Review Article

An overview on understanding the main factors involved in colon cancer and its treatment

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Abstract

Colon cancer is a leading cause of cancer related death after breast and lung cancer, in both male and female. Along with individual's lifestyle, diet and genetics are the main factors which cause proliferation of colon cells and can lead to colon cancer. Colon cancer is mainly caused by mutation or methylation of tumor suppressor genes which results in un-controlled proliferation of cells and hence cancer. Diet plays very important role in both mutations and methylation of tumor suppressor genes so these conditions can be reversed or improved by balanced diet. In addition, certain diets also have role in cancer prevention through their anti-oxidant activity and some even induce apoptosis in colon cancer cells. Diets such as fruits, legumes, cereals, fish oil, olive oil, vitamin E supplements and probiotics etc. are gaining importance with respect to their therapeutic potential in cancer patients. Moreover, according to some reports, exercise also greatly reduces the risk of colon cancer. The present study highlights the symptoms, causes, treatment and ways to prevent colon cancer.

Key words: Colon carcinogenesis: chemo-preventive diets, apoptosis, probiotics, proliferation, cancer.

Introduction

Cancer is a malignant neoplasm that possesses the ability to reproduce and spread. It is caused by multiple factors and some factors are even still unknown. Numerous environmental factors, mainly diet and genetic abnormalities result in lack of control on the rate of cell division and causes the formation of undifferentiated mass of cells, which may lead to carcinogenesis [1, 2].

According to reported evidence, the number of cancer survivors has increased due to better cancer diagnostic and treatment methods [3]. In addition, various causes of cancers have been identified by which its

probability can also be reduced, [4] but still cancer is leading cause of death worldwide [5].

One such cancer which is the cause of death is colon cancer. The mortality rate of colon cancer is 10% both in men and women in United States (US) [6] which has increased in last 20-30 years [7]. Colon carcinogenesis is a complex, multistep process which includes the progressive interruption of homeostatic mechanisms controlling intestinal epithelial cell proliferation, differentiation and apoptosis [8]. Although, cells proliferation and apoptosis are considered as vital mechanisms involved in gut replenishment

but excessive cellular division results in tumor formation [9, 10].

It is necessary to understand colon cancer's main causes so a better strategy could be devised for its treatment. According to research, mutation in cell divisions related genes are main cause of colon cancer and these mutations are mainly caused by food and environmental factors. In addition, it is very important to understand the mechanism that how certain types of food cause mutations in genes and result in cancer, so we could avoid such foods.

Symptoms

The early stage symptoms of colon cancer are like all other tumors. Usually these sign and symptoms are misunderstood which may lead to disastrous consequences. A very common symptom associated with colon cancer is constipation. Although, there are number of things which can be the cause of constipation but low fiber intake is considered as the main cause. So, high fiber intake is good for colon health and also prevents the development of cancer [11, 12].

Another common symptom found mostly in advanced stages of cancers is anorexia, which is the loss of appetite and is observed in 80% patients. It is the main cause of death, not only in the case of colon but in other cancers as well [13]. Some other symptoms, associated with advanced stages of cancer are belching, diarrhea, lack of energy, incomplete bowel evacuation, lower sexual interests and bleeding from back passage [14].

Causes

There can be numerous factors, which can result in the development of colon cancer. Reasons can be from simple intake of diet which leads to one or several mutations in genes, which are involved in cell cycle regulation. Among the numerous factors result in colon cancer, medicines are one of them as many laxative medicine have an active compound which results in colon

carcinogenesis [11]. Other causes of colon cancer can be exposure to radiations [15], obesity [16] and other diseases, like patients of diabetes mellitus are at higher risk of gastro-intestinal cancer [17]. In addition, mutations in genes which are involved in cell proliferation or in cell division can cause them to over-express [18] or under-express [19-23] respectively, which might later turn into colon carcinoma due to continued proliferation of cells.

Genes in mediating colon carcinogenesis

There are trillions of divisions in human body each day and one of the important steps in cell division is the replication of DNA. There are number of mechanisms which ensure the correct transfer of genetic information from parent to daughter cell, still, mutations occur due to the errors in replication or via carcinogens like chemical entities, radiations and infections [19].

One such mutation occurs in *p53* which is tumor suppressor gene whose mutation can cause carcinogenesis in around 50% of all tumors. Mutation causes alteration in *p53* protein [18] which dramatically reduces its quantity in effected cells. This deficiency of *p53* and other important tumor suppressor protein could cause the chemo- and radiotherapies resistant cancer cells and may even leads to cancer metastasis [20, 21]. This suppression could also result in non-functioning of *p53* induced cell cycle arrest pathway or over-expression of *mdm2* which inhibits apoptosis [18].

RAD54b belongs to the SWI/SNP genes family which is involved in chromatin remodeling. Mutation in *RAD54b* is contributing factor in colon carcinogenesis [22]. *HLTF* is another member gene of this family, which in 43% of colon cancers losses its cancer suppressing ability through hyper-methylation in promoter CpG region (Fig. 1) [23].

Promoter methylation also occurs in other genes such as *hMLH1* gene which on

methylation in CpG region causes the instability in microsatellites [24, 25]. In addition to these, *p16* is another tumor suppressor gene which also loses its functioning due to increased methylation [26]. The changes in methylation of these genes result in abnormal proliferation of cells.

SLC5A8 belongs to the SLC5 gene family and it encodes a sodium transporter. It is also a tumor suppressor gene and is reported silent in colon cancer cells. Its inactivation is found in 59% of primary colon cancers due to the methylation of its exon 1 [27]. Age related methylation is a phenomena related to pre-cancerous events in cells including colon cells. Genes such as *ERβ*, *MYOD1*, *IGF1* and *N33* are some genes whose methylation in older ages is reported to be associated with tumor formation in colon [28].

Histone de-acetylation in colon carcinogenesis

Histone acetylation is of great importance as it is directly involved in transcription and control the genes involved in cell-cycle progression, cell differentiation and programmed cell death. Number of factors causes de-acetylation of genes which affect differentiation and proliferation of cells [29]. One such factor is exogenous compounds which reach in the gut through diet. This de-acetylation process through foreign compounds cause uncontrolled cell proliferation and even lead to cancer. According to some studies, the abnormal levels of histone deacetylators cause the inactivation of tumor suppressor genes such as p21, p53 and gelsolin [30]. While some tumor activator genes like hypoxia-induced factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) are up-regulated [31].

Role of diet in mediating colon carcinogenesis

This fact is strongly supported by previous studies that diet [32] and eating frequency

[33] are directly involved in the development of colon cancer. Diets enriched in fats like meat are considered as the major reason of colon carcinogenesis as they promote the production of carcinogens through bile acid interaction [34] and even increase PKC (protein kinase C) activity and promote proliferation of colon epithelial cells [35]. TGFβRII is associated with the epithelial cells growth inhibition and it promotes the differentiation of cells [36] but its expression is greatly reduced in colon cancer cells due to some inactivating mutations and transcriptional repression [37] and also due to the increase activity of PKCβII which is antagonist of TGFβRII [38]. PKCβII over-expression represses TGFβRII and causes hyper proliferation and increased vulnerability to colon carcinogenesis [39]. PKCβII even cause the accumulation of β-catenin by inactivating enzyme GSK-3β through phosphorylation [40]. Accumulation of β-catenin causes the activation of growth related genes and cause colonocytes uncontrolled proliferation (Fig. 2) [41].

Similarly, diets which are enriched with ω-6 fatty acid like some fish are thought to promote hyper-proliferation in colon cells which can lead to colon carcinogenesis [38]. Moreover, dietary folates are involved in number of metabolic processes in the body. Its lower concentrations affect the cell growth directly and stop it, while higher concentrations cause the reduction in E-cadherin protein concentration in cells, which is responsible for cell differentiation. So, its excess and deficiency both favors the tumor formation and progression in normal colon epithelium but in already established neoplasm its deficiency has inhibitory effects [42-44]. .

Figure 1: DNA sequence of HTLF along with its promoter region. Promoter CpG islands, present at 5' site are methylated which causes HTLF gene silencing while the gene sequence remained un-methylated in colon cancer cells.

Figure-2. [A] PKC β II causes the phosphorylation of GSK-3 β which inactivates it and in turn, causes the accumulation of β -catenin. β -catenin goes into the nucleus from the cytoplasm and starts the Tcf-dependent transcription of growth-related genes which causes the colon cell proliferation and leads to tumorigenesis. [B] In the absence of PKC β II, GSK-3 β remains active and promotes the binding of APC with β -catenin and destroys it. PKC β II is important for cell growth but its increased quantities cause colon carcinogenesis.

Treatment

The first step in curing colon cancer is the early stage diagnosis. In fact, the treatment of colon cancer is directly dependent on its diagnosis [45]. Therefore, most of the treatment strategies discussed here could be the base of early stage treatment.

Gene role in suppressing colon cancer

Mutations are the main factor which causes the undifferentiated cell growth and inability of cell to identify abnormalities in cells [19]. Several measures can be taken to avoid colon cancer.

In most of the colon cancer, *HLTF* gene was found inactive due to hyper-methylation of promoter which elucidate that this gene is related to tumor suppressor function. So, if this silenced gene is reactivated by treating it with demethylator like azacytidine, then colon cancer cells growth can be inhibited [23]. Similarly, *HTLF* methylated promoter DNA sequence can be detected in blood of colon cancer patients [46] which could be used as detection marker for this cancer [47]. *SLC5A8* is another tumor suppressor gene which is inactivated in colon carcinogenesis due to methylation. Its treatment with demethylator can restore its function and its artificial introduction in areas affected with colon cancer can also inhibit the cell growth and cancer cells colony forming capability [27]. In addition, mutations in *p53* deactivate it and also the genes associated with it. As a result, uncontrolled cellular proliferation and tumor formation occurs in colon. Small molecules are discovered in cells which are known to induce re-expression of *p53* and ultimately, growth inhibition of colon cancer cells [19, 48]. Therefore, colon cancer cells are exposed to small death inducing molecules such as CP-31398 [48] and PRIMA1 [49] which cause the re-activation of *p53*. Nutlins are other small molecules which reduce the mdm-2 amount in cells and activate *p53* [50]. In addition, chartreusin is another one such compound which represses

the cell cycle progression by binding with DNA on region rich in GC and induces single stranded breaks in tumor cells [51].

Histone acetylators reactivation

Histone acetylation in DNA is very critical regarding reactivation and silencing of genes involved in carcinogenesis, cell differentiation and apoptosis [52, 53]. SCFA (short-chain fatty acids) are very important in this regard as they are found in gut and are perfect inhibitors of histone de-acetylators [54]. Butyrate, which is also a SCFA, is greatly involved as HDAC (histone deacetylase) repressors and promotes hyper-acetylation of H3 and H4 core histone and so inhibit the rapid proliferation of cells [55].

Diet chemotherapeutic properties

While diet is one of the main causes of colon cancer, still through diet, the likelihood of it can be controlled. A large amount of antioxidant vitamins, trace elements and phytochemicals are found in fruits and vegetables which are beneficial in colon cancer prevention and even in its treatment [56-58]. Diet even helps in enhancing the effects of anti-cancer drugs and on the other side, it reduces drugs associated side effects as well [59].

Diets such as fruits, legumes and cereals are rich in fiber and are considered to be beneficial in cancer prevention, [60, 61] as the result of their fermentation in gut lumen SCFA compounds are produced, mainly butyrate, which is proven to be beneficial in preventing colon carcinogenesis [62]. So, increased intake of fibrous diets causes increased production of butyrate which suppresses cell growth and may induce apoptosis [54, 63]. Propionate (SCFA) is another compound produced during fermentation and its anti-tumor properties are somewhat similar to butyrate but its effect is for longer time than butyrate. Colon

Figure-3. *n*-pufa interacts with membrane phospholipids and starts free radical chain reactions which cause the release of lipid per-oxidation products which acts as DNA adducts and binds with DNA and generates signals for cell cycle arrest for repairing and apoptosis.

cells use butyrate as energy source and it is used up there while propionate remains active until it reaches liver for metabolism [64]. Fish oil and the food supplemented with it have great amount of *n*-3 PUFA (Polyunsaturated fatty acids) in it which have anti-proliferative and pro-apoptotic effects on colon cancer cells. *n*-3 PUFA acts by incorporating itself in membrane phospholipids and induces free radical chain reactions, then, the resultant lipid per oxidation products intercalates in DNA and forms DNA adducts which send signals for cell cycle arrest and further signals for cell repairing or cell death (Fig. 3) [65-67]. Antioxidant, vitamin E is also found with *n*-3 PUFA in fish oil and its isozyme γ -tocopherol is reported to have somewhat similar pro-apoptotic effects on colon cells like *n*-3

emulsions consisting of mainly vitamin E and long chain *n*-3 PUFA, are also proven to amplify the anti-tumor effects of chemotherapeutic agents [58]. They induce apoptosis through Bax-related mitochondrial pathway and induce pro-apoptotic effects by inducing double-stranded breaks in DNA [58, 68].

PKC β II is a lipid-dependent protein kinase whose expression can be reversed through ω -3 fatty acid [69, 70]. Diets such as fish and maize oils are good source of ω -3 fatty acid which have chemo-preventive role, can directly inactivate PKC β II and re-activate TGF β RII in colon cancer cells [38]. This inhibits cancerous cells growth and induces differentiation of gut epithelial cells or signals for apoptosis [36]. Latham *et al.*, [71]

reported that ω -3 fatty acid can also repress the carcinogen induced hyper-proliferation. Olives and olive oils consists of pentacyclic triterpene compounds mainly maslinic and oleonolic acids. They are often given in diet to cancer patients as olives have ability to stop proliferation and promote apoptosis in tumor cells [72]. The presence of one extra OH-group in maslinic acid compared to oleonolic acids, results in difference of their properties [73]. Oleonolic acid has capability to cause cell growth inhibition due to its contribution in G₀/G₁ checkpoint control and DNA replication inhibition in colon cancer cells [74], while maslinic acid has ability to induce apoptosis in colon cancer cells by producing super oxide anion (ROS) which acts as pro-apoptotic signals. The production of ROS causes the release of cytochrome-c in cytosol from mitochondria which activate caspases 9, 3, 6 and 7 which in turn causes the events like protein cleavage and DNA fragmentation.

Grapes seed extracts (GSE) consists of proanthocyanidins which are chemopreventive and causes tumor suppression [75-77]. In addition to this, GSE have different anti-cancer effects on colonocytes like growth retardation, apoptotic signaling pathway markers induction and apoptosis [78].

Probiotics as anti-tumor agents

Probiotics are the group of similar or different microorganisms capable of showing positive effects on health of the host by regulating different non-cellular or cellular immune components and modify the existing microflora of the intestine [79]. *Lactobacilli* and *Bifidobacteria* are commonly found probiotics in human colons [80] which are very beneficial in inhibiting carcinogenesis in gut [81]. In fact, pre-intake of *Lactobacilli* causes reduction in tumor growth volume by inducing apoptosis and down regulation of surface proteins associated with immune responses. Its intake causes the elevation in

the caspase 3 concentrations, down-regulation of *CXCR4* expression which is a chemokine receptor and involved in metastasis and decreased expression of *MHC-1* [82, 83].

Another probiotic, *Propionibacterium freudenreichii*, present in human gut is also beneficial in causing apoptosis of colon tumor cells through its metabolites (SCFA, acetate and propionate) [84]. Yogurts are very rich source of probiotics and it's routinely intake could reduce risk of colon cancer [85].

Future concerns

Physical activity decreases the risk of colon cancer?

Physical activity is very helpful for reducing the risks of many diseases including cancer. Through continuous exercise, many immune system components like natural killer cells or macrophages can be enhanced which provide human body protection from diseases [86]. Different studies have proved that through physical activity risk of colon cancer can be reduced [87, 88] as it reduces the intestinal transport time of bowel which cause a decrease in contact of gut mucosa and any carcinogen and co-carcinogen present in bowel [89]. But the data shown by Lee *et al.* [90] are quite different from other studies as their studies do not support the hypothesis that physical activity reduces the risk of colon cancer. According to their data, physical activity has no effect on colon carcinogenesis and those who daily exercise and those who do not are equally at risk of developing colon cancer. These findings clearly show that the occurrence of this disease differs from area to area, group to group and individual to individual. May be the groups of people Lee and associates [90] have selected are not very much conscious about their diet intake and may be the others have not taken in account the parameters like alcohol consumption, exercise duration etc. Furthermore, in recent study by Morrison *et al.* [17], the physical

activity is reported to be positively linked with reduction in colon cancer.

α -catenin and plectin role in cancer

α -catenin is protein present between cells and tissues and is important in formation of adhere junctions between epithelial cells and also in formation of radial cable for stabilizing those junctions, sealing membrane and organizing epithelial sheets [91]. Plectin functions as the stabilizer and integrator of these functions [92]. It is observed that in some colonocytes in which the ER β concentrations are lower or it is thoroughly absent; undifferentiated growth of epithelial cells was observed. Still, this abnormal proliferation do not results in the tumor formation because in those cells the levels of α -catenin and plectin are remarkably lower which causes the continuous shedding of the cells along with continuous growth [93]. Thorough and advance study of these proteins role in cancer can prove fruitful in future for the prevention of colon cancer.

Conclusion

Colon cancer mortality rate is second after breast cancer and lung cancer in women and men, respectively [7]. Its early stage symptoms in majority of cases are misunderstood and remain misdiagnosed which is one of the reasons of its increased mortality rate. Diets have an important role in colon cancer as diet can act as mutagen or can also induce histone modification. So, by balancing our diet, the risk of developing colon cancer can be greatly reduced but there are some contradictory results so this area needs further thorough investigations. Likewise, new strategies must be designed to aware general public and to improve their life style as life style greatly contributes in colon cancer development.

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