

# 11 $\beta$ -Hydroxysteroid Dehydrogenase Type II is a Potential Target for Prevention of Colorectal Tumorigenesis

## Abstract

Colorectal cancer (CRC) is a leading cause of cancer death, yet primary prevention remains the best approach to reducing overall morbidity and mortality. There is a clear molecular link between cyclooxygenase-2 (COX-2)-derived prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production and CRC progression. Although selective COX-2 inhibitors as well as non-steroidal anti-inflammatory drugs (NSAIDs) reduce the number and sizes of colonic adenomas, increased cardiovascular risks of selective COX-2 inhibitors and increased gastrointestinal side-effects of NSAIDs limit their use in chemoprevention of CRC. Glucocorticoids induce apoptosis and are endogenous, potent COX-2 inhibitors. Glucocorticoids have been used for the treatment of hematologic malignancies, but not for solid tumors due to adverse side-effects such as immunosuppression and osteoporosis. In tissues, glucocorticoid actions are down-regulated by type 2 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD2), and inhibition of 11 $\beta$ HSD2 activity will elevate intracellular active glucocorticoid to levels that effectively suppress COX-2 expression. Both COX-2 and 11 $\beta$ HSD2 increase in *Apc*<sup>-/-min</sup> mouse intestinal adenomas and human colonic adenomas and either pharmacologic or genetic 11 $\beta$ HSD2 inhibition leads to decreases in COX-2-mediated PGE<sub>2</sub> production in tumors and prevents adenoma formation, tumor growth, and metastasis. 11 $\beta$ HSD2 inhibition may represent a novel approach for CRC chemoprevention by increasing tumor cell intracellular glucocorticoid activity, which in turn inhibits tumor growth by suppressing the COX-2-derived PGE<sub>2</sub> pathway, as well as other pathways, without potential side-effects relating to chronic application of COX-2 inhibitors, NSAIDs and glucocorticoids.

## Introduction

Colorectal cancer (CRC) is ranked the third most common form of cancer worldwide in terms of incidence and mortality [1]. Most patients present with advanced disease, and metastatic disease remains largely incurable. Therefore, prevention remains the best approach to reduce the overall morbidity and mortality of CRC.

In 1950, the Nobel Prize for Physiology or Medicine was awarded to Tadeus Reichstein, Edward C. Kendall and Philip S. Hench for the isolation of glucocorticoid hormones and the discovery that cortisol dramatically alleviated symptoms of rheumatoid arthritis [2]. Glucocorticoids are produced in the zona fasciculata (middle cortical layer) of adrenal gland. Glucocorticoids regulate a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions and are important for fetal development. Glucocorticoids have been used to treat diseases caused by an overactive immune system, such as allergies, asthma, and autoimmune diseases as well as a broad spectrum of hematologic malignancies, including leukemia, lymphoma, and myeloma due to their ability to induce apoptosis [3-5]. Application of glucocorticoids in solid tumors is limited due to adverse side effects such as immunosuppression, inhibition of bone

formation, suppression of calcium absorption and delayed wound healing.

## Regulation of Glucocorticoid Actions

Although circulating levels of glucocorticoids are much higher than most hormones, only about 10% of circulating glucocorticoid is free, because most of circulating glucocorticoids bind to plasma proteins, particularly the corticosteroid-binding globulin, transcortin. As protein-bound glucocorticoid is biologically inactive, protein binding leads to decreases in metabolic clear rate and attenuation of fluctuations of glucocorticoid concentration.

Circulating glucocorticoid levels are regulated by the hypothalamo-pituitary-adrenal axis [6]. Peptide corticotropin-releasing hormone (CRH) released from hypothalamus following stressors stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which in turn stimulates adrenal gland to secrete glucocorticoids. When blood concentrations of glucocorticoids rise above a certain threshold, they inhibit CRH secretion from the hypothalamus, leading to turning off of ACTH secretion and subsequent turning off of glucocorticoid secretion from adrenal gland.

Glucocorticoids act through binding to the cytosolic glucocorticoid receptor, a member of the superfamily of ligand regulated nuclear receptors [7]. Human glucocorticoid receptor, cloned in 1984, is comprised of 777 amino acids and contains a DNA binding domain (DBD), a ligand binding domain (LBD), and  $\tau$ 1 and  $\tau$ 2 activation domains. Glucocorticoid receptor exists predominantly in the cytoplasm as an inactive complex with other proteins, including hsp90 as well as immunophilins p59 and calreticulin [8,9]. In the cytoplasm, binding of glucocorticoid to the LBD of the glucocorticoid receptor causes receptor conformational change, leading to dissociation of the receptor-protein complex and translocation of glucocorticoid receptor into the nucleus, where it binds to glucocorticoid response elements (GRE) in the promoter region of targeted genes and regulates gene expression, either transactivation or transrepression. Notably, lipocortin I or annexin I, an inhibitor of arachidonic acid release from plasma membrane, is up-regulated by glucocorticoid



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[10]. Glucocorticoids can act through delayed, genomic mechanisms (as described above) as well as rapid, non-genomic effects [11].

In addition to systemic regulation, glucocorticoid effects are also regulated locally in tissues by a “pre-receptor” regulatory mechanism involving 11 $\beta$ -hydroxysteroid dehydrogenase type I (11 $\beta$ HSD1) and 11 $\beta$ HSD2 [12]. 11 $\beta$ HSD1 produces active glucocorticoids from inactive metabolites, while 11 $\beta$ HSD2 converts glucocorticoids to their inactive keto-forms. 11 $\beta$ HSD1 is expressed in key glucocorticoid target tissues such as liver and adipose tissue, while 11 $\beta$ HSD2 is expressed predominantly in mineralocorticoid responsive tissues such as the kidney, salivary gland and colon [13-16]. In addition to the activation of glucocorticoid receptors, glucocorticoids also possess the potential to activate the mineralocorticoid receptor. The concentration of glucocorticoids in the circulation is  $\sim 10^{-6}$  M, whereas the concentration of aldosterone in the circulation is  $\sim 10^{-9}$  M [17]. Therefore, the physiological function of 11 $\beta$ HSD2 is to protect mineralocorticoid receptors from activation by cortisol (human) or corticosterone (CS, rodents), thus providing mineralocorticoid receptor selectivity to aldosterone [12]. The syndrome of apparent mineralocorticoid excess (AME) is caused by mutation in exon 5 of 11 $\beta$ HSD2, which leads to a premature stop site at codon 374 instead of a normal arginine (R374), with the deletion of 32 amino acids from the C-terminus. AME, an autosomal recessive, is characterized by hypertension, hypokalaemia and suppression of the renin-angiotensin-aldosterone axis due to cortisol activation of mineralocorticoid receptors as a result of deficiency of 11 $\beta$ HSD2 activity [18]. AME is an example of human hypertension arising from a single gene defect.

### **11 $\beta$ HSD2 acts as a procarcinogenic agent through attenuation of glucocorticoid actions**

Glucocorticoids have been used for decades in the treatment of hematologic malignancies [3-5]. In recent years, glucocorticoids have also been used in the treatment of solid tumors [19]. Glucocorticoids are extensively used in combination chemotherapy of advanced prostate cancer [20]. Dexamethasone has been used to inhibit colorectal cancer hepatic metastasis [21]. In experimental studies, glucocorticoids have been shown to effectively prevent the development of lung tumors in A/J mice as well as the development of skin tumors [22-24]. Liposomal prednisolone (glucocorticoid) phosphate inhibited tumor growth dose-dependently, with 80% to 90% tumor growth inhibition of subcutaneous B16.F10 melanoma and C26 colon carcinoma murine tumor models at 20 mg/kg by single or weekly doses [25]. Dexamethasone inhibited prostate tumorigenesis through inhibition of tumor-associated angiogenesis by decreasing VEGF through activation of glucocorticoid receptors [26,27].

Recently, 11 $\beta$ HSD2 has been proposed to be a pro-proliferative agent due to its ability to attenuate the anti-proliferative effects of glucocorticoids. The most conclusive evidence for the role of 11 $\beta$ HSD2 in tumorigenesis comes from the analysis of 11 $\beta$ HSD isoenzyme expression in a large cohort of human pituitary tumors [28-30]. 11 $\beta$ HSD1 expression decreases, while 11 $\beta$ HSD2 expression dramatically increases in pituitary tumors compared with normal tissues. Inhibition of 11 $\beta$ HSD2 activity suppresses pituitary cell proliferation and augments glucocorticoids anti-proliferative effects. 11 $\beta$ HSD2 expression increases in human adrenal cortical carcinoma/

adenoma, breast cancers, and neoplastic cell lines [31-36]. In cultured cell lines, overexpression of 11 $\beta$ HSD2 stimulates cell proliferation, while inhibition of 11 $\beta$ HSD2 activity suppresses cell proliferation [32-35].

### **Cyclooxygenase-2 and colorectal tumorigenesis**

In 1934, Nobel laureate (1970, Medicine or Physiology) Ulf von Euler found that the injection of extracts of sheep vesicular gland dramatically decreased blood pressure. Extracts from human seminal fluid had a similar effect. Although von Euler named the substance prostaglandin (PG) due to the mistaken belief that it originated in the prostate gland, we did find that cyclooxygenase-2, the major enzyme to mediate prostaglandin production, is constitutively expressed in distal vas deferens of the prostate and its expression is many fold greater than in any other organs of the body and is regulated by androgens [37]. In the 1950s, Bergstrom and his associates purified two important prostaglandins, PGE and PGF, and identified their chemical structure. Bengt I Samuelsson elucidated arachidonic acid and prostaglandin metabolism and discovered endoperoxides, thromboxanes and leukotrienes. Aspirin is the most frequently used drug all over the world. Sir John Vane discovered prostacyclin and found that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) act through inhibition of prostaglandin biosynthesis. For their contributions to the crucial breakthrough in prostaglandin research, Sune Karl Bergstrom, Bengt I Samuelsson and John Vane shared the Nobel Prize in Medicine and Physiology in 1982. Until the late 1980s, only one isoform of COX had been identified (now recognized as COX-1). Needleman et al. first proposed the existence of an inducible, glucocorticoid-suppressive cyclooxygenase activity, which was then identified by two independent groups, and is now called COX-2 [38-40]. COX-1 is responsible for ‘housekeeping’ prostaglandin biosynthesis and is constitutively expressed in most tissues, while COX-2 is induced by growth factors and pro-inflammatory cytokines, and exquisitely suppressed by glucocorticoids.

During the development of CRC, prostaglandin production, particularly the E series of prostaglandins, is significantly increased in malignant tissues. Decreased polyp multiplicity and regression of polyps with long-term use of sulindac in patients with familial adenomatous polyposis (FAP) provided the first insights into the potential chemopreventive actions of NSAIDs on CRC [41,42]. Multiple studies have shown a 40-50% reduction in CRC in individuals taking NSAIDs regularly either in the context of sporadic CRC or in FAP patients [43-59]. A recent retrospective cohort study indicates that aspirin reduces cancer risk in patients with high levels of COX-2 in the tumors, and regular aspirin use after the diagnosis of colorectal cancer improves overall survival, particularly in patients with high levels of COX-2 in the tumors [60,61]. In a most recent report, regular use of aspirin is reported to be associated with longer survival among CRC patients with PIK3CA mutation [62]. NSAIDs reduce the relative risk of CRC primarily due to their ability to inhibit prostaglandin production [63]. However, the chemopreventive and therapeutic application of NSAIDs to CRC is limited due to adverse side effects (gastric ulceration and perforation) [64]. Reports estimate 10,000 to 20,000 deaths and 100,000 hospitalizations per year in the United States related to traditional NSAIDs [65]. The anti-inflammatory properties of NSAIDs are attributed to the inhibition of COX-2, while the gastrointestinal side effects of NSAIDs are attributed to the inhibition of COX-1 [63,66].

COX-2 expression increases in *Apc<sup>+/-min</sup>* (multiple intestinal neoplasms) and *Apc <sup>$\Delta$ 716</sup>* (adenomatous polyposis coli <sup>$\Delta$ 716</sup>) mouse adenomas, in azoxymethane-induced colonic cancer, and in human colorectal adenomas and cancers [67,68]. COX-2 also plays an important role in CRC metastasis [69-72]. Hepatic metastases develop more frequently when primary CRC expresses high COX-2 levels. COX-2 expression increases not only in the primary CRC, but to even higher levels in hepatic metastases [69]. Deletion of the COX-2 gene suppresses adenoma development in *Apc <sup>$\Delta$ 716</sup>* mice and *Apc<sup>+/-min</sup>* mice [73,74]. Therefore, COX-2 is involved in colorectal tumorigenesis at multiple stages. COX-2-derived PGE<sub>2</sub> promotes tumor growth and metastasis through stimulation of cell proliferation, cell migration, cell invasion, angiogenesis, and immunosuppression [70,72,75-80].

Selective COX-2 inhibitors were developed based on the hypothesis that COX-2 was the major source of prostaglandins that mediate inflammation while COX-1 was the source of cytoprotective prostaglandins in the gastric epithelium [81]. COX-2 inhibitors that reached the market (such as celecoxib) have shown similar efficacy to the traditional NSAIDs in the treatment of acute and chronic inflammatory conditions, but with fewer gastrointestinal side effects [82,83]. Selective COX-2 inhibitors have been shown to be effective in rodent models of colonic carcinogenesis [67,84-89]. Clinically, celecoxib, a selective COX-2 inhibitor, significantly reduced the number and burden of colorectal polyps in FAP patients [90,91]. However, long-term use of high doses of selective COX-2 inhibitor is currently not recommended because of the unacceptable cardiovascular side effects in certain patients, especially those with a history of atherosclerotic heart disease [92]. Possibly due to inhibition of endothelial cell-derived COX-2 activity, with selective inhibition of PGI<sub>2</sub> production, leaving COX-1-mediated production of thromboxane A<sub>2</sub> and subsequent platelet aggregation unchecked [81,93-96]. Long-term use of COX-2 inhibitors also increases blood pressure [97-99].

### **Inhibition of 11 $\beta$ HSD2 activity suppresses colorectal tumorigenesis through enhancing glucocorticoid-mediated inhibition of COX-2 pathway**

Since its discovery, COX-2 has been found to be suppressed by glucocorticoids via glucocorticoid receptors in all cell types and tissues. Activation of glucocorticoid receptors inhibits COX-2 expression primarily through three mechanisms [7]: 1. *Transcriptional inhibition:* Activation of either AP-1 or NF- $\kappa$ B leads to increased COX-2 transcription. Glucocorticoid receptors directly inhibit COX-2 expression by interaction with AP-1 and NF- $\kappa$ B; 2. *Indirect transcriptional inhibition:* Glucocorticoids induce I $\kappa$ B $\alpha$ , which directly inhibits NF- $\kappa$ B activity. Glucocorticoids also induce MAPK phosphatase-1 (MKP-1), which inhibits p38, ERK and JNK activities, leading to inhibition of NF- $\kappa$ B and AP-1. Glucocorticoids also induce glucocorticoid-induced leucine zipper, which directly inhibits transcription of both AP-1 and NF- $\kappa$ B; 3. *Post-transcriptional inhibition:* Sustained p38 activity is required for the stabilization of COX-2 mRNA. MKP-1 induced by glucocorticoids inactivates p38, leading to destabilization of COX-2 mRNA.

Recent studies have demonstrated a clear molecular link between COX-2-derived PGE<sub>2</sub> activation of prostaglandin E receptor type 2 (EP2) and colorectal cancer progression [100]. Glucocorticoids and NSAIDs inhibit prostaglandin biosynthesis through different

mechanisms. NSAIDs inhibit prostaglandin biosynthesis by non-competitive inhibition of both COX-1 and COX-2 enzymatic activity. Glucocorticoids are the most potent, endogenous, specific COX-2 inhibitors. Glucocorticoids suppress prostaglandin production through inhibiting cytosolic phospholipase A<sub>2</sub> activity and suppressing COX-2 and mPGES-1 expression [7,101,102]. The concentration of glucocorticoids in the circulation is  $\sim 10^{-6}$  M [103]. In cultured cells, COX-2 expression is inhibited by glucocorticoids in the range of  $10^{-10}$ - $10^{-7}$  M. For example, in cultured mouse medullary interstitial cells, which express high levels of COX-2 but do not express detectable 11 $\beta$ HSD2, COX-2 expression is inhibited by  $10^{-9}$  M CS [103]. We found that there is constitutive COX-2 expression in epithelial cells in the macula densa and adjacent cortical thick ascending limbs in kidney cortex [104,105]. Renal cortical COX-2 expression increases after adrenalectomy (glucocorticoid and mineralocorticoid deficiency). Glucocorticoid replacement with corticosterone reverses the cortical COX-2 elevation seen in adrenalectomized rats. Inhibition of the glucocorticoid receptors with RU486 increases cortical COX-2 expression. Inhibition of 11 $\beta$ HSD2 activity with glycyrrhizic acid leads to suppression of renal cortical COX-2 expression, which is reversed by the glucocorticoid receptor antagonist RU486 [99]. These results indicate that 11 $\beta$ HSD2 inhibition may mimic glucocorticoids to suppress COX-2 expression by attenuating glucocorticoid inactivation, thereby increasing intracellular active glucocorticoids.

11 $\beta$ HSD2 activity is higher in the colon than in the small intestine in the rat and it is primarily localized to intestinal epithelia [106]. In intestinal tumors, 11 $\beta$ HSD2 was expressed in epithelial and stromal cells [36,107,108]. Interestingly, the incidence of intestinal tumors is always higher in the colon than in the small intestine. The mechanism underlying increase in COX-2 expression in colorectal cancer is not completely understood. We recently found that 11 $\beta$ HSD2 mRNA levels were significantly higher in both human colonic small and large adenomas comparing to adjacent normal colon tissues [101]. Immunohistochemical staining showed that increased 11 $\beta$ HSD2 expression was primarily localized to adenoma epithelia and co-localized with increased COX-2 expression. Both 11 $\beta$ HSD2 and COX-2 expression also increased in adenomas of *Apc<sup>+/-min</sup>* mice. Mouse adenocarcinoma CT26 cells demonstrate significant tumorigenic activity and express high levels of COX-2, and both their proliferation *in vitro* and the size and number of CT26 tumors *in vivo* are sensitive to COX-2 inhibition [109]. CT26 cells also express 11 $\beta$ HSD2. Corticosterone treatment (the active glucocorticoid in rodents) leads to inhibition of CT26 cell COX-2 expression, which is enhanced by pharmacologic or genetic inhibition of 11 $\beta$ HSD2 activity but is attenuated by overexpression of 11 $\beta$ HSD2. Pharmacologic inhibition of 11 $\beta$ HSD2 activity with glycyrrhizic acid (GA) suppresses adenoma development and growth, associated with inhibition of adenoma COX-2 and mPGES-1 expression in *Apc<sup>+/-min</sup>* mice. Knockdown of 11 $\beta$ HSD2 in CT26 cells leads to inhibition of CT26 tumor growth associated with inhibition of CT26 tumor COX-2 and mPGES-1 expression as well as PGE<sub>2</sub> production. Pharmacologic inhibition of 11 $\beta$ HSD2 activity with GA increases active glucocorticoid levels but decreases inactive 11-keto-corticosterone levels in CT26 tumors as well as in tissues with high levels of 11 $\beta$ HSD2 activity such as kidney and colon. GA treatment leads to decreases in CT26 tumor incidence, inhibition of CT26 tumor growth, decreases in tumor phosphorylated cPLA2 (active form), COX-2 and mPGES-1 expression as well as tumor PGE<sub>2</sub> levels; all of these parameter are



reversed by treatment with RU486, an inhibitor of glucocorticoid receptors. Either genetic or pharmacologic inhibition of 11 $\beta$ HSD2 activity suppresses CT26 tumor metastasis. Human colon carcinoma HCA-7 cells constitutively express both 11 $\beta$ HSD2 and COX-2, and COX-2 activity is essential for the proliferation of HCA-7 cells [110]. On the other hand, human colon carcinoma HT-29 cells constitutively express similar levels of 11 $\beta$ HSD2 with minimal COX-2 expression, and COX-2 activity is not essential for HT-29 cell proliferation [111]. Inhibition of 11 $\beta$ HSD2 activity with glycyrrhizic acid has no effect on HT-29-derived tumor growth, but significantly inhibits HCA-7-derived tumor growth, which is associated with decreases in tumor COX-2 and mPGES-1 expression. Therefore, 11 $\beta$ HSD2 inhibition represents a novel approach for colorectal cancer chemoprevention and therapy by increasing tumor glucocorticoid activity, which in turn selectively blocks local COX-2 activity.

### Other mechanisms by which 11 $\beta$ HSD2 inhibition may suppress colorectal tumorigenesis

In addition to inhibition of COX-2 pathway, increased intracellular glucocorticoids due to 11 $\beta$ HSD2 inhibition may suppress proliferation and induce apoptosis and differentiation through other mechanisms. The retinoblastoma protein (Rb), a tumor suppressor, inhibits cell proliferation through its interaction with the E2F family of transcription factors and the resultant regression of genes that are essential for DNA synthesis [112]. The inactivation of retinoblastoma protein is a prerequisite for cell proliferation. Inactivation of Rb is achieved through cyclin-dependent protein kinase-mediated phosphorylation during cell cycle progression. Glucocorticoids inactivate Rb through p53-mediated induction of p21<sup>waf1/cip1</sup>, an inhibitor of cyclin-dependent kinase [105,113-116]. Glucocorticoids may also inhibit tumor growth through suppression of the mTOR signal pathway [117,118]. REDD1 (RTP801, an mTOR complex 1, mTORC1, repressor) is a novel stress-induced gene linked to regression of mTOR signaling and is induced by glucocorticoids [119-121]. Recently, we generated *Apc<sup>+/-min</sup>* mice with selective deletion of 11 $\beta$ HSD2 in intestinal epithelial cells, and intestinal tumorigenesis is inhibited in these mice (our unpublished data). In adenomas from these mice, p53 and p21 levels increased, with concomitant decrease in phosphorylation of retinoblastoma protein. In addition, adenoma REDD1 increased, while the activity of mTOR signaling pathway was inhibited.

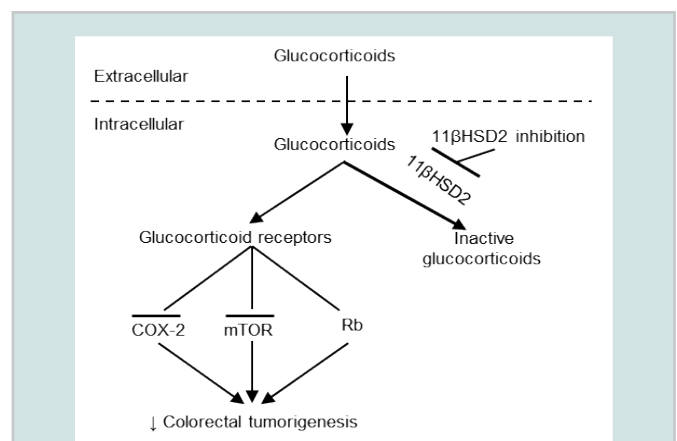
The lipoxygenase (LOX) pathway is another important pathway for metabolism of arachidonic acid. 5-LOX and its metabolite, leukotriene B<sub>4</sub>, have been reported to stimulate the proliferation of several human colon carcinoma cell lines [121-123]. 5-LOX increases in human colorectal tumors, and inhibition of the 5-LOX pathway suppresses the proliferation of human colon carcinoma cell lines and inhibits CRC development [124-127]. Dexamethasone has been reported to inhibit 5-LOX expression and its metabolite production [128-130].

### Conclusion and Future Perspective

We propose that inhibition of 11 $\beta$ HSD2 activity may provide a new target for chemoprevention and/or adjunctive therapy for colorectal cancers, particularly for patients with increased risk, such as familial adenomatous polyposis (FAP) patients, as a result of increased

tumor intracellular active glucocorticoids, because of the following advantages (Figure 1): 1) Glucocorticoids selectively inhibit COX-2, but not COX-1, mediated prostaglandin production. Therefore, inhibition of 11 $\beta$ HSD2 activity has the beneficial effects of traditional NSAIDs to prevent and regress colorectal cancers without the gastrointestinal side effects associated with COX-1 inhibition; 2) Physiologic 11 $\beta$ HSD2 expression is most highly expressed in colon and kidney. Therefore, inhibition of 11 $\beta$ HSD2 activity is not expected to incur the cardiovascular risk posed by COX-2 inhibitors that suppress COX-2-derived PGI<sub>2</sub> production in vascular endothelial cells; 3) Increased levels of intracellular active glucocorticoids are observed only in tissues with elevated 11 $\beta$ HSD2 expression. Inhibition of 11 $\beta$ HSD2 will not produce immunosuppression or other systemic side effects of conventional glucocorticoid therapy; 4) Increased tumor active glucocorticoids also inhibit tumor development and growth through induction of G1 cell cycle arrest and inhibition of the mTOR pathway; 5) Tissue prostaglandin levels are determined by both biosynthesis and catabolism. A key enzyme in prostaglandin catabolism, 15-hydroxyprostaglandin has been reported to be decreased in *Apc<sup>+/-min</sup>* mouse intestinal adenomas and in human colorectal cancer [131], and glucocorticoids have been shown to induce 15-hydroxyprostaglandin in A549 human lung adenocarcinoma cells [132]. Therefore, it is possible that increased intracellular active endogenous glucocorticoids may inhibit colorectal tumorigenesis not only by inhibiting prostaglandin synthesis, but also by enhancing prostaglandin degradation; and finally 6) Glucocorticoid also inhibit the activity of cPLA<sub>2</sub>, preventing potential shunting of arachidonic acid metabolism to other procarcinogenic metabolizing pathway such as 5-lipoxygenase lipoxygenase pathway [128-130,133]. In addition, glucocorticoids inhibit both COX-2 and 5-LOX.

Glycyrrhizic acid and its analogs are excellent prototypes for 11 $\beta$ HSD2 inhibitors. It is a natural compound contained in licorice, a natural botanical antiinflammatory agent and a powerful 11 $\beta$ HSD2 inhibitor.



**Figure 1: Proposed mechanism underlying epithelial cell 11 $\beta$ HSD2 activity and colorectal tumorigenesis.** In tumor epithelial cells, glucocorticoids are converted to inactive 11-keto-forms, reducing glucocorticoid receptor activity, while 11 $\beta$ HSD2 inhibition leads to increased levels of epithelial intracellular active glucocorticoid. The subsequent inhibition of the COX-2 pathway and induction of G1 cell cycle arrest through activation of retinoblastoma protein (Rb, a tumor suppressor) and inhibition of the mTOR pathway lead to inhibition of colorectal tumorigenesis.

Long-term excessive ingestion of licorice has been reported to induce hypokalemia and elevation of blood pressure in a subset of people [134]. Although we did not see these side effects in our experimental animals with glycyrrhizic acid treatment, we did observe that the concentrations of glycyrrhizic acid used in our studies increased levels of active glucocorticoid levels in the kidney [101]. Therefore, it is likely that if comparable doses of glycyrrhizic acid were used in humans, some percentage of patients would develop hypertension and/or hypokalemia, requiring treatment with the potassium-sparing diuretic amiloride. However, the need to monitor for these potential side effects should not necessarily preclude the use of 11 $\beta$ HSD2 inhibition as a strategy to inhibit colorectal tumor COX-2 expression and growth. In addition, development of locally acting enteric 11 $\beta$ HSD2 inhibitors that are not systemically absorbed would be a potential therapeutic means to prevent colorectal tumorigenesis [6].

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