly upregulated production of PGE_2, down-regulated expression of a great number of inflammatory cytokines and rapidly resolved the DSS-induced inflammation (182). This study indicated that PGE_2 was protective against chronic inflammation, rather than a promoter of inflammation. Several other studies support this conclusion. DSS treatment of knockout mice that lack the EP4 PGE_2 receptor caused a more severe colitis, greater mucosal damage, and enhanced proliferation of inflammatory $CD4^+$ T cells when compared with EP4 wildtype mice (183). In rats, rectal injection of PGE_2 following treatment with DSS caused the inhibition of tissue damage and the down-regulation of inflammatory cytokines (184). This finding was in agreement with the low levels of PGE_2 observed upon enterocolitis relapse in the Lewis rat strain that is susceptible to relapse (185). PGE_2 can also suppress Th_1 cytokine production (IL-12, IFN-gamma) in intestinal macrophages, thereby helping to maintain resident macrophages in an anti-inflammatory mode (186,187). Indeed, COX-2 may have anti-inflammatory properties (188), and a number of studies suggest that inhibition of COX-2 and PGE_2 can actually exacerbate IBD in mice. For example, in the IL-10 knockout model of colitis and inflammatory colon cancer, treatment of IL-10 knockout mice with the COX-2 selective inhibitors celecoxib and rofecoxib greatly increased the incidence of colitis (189). Similarly, anti-inflammatory n-3 polyunsaturated fatty acids obtained from fish oil that can reduce intestinal tumorigenesis in mouse models (190-192), also significantly enhanced colitis in the IL-10 knockout mouse in comparison with mice fed n-6 fatty acids (193). Thus, the specific context is a major determinant of whether a molecule acts in an inflammatory or anti-inflammatory fashion and this is clearly true for PGE_2. C57BL/6 mice that overexpress a wildtype $sPLA_2$-IIA transgene (a concatemer of 9 transgene copies derived from the AKR strain) that is driven by its endogenous promoter do not develop any spontaneous inflammatory phenotypes. We have worked with this transgenic strain for more than 10 years and we have not observed (under normal husbandry conditions) any phenotypic differences between transgenic mice and their B6 littermates. Moreover, we have recently observed that expression of this transgene prevents both IBD and related carcinogenesis in the C57BL/6-IL-10 knockout model, in Muc2 knockout mice, and in both spontaneous and induced IBD in $Apc^{Min/+}$ mice (unpublished observations). Previous reviews have also proposed that $sPLA_2$-IIA does not play a significant role in inflammatory processes in mice (39,58,194,195), with the single exception of skin inflammation observed in B6 mice that expressed a human $sPLA_2$-IIA transgene driven by a human gene promoter. Indeed, experiments with supposedly $sPLA_2$-IIA-specific chemical inhibitors that ameliorate inflammation in C57BL/6 mice suggest that other enzymes are the target of inhibition (194).

How might the activities of $sPLA_2$-IIA prevent or resolve inflammation in the GI tract? We have already discussed the role of normal bacterial flora in the development of colonic inflammation and inflammation-dependent and independent intestinal cancer, so it is quite likely that bactericidal factors such as $sPLA_2$-IIA that are active in the intestinal crypt lumen may play an important role in management of gut flora, especially in the context of mucosal damage. Eicosanoids that are produced downstream of $sPLA_2$-IIA are involved in both the initiation and resolution stages of inflammation and wound repair. This biphasic response involves multiple interacting factors that include PGE_2, PGD_2, PGJ_2, plus the level of immune cell activation (52,195-197). This was demonstrated in a carrageenin-induced pleurisy model in rats where $sPLA_2$-IIA was maximally produced in the resolution stage where it was associated with production of PGD_2 and PGI_2. $sPLA_2$-IIA has been associated with upregulation of lipoxin A4, a suppressor of leukocyte function that reduces inflammation and leukocyte infiltration, and platelet activating factor (PAF), a molecule that enhances macrophage phagocytosis of apoptotic cells (196). Recent studies of sepsis also call into question a pro-inflammatory role for $sPLA_2$-IIA. In human clinical trials, $sPLA_2$-IIA inhibition produced a negative survival trend in sepsis patients. Sepsis has traditionally been treated as an inflammatory disease, but very recent work indicates that elevated levels of inflammatory cytokines actually improve clinical outcome. Consistent with this observation studies in rodent models of peritonitis showed that blockage of TNF-alpha resulted in a reduction in animal survival. Similarly, combination therapy against TNF-alpha and the IL-1 receptor was fatal in a rodent model of sepsis, an effect that was also observed in a clinical trial of TNF-alpha antagonists. In a review by Menschikowski and colleagues, the authors proposed that increased levels of $sPLA_2$-IIA act bactericidally in inflammations, and that its inhibition may be counter productive in specific situations. They cited the significant clinical improvement of sepsis patients treated with statins, a molecule that induces both $sPLA_2$-IIA and pro-inflammatory cytokines (52). All together, these data indicate that $sPLA_2$-IIA stimulates the resolution of inflammatory responses, thereby contributing to the prevention of chronic inflammation.

3.4. $sPLA_2$-IIA, arachidonic acid, and apoptosis.

As mentioned before, $sPLA_2$-IIA has a preference for negatively charged lipid membranes containing anionic lipids like phosphatidylserine, due to its cationic nature (pI > 10.5). The outer leaflets of lipid bilayers in unperturbed cells have a neutral lipid composition enriched in phosphatidylcholines, sphingomyelin, and cholesterol, and form a poor substrate for $sPLA_2$-IIA. However, cells that are undergoing apoptosis lose their membrane asymmetry, resulting in exposure of anionic lipids to the outer leaflet (42,198), thereby increasing the affinity for $sPLA_2$-IIA binding. Moreover, perturbed cell membranes are also found in cancer cells (199). Apoptosis of transformed cancer cells is an important defense mechanism against neoplastic development, and PLA_2 enzymes both promote and prevent apoptosis in different cell types under different pathological conditions. Addition of phospholipid scramblase, an enzyme that alters membrane symmetry, to HEK293 cells, caused an increase in $sPLA_2$-IIA activity, resulting in an increase in arachidonic acid release and a slowing in cellular proliferation (44). Arachidonic acid produced by $sPLA_2$-IIA enzymatic activity promotes apoptosis in colon cancer cells and in many other types of normal and cancer cells (200-206). Exogenous arachidonic acid is cytotoxic to HCT-116 colon cancer cells and causes an increase in the expression of pro-apoptotic genes such as caspase-3, AP-1 and c-Jun and a decrease in the expression of genes that promote cancer cell survival (207). In another study, exogenous arachidonic acid caused a dramatic increase in rates of apoptosis in HCT-116 and SW480 colon cancer cells, a phenotype that was associated with the activation of neutral sphingomyelinase resulting in the greatly enhanced production of ceramide (200). Sphingomyelinases and their sphingolipid products confer resistance to colon cancer, including the suppression of tumorigenesis in $Apc^{Min/+}$ mice (208-210). Sphingomyelin in the outer leaflet of healthy cells is not a substrate for $sPLA_2$-IIA and inhibits its activity (211). It is possible that sphingomyelinase may stimulate $sPLA_2$-IIA by the removal of inhibitory sphingomyelins. It has been reported that TNF-alpha induces sphingomyelinase hydrolysis, resulting in $sPLA_2$-IIA activation and arachidonic acid release (212). Cytosolic PLA_2's resistance to AOM-induced colon tumors was associated with a sharp increase in ceramide production in $cPLA_2$ wildtype mice versus knockout mice. A similar mechanism may contribute to $sPLA_2$-IIA's mode of action as has been shown with other $sPLA_2$'s (213). Interestingly, factors that promote release of arachidonic acid from cells, such as Vitamin D_3 and other nuclear receptor agonists promote apoptosis despite the concurrent increase in lipoxygenases (214). Secretory PLA_2-IIA also induces apoptosis in a wide range of non-colonic cells (215) including neurons (192,216), astrocytes (217), fibroblasts (218), murine macrophages (219) and NK and cytotoxic T cells (220). Finally, while PGE_2 is generally described as a pro-cell-survival factor (221), it does induce apoptosis in certain cell types such as brain glial cells where PGE_2 activates BAX (222).

3.5 $sPLA_2$-IIA, $cPLA_2$, and cancer

While $sPLA_2$-IIA is upregulated in a range of human tumors (43) its role in cancer has been examined most thoroughly in prostate, gastric, ovarian and intestinal cancers. Several studies of human prostate cancer cells and analysis of human prostate cancer tissues support an oncogenic role for $sPLA_2$-IIA, in particular in its progression to advanced cancer (223-225). In advanced ovarian cancers, $sPLA_2$-IIA was found to be upregulated prior to chemotherapy but significantly down-regulated post-chemotherapy (341). In contrast, in human gastric cancer, patients expressing high levels of $sPLA_2$-IIA showed a highly significant survival advantage (89,226). In studies of intestinal cancer, overexpression of a $sPLA_2$-IIA transgene in C57BL/6 mice conferred resistance to tumorigenesis in $Apc^{Min/+}$ mice where its relative resistance is strongest in the large intestine (21,22). Secretory PLA_2-IIA is also the proximal element of the Modifier of Min-1 (*Mom1*) locus, a region of ~ 15 kb on distal mouse chromosome 4 that was isolated from the tumor resistant AKR strain and made congenic on the B6 genetic background. *Mom1* acts semi-dominantly, with one copy of *Mom1* reducing tumor multiplicity by greater than 50%, and two copies of *Mom1* causing a reduction in tumor number of ~ 80% in $Apc^{Min/+}$ mice. Recent work by our group has shown that $sPLA_2$-IIA confers resistance to intestinal tumorigenesis independent of *Apc*-germline mutations. We have found that expression of the same $sPLA_2$-IIA transgene strongly reduces tumors in the AOM carcinogen-induced model and in the *Muc2* knockout mouse (manuscripts submitted), as well as in the *IL-10* knockout mouse model where $sPLA_2$-IIA also reduces the incidence and extent of IBD (manuscript in preparation). Comparison of inbred strains of mice that express wildtype alleles of the *Pla2g2a* gene indicate that its tumor resistance corresponds to the amount of $sPLA_2$-IIA protein expressed and its enzymatic activity (227,228). However, the tissue context of $sPLA_2$-IIA's activity appears to be critical to its tumor resistance. Belinsky and colleagues expressed wildtype alleles of $sPLA_2$-IIA obtained from SWR and AKR mice in human colon cancer cells and found that while $sPLA_2$-IIA slowed the growth of the cancer cells in culture it required the presence of the substrate, palmitoyl-arachidonoyl-phosphatidic acid (PAPA). Even more interesting, they found that introduction of the $sPLA_2$-IIA-expressing cancer cells as tumor explants in nude mice caused a significant increase in the size and aggressiveness of the cancer cells suggesting that the tissue context of $sPLA_2$-IIA expression is a key determinant of its ability to reduce tumorigenesis (229).

The role of $cPLA_2$ is also complex in mouse and human intestinal cancer. Like $sPLA_2$-IIA, $cPLA_2$ is upregulated in a significant number of human colorectal cancers and in virtually all reviews it is characterized as an oncogene (230-233). Two studies of $cPLA_2$ knockout mice generated on the C57BL/6 background showed that loss of $cPLA_2$ strongly reduced the multiplicity of small intestinal tumors in both the $Apc^{Min/+}$ and $Apc^{\Delta 716}$ mouse models but had no significant effect in the large intestine (234,235). However, when the $cPLA_2$ mutation was made congenic on the Balb/c genetic background, knockout mice treated with AOM developed significantly more colon tumors than their wildtype littermates (236). Interestingly, expression of $cPLA_2$ was also shown to suppress carcinogen-induced lung tumors in Balb/c mice, again showing the opposite

phenotype as that observed in B6-cPLA$_2$ knockout mice (237). The cPLA$_2$ knockout in Balb/c mice resulted in a reduction in apoptosis in the colon that was accompanied by a sharp decrease in PGE$_2$ production, even in COX-2 overexpressing tumors. These results support the view that eicosanoids produced by arachidonic acid release have complex downstream effects on cellular processes such as apoptosis, tissue repair and neoplastic development that may be mediated by the specific nature of challenges to the tissue and the presence of co-factors. In that regard, in comparing the cPLA$_2$ knockout studies in C57BL/6 and Balb/c mice we note that Balb/c mice express a wildtype allele of sPLA$_2$-IIA (unlike C57BL/6) and as there is evidence that sPLA$_2$-IIA activity is dependent in some contexts on prior action of cPLA$_2$ and 12/15-LOX (108). The significant increase in colon tumors in AOM-treated Balb/c mice might be due in part to a dysregulation of sPLA$_2$-IIA activity. The same group of researchers at the University of Connecticut had previously shown that resistance to AOM-induced colon tumors corresponded to the relative expression levels and enzymatic activity of sPLA$_2$-IIA (227).

In human colorectal cancers, the role of sPLA$_2$-IIA is more controversial. While there is at least one report of a family with susceptibility to colon cancer that carries a mutant allele of sPLA$_2$-IIA (238), overall, since the discovery of its function as a suppressor of intestinal cancer in the mouse there has not been a confirmation of a similar role for sPLA$_2$-IIA in human intestinal cancer, notwithstanding its location on human chromosome 1p35-36, a region that is frequently deleted in human cancers, including colon cancer (29-34). There has been more speculation that sPLA$_2$-IIA may act as an oncogene in human colon cancer. For example, several studies have reported that sPLA$_2$-IIA is upregulated in human colon adenomas from FAP patients (43,239) and in sporadic colonic adenocarcinomas (230), although in another study it was reported that while sPLA$_2$-IIA was upregulated in microsatellite instable-high (MSI-H) human colon cancers, sPLA$_2$-IIA was significantly downregulated in microsatellite stable (MSS) colon cancers (240). As we discuss below, sPLA$_2$-IIA is a beta-catenin target gene that appears to be upregulated upon dysregulation of Wnt/beta-catenin signaling, as often occurs in *Apc*-deficient cells. Thus it is not surprising that sPLA$_2$-IIA might be more highly expressed. However, it is not likely that this upregulation contributes to an overall selective advantage to cancer cells. The expression of sPLA2-IIA might involve an autoregulatory loop in the GI tract, similar in that regard to Tcf-1, betaTrCP, Axin-2, EphB2, and Cdx2, which are all beta-catenin target genes that are upregulated in early neoplastic intestinal lesions but which have been demonstrated to act as suppressors of intestinal tumorigenesis in multiple assays, including genetic experiments in mice. This idea is further supported by the reports of significant upregulation of sPLA2-IIA mRNA expression in intestinal tumors from B6-ApcMin/+ mice (241,242). As B6 mice are naturally mutant for sPLA2-IIA, this upregulation could not confer a selective advantage to tumor cells, at least not for any activity of sPLA2-IIA dependent on its enzymatic hydrolysis of phospholipids.

Finally, the linkage between sPLA$_2$-IIA, COX-2 and PGE$_2$, and the role of COX-2 and PGE$_2$ in colon cancer needs to be addressed. COX-2 and PGE$_2$ are elevated in both familial and sporadic human colon cancers (243-247). Studies in human colon cancer cells indicate that PGE$_2$ inhibits apoptosis and promotes proliferation, and mouse genetic studies of knockouts of the PGE$_2$ cell surface receptors, in particular EP2, demonstrate that these receptors play a role in polyp formation (60,248,249) and that PGE$_2$ is associated with the promotion of inflammation-induced cancers (250). One study in *Apc*$^{Min/+}$ mice indicates that PGE$_2$ promotes colorectal adenoma growth via transactivation of the PPARδ nuclear receptor (244). In addition, *Apc*$^{Min/+}$ mice show elevated levels of PGE$_2$ at 15 weeks of age (251). Obviously, the elevated levels of PGE$_2$ in B6-*Apc*$^{Min/+}$ mice are not due to the enzymatic activity of sPLA$_2$-IIA since B6 mice carry a catalytically mutant allele, implicating other PLA$_2$ enzymes (cPLA$_2$ or perhaps sPLA$_2$-X) in this increase in PGE$_2$. Overall, it is clear that PGE$_2$ is upregulated in response to tissue damage and inflammation, and that it has potent signaling effects within the intestinal lamina propria where it mediates tissue repair and regulates the immune response, as we will discuss in the next section.

3.6. Does sPLA$_2$-IIA Play a Role in Signaling between the Intestinal Epithelia and Stroma?

Signaling between the epithelia and underlying stromal cells is essential for the development of the gut, maintenance of homeostasis and prevention of disease. Both normal intestinal epithelia and intestinal cancer cells exist in a complex microenvironment that includes the extracellular matrix, fibroblasts, endothelial cells, and cells of the immune system such as macrophages, mast cells, B cells and T cells. The organized bi-directional transfer of diffusible signals (both autocrine and paracrine, consisting of growth factors and other signaling molecules such as cytokines, lipids, energy substrates, *etc.*) between these compartments is essential for the development of the gut and the proper maintenance of homeostasis in the adult animal, including lineage specification in the epithelial mucosa. Key regulatory signaling pathways that interact in this network are Wnt/beta-catenin, Notch, Hedgehog and Tgf-beta/BMP (252-259).

3.6.1. sPLA$_2$-IIA, PGE$_2$, and Wnt signaling.

Wnt/beta-catenin signaling is required to maintain the stem cell compartment in the crypts of the intestines. Mutations in the Wnt/beta-catenin pathway, including mutations in the *APC* tumor suppressor gene, disrupt normal development of the intestines and lead to tumorigenesis (253,260). As previously mentioned, expression of sPLA$_2$-IIA has been correlated with activation of the Wnt/beta-catenin pathway (88,242). Interestingly, Wnt/beta-catenin signaling is also required for maturation of Paneth cells (261), a subset of secretory cells in the intestinal tract with very high expression of sPLA$_2$-IIA (22). Together, these data suggest that sPLA$_2$-IIA is a transcriptional target of Wnt/beta-catenin signaling.

One of the effects of sPLA$_2$-IIA that was previously discussed is its function in inflammation, and its

capacity to enhance the induction of COX-2, thereby increasing the production of various prostaglandins. PGE_2 is one of the most abundantly produced prostaglandins in the intestinal tract. Among many other functions, PGE_2 has been shown to enhance Wnt/beta-catenin signaling through binding to its EP2 receptor (262), and was recently shown to increase (hematopoietic) stem cell numbers (263). This suggests that production of PGE_2 by inflammatory cells may stimulate intestinal epithelial stem cells to repair epithelial damage and may explain why colon carcinomas 'benefit' from constitutive expression of COX-2 and high levels of PGE_2 production, which stimulates their proliferation and survival. Considering the role of $sPLA_2$-IIA, it may be both a target and an activator of the Wnt/beta-catenin signaling pathway, and the up-regulation of $sPLA_2$-IIA in GI cancers may reflect its role in an autoregulatory loop, similar to what has been proposed for several other Wnt/beta-catenin target genes that have been demonstrated to suppress GI cancer progression.

3.6.2. Contrasting roles of intestinal macrophages

Pathologies of the GI tract, such as infection, inflammation and cancer, almost inevitably involve dysregulation of the network of signaling between the epithelial cells and their underlying non-epithelial cells (264-266). The GI tract contains the greatest number of resident macrophages in the mammalian body. Intestinal macrophages located in the lamina propria comprise a key early response component of the innate immune system in the gut where they are the first defender cells to encounter microorganisms and microbial products that have penetrated through the epithelial barrier (267,268). Resident intestinal macrophages are relatively long-lived, are non-proliferating, and in their function as regulators of homeostasis they mostly act in an anti-inflammatory manner (269,270). An important function of resident macrophages is the repair of tissue damage, where they are recruited to the epithelia stem cell niche and participate in the regenerative response of stem cells to epithelial damage (271), a process that involves signaling by the Toll-like receptor pathway, including TLR2 and TLR4, and the MyD88 adaptor protein (272). Thus, the resident macrophages adapt to the presence of commensal flora to maintain intestinal homeostasis.

Macrophages phagocytose and destroy foreign pathogens. Upon recognition of pathogens, macrophages acquire a 'pro-inflammatory' phenotype and initiate inflammatory responses by activation of the transcription factor NF-kappaB, induction of COX-2 and iNOS gene expression followed by secretion of prostaglandins and nitric oxide, and by secretion of various pro-inflammatory cytokines and chemokines such as TNF-alpha, IL-12, and CXCL-8 (273-275). Moreover, during inflammatory conditions like in IBD, pro-inflammatory cytokines secreted by other immune cells like activated T cells and dendritic cells cells may further stimulate macrophages to secrete potent inflammatory cytokines such as TNF-alpha and IL-1. Macrophages are also the main type of cell responsible for the orderly clearance of apoptotic cells and cell debris. Upon uptake of apoptotic cells, macrophages acquire an 'anti-inflammatory' phenotype by actively suppressing inflammatory responses in order to prevent tissue damage (276-278). Importantly, both these pro- and anti-inflammatory macrophage functions are mediated through a variety of pattern recognition receptors (PRRs) that can recognize pathogen-derived exogenous ligands as well as host-derived endogenous ligands, such as expressed on apoptotic cells, indicating that signaling through these receptors does not simply result in straightforward pro- or anti-inflammatory responses but depends on a balance of receptor stimulation by external factors (279). This versatility of macrophage activation pathways and responses illustrates the capacity of macrophages to shape their local environment in different directions. The factors that determine whether macrophages act in a pro- or anti-inflammatory fashion are not fully understood but lipid mediators and their effectors are clearly among the factors that most influence macrophage activation, including PPAR-gamma (86,280-284), $cPLA_2$ signaling via TLR-4 285, PGE_2, and $sPLA_2$-IIA (143). In the context of tumor development, different types of macrophage activation can contribute both positively and negatively to different stages of tumorigenesis by influencing processes like angiogenesis, tissue remodelling, and subversion of anti-tumor immunity (286-288).

The observation that $sPLA_2$-IIA stimulates both the recognition of pathogens ('pro-inflammatory response') and the recognition of apoptotic cells ('anti-inflammatory response') by macrophages further underscores the complexity of molecular pathways in which $sPLA_2$-IIA is involved. The fact that similar PRRs are involved in both processes emphasizes that the molecular and cellular context is of crucial importance. On the one hand, $sPLA_2$-IIA mediated recognition of pathogens enhances the induction of COX-2 and production of PGE_2, and is accompanied by activation of pro-inflammatory transcription factors like NF-kappaB that in turn increase $sPLA_2$-IIA transcription. On the other hand, $sPLA_2$-IIA mediated recognition of apoptotic cells may enhance the induction of COX-2 and production of 15-deoxy-^{12,14}-PGJ_2 (15d-PGJ_2), thereby inhibiting 'pro-inflammatory' NF-kappaB activity (289) and activating 'anti-inflammatory' PPAR-gamma (95), which is also capable of stimulating $sPLA_2$-IIA transcription. Overall, a picture emerges in which $sPLA_2$-IIA enhances the efficiency of both pro- and anti-inflammatory responses, supporting the notion that it may play an important role both in the initiation and resolution of inflammatory responses (290,291). As such, we emphasize that one of the main general functions of $sPLA_2$-IIA may be the prevention of chronic inflammation.

3.6.3. Eicosanoids link innate to adaptive immunity

Like macrophages, dendritic cells (DCs) are also present in the intestinal lamina propria, waiting to capture and phagocytose invading microorganisms. Upon recognition of pathogens DCs become activated and migrate to (intestinal) lymphoid organs where they present antigens to T lymphocytes. Several eicosanoids like PGD_2, PGE_2, LTB_4, and LTC_4 mediate DC differentiation, activation, migration, and antigen presentation, and therefore play a crucial role in the crosstalk between innate and adaptive immune responses (197). The context in which stimulation of DCs takes place, *i.e.* the interaction

between eicosanoids, cytokines and chemokines, influences the type of instructions that will be provided by DCs, thereby affecting T cell differentiation. For instance, PGE_2 can interact in DCs (and macrophages) with proinflammatory cytokines such as TNF-alpha, IL-1, IL-23 and IL-6, but it can also induce anti-inflammatory cytokines such as IL-10. The effect on inflammation depends on the maturation stage of the cells, the presence of co-factors and the nature of the challenge to the immune system (292-295). As $sPLA_2$-IIA influences eicosanoid generation, it may affect DC migration and maturation, thereby changing the capability of DCs to respond adequately to pathogenic challenges and/or shift the balance between developing a pro- or anti-inflammatory response.

T lymphocytes of several types are also critically involved in regulation of intestinal inflammation and neoplasia. One dramatic example is the conditional knockout of Smad4 in T cells that resulted in intestinal cancer throughout the GI tract, while a conditional knockout of Smad4 in epithelial cells had no cancer phenotype (296). Furthermore, treatment of gamma-delta T cell-deficient mice with AOM enhanced the number of colon tumors (297), and proinflammatory CD4+CD45RB hi lymphocytes promote tumorigenesis in *Apc*$^{Min/+}$ mice (298). Another interesting group of T cells are the CD4+CD25+ Foxp3+ T cells (299), which act as intestinal regulatory T cells that are important for prevention of inflammation (300-302), and the inhibition of microbially-induced colon cancer (303). PGE_2 promotes Foxp3 expression *in vitro* and activates CD4+CD25+ regulatory T cells (304,305). PGE_2 is also a potent inducer of Runx1 transcription (263), which has been shown to control regulatory T cells via its interactions with Foxp3 (306). The anti-inflammatory action of FoxP3+ regulatory T cells involves interactions with the NFATc transcription factor to cooperatively repress the pro-inflammatory transcription factor NF-kappa B and the production of pro-inflammatory cytokines (307,308). As discussed previously, NFATc acts in a gene expression regulatory loop with PPAR-gamma, and NFATc promoter binding transactivates the expression of $sPLA_2$-IIA. Thus, $sPLA_2$-IIA may be capable of transcellular regulation of regulatory T cells via its stimulation of secreted lipid mediators.

All together, $sPLA_2$-IIA may be involved in coordination of the network of intestinal epithelial-stromal signaling. It is expressed in the secretory cells (Paneth and goblet cells) of the epithelia and is expressed in cells of the monocyte lineage (macrophages, mast cells) in the underlying stroma. Secretory PLA_2-IIA and its lipid mediators such as arachidonic acid and prostaglandins can act transcellularly (309), and thus they are ideally positioned to transduce signals between the two compartments.

3.7. $sPLA_2$-IIA, a link between Wnt and Notch Signaling?

Recently we compared by microarray analysis the expression of genes in normal healthy colon of B6 mice, which are 'natural mutants' for $sPLA_2$-IIA, to colon of transgenic mice that carry the functional AKR-derived $sPLA_2$-IIA gene on the B6 background (manuscript submitted). Our data showed that $sPLA_2$-IIA significantly affects the expression of more than 400 genes and transcripts (out of > 40,000 on the oligo arrays), and confirms that $sPLA_2$-IIA plays a role in inflammation and immune responses, in apoptosis, and in lipid metabolism (Figure 1). In addition, the differentially expressed genes also included a number of genes involved in the pathways that are crucial for gut development and homeostasis, *i.e.* the Wnt/beta-catenin, Notch, Hedgehog, and TGF-beta/BMP pathways (Figure 1). Of the Wnt/beta-catenin genes, an association between expression of $sPLA_2$-IIA and *EphB2*, a member of the Eph family of receptor tyrosine kinases that mediates the position of cells in the intestinal crypts (310), has previously been described. Secretory PLA_2-IIA and *EphB2* are co-regulated in both human colorectal and gastric cancers (88), $sPLA_2$-IIA and *EphB2* are both up-regulated in mouse intestinal adenomas (241,242), and EphB2, like $sPLA_2$-IIA, has also been demonstrated to be a potent tumor suppressor in mouse colon cancer models (311,312). Moreover, downstream signaling of EphB2 is mediated by Rgs3 (regulator of G protein signaling-3) (313), a gene that is also highly upregulated (> 4-fold) by $sPLA_2$-IIA according to our data. Hence, our data confirm that there is a functional link between $sPLA_2$-IIA and the Wnt-signaling pathway.

A new finding revealed by our microarray expression data was the potential link between $sPLA_2$-IIA and Notch signaling. One of the genes that was most highly induced by $sPLA_2$-IIA was Runx1 (>15-fold), a major downstream mediator of the Notch signaling pathway (314,315). Notch signaling is essential for maintenance of intestinal homeostasis, where it acts in concert with the Wnt/beta-catenin pathway (316-318). Runx1 is a transcription factor that can either activate or repress target genes depending on the cellular context, and the presence or absence of coactivator and corepressor molecules (319,320). While Runx1 is best known for the oncogenic fusion proteins that give rise to leukemias (321), the gene is widely expressed in mammalian tissues and has a diverse group of target genes. For example, Runx1 controls the CSF-1 dependent and independent growth and survival of macrophages (322), Runx1 physically interacts with the hypoxia-inducible factor Hif-1alpha and inhibits several of its transactivation targets (323), and as previously mentioned, Runx1 was recently shown to physically interact with Foxp3 in regulatory T cells, thereby suppressing IL-2 and IFN-gamma production (306). In human gastric cancer Runx1 has been reported to be down–regulated, and exhibits growth inhibitory functions in gastric epithelium similar to Runx3 (324). Although little is known about the functions of Runx1 in the normal intestine or in intestinal cancer, the effects of Notch signaling have been investigated. Mice that are deficient for Hes1, a Notch pathway protein that may physically interact with Runx1, develop a secretory cell phenotype in the intestine with crypts largely populated by goblet cells (325). A similar phenotype is observed upon treatment of mice with a gamma-secretase inhibitor, as gamma-secretase is essential for the proper cleavage of the Notch intracellular domain

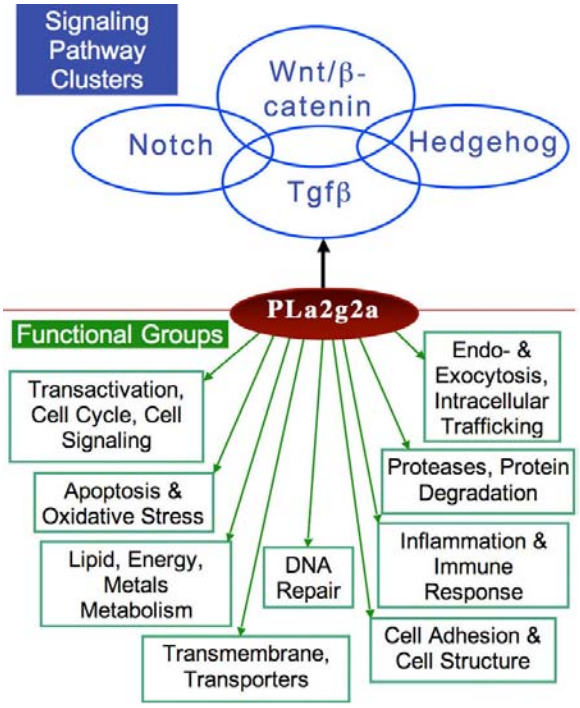

Figure 1. Effects of Pla2g2a on gene expression, functional groups and key signaling pathways in colon (tumorigenesis). Comparison by microarray analysis of gene expression in normal healthy colon from C57BL/6 mice, which are 'natural mutants' for sPLA$_2$-IIA, *versus* healthy colon of transgenic mice that carry the functionally active AKR-derived sPLA$_2$-IIA gene on the B6 background (manuscript submitted) revealed more than 400 genes and transcripts that were differentially expressed. Clustering of genes into functional groups confirms that sPLA$_2$-IIA plays a role in inflammation and immune responses, in apoptosis, and in lipid metabolism. In addition, the differentially expressed genes also included a number of genes involved in molecular pathways that are crucial for gut development and homeostasis, *i.e.* the Wnt/beta-catenin, Notch, Hedgehog, and TGF-beta/BMP pathways. Hence, there emerges a pattern of coordinated gene expression in the intestine in which sPLA$_2$-IIA seems to play an important role. However, it remains to be elucidated how the elements of this network cooperate to suppress tumorigenesis.

(NICD) signaling element (326-328). In contrast, loss of the bHLH transcription factor Math1 shifts the cell lineage towards an entirely absorptive cell type, with a complete absence of secretory cell such as goblet cells (329). *Math1*, in turn, is a target of Notch signaling that is negatively regulated by Notch genes such as *Hes5, Notch1*, and *Jagged 2* (330,331), and possibly by antagonists of BMP signaling (332). Interestingly, Runx1 is activated by Bmp4, also part of the TGF-beta pathway that interacts directly with the Wnt/beta-catenin pathway (333,334).

Considered together, there emerges a pattern of coordinated gene expression in the intestine that ties together elements of the Wnt/beta-catenin, Notch, and possibly the Hedgehog and TGF-beta signaling pathways. A diagram of major sPLA$_2$-IIA target genes and functional pathways is depicted in Figure 1. That these four signaling pathways are represented in the list of sPLA$_2$-IIA-target genes should not be surprising since Notch, Wnt, Hedgehog and BMP pathways are all involved in lineage specification in the mammalian intestine, and homeostasis is maintained by crosstalk between these pathways (252,253,335-339). Importantly, sPLA$_2$-IIA seems to play a role in the organization of this network, although it remains to be elucidated how the elements of this network cooperate to suppress tumorigenesis.

4. BUILDING MODELS OF sPLA$_2$-IIA TUMOR RESISTANCE

Here, we propose three models to explain the intestinal tumor resistance of sPLA$_2$-IIA in mice based on the experimental data to date. In model #1, sPLA$_2$-IIA protects the mucosa from bacterial damage and prevents chronic inflammation (Figure 2A, 2B). In model #2, sPLA$_2$-IIA promotes apoptosis of cancer cells (Figure 3). And in model #3, sPLA$_2$-IIA maintains the balance between Wnt, Notch, and other key signaling pathways that are relevant for gut development and homeostasis (Figure 4). These models are not meant to be exclusive of each other, thus sPLA$_2$-IIA may be protective at both early stages of neoplastic development in the resolution of chronic inflammation and at later stages, such as the apoptotic removal of cancer cells. Signaling pathways are also likely to overlap in the various models, for example, Runx1 may be involved in both the anti-inflammatory action of FoxP3+ regulatory T cells and in the establishment of lineage boundaries in the epithelial compartment. Finally, we recognize that the activity of some mediators of sPLA$_2$-IIA may have different roles in different models. For example, we propose that PGE$_2$ helps resolve chronic inflammation in model #1 through its promotion of wound repair and its regulation of FoxP3+ regulatory T cells. However, PGE$_2$'s pro-survival characteristics may also be anti-apoptotic and thus counteract the potential effect of sPLA$_2$-IIA on apoptosis of intestinal cancer cells that is described in model #2.

4.1. Model 1: sPLA$_2$-IIA protects the mucosa from bacterial damage and prevents chronic inflammation

4.1.1. Bactericidal properties

Secretory PLA$_2$-IIA is expressed by secretory cells such as Paneth and goblet cells, thus secreted sPLA$_2$-IIA is optimally localized to manage luminal bacteria. In the small intestine Paneth cells secrete a host of defensive molecules against pathogens and they are also ideally located to defend the neighboring stem cells at the base of the crypt (90,91). In the extracellular environment sPLA$_2$-IIA manages both Gram-positive and Gram-negative bacteria. sPLA$_2$-IIA binds tightly to the anionic surface of Gram-positive bacteria, penetrates the cell wall and hydrolyzes membrane phospholipids, causing cell death (Figure 2A). The bactericidal activity of sPLA$_2$-IIA against Gram-negative bacteria is likely mediated via receptor-mediated signaling events (Figure 2B). In the large

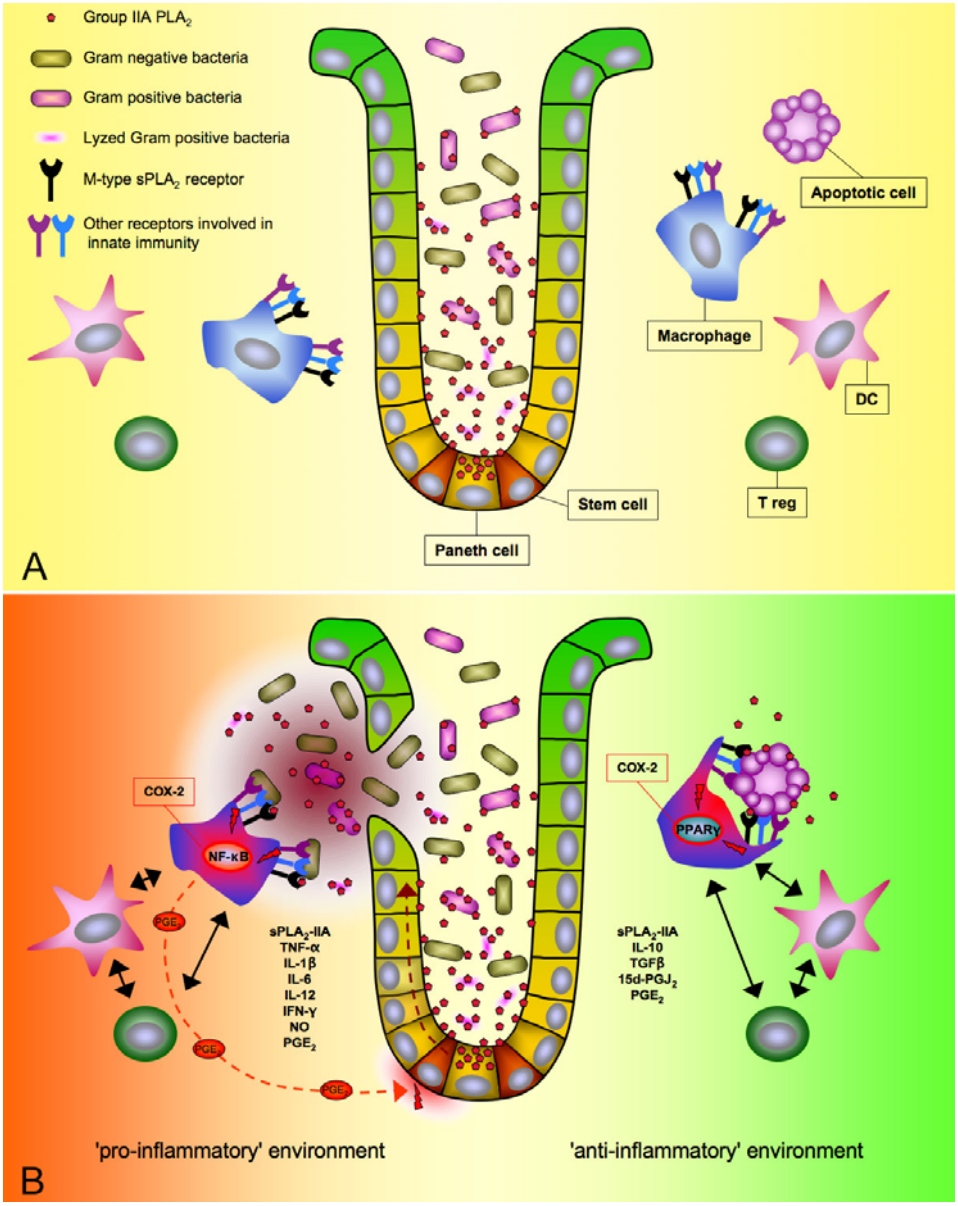

Figure 2. Model 1: sPLA2-IIA protects the mucosa from bacterial damage and prevents chronic inflammation. Enzymatic activity of $sPLA_2$-IIA forms a first line of defense against Gram-positive bacteria (Figure 2A). Secretory PLA_2-IIA is also protective against Gram-negative bacteria, although this effect may be largely mediated by the M-type $sPLA_2$ receptor in combination with other receptors from the innate immune system (Figure 2B). Pathogenic activation of cells of the innate immune system like macrophages results in activation of the 'pro-inflammatory' transcription factor NF-kappaB, the induction of COX-2, and the production and secretion of various mediators of inflammation, including $sPLA_2$-IIA. Eicosanoids that are produced downstream of $sPLA_2$-IIA mediate DC differentiation, activation, migration, and antigen presentation, and therefore play a crucial role in the crosstalk between innate and adaptive immune responses. PGE_2 has potent signaling effects within the intestinal lamina propria where it mediates tissue repair and regulates the immune response, *e.g.* by promoting Foxp3 expression and activation of CD4+CD25+ regulatory T cells. Alternatively, upon uptake of apoptotic (non-pathogenic) cells, macrophages acquire an anti-inflammatory phenotype by actively suppressing inflammatory responses in order to prevent tissue damage. Secretory PLA_2-IIA mediates recognition of apoptotic cells and enhances the induction of COX-2 and production of prostaglandins such as 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), thereby inhibiting 'pro-inflammatory' NF-kappaB activity and activating 'anti-inflammatory' PPAR-gamma, which, like NF-kappaB, is also capable to increase the production of $sPLA_2$-IIA. Overall, a picture emerges in which $sPLA_2$-IIA enhances the efficiency of both pro- and anti-inflammatory responses, thereby stimulating appropriate responses to both non-pathogenic and pathogenic challenges. As such, we emphasize that one of the main general functions of $sPLA_2$-IIA may be the prevention of chronic inflammation.

intestine $sPLA_2$-IIA that is secreted from goblet cells is found in the mucinous matrix of the crypt lumen where it cooperates with proteins such as Muc2 and decorin in management of microflora.

4.1.2. Anti-inflammatory properties

Secretory PLA_2-IIA protects against chronic inflammation in the GI tract by the direct management of microflora and by the intracellular and transcellular induction of lipid mediators that can act at multiple levels such as binding to cell surface receptors and the transactivation of target genes. These effects can occur within the intestinal epithelium and via crosstalk between epithelia and the cells of the underlying microenvironment such as cells of the monocyte lineage and T lymphocytes. In the acute phase of inflammation $sPLA_2$-IIA can promote the expression of pro-inflammatory factors such as TNF-alpha to destroy sources of inflammation such as invading pathogens. Perhaps, more important for prevention of tumorigenesis $sPLA_2$-IIA promotes the resolution of chronic inflammation via local production of PGE_2 and other lipid mediators to assist in tissue repair and the transcellular recruitment of anti-inflammatory resident macrophages to sites of inflammation. Through lipid mediators such as PGE_2, $sPLA_2$-IIA prompts the conversion of CD4+ T cells into anti-inflammatory FoxP3+ regulatory T cells. This conversion may be indirectly mediated by promoting the maturation of dendritic cells. Secretory PLA_2-IIA can also regulate the action of FoxP3+ regulatory T cells via PGE_2 and the induction of Runx1, a key $sPLA_2$-IIA target gene that physically interacts with FoxP3 and is essential for regulatory T cell activity.

4.2. Model 2: $sPLA_2$-IIA promotes apoptosis of cancer cells

4.2.1. Arachidonic acid produced by $sPLA_2$-IIA promotes apoptosis

Arachidonic acid is a major hydrolytic product of $sPLA_2$-IIA's enzymatic activity. Studies in rodents and in human colon cancer cells have shown that AA can cause a dramatic induction of apoptosis that is correlated with activation of sphingomyelinases and production of ceramide. Sphingomyelinases may also activate sPLA2-IIA. Thus, arachidonic acid produced by $sPLA_2$-IIA can directly cause the apoptosis of cancer cells and potentially act in a positive feed back loop with sphingomyelinases to maintain $sPLA_2$-IIA activity (Figure 3, section 1).

4.2.2. Loss of membrane asymmetry in cancer cells activates $sPLA_2$-IIA and can lead to apoptosis

Secretory PLA_2-IIA enzymatic activity at the cell surface of normal healthy intestinal cells is relatively low due to the predominance of cationic or neutral phospholipids such as phosphatidylcholine (PC), sphingomyelin and cholesterol in the outer leaflet. In contrast, the inner leaflet is rich in anionic phospholipids such as phosphatidylserine (PS) and phosphatidylethanolamine (PE). This asymmetry helps maintain cellular homeostasis with one effect being the prevention of harmful indiscriminate phospholipid hydrolysis by $sPLA_2$-IIA. However, the asymmetric phospholipid distribution is perturbed in a range of pathologies, including neoplasia. For example, many cancer cells, including epithelial colon cancer cells, demonstrate sharply elevated levels (up to five fold more) of phosphatidylserine at their cell surface than normal differentiated cells (199). Increased levels of PS can activate $sPLA_2$-IIA and lead to the hydrolysis of outer leaflet phospholipids and cellular blebbing, and contribute to cancer cell apoptosis (Figure 3, section 2). This mechanism has been confirmed *in vitro* via expression of phospholipid scramblase, a lipid transporter that shuttles phospholipids between the two monolayers. Overexpression of scramblase in cell culture resulted in the activation of $sPLA_2$-IIA and the slowing of cellular proliferation. Interestingly, exposure of PS on the outer surface of cancer cells is correlated with their recognition by phagocytic macrophages, a recruitment that may be aided by the presence and/or activity of sPLA-IIA.

4.2.3. $sPLA_2$-IIA induces the expression of pro-apoptotic molecules

Secretory PLA_2-IIA causes the induction of apoptosis in various cells in culture. In our microarray analysis, we discovered almost 2 dozen $sPLA_2$-IIA target genes that are involved in apoptosis, oxidative stress and mitochondrial function. Thus, $sPLA_2$-IIA appears to regulate intrinsic signaling pathways that promote apoptosis.

4.3. Model 3: $sPLA_2$-IIA maintains a balance between Wnt, Notch and other key signaling pathways

Figure 1 provides lists of $sPLA_2$-IIA target genes that are associated with Wnt/beta-catenin, Notch, Hedgehog and TGF-beta/BMP signaling, key pathways that govern lineage specification, cellular proliferation, cellular migration, inflammation and susceptibility to cancer in the GI tract. These pathways are active in both the epithelial and stromal compartments and crosstalk between these pathways is essential for intestinal homeostasis. The Notch pathway gene *Runx1* is significantly up-regulated (> 15-fold) by the expression of $sPLA_2$-IIA in colon. *Runx1* is expressed by stromal cells such as Foxp3+ regulatory T cells and Runx1 also may be expressed by epithelial cells. We propose that $sPLA_2$-IIA's regulation of Runx1 and Notch signaling permits the establishment of proper cell lineage boundaries that can limit the size of the stem cell niche and promote gut homeostasis (Figure 4). On the one hand, these effects of $sPLA_2$-IIA may be restricted to the epithelial crypts of the intestines, where $sPLA_2$-IIA itself appears to be a Wnt/beta-catenin target gene. On the other hand, similar mechanisms may also influence the characteristics of hematopoietic stem cells and affect their differentiation into anti-inflammatory regulatory T cells, which play an important role in maintenance of homeostasis in the intestine and prevent tumorigenesis (296,298,340).

5. CONCLUSIONS AND PERSPECTIVES

We began our review by indicating that $sPLA_2$-IIA has been identified as a susceptibility gene for intestinal cancer, through an unbiased forward genetics

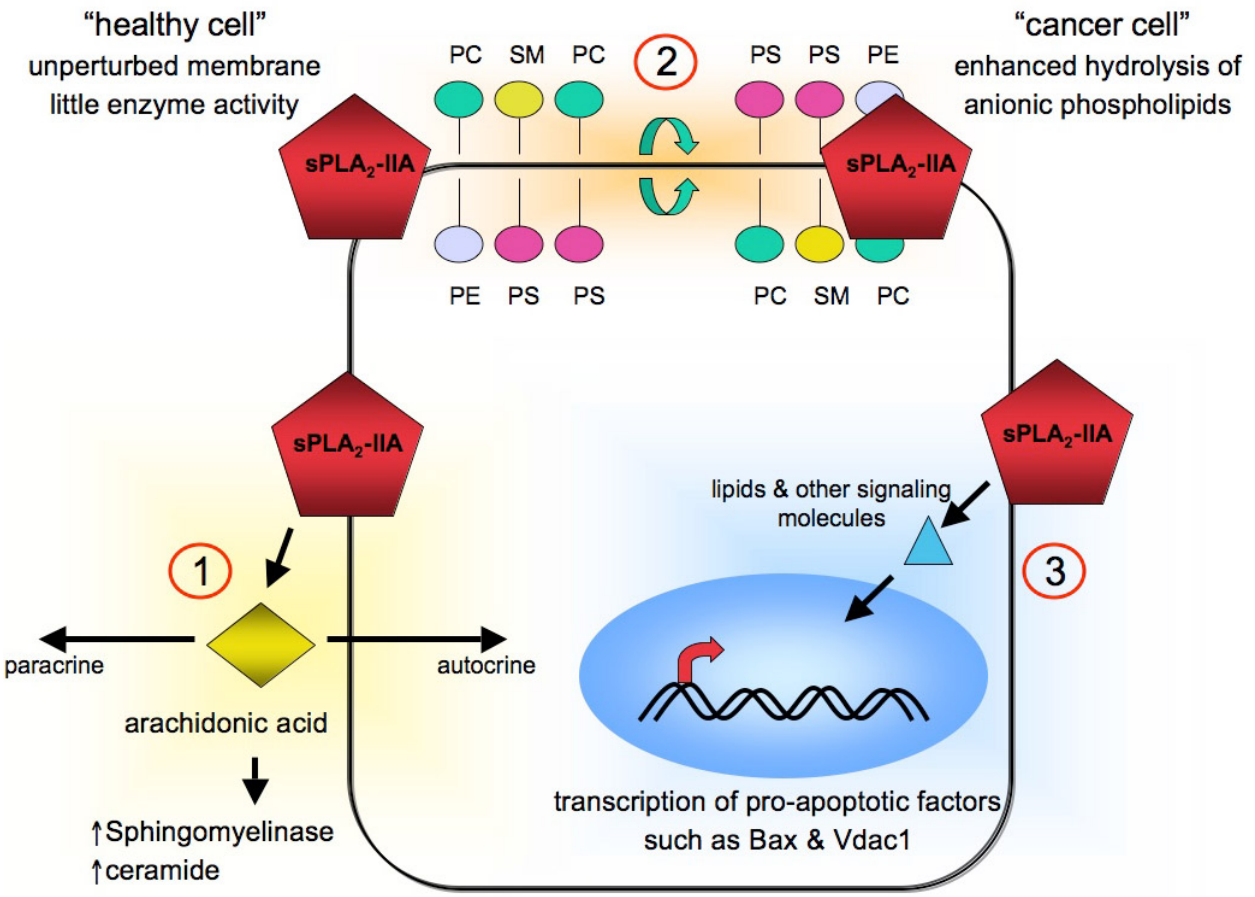

Figure 3. Model 2: sPLA2-IIA promotes apoptosis of cancer cells. Section 1: Arachidonic acid is a major hydrolytic product of $sPLA_2$-IIA's enzymatic activity, and can cause a dramatic induction of apoptosis that is correlated with activation of sphingomyelinases and production of ceramide. Sphingomyelinases may also activate sPLA2-IIA. Thus, arachidonic acid produced by $sPLA_2$-IIA can directly cause the apoptosis of cancer cells and potentially act in a positive feed back loop with sphingomyelinases to maintain $sPLA_2$-IIA activity. Section 2: The outer leaflets of unperturbed mammalian cells are characterized by neutral lipid composition enriched in phosphatidylcholines (PC), sphingomyelin (SM) and cholesterol, thus unperturbed cells are poor substrates for extracellular $sPLA_2$-IIA, thereby preventing indiscriminate hydrolysis of healthy cells. Perturbation of cell membranes, which also occurs in cancer cells, alters membrane symmetry and increases $sPLA_2$-IIA activity and arachidonic acid release. Section 3: In our microarray analysis we discovered almost 2 dozen $sPLA_2$-IIA target genes that are involved in apoptosis, oxidative stress and mitochondrial function, indicating that $sPLA_2$-IIA regulates intrinsic signaling pathways that promote apoptosis.

approach. As such, $sPLA_2$-IIA defines a promising 'anchorpoint' to identify and investigate new molecular pathways that modulate tumor development. Our group and others have shown that $sPLA_2$-IIA confers resistance to tumorigenesis in multiple mouse models of intestinal cancer, including carcinogen-induced (AOM) and genetically-modified models ($Apc^{Min/+}$, *Muc2*-knockout, *IL-10*-knockout), as well as in mice that develop inflammatory cancer (*IL-10*-knockout). Therefore, we propose that $sPLA_2$-IIA influences very fundamental mechanisms of tumorigenesis that are shared by all of these models. Understanding how murine $sPLA_2$-IIA prevents neoplastic development will provide valuable insights into the complex activity of lipid mediators in colon cancer, with the potential of therapeutic applicability to human disease.

We here offered three models to indicate how $sPLA_2$-IIA might suppress tumorigenesis: 1) By its bactericidal activity in conjunction with its potential modulation of the activity of stromal cells such as macrophages and regulatory T cells, thereby preventing chronic inflammation; 2) By promotion of apoptosis of cancer cells, via its production of arachidonic acid and/or the preferential hydrolysis of the phospholipid membrane of cancer cells, and by its regulation of the expression of a number of genes that promote apoptosis; and 3) By its regulation of expression of genes that are critical components in the four key signaling pathways in the GI tract – Wnt/beta-catenin, Notch, Hedgehog and TGF-beta/BMP – resulting in the proper maintenance of cell lineage boundaries and cellular proliferation.

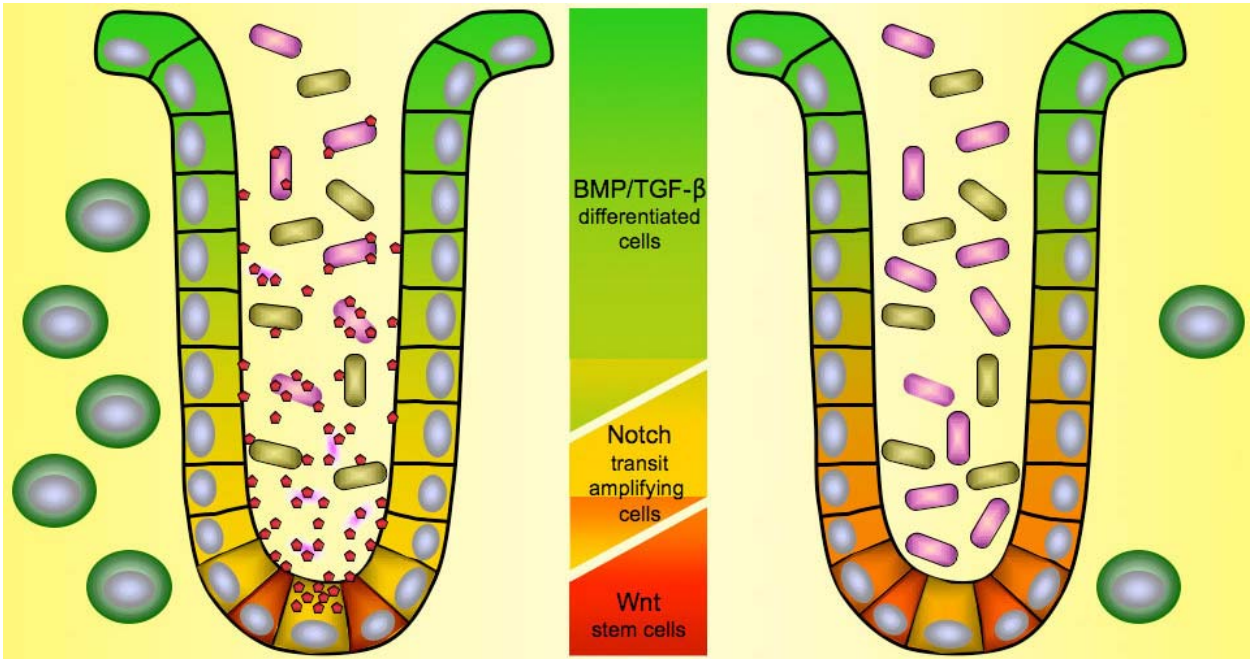

Figure 4. Model 3: sPLA2-IIA maintains a balance between Wnt, Notch and other key signaling pathways. Our microarray analysis revealed $sPLA_2$-IIA target genes that are associated with Wnt/beta-catenin, Notch, Hedgehog and TGF-beta/BMP signaling, key pathways that govern lineage specification, cellular proliferation, cellular migration, inflammation and susceptibility to cancer in the GI tract. We propose that $sPLA_2$-IIA's regulation of Runx1 and Notch signaling permits the establishment of proper cell lineage boundaries that can limit the size of the stem cell niche and promote gut homeostasis. On the one hand, these effects of $sPLA_2$-IIA may be restricted to the epithelial crypts of the intestines, where $sPLA_2$-IIA itself appears to be a Wnt/beta-catenin target gene. On the other hand, similar mechanisms may also influence the characteristics of hematopoietic stem cells and affect their differentiation into anti-inflammatory regulatory T cells, which play an important role in maintenance of homeostasis in the intestine and prevent tumorigenesis.

There are still many outstanding questions, such as the relative contributions of $sPLA_2$-IIA's autocrine and paracrine signaling to its cancer prevention, the role of $sPLA_2$-IIA binding to its cell surface receptor and the possible signaling pathways that may lie downstream of that binding, and the identity of intestinal cells that express Runx1 upon activation by $sPLA_2$-IIA. Further experiments are underway to confirm and expand upon these putative mechanisms for $sPLA_2$-IIA's tumor resistance. Overall, $sPLA_2$-IIA appears to be an example of a murine genetic predisposition factor that links cancer risk to management of normal and tumor microenvironment. Nevertheless, the role of $sPLA_2$-IIA in intestinal cancer remains quite ambiguous, as it may function in multiple pathways implicated in tumor development (proliferation, apoptosis, energy metabolism, cell adhesion and migration, DNA repair, protein degradation, *etc.*). This rather complex result was obtained despite the fact that our experiments were based on non-pathogenic colon tissue using mice that differed in only one gene, *i.e. Pla2g2a*, and indicates that gut development and homeostasis is influenced by multiple complex epithelial-stroma interactions involving hundreds if not thousands of genes. We expect that many genes may affect cancer risk through these mechanisms, and emphasize that context is key in understanding the activity of $sPLA_2$-IIA and many other mammalian genes in carcinogenesis.

6. ACKNOWLEDGEMENTS

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Abbreviations: APC (adenomatous polyposis coli); Min (multiple intestinal neoplasia); CRC (colorectal cancer); FAP (familial adenomatous polyposis); Mom1 (modifier of Min-1); IL (interleukin); COX (cyclooxygenase); PGE2 (prostaglandin E2); IBD (inflammatory bowel disease); PPAR (peroxisome proliferator activated receptor); LPS (lipopolysaccharide); IFN (interferon); DSS (dextran sulfate sodium); AOM (azoxymethane); QTL (quantitative trait locus)

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