

Aspirin and its Preventative Role in Colorectal Cancer

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Abstract— It has been evident for a number of years that aspirin (ASA) has held a strong position in the prevention of cardiovascular related events but is now becoming a prominent preventative measure in colorectal cancer. This paper has sought at what molecular levels these new preventative results are stemming from.

Index Terms— Apoptosis, Aspirin, B-Catenin, Colorectal Cancer, MTOR, NSAIDS, PIK3CA, Signaling pathways

1 INTRODUCTION

In today's modern world, there are substantial opportunities for many to live longer, healthier lives. From exercise and organic foods, people are choosing ways to prolong life. With such trends we are witnessing more diseases and cancers like colon cancer that increase with increasing age. Thus it has been a growing topic of interest in how colon cancer, that is rising as one of the leading cancers in both sexes in the developed world can be prevented.

In addition to its rise in cases throughout the years, it has also gained attention due to its relation to various gene mutations namely, Adenomatous polyposis coli (APC) gene, oncogenes like Phosphoinositide 3-kinase (PIK3) and syndromes that are highly common in colorectal cancers like hereditary nonpolyposis colorectal cancer (HNPCC or Lynch Syndrome) and familial adenomatous polyposis (FAP). As Burn et al. mention "people with monogenic predisposition to cancer offer an ideal focus for chemoprevention" (Burn et al., 2011) for it targets a subset of the growing population by which measures can be taken to prevent those at high risk and susceptible to untimely deaths. Thus aspirin (ASA), a common drug used by both sexes in the primary prevention of cardiovascular events by decreasing risk of ischemic stroke (Berger et al., 2006), and still considered the drug of choice in peripheral arterial diseases (Poredos and Jezovnik, 2013) has been advocated as a means of preventing cancers. For why not use a drug so readily available and common to the general public for something more?

At its most basic level ASA prevents inflammatory reactions by blocking COX-1 and COX-2 and by doing so it limits chronic inflammation that promotes the occurrence of cell growth, new blood vessel formation and thus malignancies (National Cancer Institute, 2014). Therefore many studies have searched for deeper molecular mechanisms by which aspirin works and "the favorable outcome that has been associated with aspirin use after colorectal cancer is diagnosed suggests that aspirin in a promising agent for adjuvant therapy" (Liao et al., 2012).

2 METHODS

Literature for this paper was sought using PubMed and WorldCat databases as well as supplementary articles found through Google scholar. Keywords used to limit articles were "Aspirin AND colorectal cancer", "Aspirin AND cardiovascular diseases". Once various article titles emerged extra keywords based on molecular mechanisms were added in order to include more specific data into the searches such as "MTOR", "PIK3CA", "B-Catenin", "apoptosis" and "experiment". In order to limit the studies to only very recent articles, only those from the last 5 years were included with a few exceptions to gain background on the recent findings. Filters were also included to exclude articles that did not fit search to "Clinical Trials", "Meta-Analysis", "Review", "Peer reviewed" and "Full free text available". Once articles were collected, studies were chosen based on

their ability to relate to one another on 1 or 2 molecular levels. This allowed this particular paper to only reflect upon a few of the molecular mechanisms by which aspirin could act.

3 RESULTS

The available literatures suggest that there are different mechanisms by which aspirin works to inhibit cancer cells and reduce colorectal cancer. One gene of high interest is the *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)*. This gene gives specific instructions for making protein *p110 alpha*, which is one subunit for the enzyme known to many as phosphatidylinositol 3-kinase (PI3K), a downstream target of the Arachidonic acid pathway. PI3K phosphorylates various signaling molecules and is responsible for a variety of processes that determine cell growth, migration of cells and overall cell survival (US National Library of Medicine, 2014). Two papers specifically rose to attention during research, a review study and one prospective cohort that demonstrated patients with colorectal cancers who had mutated PIK3CA had an unfavourable outcome where as patients with wild type PIK3CA (WT-PIK3CA) did not have such effects (Liao et al., 2012). It has been described that PIK3CA mutations are involved in many types of cancer. Moreover PIK3CA mutations in cancer are somatic, thus they are acquired throughout ones lifetime and present only in tumor cells. The mutation actually changes a single amino acid in the p110 alpha subunit, and this leads to unregulated PIK3 signaling (US National Library of Medicine, 2014). Secondly other experiments have shown that upstream regulators of PIK3 such as PGE2 promote cell growth and inhibition of apoptosis and demonstrated how aspirin has the ability to alter signaling and decrease the levels of such regulators (Smartt et al., 2012). Some studies further showed that aspirin promotes apoptosis and autophagy by means of down regulation of IL-6-STAT3, an inflammatory mediator as well as MTOR, another gene related to the PIK3 pathway (Tian et al., 2011 and Din et al., 2012).

3.1 Tumor PIK3CA mutations

In 2012 Liao et al. performed a prospective cohort study that demonstrated that ASA had the ability to increase survival and prognosis in patients that had cancers with a mutated PIK3CA and that there was no increase in colorectal cancer-specific survival in patients with WT-PIK3CA and regular use of aspirin after diagnosis. ASA acts by inhibiting prostaglandin-endoperoxidase synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX2) leading to the down regulation of PIK3. As mentioned, PIK3 has the ability to phosphorylate targets which effects cell growth, migration, invasion, anti-apoptosis and angiogenesis. Thus by down regulating PIK3, ASA has the ability to alter these cancer favorable outcomes (Fig 1., Langley and Rothwell, 2013). After following 964 patients with rectal or colon cancer, patients identified with mutated-PIK3CA colorectal cancers and usage of ASA regularly after their diagnosis, were found to have a superior colorectal cancer-specific survival rate. In comparison, the use of ASA regularly in patients with colorectal cancers and the WT-PIK3CA did not show any significant cancer-specific survival (Liao et al., 2012). This suggests that the biomarker of a mutated PIK3CA in cancer "predict(s) the

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response to the initiation of ASA therapy in patients with newly diagnosed colorectal cancer" (Liao et al., 2012).

3.2 Increased prostaglandin- E2 (PGE2) regulates PI3K and how β -catenin can decrease both

As seen in Fig 1., by Langley and Rothwell (2013) we can understand how exactly inhibition of COX2 can produce an immediate downstream effect on many mediators and targets. This is further demonstrated in an experimental study done by Smartt et al. (2012) that looks deeper at how β -catenin negatively regulates prostaglandin (PG) transporter PGT which usually catabolizes PG. β -catenin is a protein regulating the coordination of cell adhesion and gene transcription and also acts as a signal transducer in the Wnt-signaling pathway (Wikipedia, 2014) and as MacDonald et al. states "given the roles of Wnt/ β -catenin signaling in development of homeostasis it is no surprise that mutations of the Wnt pathway components are associated with hereditary disorders, cancers and other diseases" (MacDonald et al., 2009). Smartt et al. (2012) demonstrated by obtaining colon cancer-derived cell lines and immunohistochemical staining using a polyclonal PGT antibody and inducible intestinal β -catenin ablation in mice, that deletion of β -catenin in intestinal epithelium up-regulated PGT protein in crypt epithelium. Crypt epithelium was the chosen site for this staining because this is where Wnt/ β -catenin transcriptional activity is known to be highest and where it has been demonstrated that knockout of β -catenin up-regulates PGT in both colorectal adenoma and carcinoma. Their staining proved their hypothesis that β -catenin repressed PGT in intestinal epithelial cells (Fig 1E,F). Furthermore they also showed that β -catenin could also decrease PGT expression in human colorectal cancer cells through 15-hydroxyl prostaglandin dehydrogenase (15-PGDH) a "PG-degrading enzyme that physiologically antagonizes COX-2" (Yan et al., 2004). As demonstrated in their previous paper, Smartt et al., "showed that β -catenin activated at the earliest stages of colorectal neoplasia, can repress expression of the colorectal tumor suppressor and the 15-PGDH" (2012). Lastly ablation of β -catenin increased the expression of PGT thus increasing PG levels in both adenoma and carcinoma-derived cell lines (Smartt et al., 2012). Thus it was demonstrated that down-regulation of PGT, in addition to 15-PGDH, through β -catenin increases PGE2 levels, resulting in PGT expression that resembles "very early stage(s) of colorectal neoplasia in sporadic tumors as well as those arising in the context of germline APC mutation" (Fig 2B and C, Smartt et al., 2012). This indicates the crucial nature of ASA to thus inhibit elevated COX1 PG levels during early colorectal cancer before COX-2 and PGE2, further explaining the "particular efficacy in colorectal cancer chemoprevention of non-selective NSAID aspirin" (Smartt et al., 2012).

3.3 ASA promotes apoptosis

Signaling pathways involving interleukin-6 transcription factor – signal transducer and activator of transcription factor 3 (IL-6-STAT3) are included in previous mentioned pathways such as Wnt/ β -catenin, which are associated with colorectal carcinogenesis. It has also been noted that besides activation of STAT3, IL-6 a pro-inflammatory mediator that helps initiate synthesis of PGE2, which has already been discussed above as a pro-colorectal cancer mediator as well. Thus ASA, which inhibits both of these, is proven to play an important role in prevention of colorectal tumorigenesis (Tian et al., 2011). By inducing a mouse model for inflammation-related colorectal cancer and visualizing ASAs effect on tumor number and size of colorectal cells were quantified by investigating molecules such as STAT3, IL-6 and their downstream anti-apoptotic genes Bcl-2 and Bcl-xl through

ELISA and western blots. In order to identify apoptosis, an apoptotic index (AI) was determined that examined stained sections and for each, five fields of non-necrotic areas of carcinoma were studied and apoptotic nuclei were counted. All of these analyses were considered significant with p-value <0.05. From their results they concluded that specimens induced with inflammation-related colorectal cancer plus aspirin had comparatively greater AI than those simply induced with colorectal cancer alone with p=0.01 (Fig. 3, Tian et al., 2011). It was further revealed that aspirin also highly promoted the apoptosis of colorectal cancer cells through a specific terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end label (TUNEL) staining. Such staining were able to visualize extended areas of epithelial apoptosis in the tumor tissue (p<0.01), which did not show results in those that were treated with aspirin in non-tumor tissues. In addition to apoptosis, they were able to identify that IL-6 levels were much lower in those induced with inflammation-related colorectal cancer plus aspirin versus those of the control group (fig 4) at a p<0.01. Such findings were lastly continued further to include that ASA treatment also suppressed the expression of Bcl-2 and Bcl-xl anti-apoptotic genes. It was significantly proven that both genes had decreased levels in those induced with inflammation-related colorectal cancer plus aspirin groups in comparison to those without aspirin treatment (p<0.01). Thus it was confirmed that ASA plays a significant role in induction of apoptosis by means of down-regulating IL-6-STAT3 in colorectal cancers (Tian et al., 2011). Furthermore, to tie this with the previous study regulating PGE2, ASA thus has the ability to work simultaneously to decrease IL-6, reduce anti-apoptotic mediators and those that promote cell growth.

3.4 ASA on mTOR inhibition and activation of AMP-activated protein kinase

Similarly an experimental study examined how aspirin targets yet another molecule, adenosine monophosphate-activated kinase (AMPK) and signaling via target of rapamycin (mTOR). mTOR is an important target that "controls cell survival and regulation of metabolism pivotal in assimilating growth factor, [as well as] nutrient and signaling stimuli that regulate protein synthesis and growth" (Din et al., 2012). Din et al., obtained colorectal cell lines and AMPK knockout mouse embryonic fibroblasts, and through immunoblotting were able to measure the effects by which ASA reduced mTOR signaling and changed nucleotide ratios of AMPK in colorectal cells. Thus ASA induced autophagy, which results from inhibition of mTOR (Din et al., 2012). They demonstrated for the first time how aspirin inhibits downstream effectors mTORC1: S6K1, 4E-BP1 and S6, mTORC1 being 1 of the 2 distinct complexes that forms mTOR, that is involved in integration of growth factor and nutrient signals in order to influence protein synthesis, growth and autophagy (Din et al., 2012). The mTORC1 also has the ability to regulate the other complex making up mTOR (mTORC2) via phosphorylation of S6 kinase 1 (S6K1). This pathway ties in substantially with PIK3CA mutations, for "substantial evidence implicates dysregulated PIK3/mTOR signaling in cancer development, including colorectal cancer (CRC), where PIK3 signaling mutations occur in 40% of CRC (Din et al., 2012). Furthermore activation of AMPK, which is an important cellular energy sensor, can lead to mTOR suppression. Thus if this is mutated, it does not act as a tumor suppressor and this is in fact what is inactivated by germline mutations in Peutz-Jeghers syndrome; another CRC susceptibility disorder (Din et al., 2012). It was also shown that a striking decrease in S6K1 phosphorylation occurred within 10 minutes and that overall decrease after 16 hours was produced in all CRC cell lines after aspirin (Fig. 5, Din et al., 2012). They also tied in AMPKs activation effects on mTOR by showing that ASA increased phosphorylation of AMPK thus

increasing AMPK's activity at 10 minutes in all CRC cell lines (Fig. 6, Din et al., 2012). Lastly once it was established that ASA changes the mTOR signaling within CRC cell lines, it was also found that "aspirin increased cleaved caspase-3 and reduced proliferating cell nuclear antigen (PCNA) levels in CRC cells" (Fig. 7, Din et al., 2012) which is what occurs with apoptosis and inhibition of proliferation. It was also observed that that LC3 (common autophagy marker) and its processed form LC3-I and post-induction levels of LC3-II, increased after ASA usage in CRC cell lines (Din et al., 2012).

4 DISCUSSION

It is evident that there is overwhelming evidence that ASA is no longer a drug just for cardiovascular event prevention but also works at a variety of molecular levels to prevent and prolong survival in colorectal cancers. Ranging from the top of the inflammatory pathway, down to PG alteration, ASA clearly affects tumorigenesis on a variety of levels.

4.1 Interpretation of PIK3CA tumor mutations

Liao et al. (2012) as well as Langley and Rothwell (2013) identified PIK3CA as a target biomarker in those with a mutated form in their CRC. It was demonstrated that "specifically among patients with mutated-PIK3CA tumors, regular aspirin after diagnosis was associated with significantly increased survival" (Liao et al., 2012). Such data as reported by Langley and Rothwell (2013) and Liao et al. show there is evidence that regular aspirin use is suitable for testing as an adjuvant treatment and that identifying mutated-PIK3CA cancers is how we can further predict response and survival (Liao et al., 2012, Langley and Rothwell, 2013).

4.1.2 Limitations

In the study by Liao et al. limited amount of colorectal cancer specific deaths (3/66 patients with mutated PIK3CA) makes it hard to extrapolate to what degree colorectal cancer deaths are prevented due to ASA usage. Furthermore they discuss and note that their data on cancer treatment was limited (Liao et al., 2012), and that some assumptions based on time of diagnosis and chemotherapy had to be made in regards to PIK3CA mutation status. Langley and Rothwell (2013) further emphasize that although ASA has been shown to reduce the development of distant metastasis and its use as adjuvant therapy is quite effective, that "the mechanism of action is unclear and the benefits of starting aspirin after a diagnosis of cancer have not yet been determined in randomized trials" (Langley and Rothwell, 2013). Langley and Rothwell, opine that the definition of "regular aspirin" was broad and given the low frequency of PIK3CA mutations in patients, it seems quite unlikely that the effect of PIK3CA alone is enough to explain the larger effects of aspirin on colorectal cancers (Langley and Rothwell, 2013).

4.1.3 Future Directions

Both studies reflect on the opportunity of PIK3CA becoming a regular biomarker for the use of aspirin after diagnosis of colorectal cancer and how it is associated with increased survival among patients with mutated PIK3CA tumors specifically. Yet it is necessary that it be acknowledged as only part of the anticancer effects of aspirin. Furthermore, Langley and Rothwell mention that "increasing knowledge about the epidermal growth factor receptor (EGFR) pathways (that include PI3K), which promote cell growth and survival in colorectal cancer, provide an opportunity to individ-

ualize therapy" but that it is also essential that randomized clinical studies be performed in order to determine aspirin's efficacy (Langley and Rothwell, 2013).

4.2 Interpretations on increased (PGE2) regulating PI3K and β -catenin regulating both

Smartt et al. (2012) were able to identify a crucial link between two important pathways found in colorectal cancers, Wnt/ β -catenin and PGE2 via a negative regulation of PGT expression. These results point out another molecular target that can be sought out in colorectal cancer patients and thus downregulated at early stages of those with sporadic as well as APC germline mutation forms. In addition, by looking further into how β -catenin is influencing 15-PGDH, they were able to identify two important regulators of PG catabolism, which in turn relates to ASA and its ability to shut-down COX pathways and thus PG synthesis in the prevention and survival of cancer patients.

4.2.2 Limitations

Due to the study design performed in Smartt et al., (2012) the immunohistochemical studies and interpretations do not demonstrate any clear quantifiable results. But rather results are based on gradients and "quantitative real-time PCR". As with any experimental results, human error could have occurred that limit such results. Furthermore results were representative on a minimum of three independent repeat experiments, and they did not state in their study at what p-value or confidence interval they were interpreting their findings.

4.2.3 Future Directions

Smartt et al. showed how β -catenin plays a role in repressing PGT and 15-PGDH. Since both catabolize PG, and elevated PG levels have been seen in early colorectal neoplasia, there is another means by which ASA could be working to reduce PG as well and why it has helped in patients. Yet there still needs to be further investigation on what exact dose of aspirin is in fact needed to reduce PG levels enough to be chemopreventative.

4.3 Interpretations of ASA promoting apoptosis

After reading various articles on the different plausible mechanisms by which ASA works to reduce cancer, it was interesting to come across ones that directly link apoptosis to aspirin's effects. Tian et al. (2010) induced chronic inflammation within mice, mimicking how ASA is reducing inflammatory changes and increasing apoptosis. Unlike the previous studies mentioned Tian et al. (2010) were able to show that "long-term consistent use of aspirin... is necessary to achieve a protective effect against CRC [with prescriptions lasting five or more years] and that in their mouse study a "dose of aspirin is equivalent to a dose of 80-110 mg/day in humans" (Tian et al., 2010). In addition they were able to identify that IL-6-STAT3 trans-signaling pathway is a more selective therapeutic approach to treating CRC and that Bcl-2 and Bcl-xl, both decreased in aspirin-treated groups (Tian et al., 2010). Thus Tian et al. expanded the current discussion by introducing apoptosis as another underlying molecular mechanism of ASA on colorectal cancer.

4.3.2 Limitations

Unlike the other studies mentioned in this paper, Tian et al. (2010) did not

provide a molecular target that a subset of colorectal patients may use to see ASA results. Rather it simply reveals another molecular mechanism that ASA could be working on in addition to the ones already noted. Yet although not providing a distinct mutation of target such as PIK3CA, it does reflect on the idea that added inflammation of the bowels can trigger and cause CRC, and that this alone should be limited in order to add chemoprevention through ASA. They also acknowledge that "the exact downstream effects from constitutively activated STAT3 that promote tumorigenesis are not fully revealed [and that] STAT3 has the capacity of inducing expression of genes that promote angiogenesis, cell cycle progression and cell survival" (Tian et al., 2010). Thus as in the other papers, the study did not completely rule out that ASA does not act by other mechanisms to produce the effects.

4.3.3 Future Directions

Tian et al. (2010) emphasized that the IL-6-STAT3 signaling pathway activates many genes as well as apoptosis of CRC cells. Yet there is still a need for more studies to gain a better understanding of the therapeutic mechanism of ASA (Tian et al., 2010) and what other possible signaling pathways are activated and inactivated by ASA means.

4.4 Interpretation on mTOR inhibition and activation of AMP-activated protein kinase

Din et al. (2012) showed that aspirin works by another mechanism, inhibition of mTOR in CRC cells and its activation of AMPK. This is in addition to Tian et al., (2010) discussing the effect of ASA on apoptosis and autophagy, which occurs as a characteristic of mTOR inhibition caused by ASA (Tian et al., 2010). This is important for "constitutively activated mTOR signaling has been shown previous in CRC" (Din et al., 2012) and that targeting mTOR through inhibition decreases adenoma formation in mice with FAP and inhibition of CRC cell growth. This again provides another mechanism by which susceptible colorectal individuals could be targeted with ASA preventative therapy.

4.4.2 Limitations

Din et al. (2012) highlight that an alternative mechanism causing inhibition of mTORC1 via RAG (guanosine triphosphate inhibition) has arisen. Again as in the other limitations mentioned previously, aspirin may be working on a variety of levels leading to their findings and that "upstream mechanism underlying aspirin-induced AMPK activation merit further investigation" (Din et al., 2012).

4.4.3 Future Directions

As ASA research in relation to colorectal cancer prevention grows, Din et al. (2012) mention, "combination treatment is [a] particularly attractive strategy to combat the metabolic syndrome, characterized by hyperinsulinemia, insulin resistance, obesity, type 2 diabetes and hypertension [which plays] a strong association with colorectal neoplasia" (Din et al., 2012). Furthermore, they discuss that there is current evidence that shows that physical inactivity is associated with cancer risks and because exercise activates AMPK, there are links that tie AMPK and mTOR to cancer-protection effects of exercise (Din et al., 2012). This leads to the implication that colorectal cancer prevention can be targeted through a combination of ASA and its molecular mechanisms as well as exercise in the future.

6 CONCLUSION

Colorectal cancer is rising to one of the most common cancers today. In our aging population, we are always searching for new ways to reduce cancers that increase with age such as colorectal. Aspirin has been a useful drug in the prevention of cardiovascular events that also rise in an aging population. Thus it made sense to start looking into how aspirin works at the molecular level and if it also pertains to prevention of colorectal cancer.

It is evident that there are a variety of molecular mechanisms by which aspirin acts in the prevention of colorectal cancer and is important to begin implementing the knowledge presented in this paper in those with susceptible genes and markers. With such steps, we may begin to better understand how a combination of preventative measures could possibly reduce this rising cancer.

7 FIGURES AND TABLES

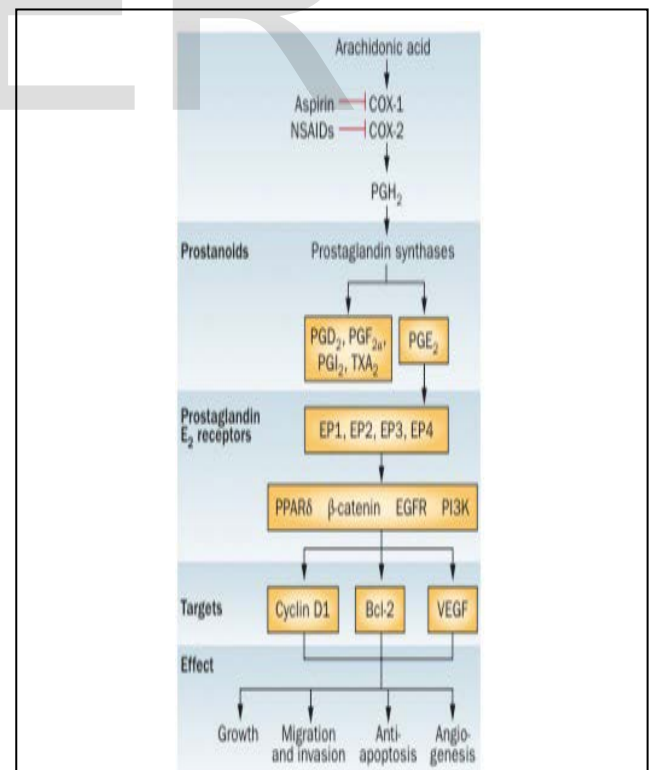
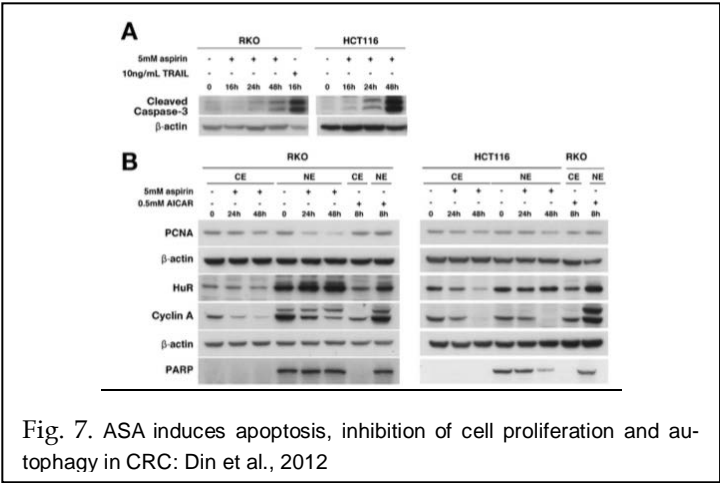
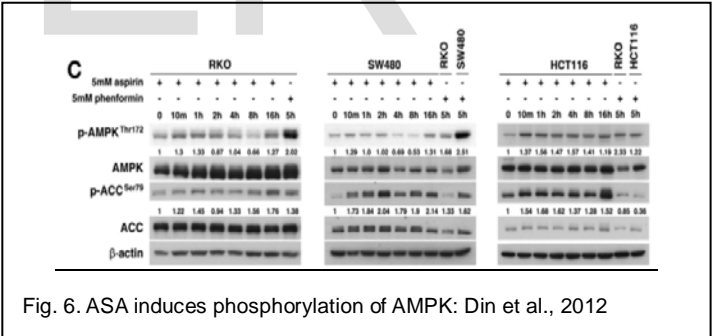
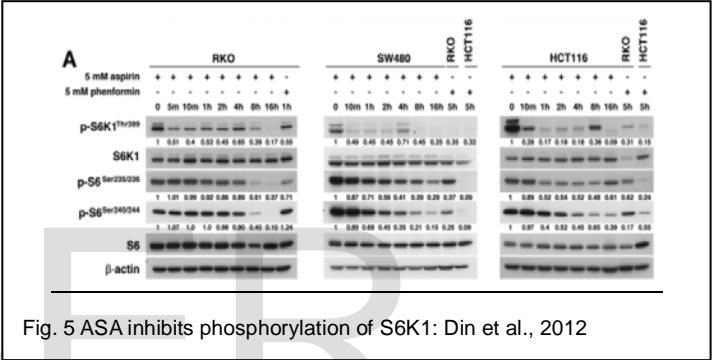
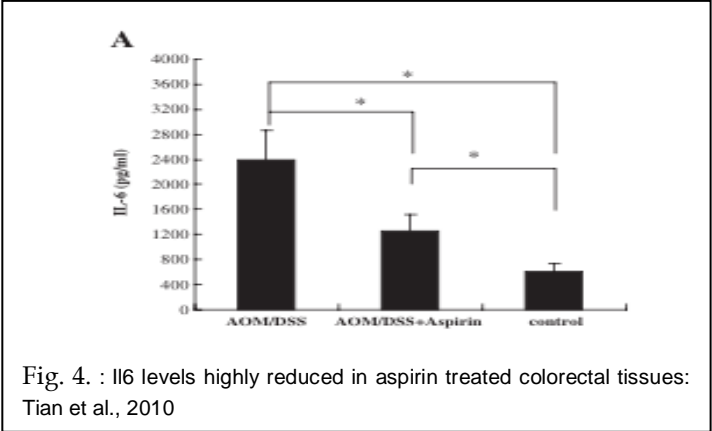
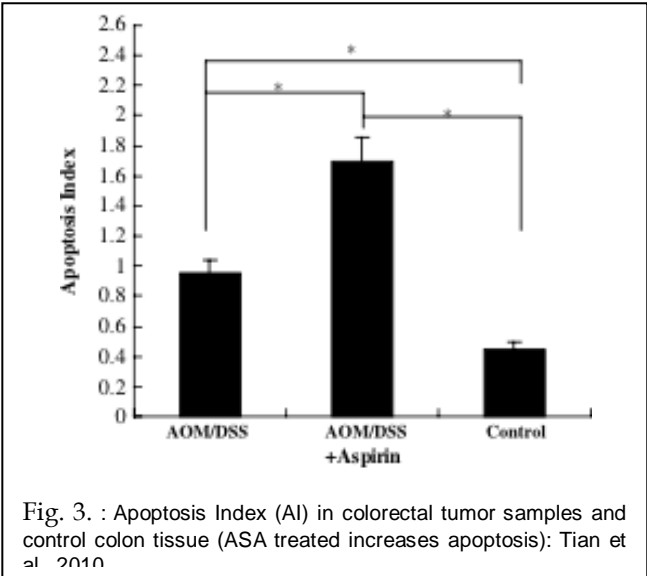
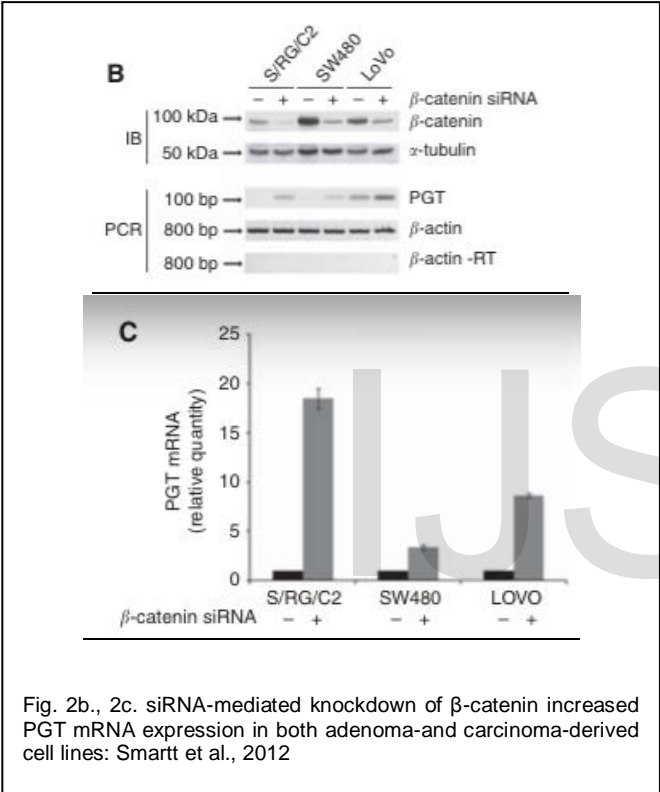
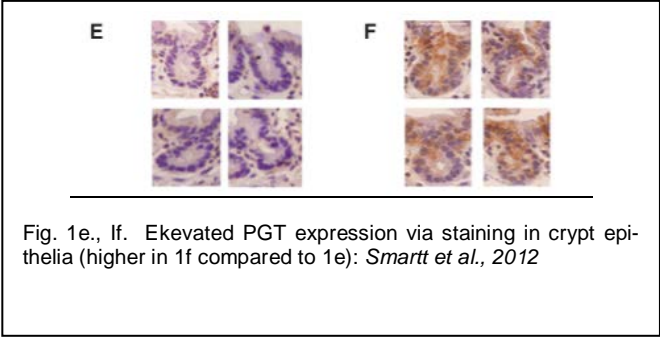


Fig. 1. Summar of Arachidonia Acid Pathway: Langley and Rothwell 2013



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