

Antidepressant fluoxetine and its potential against colon tumors

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Core tip: It is currently thought that aerobic glycolysis is key for understanding cell survival in the hostile tumor microenvironment. Then, the antidepressant fluoxetine (FLX) has been shown to reduce colon tumor growth in animals and colon cancer incidence in humans. Here, we explore new perspectives of FLX reducing the development of colon tumors through a blockage in tumor metabolism. This perspective review is based on our current unpublished experimental dataset which shows FLX as a potential co-chemotherapeutic agent for colon cancer therapy.

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Abstract

Colon cancer is one of the most common tumors worldwide, with increasing incidence in developing countries. Patients treated with fluoxetine (FLX) have a reduced incidence of colon cancer, although there still remains great controversy about the nature of its effects. Here we explore the latest achievements related to FLX treatment and colon cancer. Moreover, we discuss new ideas about the mechanisms of the effects of FLX treatment in colon cancer. This leads to the hypothesis of FLX arresting colon tumor cells at the G_1 cell-cycle phase through a control of the tumor-related energy generation machinery. We believe that the potential of FLX to act against tumor metabolism warrants further investigation.

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INTRODUCTION

Colon cancer is one of the most common human malignancies worldwide and much effort has been applied to understand its development. The discovery of new therapeutical strategies or potential co-therapeutical agents against it might reduce the suffering of millions of people. A growing body of evidence suggests that the use of fluoxetine (FLX), an antidepressant belonging to the selective serotonin reuptake inhibitors (SSRIs), may be related to a reduced colon cancer incidence. However, its activity is not completely understood and potential new mechanisms are unknown to date.

Here, we discuss our recent published and unpublished data regarding the activity of FLX against colon cancer. This review takes a fresh view of the material, mainly of how FLX acts to block malignant metabolism,

reducing colon tumors.

COLON CANCER

The American Cancer Society estimates the number of new cases and expected deaths for cancer in the United States every year^[1]. About 1.5 million cases and 569490 deaths of cancer were expected in 2010. This ranked colon cancer as the third most common cancer in the United States, with almost 50000 deaths per year^[1,2]. In this year, it is expected that more than 143460 patients will be newly diagnosed with colon cancer in the United States^[3]. Although survival has increased during the 5 years after diagnosis^[2], a 60% increase for newly diagnosed cancer cases is projected for developing countries until 2030^[4]. This highlights colon cancer as one of the major human malignancies worldwide and a great challenge for cancer therapy^[5-7].

Adenoma-adenocarcinoma sequence model

The adenoma-adenocarcinoma sequence model is the most well-known and accepted hypothesis for the development of colon cancer^[8]. It is thought that a sequence of mutations of the epithelial stem cell niche induces the development of colon tumors through different stages, such as initiation, promotion and progression^[8]. Initiation is known as an irreversible step, where mutations in one or two gatekeeper genes occur in a single cryptal stem cell. This will then disrupt cell proliferation, leading to the expansion of malignant clones, a process termed promotion^[9,10]. Mutations are thought to derive from cell exposure to carcinogenic compounds which directly attack the DNA or lead to increased oxidative stress (OS) with the generation of reactive oxygen species (ROS), which would then attack the DNA basis inducing mutations^[11,12]. Clever's research group has elegantly generated *Lgr5^{-EGFP-IRES-oriERT2} / Apc^{fllox/fllox}* mice, which have a stem cell-specific knockin reporter for tamoxifen-inducible loss of the adenomatous polyposis coli (APC) sequence, and found that this genetic deletion in epithelial stem cells leads to their transformation within days, which was due to β -catenin accumulation^[13]. This further supports the idea that a monoclonal propagation of acquired stem cell mutations occurs during the initial steps of colon carcinogenesis^[9]. The manifestation of mutations in colon epithelia seems to be closely related to hyperproliferation^[13-15]. In fact, mutations in the *APC* gene sequence at cryptal stem cell niches activate hyperproliferation due to an increase in β -catenin transcriptional activity which blocks p53 activity^[15-17].

Subepithelial cells and their role in carcinogenesis

The cancer-enhancing activity of the microenvironment has been a matter of discussion since recent reports showed that disrupting key genetic sequences in stromal cells abrogates epithelial homeostasis, which then induces tumors^[18-20]. An elegant report has specifically shown that epithelial tumors have arisen in forestomach after disrupt-

ing the transforming growth factor- β (TGF- β) signaling within the subepithelial compartment^[21]. Previous studies had already shown that the subepithelial TGF- β signaling has tumor promoting potential on epithelial cells, due to its control over proliferation^[22,23]. Nevertheless, under inflammatory conditions, subepithelial cells seem to be able to transform epithelial progenitor cells towards malignancy^[20]. These ideas have actually been applied to colon carcinogenesis, confirming the malignant participation of subepithelial cells in the development and manifestation of colon tumors^[20,24,25].

TUMOR METABOLISM

Hyperproliferation enables the clonal expansion of mutated cells, which further drives tumor growth^[14,15,17,26-29]. For this, tumor cells require: high and fast adenosine-5'-ATP generation; a tightened maintenance of the cell redox status to overcome the stressful tumor microenvironment; and enhanced biosynthesis of macromolecules. Basically, tumor cells shift their energy generation machinery from oxidative phosphorylation to an aerobic-glycolytic metabolism^[30,31]. This allows tumor cells to keep a high ATP generation and at the same time to avoid the negative feedback regulation from overusing glycolysis, which would otherwise activate metabolic and cell-cycle inhibitors, such as p53^[30]. This was extensively discussed by Cairns *et al.*^[31]. Specifically, glycolysis-related mechanisms enhance the synthesis of nucleotides and DNA repair^[30,31]. However, high proliferation enlarges the distance between cells and microvessels, which reduces the oxygen and nutrient supplies to the cells and creates a hypoxic microenvironment. While hypoxia generally promotes the expression of growth factors, inducing neovascularization, hypoxic areas in tumors may persist due to the chaotic and malformed structures of tumoral vessels and microvessels^[14,32-34].

Moreover, hypoxic tumor cells are known to use glycolysis in order to increase energy generation (Figure 1). This requires an over-activation of glucose transporters (*i.e.*, GLUT1), lactate transporters (*i.e.*, MCT4) and lactate dehydrogenase A (LDH-A) through the hypoxia-inducible factor 1 (HIF-1) transcriptional activity. By inhibiting the degradation of HIF-1, a transcription factor which upregulates the glycolysis-related molecular activities, tumor cells increase the conversion of pyruvate to lactate^[32,35]. Because tumor cells would then suffer from the hypoxia-induced and glycolysis-related acidosis, they alkalinize their intracellular pH (ipH) on their way to survival and proliferation. This is achieved *via* hyperactivation of HIF-1 activity, which enhances the hydration of carbon dioxide to bicarbonate by the catalytic activity of carbonic anhydrase IX and XII enzymes and promotes the activity of MCT-4 to extrude lactate and H⁺ ions, both supporting an ipH alkalinization^[32,36]. Overall, tumor cells undergo deep metabolic changes on their way to survival in the stressful tumoral microenvironment^[31].

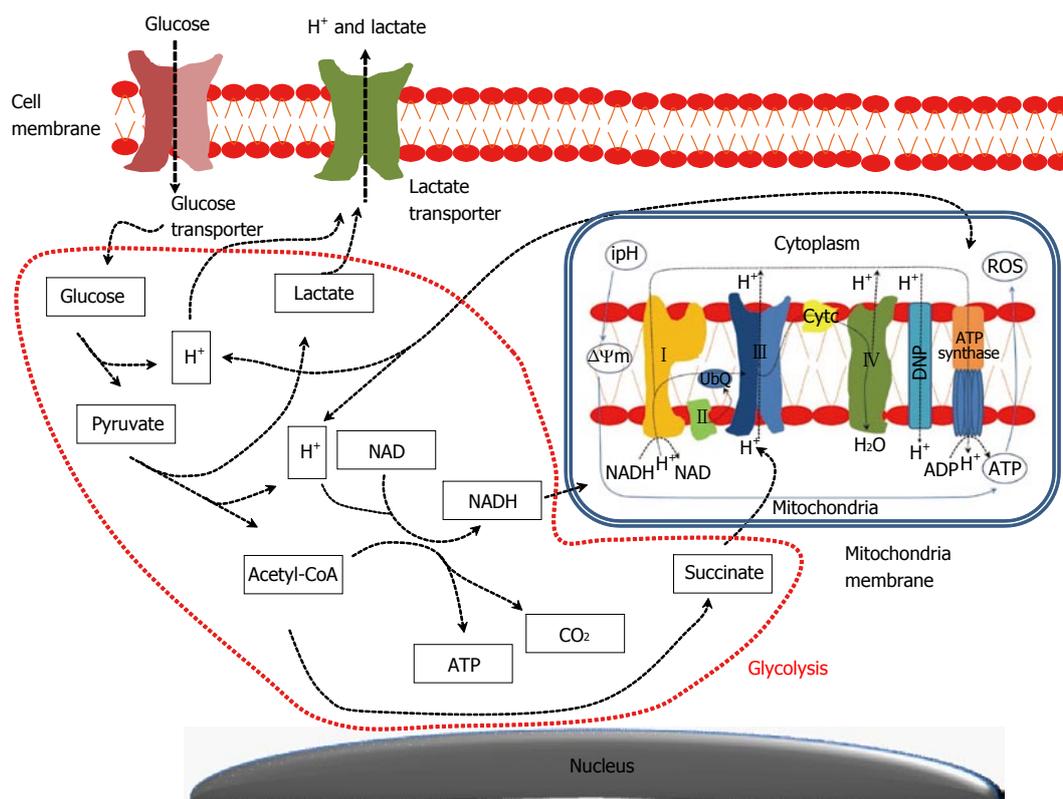


Figure 1 Main metabolic interactions lead to formation of the aerobic glycolytic metabolism in colon tumor cells. The increased biosynthetic activity of cancer cells, as related to the activation of the aerobic glycolytic metabolism or “Warburg effect”, is based on the activation of glucose and lactate transporters supplying tumor cells not only with vast amounts of energy (glucose), but further reducing blockage-associated mechanisms due to glycolysis over usage. It seems that the lactate overproduction is compensated by the hyperactivation of lactate transporters allowing a rapid transport of this molecule across the plasma membrane together with H⁺ atoms, which results in an intracellular alkalinization. This event hyperpolarizes the mitochondrial membrane potential ($\Delta\Psi_m$) and induces a higher uptake of NADH by the first and succinate by the second mitochondrial complexes enhancing the oxidative mitochondrial phosphorylation (Krebs cycle). All together, this means that tumor cells are prone to produce higher energy amounts (ATP) than found in a normal tissue. ROS: Reactive oxygen species; CO₂: Carbon dioxide; ipH: intracellular pH. NADH: Nicotinamide adenine dinucleotide phosphate-oxidase.

ANTIDEPRESSANT FLX MODULATES OXIDATIVE STRESS

FLX was first reported by a research group from the Eli Lilly Company in 1974 as a SSRI^[37]. In 1978, the United States Food and Drug Administration approved FLX for the treatment of patients with depression, anxiety and insomnia; this medication became known worldwide as Prozac^[38,39]. This antidepressant exhibits higher safety and fewer side effects than other groups of antidepressants^[38-41]. FLX was characterized as a lipophilic weak base, which when administered orally experiences a direct contact with epithelial cells in the intestines. In these epithelial cells, it induces an increase in serotonin (5-HT) levels by blocking L-monoamine oxidase and serotonin reuptake transporters^[41-43].

On the other hand, FLX has been shown to interfere with the OS machinery in experimental models and humans^[44-55]. Treatment with FLX was found to reduce malondialdehyde (MDA) and carbonyl levels in stressed rats, whilst it enhanced superoxide dismutase (SOD), catalase, glutathione S-transferase, glutathione reductase and glutathione contents^[45,46]. Similar findings

were reported by another research group^[48]. Then, this compound showed neuroprotective effects, decreasing the translocation of p67 protein and ROS generation (by suppressing the activation of nicotinamide adenine dinucleotide phosphate-oxidase and inducible nitric oxide synthase) in rats exposed to lipopolysaccharide^[47]. In depressive patients, FLX was found to decrease serum MDA, SOD and ascorbic acid levels^[44].

FLX AND TUMORS

Tutton and Barkla first revealed the anticancer potential of FLX against colon tumors^[56]. However, in 1992, Brandes and colleagues reported a 40% increase of the numbers of mammary fibrosarcomas among mice treated with FLX for 5 d, which was followed by findings of a 95% enhancement in breast cancer incidence after 15 wk^[57]. Opposite to that, Volpe *et al.*^[58] showed that treating human and murine breast tumor cell lines with FLX *in vitro* did not stimulate tumor cell proliferation, DNA synthesis or colony formation. Jia *et al.*^[59] reported that FLX did not enhance the growth of pancreatic tumors. Moreover, this treatment was further found to reduce lymphoma growth, modulating the T-cell-mediated im-

munity reaction through a 5-HT-dependent activity^[40].

In patients, FLX treatment was reported to reduce the risk of colon cancer to almost 50%^[60]. Chubak *et al*^[61] also observed that FLX reduced the risk of colon cancer in humans, while one meta-analysis study suggested that FLX does not act on colon cancer^[62]. Studies with animal models support the idea of FLX reducing colon cancer incidence in different animal models, such as carcinogen induced preneoplastic lesions and tumors in rats and mice, and xenograft tumors in immunosuppressed rats^[38,63-65]. These studies have mainly been focused on the antiproliferative effects of FLX treatment in colon tumorigenesis^[38,63-65]. In cell culture models, FLX was reported to not only inhibit multidrug resistance and increase the intracellular doxorubicin concentration^[66], but also to induce a further nuclear distribution of this chemotherapeutic drug^[67].

FLX reduces preneoplastic lesions acting on colonic microenvironment

We have reported that FLX treatment counteracted the carcinogen-induced dysplasia in two different experimental colon cancer models^[64,65]. Our first report revealed FLX as a chemopreventive compound against colonic dysplasia since treatment with FLX was started before the treatment with the carcinogen^[65]. We then reported that FLX could also reduce pre-existent colon preneoplastic lesions^[64]. Our findings suggested that FLX takes the carcinogen-induced preneoplastic changes under control by reducing epithelial proliferation^[38,56,60,61,64,65].

Besides the fact that FLX treatment reduced dysplasia and preneoplastic angiogenesis, decreasing the epithelial and subepithelial proliferation^[64,65], our unpublished dataset further suggests that by suppressing the NF- κ B nuclear activity, through increased expression of cytoplasmic NF- κ B-inhibitor I κ B- α and I κ B- β proteins, FLX reduced *c-Myc* expression and then stromal proliferation (Figures 2 and 3). As we will discuss next, FLX treatment seems to take preneoplastic angiogenesis under control by reducing the proliferation of subepithelial cells (Figure 4). Indeed, NF- κ B-transcriptional activity was reported to induce the transformation of subepithelial cells from normal to reactive phenotypes, enhancing the expression of pro-inflammatory molecules and periendothelial cell numbers^[68,69]. Koh *et al*^[38] reported that FLX inhibited NF- κ B signaling in colonic epithelial tumor cells. Inhibition of the NF- κ B-transcriptional activity actually yields reduced expression of its downstream genes *c-Myc* and vascular endothelial growth factor, which blocks the proliferation of colon cancer cells^[70,71].

The activity of FLX on the colonic preneoplastic microenvironment further includes the question whether this treatment could directly act upon angiogenesis-related cell phenotypes^[64,65]. We have demonstrated that the anti-angiogenic potential of FLX could be related to its control over the differentiation and further transition of endothelial cells through different angiogenesis-related stem cell markers in colon preneoplastic lesions

(Figure 4)^[64]. This idea was abetted by the discovery of a small subset of stromal spindle cells expressing CD133 and CD34 in angiofibromas, which suggests tumors promoting subepithelial resident cells to transit towards endothelial cell phenotypes^[72]. Endothelial progenitor cells were then shown to lose, in a process related to high proliferation^[73], the expression of CD133 during their differentiation into vascular cells, while the expression of CD34 was increased^[74-76]. Considering that CD31-positive cells have been designated as mature endothelial lineage promoting microvessels^[77], vascular smooth muscle cells were found to increase the expression of CD31 during their differentiation process, whilst a simultaneous decrease of CD133 and CD34 progenitor markers was previously observed^[78,79].

FLX TAKES ENERGY GENERATION UNDER CONTROL TO REDUCE MALIGNANT EXPANSION

Here, we should pull a few points together about malignancy, ROS production and energy generations, as: (1) unbalancing the machinery for energy generation induces ROS production; (2) ROS production is one of the main known events inducing DNA damage and mutation; (3) ROS generation promotes genetic mutations leading to the manifestation of preneoplastic lesions; (4) tumor cells undergo deep metabolic changes to survive and promote malignant expansion; (5) tumors enhance ROS production to promote growth through malignant molecular signaling; and (6) malignant metabolism seems to be the Achilles' heel in tumors. These few remarks give us the notion that metabolism, or energy generation, is a key for malignant transformation, tumor manifestation and growth, as well as a valuable tool for anticancer therapy^[35,80-82].

As a lipophilic weak base^[42], FLX quickly diffuses through multiple body-sites^[83]. We have already demonstrated that FLX treatment arrested colon tumor cells within the G₀/G₁ cell-cycle phase without inducing DNA damage^[64]. Then, FLX was shown to reduce ROS generation, reversing the melanoma-induced tissue oxidation in mice^[50]. In brain tissue of tumor-bearing mice, FLX treatment further reduced OS, enhancing the SOD activity^[49]. Actually, FLX was twice reported to stimulate Ca²⁺ flux reducing the B-cell lymphoma 2 (*bcl-2*) expression and mitochondrial membrane potential ($\Delta\Psi$ m), which induced DNA fragmentation and apoptosis in Burkitt's lymphoma cells^[52,53]. Another lipophilic weak base ([Z]-5-methyl-2-[2-(1-naphthyl) ethenyl]-4-piperidinopyridine [AU-1421]) was also reported to uncouple mitochondrial oxidative phosphorylation, dissipating the proton motive force during its energized state, which inhibited ATP synthesis^[84]. It is known that lipophilic weak bases, such as FLX, reduce $\Delta\Psi$ m (or extra- and intra-mitochondrial motions of H⁺ atoms generating positive charges in the mitochondrial membrane) in their energized or proton-

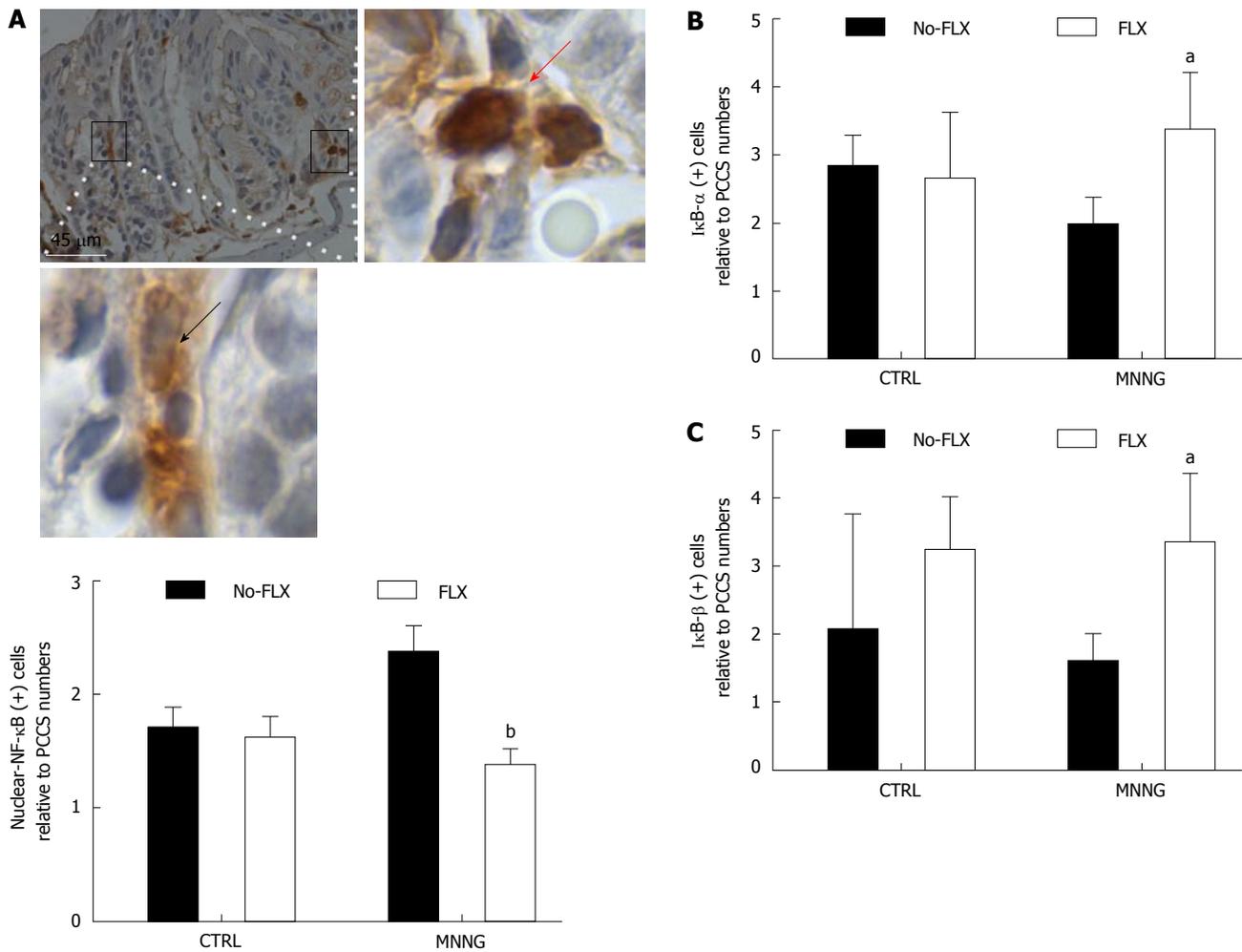


Figure 2 Fluoxetine modulates nuclear factor kappa-B nuclear activity among subepithelial colonic cells. For this figure, groups of female C57BL/6 mice (25 g) consisted of control (CTRL) animals or received methylnitrosoguanidine (MNNG) treatment [four successive doses of MNNG (5 mg/mL; intrarectal deposits of 100 µL) twice a week for 2 wk], FLX treatment (30 mg/kg per day; intraperitoneal, *ip*) or MNNG + FLX treatment. FLX treatment was started after 2 wk from the end of MNNG treatment, and continued for the next 4 wk. All mice were euthanized by CO₂ exposure at week 8. Individual autopsies were performed and colon tissue samples were fixed in paraformaldehyde buffer (4%; 24 h). All experimental protocols were approved by the Internal Animal Care, Ethical and Use Committee (n° 068/2012). Immunohistochemistry was performed with anti-nuclear factor kappa-light-chain-enhancer of activated B cells [nuclear factor kappa-B (NF-κB), p50; clone C-19], nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB), alpha (IκB-α; clone N-20), beta (IκB-β; clone H-4). Antibodies were acquired from Santa Cruz Biotechnology (Heidelberg, Germany). A: Representative histological image of a colonic-longitudinal section labeled with anti-NF-κB antibody, picture taken at × 400 magnification and scale bar of 45 µm inserted. A cytoplasmic anti-NF-κB antibody positively cell detected within cryptal area (inset below; × 1000 magnification of the boxed region, middle-left). Nuclear-NF-κB protein detected in stromal cells (inset right-side; × 1000 magnification of the boxed region, middle-right). Graph shows the relative number of nuclear-NF-κB positive cells within colonic subepithelial areas (PCCS; ^a*P* < 0.01 vs MNNG without FLX, *n* = 4; FLX + MNNG, *n* = 4); B: Relative number of IκB-α positive cells (^a*P* < 0.05 vs MNNG without FLX, *n* = 4; FLX + MNNG, *n* = 4); and C: IκB-β positive cells within colon stromal areas (^a*P* < 0.05 vs MNNG without FLX, *n* = 5; FLX + MNNG, *n* = 4). FLX: Fluoxetine. PCCS: Pericryptal colonic stroma.

ated state, which reduces mitochondrial respiratory rate and energy generation^[84-86]. FLX was also found to induce ROS generation in human ovarian cancer cell lines, which induced apoptosis through mitochondrial bcl-2-associated X protein, cytochrome c release, caspase-3 activation and p53 expression levels, whilst this treatment further reduced ΔΨ_m, BH3 interacting-domain death agonist and bcl-2 levels^[54]. Similar findings were reported in human neuroblastomas^[55].

Comparing those reports that describe how FLX modulates tumor metabolism^[49,50,52-55] with others describing its activity against tumor growth^[40,58,87-92], it becomes clear that FLX blocks tumor cell proliferation by impair-

ing the malignant energy generation. The anti-tumor proliferative effects of FLX^[40,56,92,93] have been related to different causes, such as delays in cell-cycle progression by inhibiting DNA synthesis and also to a possible binding directly to DNA *via* groove mode and high attraction force^[58,87-90,94]. On a molecular level, FLX was shown to arrest breast tumor cells at G₀/G₁ phase by disrupting skp2-CKS1 assembly, which is required to enable cell cycle progression^[91]. Recent reports have been supporting the idea of FLX acting against tumor proliferating cells by reducing *c-Myc* and cyclins (D1, D3, E, B and A), whereas cell-cycle checkpoints (p15, p16, p21, p27 and p53) were enhanced^[40,91,92].

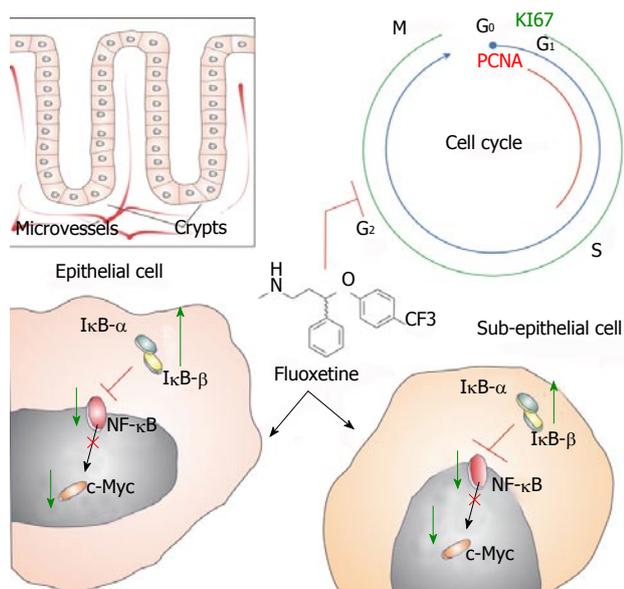


Figure 3 Schematic illustration shows fluoxetine antiproliferative activities in colon tissue. Boxed figure shows the clear division between epithelial and subepithelial colonic areas. Considering that crypts compose the colonic epithelia, it is known that microvessels surround these gland structures. Fluoxetine (chemical structure represented at the center) blocks cell-cycle (blue line and letters) in colonic tissue. We have observed that fluoxetine treatment reduced two proliferative markers, named proliferating cell nuclear antigen (PCNA, red line) and KI67 (green line). These effects of fluoxetine treatment might be related to its enhancement on IκB-α and IκB-β proteins. This could arrest nuclear factor kappa-B (NF-κB) protein in the cytoplasm, reducing its transcriptional activity which, due to its activation over c-Myc transcription factor, would decrease this protein activation and proliferation. We believe that a similar mechanism could take a place in epithelial and subepithelial cells.

Perspectives in FLX treatment acting against colon cancer

The application of FLX for tumor patients has so far been limited to its use as an antidepressant, but it might provide much more benefit, potentially making it an interesting co-chemotherapeutic agent. FLX treatment seems to block tumor growth by breaking the malignant metabolism down^[49,52-55]. While the pieces for this puzzle are slowly being pulled together, there are already several reports which have given the ground ideas for following investigations^[38-40,49,50,52-56,58,60,61,64-67,87-92,95]. Besides the specific idea of FLX acting against the tumor metabolism, there is an open question regarding the effects of FLX treatment against the “reverse Warburg effect”. Pavlides *et al.*^[96] have suggested the idea of a reverse Warburg effect taking place in tumors; this idea argues that epithelial cancer cells induce the subepithelial cells to undergo aerobic-glycolysis and secrete lactate and pyruvate, which malignant cells would take up to enhance their tricarboxylic acid cycle, not only to generate more energy through mitochondrial phosphorylation, but further increase redox mechanisms which in turn corroborates with tumor cell survival and proliferation^[30,31,82]. Schulze and colleagues have extensively reviewed this topic^[30,82]. Such a mechanism would efficiently ensure enough energy pro-

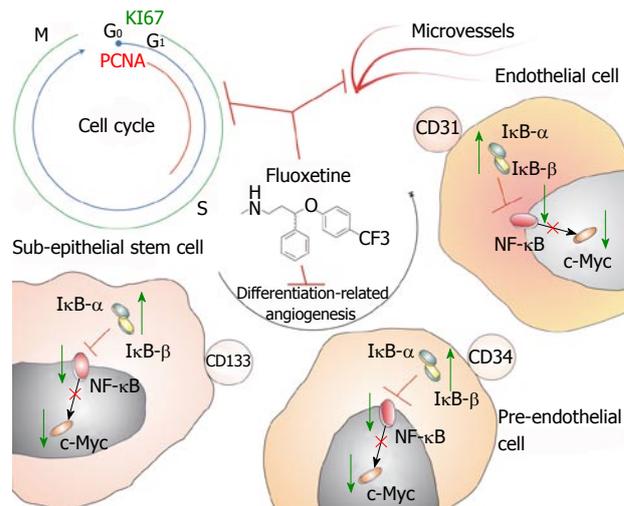


Figure 4 Schematic illustration shows fluoxetine anti-angiogenic potential in colon preneoplastic tissue. This means that by reducing proliferation of subepithelial cells, blocking their cell-cycle, fluoxetine would reduce microvessel density. This anti-angiogenic potential was observed in a direct relationship with reduced differentiation-related angiogenesis of subepithelial stem cells. This suggests that fluoxetine would reduce the differentiation of CD133 positive cells into a CD34 phenotype, which would also not differentiate in endothelial cells, as CD31. This sequence of events would mainly be associated with the control of fluoxetine treatment on nuclear factor kappa-B signaling, as reducing proliferation and preneoplastic angiogenesis. PCNA: Proliferating cell nuclear antigen.

duction for malignant cells within the hostile tumor environment, allowing not only high proliferative rates, but the enhancement of malignant angiogenesis^[97-101]. These authors have further shown that enhancing the subepithelial NF-κB signaling is closely associated with “reverse Warburg effect” in tumors^[96].

Our findings, that FLX treatment reduced the nuclear detection of NF-κB protein among preneoplastic subepithelial cells (as related to reduced angiogenesis due to fewer subepithelial cellular proliferation^[64,65]), lead towards the idea of FLX treatment having similar effects on subepithelial cells which surround epithelial cells in colon tumors. Figure 5 illustrates that malignant microvessels show high-cytochrome C oxidase activity in colon xenograft tumors. Moreover, our new experiments (unpublished dataset) argue that FLX treatment, in different colon tumor models, takes the malignant metabolism-related energy generation in epithelial cells under control to shrink tumors. We strongly believe that FLX counteracts aerobic glycolysis reducing the activity of lactate transporters that inhibits oxidative phosphorylation due to increased intracellular levels of lactate. This might bring down the pH values blocking the tumor energy generation machinery. After having this hypothesis challenged in experimental models and by different research groups, we could think of clinical trials for FLX as a co-chemotherapeutic agent in colon cancer patients. Because of the low costs of FLX, this would also be transferable to developing countries with their tightly limited budget for cancer therapy.

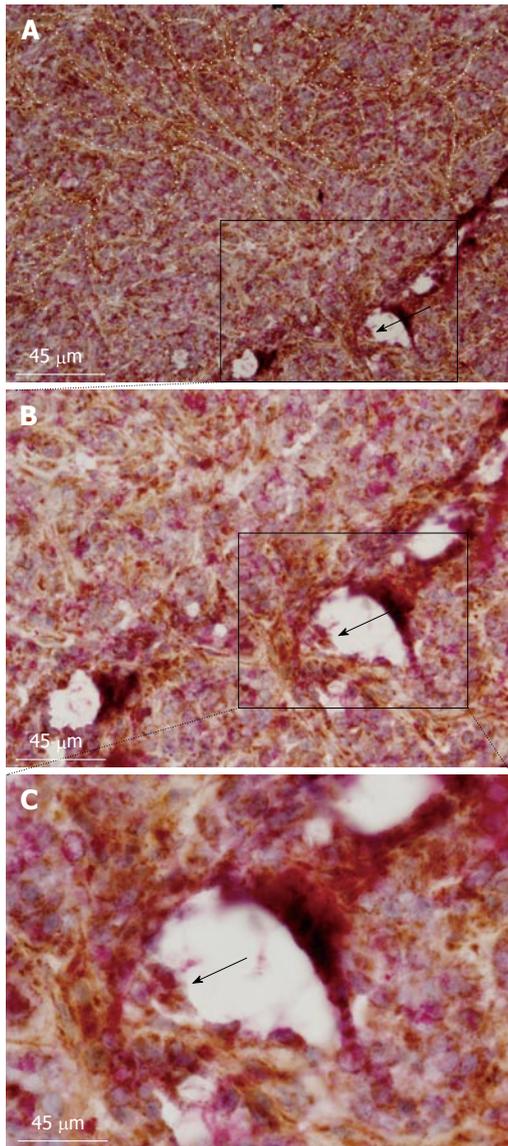


Figure 5 Tumor metabolism and malignant angiogenesis. Histopathological images show double staining between cytochrome C oxidase (COX) and anti-CD31 antibody (clone 1A10 at 1:100; Novocastra, United States). Microvessel walls are traced with sectioned white lines (horizontal view of sectioned tumor microvessels). Black arrow indicates a microvessel lumen with double-stained cells (boxed region; transversal view of a tumor microvessel). Picture was taken at $\times 100$ magnification and 45 μm scale bars are inserted in all images. Inset (C) shows the same boxed region at $\times 200$ magnification. Double-stained cells are pointed out by a black arrow at the microvessel wall (inset; B). Sectioned green line circulates a niche of double-stained endothelial cells at the edge of a microvessel bifurcation. To build these images, 5 wk (20 ± 2 g) nonobese diabetic, severe combined immunodeficient mice (NOD/SCID) were subcutaneously transplanted with HT29 cells (1.5×10^6 cells per mice) in agreement with the protocol approved by the Internal Animal Care, Ethical and Use Committee (n° 121/2012). All mice were acclimated for 1 wk before starting the experiment and maintained under specific pathogen-free conditions. Tumor volume was monitored throughout the whole experimental period by measures with a caliper. Mice were sacrificed under general anesthesia (1.5% Forane in 98.5% oxygen; 2l min). Tissue samples were frozen within TissueTek (Sakura, Germany) and kept at -80°C for immunohistochemical analyses. Double-staining was performed according to our standard methods.

CONCLUSION

To summarize, research data concerning the activity of

FLX treatment against tumor metabolism are still very limited but exciting enough to warrant new investigations. The fact that FLX was designed as an antidepressant but was further found to act against tumors already highlights that new drugs can be developed from it. Additionally, cancer therapy lacks alternative strategies to overcome chemoresistance. In many cases, chemoresistance is closely associated with tumor metabolism. It seems reasonable to suggest that treatments disrupting metabolic events, as might be possible with FLX, could effectively not only reduce chemoresistance, but also malignant angiogenesis. Whether these new perspectives for FLX treatment will be applicable for colon cancer patients are a matter of time, discussion and deeper research efforts. We strongly suggest that FLX is a promising target for further studies in cancer research.

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