Recent Advances in Understanding the effects of Obesity on Stem Cell Proliferation and the Development of Colon Cancer

*Bridgette King, Chaodong Wu

Department of Nutrition and Food Science, Texas A&M University, College Station, TX 77843, USA

ABSTRACT

Several studies have determined the effect of a high fat diet on the development of obesity and colon cancer. Researchers are now working to determine the mechanistic link of obesity on stem cell proliferation and the development of colorectal cancer. Lgr5+, a marker protein to determine stemness in mice is analyzed using several techniques to determine the effect of high fat diet on stem cell proliferation. Techniques such as RT-PCR, immunohistochemistry, flow cytometry, fluorescence microscopy, and mRNA and RNA analysis allow researchers to determine the effect of diet on Lgr5+ stem cell populations. A high fat diet increases body mass in mice and results in obesity. This increases the amount of hormones circulating the body such as leptin, adiponectin, interleukin-6, and tumor necrosis factor alpha, all of which have been linked to inflammation and cancer. High fat diets create mutations in genes such as the Apc gene, a major target of the Wnt cellular pathway. Mutations causing upregulation leads to high amounts of activity in the pathway and the development of colon cancer. While the results of many studies indicate that a high fat diet causes obesity and inflammation, more research is required to determine the exact mechanism for the effect of obesity on colon cancer.
INTRODUCTION

According to the Center for Disease control, 93.3 million adults are considered obese in the United States \[1\]. Obesity increases the risk of colorectal cancer by 30% compared to normal weight individuals \[2, 3\]. Many studies report a link between obesity and the development of colon cancer \[4, 5\], however the exact mechanisms underlying this link still remain unclear. While researchers are still exploring this topic, it is relatively clearer on obesity regulation of inflammation \[6, 7\]. Mounting evidence indicates that obesity increases the mass adipose tissue, causing inflammatory responses of various types of cells. This leads to the release of inflammatory mediates from immune cells, as well as non-immune cells of a wide range of tissues including adipose tissue, intestine, and the brain. Of note, increased inflammation appears to lead to the proliferation of stem cells and the development of tumors \[8-12\]. To study the effects of obesity, researchers have examined how a high fat diet influences stem cell proliferation, and identified important stem cell marker(s). For example, several studies have determined an increase in proliferation of leucine rich G protein coupled receptor-5 (Lgr5+) protein located in stem cells (Figure 1A) \[13\]. Lgr5+ is located in colonic crypts acting as a marker for stem cells \[14\]. This protein directly targets the Wnt signaling pathway and regulates gene expression and cell functions \[15\]. Increased proliferation of Lgr5+ may cause dysregulation in the pathway and increase the chance of tumor formation. The objective of this review is to analyze how obesity influences inflammation and stem cell proliferation to allow researchers to delineate the mechanisms for the development of colon cancer during obesity.

OBESITY AND ASSOCIATED RISK FACTORS

Pathogenesis of Obesity

Obesity occurs when energy intake is greater than energy expenditure \[16, 17\]. This leads to an increase in body fat storage in adipose tissues. Many studies provide a link between high fat diets and the development of obesity. Also, researchers have shown that fatty acid composition in the diet may play a role in regulating body weight and adipose tissue \[18, 19\]. Studies indicate that saturated fatty acids are more likely to cause obesity than polyunsaturated fatty acids and lead to a greater accumulation of body fat. Saturated fatty acids are a poor source of energy due to slower oxidation rates and remain stored as triglycerides in adipose tissue. Poly- and mono-unsaturated fatty acids are stored less in fat tissue and are a much better source of energy for the body. An increase in storage of fatty acids without being broken down for energy leads to the development of obesity.

Another mechanism for the development of obesity from a high fat diet includes the effect of saturated fatty acids on decreased satiety in the diet. Poly-unsaturated fatty acids have the greatest appetite-suppressing effects, much greater than mono-unsaturated fatty acids and saturated fatty acids \[12, 20\]. This is likely due to the increased release of cholecystokinin with high amounts of poly-unsaturated fatty acids. Cholecystokinin is a hormone that signals satiety in the humans \[12, 21, 22\]. Studies also determined that energy from fat sources increases the effect of weight gain. While building fatty acids requires energy, the consumption of dietary fat contains preformed fatty acids chains that require no energy. The mechanisms described help researchers determine the effect of a high fat diet on the development of obesity.

Several hormones play a role in the effect of a high fat diet on the development obesity. Fats are stored in the body and release hormones such as leptin, an important regulator of metabolism. Leptin is a hormone that controls food intake \[23\]. Increased levels of leptin results in decreased intake of food and increased satiety \[24\]. Obese individuals consuming high fat diets have excessively high levels of leptin which leads to resistance of the hormone \[25, 26\]. Increasing amounts of leptin signals to other tissues to stop secreting the hormone. As leptin increases, suppressor of cytokine signaling (SOCS-3) increases \[27\]. SOCS-3 inhibits and blocks the effects of leptin in
regulating metabolism. This reduces the appetite suppressing effect of leptin and is linked to the development of obesity.

Ghrelin is another hormone important in fat metabolism. Ghrelin levels rise after food intake. In obese individuals, studies have shown that ghrelin secretion is suppressed after a meal. Ghrelin-simulated secretions from the hypothalamus increase the intake of food and decrease the oxidation of fat. High fat diets cause down regulations in this mechanism. Because ghrelin increases satiety in humans, down regulation may influence the development of obesity following the intake of a high fat meal. This leads to overconsumption and the accumulation of fat.

Insulin is also a hormone involved in fat metabolism. High fat diets have shown to cause insulin insensitivity and an increase in blood glucose levels. Studies show that a high fat diet decreases insulin sensitivity due to various mechanisms associated with excessive amount of adipose tissue. Accordingly, dysfunctional adipose tissues release cytokines, which work with or without increased levels of circulating free fatty acids and other mediators to decrease the responsiveness of key metabolic cells such muscle cells and hepatocytes to insulin. This state is termed insulin resistance, which is characterized by hyperinsulinemia. To be noted, during insulin resistant states, there is impairment of insulin actions on suppressing glucose production from the liver and stimulating adipose and muscle glucose uptake. However, hyperinsulinemia can still function to stimulate fat synthesis and storage. This contributes to, in large part, increases in fat deposition and ultimately the development or exacerbation of obesity. Obesity further leads to the development of multiple types of diseases including colon cancer.

Obesity and Metabolic Inflammation

According to the Center for Disease Control, 36.5% of Americans are obese (BMI > 30). Obesity causes inflammation, which in turn contributes to the development and progression of certain types of cancers. Several hormones are affected by obesity and cause inflammation. Adiponectin is an important hormone causing an increase in the amount of free fatty acid oxidation in multiple tissues. This hormone is essential for proper health and has been shown to decrease in overweight and obese individuals. Scientists propose this decrease in adiponectin is caused by an increase in size of adipose tissue which downregulates synthesis and secretion of adiponectin. This reduction in adiponectin sensitivity leads to insulin resistance and hyperinsulinemia. In contrast, weight loss following gastric bypass surgery results in increased circulating levels of adiponectin and decreased circulating levels of interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP), indicating that weight loss is followed by a significant improvement of systemic inflammatory state. One study confirms this by measuring the increase in adiponectin levels following a 10 percent weight loss in severely obese individuals (BMI > 40). This increase in adiponectin levels increase anti-inflammatory functions while a decrease inhibits tumor necrosis factor alpha (TNF-alpha) expression and leads to the production of macrophages, causing an immune response. Obesity also increases the amount of pro-inflammatory factors released in the body that play critical roles in increasing the inflammatory state in the body.

OBESITY AND COLON CANCER

Inflammation and Tumor Development

Stimulation of the development of tumors by inflammation involves the effects of pro-inflammatory mediators and growth factors. Much evidence demonstrates that inflammation causes the proliferation of stem cells due to the increased presence of immune cells. These immune cells initiate local inflammatory responses, resulting in the release of cytokines such as IL-6 and, TNF-alpha. These factors attract monocytes to the damaged tissues which mature into macrophages. This mechanism accelerates the growth and proliferation of cells, including stem cells.
The chronic growth in stem cells due to inflammation leads to changes in gene expression and ultimately affects stem cell division.

One mechanism contributing to the growth of tumors involves the Wnt Pathway. Researchers have determined inflammation to increase activity in the Wnt pathway causing mutations in the tumor suppressing Apc gene and beta-catenin. Cytokines released from excess adipose tissue over-stimulate the pathway and cause mutations in both the Apc gene and beta-catenin. Another factor overstimulating this pathway is TNF-alpha which is also increased in adipose tissue. Mutations cause dysregulation of the Wnt pathway and lead to the mutation of beta-catenin. The mutated beta catenin is translocated into the nucleus and causes the transcription of genes involved in the genesis of cancer \[46\]. However, further studies are needed to better elucidate the link between obesity-induced inflammation and the development of colon cancer.

**Lgr5\(^+\)** cells in Colon Cancer

Colorectal cancer is one of the most studied forms of cancer in treatment research, and already one million lives have been saved due to continued research on this disease \[47\]. Current research focuses on the effect of diet on stem cell proliferation by analyzing the Lgr5\(^+\) stem cell marker in colonic crypts (Figure 1A). Studies use several methods to analyze the effect of proliferation during each stage of tumor development. Results have determined that Lgr5\(^+\) stem cell expression is increased in the crypt bases during the development of cancer (Figure 1B) \[48\]. The cells shift from the surface of the lumen to the crypts and may play a role in the progression of colon cancer. These results may be related to the metastasis of colon cancer stem cells \[49\]. Lgr5\(^+\) marks increased cycling in stem cell lines and plays a role in Wnt cascade signaling. Current research indicates that Lgr5\(^+\) is overexpressed in colorectal cancer and may acts as a surface marker for colon cancer stem cells. Lgr5\(^+\) stem cells may also play a role in radiation and chemotherapy resistance and the reoccurrence of cancer following treatment. Studying the effect of a high fat diet on stem cell proliferation will allow researchers to target the Lgr5\(^+\) gene in many types of cancer treatment to prevent the growth and metastasis of colon cancer.

Several researchers have proven that Lgr5\(^+\) directly targets the Wnt pathway \[50\]. This marker protein has also been discovered in abundance in several different types of cancers. Researchers hypothesize that obesity increases inflammatory mediators, leading to extreme proliferation of stem cell populations. The Lgr5\(^+\) gene is located in stem cell crypts and acts as a target to increase activity in the Wnt pathway. The proposed mechanism involves the binding of Lgr to R-spondin in the cell membrane which increases the activity of Lrp6 and Frizzled at the surface of the cell \[50\]. This increases the activity of the Wnt pathway and causes gene mutations and ultimately leads to the production of tumors. While researchers believe this mechanism to be true, no research has determined the exact mechanism of obesity on Lgr5\(^+\) stem cell proliferation and the development of colon cancer.

**High Fat Diet on Inflammation and Cancer Development**

While much research is still dedicated to determine the mechanistic link between the effect of obesity on stem cell proliferation and the development of colon cancer, several researchers have determined the effects of a high fat diet on inflammation. Because inflammation affects tumor development, results from experiments will help determine the link between obesity and colon cancer. Researchers found an increase in the production of IL-6 in the colons of mice given high fat diets over the course of the study indicating an increase in inflammation. The results also indicated an increase in the amount of leptin levels in mice given a high fat diet. This caused changes in the CYP2E1 enzyme in the liver that may be related to the development of certain forms of obesity related cancers. Increased levels of leptin have also been linked to the proliferation of the Apc gene which is important in the Wnt signaling pathway \[4,51\]. Activity of this enzyme is increased when altered by a high fat diet and leads to the release
of certain carcinogens. These carcinogens are thought to influence the colon and contribute to obesity-related colon cancer.

**High Fat Diet Increases Lgr5-GFP Stem Cell Proliferation**

Because Lgr5⁺ expression increases in cancer patients, researchers studied the effect of a high fat diet on stem cell proliferation. Some studies found an increase in the expression of Lgr5⁺ in colonic crypts. While the distal colon showed no significant difference, the proximal colon showed significantly elevated levels of Lgr5⁺ stem cell expression in mice given a high fat diet [52]. These results indicate that a high fat diet may increase the proliferation of stem cells which is linked to the formation of colon cancer. While this has been shown in several studies, more research is required to determine the exact mechanisms for this increased proliferation of stem cells. The finding that a high fat diet increases Lgr5⁺ stem cell proliferation provides a likely link between obesity, stem cell proliferation, and tumor formation. Considering the presence of elevated levels of inflammatory mediators during obesity, inflammation is speculated to increase stem cell proliferation. This, in turn, may further lead to the development of colon cancer. As illustrated in Figure 1B, key mechanisms underlying the link between obesity and colon cancer are integrated at the level of Lgr5 stem cells.

![Figure 1. Lgr5⁺ stem cells and its role in obesity-related colon cancer](image)

(A) Location of Lgr5⁺ stem cells in paneth cell zone. (B) During obesity, increases in adiposity-related proinflammatory cytokine, e.g., TNFα, and saturated fatty acids act to activate Wnt signaling. In contrast, a decrease in adiponectin leads to decreased inhibitory effect on Wnt signaling. These effects, in combination, promote the stemness of Lgr5+ cells, thereby the pathogenesis of colon cancer.

**STEM CELL TARGETED THERAPY**

**Lgr5 Targeted Stem Cell Therapy**

Based on current research, scientists have begun targeting the Lgr5⁺ marker protein in stem cells as a treatment for colon cancers. Because Lgr5⁺ cells are highly expressed in colon cancer, one method of treatment involves the destruction of Lgr5⁺ cells before they metastasize. One of the goals of stem cell therapy is to target tumors and
destroy them without damaging other tissues. Lgr5* is highly expressed in cancers but also has low levels of expression in normal tissues. To target the antigen located in the Lgr5* cells, researchers use an antibody drug component (ADC) \(^{[53-56]}\). Once targeted, the antibody binds to and destroys the colon cancer stem cell (CSC). Scientists discovered decreased tumor size and proliferation in mouse models using a specific ADC (anti- Lgr5*). This therapy targets colon cancer at the source, prevents reoccurrence of new tumors, and leads to greater incidences of remission in cancer patients \(^{[14]}\).

**Nutritional Therapy**

Researchers determined bioactive compounds in fruits and vegetables to positively affect cancer chemoprevention. The mechanism directly includes colon cancer stem cells. Chemoprevention involves the use of natural agents to reverse or suppress tumors \(^{[52]}\). Bioactive compounds sensitize cancer stem cells to chemotherapy treatments and inhibit self-renewal and metastasis. Several studies show curcumin eliminates cancer stem cells and prevents self-renewal. Curcumin inhibits cell proliferation and suppresses activity of the Wnt pathway \(^{[57-59]}\). Other bioactive compounds such as resveratrol show suppression of cell proliferation and cellular pathway activity involved in colon cancer \(^{[60,61]}\).

**Wnt Pathway Targeted Therapy**

Wnt pathway signaling regulates stem cell proliferation and death \(^{[62,63]}\). High activity leads to increased cell division and tumor formation. Scientists believe the major force of colorectal cancer involves dysregulation of the Wnt pathway. Researchers continue to explore ways to block the pathway and prevent this dysregulation. One method involves the use of small molecules to inhibit receptors on the pathway, block signaling, and reduce stem cell proliferation and metastasis \(^{[64]}\). This prevents dysregulation of the Wnt pathway and may prevent the development of colon cancer. While many researchers continue to study stem cell proliferation mechanisms in colon cancer, a better understanding will allow more efficient treatment options and better outcomes in colon cancer patients.

**CONCLUSION**

Colon cancer research continues to progress towards better treatment and prevention options. Understanding this disease and the effects associated with obesity remains the most important goal for researchers. As research continues, scientists determine important mechanisms related to cancer and obesity. The results from this review provide evidence of the effects of obesity on stem cell numbers and gene expression. However, the mechanism for increased stem cell expression remains unclear. Future research should analyze the effects of a high fat diet on regulation of certain pathways involved in colon cancer. This will allow researchers to determine the exact mechanism behind the formation of colon cancer and provide stem cell targeted therapy for patients.

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