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Anticancer Activities of Resveratrol in Colorectal Cancer

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Abstract

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a dietary polyphenolic phytochemical that has demonstrated health benefits such as cardioprotection, the prevention of neurodegeneration and chemoprevention. Resveratrol has shown great potential in the prevention and treatment of carcinomas and clinical trials support resveratrol as anticancer compound in colorectal carcinoma. Colorectal cancer remains a major cause of cancer-related deaths for both men and women in industrialized countries. Because of this widespread prevalence, identifying major risk factors and initiating colorectal screening procedures provide the distinct advantage for recognizing early disease and addressing treatable forms of CRC. Epidemiological studies of fruit and vegetable consumption in relationship to developing CRC have led to the notion that safe and inexpensive chemopreventive agents might be a valuable tool in diminishing the morbidity and mortality of CRC. While clinical trials and *in vivo* data show positive effects of resveratrol in CRC, the mechanism of action is relatively unclear. In this review, we will evaluate the current literature on the actions of resveratrol in CRC and provide a more mechanistic view of resveratrol in relationship with CRC.

List of Abbreviations:

CRC: Colorectal Carcinoma; CIN: Chromosome Instability; MSI: Microsatellite Instability; CIMP: CpG Island Methylator Phenotype; FAP: Familial Adenomatous Polyposis, HNPCC: Hereditary Nonpolyposis Colorectal Cancer; APC: Adenomatous Polyposis Coli; MMR: Mismatch Repair; ACF: Aberrant Crypt Foci

Keywords: Resveratrol; CRC; Colorectal cancer

Introduction

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural polyphenol that can be found in several nutritional sources, including grape skin, peanuts and berries. Resveratrol was first identified by Michio Takaoka in 1940 in *Veratrum grandiflorum* O. Loes [1] and was hypothesized in 1992 to be responsible for the cardioprotective effects of red wine [2]. However, resveratrol became of major interest to the scientific community after a study of Pezzuto et al., who showed that resveratrol interfered with the initiation of 7,12-dimethylbenzanthracene (DMBA)-induced skin cancer and progression of breast cancer in mice [3]. Many publications have since followed and provide evidence that resveratrol exhibits various beneficial health effects, such as cardiovascular protection [4] and chemoprevention [5]. The use of resveratrol for the treatment of CRC seems to be of most current interest; two completed clinical studies have shown direct positive changes in colorectal tissues after oral resveratrol intake [6,7].

Colorectal cancer (CRC) is a major cause of cancer-related deaths for both men and women living in industrialized countries. Because of this widespread prevalence, identifying major risk factors and initiating colorectal screening procedures provide the distinct advantage for recognizing early disease and addressing treatable forms of CRC. While CRC screening procedures have the highest impact for cancer prevention, safe and inexpensive chemopreventive agents are being developed that exhibit great value in diminishing the morbidity and mortality of CRC. Enthusiasm for this approach is based on epidemiological studies of fruit and vegetable consumption, and reduction in risk for CRC [8,9], combined with animal investigations that examined dietary phytochemicals in CRC prevention.

Resveratrol has been characterized as a pleiotropic agent that exhibits multiple targets in cancer cells with marginal effects on non-mitotic cells [10-12]. Included among the responsive cellular processes are cell cycle checkpoints, apoptotic and angiogenic pathways, host immune responses, and genomic instability. Since resveratrol may have multiple actions in diseased tissue [13,14], it is important to evaluate the effectiveness of resveratrol in CRC. In this review we summarize the recent literature to assess the mechanisms of actions of resveratrol in CRC.

Resveratrol

The role of nutrition in cancer prevention is well supported by animal studies and clinical trials as well as epidemiological findings. Chemoprevention is based on the general concept that naturally-occurring plant or animal substances and even synthetically produced derivatives can prevent, suppress, or reverse carcinogenic progression to metastatic disease. The importance of the effect of diet on cancer prevention and control is evidenced by the fact that approximately 274 clinical trials in the USA have recently been completed or are currently ongoing (clinicaltrials.gov: accessed on May 5th, 2016).

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a polyphenolic phyloalexin present in over 70 plant species and found in particular within the skin or peel of grapes, peanuts and berries [15,16]. Its pleiotropic properties have been observed in a wide variety of cancers including breast, prostate, lung, skin, and colon and of the nine clinical chemoprevention trials; six involve resveratrol and GI cancers. The

flurry of recent research activities on resveratrol largely stems from the seminal report by Pezzuto et al. in 1997, showing its anticancer properties [3].

The anti-tumorigenic activity of resveratrol is supported by *in vitro* studies [17,18], animal experiments [10,19], and clinical trials [20-23]. Patients diagnosed with CRC and fed resveratrol show statistically significant reductions in tumor proliferation [6,7]. This effect correlates with HPLC/UV/Mass Spectrometry-validated increases in resveratrol and its metabolites in resected colorectal tissues and suggests that, unlike cells located within internal organs, resveratrol metabolites can reach sufficiently high, anti-carcinogenic levels in responsive target cells within the gastrointestinal tract [6,24].

Bioavailability of resveratrol

One of the major limitations encountered in studies on resveratrol is its availability in humans. This is related to high resveratrol excretion rates in intestinal cells and the various resveratrol modifications that occur after resveratrol uptake into intestinal cells.

Resveratrol uptake and transport: Mechanisms of resveratrol uptake following oral ingestion have not been completely elucidated. Several *in vitro*, *ex vivo* and *in vivo* studies have suggested that a passive uptake and an active extrusion process are responsible for resveratrol uptake and excretion in the intestine. Cell culture studies using Caco-2 cells show that trans-resveratrol diffuses into enterocytes and that trans-resveratrol is actively transported by the sodium-glucose linked transporter (SGLT1) [25]. Subsequent studies show that trans-resveratrol can also be transported via clathrin-independent endocytosis [26].

Ex vivo studies, that use organ perfusion techniques, and *in vivo* studies using rats have shown that resveratrol can be passively absorbed and conjugated with glucuronic acid or sulfate to form resveratrol glucuronide or resveratrol sulfate [27,28]. These conjugates are formed by UDP-glucuronosyltransferases (UGT) and sulfotransferases (SULT), respectively [29], which convert resveratrol to hydrophilic derivatives, and facilitate renal and biliary secretion. In rats, a large fraction of these metabolites undergo enterohepatic circulation which can reintroduce as much as 50% of the initial amount of resveratrol absorbed [29]. Extrusion of resveratrol glucuronide and, to a lesser extent, resveratrol sulfate into the intestinal lumen is mediated by three ABC-transporters: P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC) and the breast cancer resistance protein (BCRP/ABCG2) [29,30]. Residual intracellular resveratrol glucuronide is released through the serosa into the bloodstream by another multidrug resistance-associated protein 3 (MRP3). MRP3 is an ABC-transporter that transports resveratrol and other polyphenolic compounds as well riboflavin and biotin into the bloodstream [31]. Once in the blood, resveratrol can be transported as the glucuronide bound to albumin [26,32].

Notably, these transporters and modifying enzymes are differently distributed along the intestinal tract, but expression is also slightly dependent on the individual. Data, mostly obtained by studies in rat intestine, suggest that MRP2 and ABCG2 transporters are mainly expressed in the duodenum and jejunum, and gradually decrease further down the intestinal tract [33,34]. By contrast, ABCB1 expression is low in the initial parts of the intestine, but higher in the ileum and colon. The expression of MRP3 is high in duodenum and colon, and lower in all other parts of the intestine [35]. UGT proteins are abundantly expressed throughout the entire intestine, but are most prominent in the duodenum and jejunum, after which they gradually decrease in abundance towards the rectum. The expression of UGT is both constitutive and inducible, where the level of induction is highly variable in different parts of the intestine, but also in different individuals [36]. SULT have several isoforms of which the distribution is not as clearly identified as the transporters and enzymes described above. Several SULT isoforms are expressed in the human intestine. SULT1B1 seems to be the most abundantly expressed isoform in the small intestine, followed by SULT1A3 and SULT1A1 [37]. Aside from the many isoforms that exist, quantification of SULT activity is complicated by the existence of several SULT polymorphisms. Polymorphisms in e.g. SULTA1 have been shown to exhibit different enzyme kinetics towards resveratrol metabolites [38].

These studies imply that resveratrol uptake and metabolism in the intestine is regionally- and individually-specific. The relatively high concentration of UGT, MRP2 and ABCG2 in the initial parts of the intestine, (duodenum and jejunum) might form a first line of defense against ingested xenobiotic compounds, while the presence of MRP3 in later portions of the intestine (ileum and colon) may ensure the absorption of specific nutrients and beneficial compounds. This hypothesis is important in determining the efficacy of resveratrol in ameliorating intestinal carcinomas. When considering dietary intake, the duodenum and jejunum probably receive the highest concentration of unmodified resveratrol, but modify and excrete resveratrol soon after it enters the cell, due to the high activity of UGT, MRP2 and ABCG2, aka BCRP. By contrast, regions in the ileum and colon may be subject to less unmodified metabolites, but also contain less metabolizing enzymes, which could make these regions more responsive to resveratrol supplementation. Other factors that influence the availability of resveratrol may involve the distribution, titer, and types of microbial flora along the GI tract.

Bioactivity of resveratrol: Different studies in healthy volunteers have examined the bioavailability of resveratrol after oral and intravenous administration. Generally, peak levels of resveratrol are observed after 0.5 to 1.5 hours following ingestion. Due to use of different resveratrol doses, peak plasma levels of resveratrol have been reported to vary from 3.89 μg/L to 750 μg/L [39-43]. Two studies reported similar peak serum concentration around 470-490 μg/L after 25 mg/70 kg intake resveratrol [41,42]. The major metabolites observed were conjugated forms of resveratrol. While some studies reported small amounts of unmodified resveratrol [39,42], others found no traces of unmodified resveratrol in the plasma [41]. Half-lives of resveratrol *per se* were found to be short. Resveratrol is excreted within 12-24 hours post oral administration. One study reported that the amount of resveratrol found in urine after 12h of ingestion was as high as 85% of the ingested dose [41].

Interestingly, two studies reported that intakes of higher doses of resveratrol lead to smaller fractions of excreted resveratrol compared to low-dose administration. Meng et al. [43] found that ingestion of 0.03 and 1 mg/kg body weight resveratrol in two subjects resulted in recovery of 52% and 26% of the administered dose in urine after 24 hours, respectively. Similarly, Boocock et al. [39] reported that while approximately 20% resveratrol was excreted at a dose of 0.5 gram, less than 10% was excreted at a dose of 5 gram resveratrol. Additionally, even though the half-life of total resveratrol varied greatly among individuals, the half-life of intravenously administered resveratrol was approximately an hour longer than orally administered resveratrol [41].

Although differences were small, absorption of resveratrol was greater when given in grape juice, compared to white wine or vegetable juice [42]. It has been hypothesized that resveratrol uptake may depend on the presence of other dietary factors. For example, the bioavailability of resveratrol is increased by combining its intake with piperine, a compound abundant in black pepper, which inhibits several ABC transporters, including resveratrol-excreting ABCB1, MRP2 and ABCG2 [44-46]. This hypothesis was further supported by the observation that resveratrol pharmacokinetics show a circadian rhythm, where resveratrol bioavailability was highest in the morning [40]. Presumably, the digestion or presence of other foods could interfere with the bioavailability of resveratrol, which is prevalent during the day, but of less importance after a night of fasting.

Bioactivity of resveratrol conjugates: Since the availability of unmodified resveratrol is low in humans, it has been hypothesized that resveratrol conjugates could have biological functions similar to those of the parent compound. There are some indications in the literature which support this hypothesis. In an *in vitro* experiment, using fibroblasts from patients with an inborn enzyme deficiency of mitochondrial fatty acid beta-oxidation, it has been shown that cis-resveratrol, trans-picied and dihydroresveratrol improved fatty acid oxidation [47], potentially through stimulating mitochondrial biogenesis or via competition for cellular extrusion of riboflavin, which is the precursor for mitochondrial FAD, the coenzyme of acyl-CoA dehydrogenases critical for fatty acid β-oxidation [48]. However, the effects of major resveratrol metabolites – resveratrol-3-O-glucuronide, resveratrol-4-O-glucuronide and resveratrol-3-O-sulfate – on fatty acid oxidation was modest to non-existent [47]. Another study evaluated the biological activities of five resveratrol sulfate metabolites in several *in vitro* assay systems. Even though two of these metabolites showed induction of NADPH:quinone oxidoreductase 1 (NQO1), inhibition of COX-1 and COX-2, and decrease in NO production in a cell-free system, cellular uptake of these resveratrol metabolites was insignificant *in vitro* [49]. A large study, also using various *in vitro* assays, tested 92 resveratrol derivatives and reported that several metabolites had activities that were even more potent than the parent compound in specific assays. However, it should be noted that resveratrol-glucuronide was not included and that only one resveratrol sulfate metabolite was tested [50].

It has also been proposed that sulfated metabolites of resveratrol provide an intra-cellular pool of resveratrol that can be accessed by desulfation. Additionally, although resveratrol-glucuronide does not seem to exhibit effects on CRC cell proliferation, resveratrol-sulfate is able to inhibit CRC cell proliferation by 20% *in vitro*, while having no effect on normal epithelium-derived colorectal cells. The desulfation of resveratrol was confirmed in mice: hydrolysis of the sulfate group yielded free resveratrol which accounted for 2% of the total resveratrol content in the plasma. The concentration of resveratrol-sulfate on the serosal side of the human intestine can reach approximately 640 μM, which is much higher than the concentration used in the *in vitro* experiments that showed a reduction in proliferation of 20% [24]. Through these findings, one may hypothesize that the observed beneficial effects of resveratrol in reducing CRC proliferation is mediated partially through resveratrol sulfate and partially through desulfation events, which yield the parent compound.

All in all, it seems that concentrations of unmodified resveratrol available following oral ingestion are much less than those used in experimental studies. However, since the intestinal tract is directly exposed to resveratrol from the diet, gastrointestinal cells may be exposed to concentrations much higher than observed plasma levels, as found by Patel et al. Additionally, one of the major resveratrol derivatives, resveratrol sulfate, seems to exert activities in colorectal tissue. These findings support the use of resveratrol in the treatment of intestinal diseases and may contribute to the observed amelioration of CRC after resveratrol supplementation [6,7]. Resected colorectal tissue indeed contains traces of resveratrol and its metabolites, suggesting that, unlike cells located within internal organs, resveratrol metabolites can reach sufficiently high, anti-carcinogenic levels in responsive target cells within the gastrointestinal tract [6,24].

Colorectal carcinoma (CRC)

CRC is the third and second most common cancer in males and females worldwide, respectively. Over the past few decades, a decline in mortality (2.5%) and incidence rate (4.3% among adults 50 years of age or older) has been observed in CRC; nevertheless, this disease remains a major contributor to cancer cases and was the second leading cause of cancer-related deaths in 2015 (8.4%) for both men and women, after lung carcinoma.

The GI tract is highly predisposed to developing cancerous lesions, partly because of rapid epithelial cell turnover and exposure to dietary toxins. Not surprisingly, adenoma formation is not uncommon in individuals younger than 50 years of age. Colorectal screening reveals that 12% of women and 24% of men develop adenomas. In women and men 80 years and older, formation rates of adenomas increase to 27% and 40%, respectively [51]. Progression of CRC from adenoma to carcinoma originates over an extended period of time within a colony of progenitor cells. The etiology of disease progression is thought to be heterogeneous characterized by unique genomic abnormalities, molecular changes, and distinct pathological profiles [52,53].

In vivo evidence for chemoprevention of CRC

Different animal models have been used in the evaluation of resveratrol supplementation on CRC. Using mouse models of CRC, investigators have reported different outcomes with resveratrol. In a mouse model of KRAS-activated sporadic CRC, Saud et al. showed that resveratrol prevented tumorogenesis by inhibiting KRAS expression. In a study using five week old mice consuming drinking water with 0.01% resveratrol, Schneider at al. showed that cyclin D1 and D2 expression decreased in CRC tissue and that there was 70% reduction in small intestinal tumors. However, resveratrol supplementation in the form of 4, 20 or 90 mg/kg body weight in the diet failed to show effects on CRC tumorigenesis in 43 day old mice [54]. Additionally, Sale et al. found a reduction in the number of colorectal adenomas at a dose of 240 mg/kg body weight resveratrol in 4 week old mice, but did not find any significant reductions in adenoma number at a dose of 60 mg/kg body weight resveratrol. These differences in findings may be due to the method of dosing, e.g. injection vs. diet exposure in the drinking water.

Several *in vivo* studies in rats, with varying protocols, have shown that resveratrol reduces the manifestation of aberrant crypt focus (ACF), which are early precursors of CRC, compared to control rats [55-57]. Also in rats, Tessitore et al. reported an increase in expression of BAX, a pro-apoptotic protein, in CRC tissue after resveratrol supplementation, which suggests activation of apoptosis in CRC after resveratrol supplementation. This hypothesis is further supported by the finding that resveratrol increases the expression of activated caspase-3, a central player in the activation of apoptosis, in CRC in rats

[58]. Thus, mouse and rat models seem to support the claim that resveratrol has beneficial effects in the prevention and reduction of CRC lesions.

Molecular targets of resveratrol

Inflammatory factors: Persuasive lines of evidence indicate that expression of proinflammatory mediators correlate with progression of cancer [59]. At the molecular level, inflammation can be measured by the expression of inflammatory transcription factors (e.g., NF-κB, AP-1, STAT3), inflammatory enzymes (e.g., COX2, MMP-9, PPAR), inflammatory cytokines (e.g., IL-6, IL-8, and TNF-α), and chemokines (CCL3, CCL4, CCL5) [60]. Because of its pleiotropic activity, resveratrol is able to suppress or to interfere with these key inflammatory molecular targets almost instantly [61]. In addition, recent interest in resveratrol has focused on its ability for modulation of tumor-suppressive miRNA by inducing apoptotic cell death [62,63]. In absence of a clear mechanistic link, the activity of resveratrol may be partially dependent upon an impaired expression of certain miRNAs associated with inflammatory and tumor suppression effects (miR-663), proinflammatory effects (miR-155), or oncogenicity (miR -21).

This role of resveratrol in inflammation is especially important to CRC, since inflammation has been linked to the development of CRC. This is especially visible in inflammatory bowel diseases (IBD), including Crohn disease and ulcerative colitis, which pose a significant increase in risk for CRC. Patients diagnosed with IBD below the age of 30 exhibit a 4 times higher risk to develop CRC than are patients diagnosed at a later age [64]. The modulation of inflammatory agents by resveratrol may therefore reduce the risk of developing CRC and resveratrol potentially acts as a chemopreventive agent.

Antioxidants and phase II detoxification: Detoxifying enzymes are extremely important in cancer prevention. Each individual is exposed to many potential carcinogens every day, most of which undergo oxidative metabolism by detoxifying enzymes. Phase I enzymes, such as the cytochrome P450 (CYP) family, form a first-line defense towards eliminating toxic substances and produce products that are eliminated though conjugation reactions by phase II enzymes, exemplified by glutathione S-transferase (GST), NQO1 and heme oxygenase-1 (HO-1). However, pro-carcinogenic substances can also be activated by phase I enzymes. This occurs for example during the processing of polycyclic aromatic hydrocarbons (PAH). Resveratrol has been shown to reduce this unfavorable activation by inhibiting CYPA1, an enzyme involved in metabolic activation, in human mammary epithelial cells [65], human hepatoma cells and human mammary carcinoma cells [66]. Aside from affecting selectively phase I enzymes, resveratrol also stimulates the expression and activity of phase II enzymes. Supplementation of resveratrol in the medium of different cell types resulted in increased expression and activation of NQO1 [67], increased HO-1 expression and increased cellular glutathione content [68,69].

Intestinal cells are constantly exposed to toxic substances that are ingested through food. The constant renewal of the intestinal epithelium prevents these toxic compounds from having a permanent effect. However, intestinal stem cells are permanent residents of the intestine and need to prevent the accumulation of toxic substances. Thus the stimulation of the detoxification of toxic substances by resveratrol is another potential mechanism by which resveratrol exerts its chemopreventive actions.

Cell cycle: Failure to undergo cell cycle arrest is one of the hallmarks of cancer. Resveratrol has been shown to elicit cell cycle arrest in various types of cancer. For example, in a cellular model system of hepatocellular carcinoma, HepG2 cells, resveratrol induces cell cycle arrest in G1 and G2/M [70]. In a different hepatoma model system, Huh-7 cells, resveratrol induced cell cycle arrest, which was manifested through inhibition of cyclin E, cyclin A, and cyclin-dependent kinase (CDK)-2 [71]. Other model systems for cancer have also shown resveratrol-induced cell cycle arrest. Cell lines representing lung cancer were arrested in G0/G1, potentially through down-regulation of cyclin D1, CDK4 and CDK6, accompanied by the up-regulation of CDK inhibitors, p21 and p27 [72]. Studies in prostate cell lines reported cell cycle arrest at the G0/G1 phase through down-regulation of cyclins and CDKs and upregulation of CDK inhibitors [73].

As for any cancer, the inhibition of the cell cycle in CRC will slow lesions that have formed already and may cause cell cycle arrest in precancerous cells, preventing further progression. Since CRC develops through an extended process of acquiring mutations, interventions in successive steps of CRC formation may prevent lesions to reach the final steps of tumor formation. The literature is replete with studies providing evidence for the inhibitory effects of resveratrol at several phases of the cell cycle, supporting this additional aspect in the chemopreventive effects of resveratrol in CRC.

Angiogenesis and metastasis: As carcinomas grow, a hypoxic environment often arises within the center of the tumor because of increased metabolic activities and an increased requirement for molecular oxygen. To maintain a viable milieu, tumor cells overexpress hypoxia inducible factor (HIF), which acts as a regulator of cellular oxygen homeostasis by inducing the transcription of several hypoxia-responsive genes. Factors that are synthesized as a result include vascular endothelial growth factor (VEGF), iNOS and erythropoietin. Consequently, angiogenesis will enable tumor cells to continue to grow and potentially invade other tissues. Resveratrol disables this mechanism by blocking expression of HIF-1α and VEGF [74,75]. In a manner similar to that seen in inflammation, resveratrol inhibits iNOS in these cell lines as well.

Metastasis is a central mechanism to tumor progression, which often limits treatment options and reduces survival rate. Therefore, prevention of metastatic events would greatly improve chances of survival. It has been shown that resveratrol is capable of inhibiting mediators of metastasis, which are believed to include lysophosphatidic acid (LPA), transforming growth factor (TGF) and focal adhesion kinase (FAK). LPA induces the expression of e.g. HIF-1α and VEGF, and promotes cell migration. These events were efficiently blocked by resveratrol in a human ovarian carcinoma cell line [76]. Additionally, the induction of cell adhesion, migration and invasion of lung cancer cells, mediated by TGF-β1, was also greatly inhibited by resveratrol in A549 lung cancer cells [77].

As for other carciomas, angiogenesis and metastasis are pertinent to CRC as well, since CRC often metastasizes to the liver through the portal vein. Metastasis to the liver is found in 10-25% of CRC patients who have surgery for CRC [78]. Prevention of metastases and larger tumor masses in CRC are a valuable treatment option, since smaller CRC lesions can be dissected surgically, resulting in a 5-year survival rate of 90%. However, CRC that is detected at more advanced stages have survival rates ranging from 13-71% after 5 years. Unfortunately, only 40% of CRC cases are diagnosed at early localized stages, reducing the total survival rate significantly [79]. Thus, by decreasing angiogenesis and metastasis, resveratrol could increase the amount of

patients that develop CRC to a localized stage, resulting in higher overall survival rates for CRC.

Future perspectives

In this review we have evaluated the current literature on the actions of resveratrol in CRC. Various reports support the notion that resveratrol is a potential natural compound that has anticancer properties in CRC. Mechanistically, several molecular targets have been proposed, including those involved in inflammation, detoxification, cell cycle and angiogenesis.

However, it is generally known that CRC evolves through distinct etiologies. Three genetic profiles, namely chromosome instability (CIN), CpG island methylator (CIMP) and microsatellite instability (MSI), are discriminated; each with distinct and significant contribution to the development of CRC. Although a distinction between different etiologies is useful in assessing treatment options [80,81], few reports focus on the potential different actions of resveratrol in these defined etiologies. Resveratrol might affect each etiology differently, potentially doing more harm than good in certain etiologies. Therefore, we proposed that future research evaluate resveratrol in the light of the aforementioned CRC etiologies. As a first step in this process, we have included data on the distinction of CRC etiologies in CRC (Figure 1).

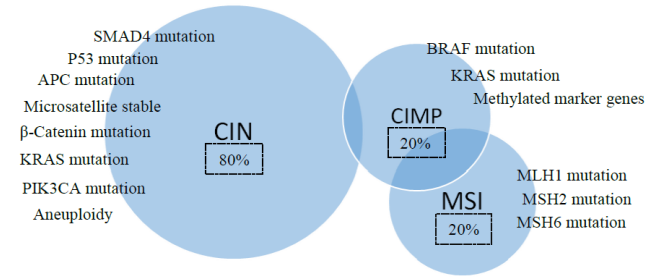

Figure 1: Graphical representation of CRC etiologies. The three major CRC etiologies are CIN, CIMP and MSI. Each etiology is characterized by several mutations, which are depicted at the borders of each etiology. The prevalence of each CRC etiology [82] is enclosed in boxes; the CIMP and MSI etiology frequently overlap [83].

The chromosome instability (CIN) pathway: The CIN pathway is regarded as the 'traditional' or 'suppressor' pathway in CRC progression; an estimated 80% of CRCs develop via the CIN pathway [84]. Appearance of a dysplastic ACF, a mucosal lesion that precedes the development of a polyp [85] is the earliest identifiable lesion in the CIN pathway. Among the initial events in the CIN pathway leading to CRC are the mutations in the adenomatous polyposis coli (APC) gene and/or loss of chromosome 5q, loss of chromosome 18q and deletion of chromosome 17p, which contains the important tumor suppressor gene P53 [84]. Other frequently found mutations in CIN include those in KRAS, P53, SMAD4 and PIK3CA [86]. However, only a very small portion of CRC patients display all of these alterations concurrently [87].

The microsatellite instability (MSI) pathway: The MSI pathway is characterized by alterations in the MMR system, which can lead to failure of normal surveillance and post-replication DNA repair [88]. Impairment of the MSI pathway can result in accumulation of mutations and potentially, genomic instability. Deficiency in the MMR also implicates deficiency in repairing errors in microsatellite DNA replication [89], mediated by DNA polymerases δ and ε [90,91]. Several modifications in microsatellites are observed within CRC implicated genes; e.g. Transforming Growth Factor, Beta Receptor II (TGFRII) [92], Bax [93] and CASP5 (caspase 5) [94]. The most common mutations in the MMR system itself are MLH1, MSH2 and MSH6 mutations. Additionally, frame shift mutations are also observed in a selective number of microsatellites (BAT25, BAT26, D5S346, D2S123 and D17S250) [95]. A classic example of the MSI etiology is Hereditary Nonpolyposis Colorectal Cancer (HNPCC), in which inherited mutations in the MMR system significantly increase the risk of developing CRC [96].

CpG island methylator phenotype (CIMP) pathway: The CIMP pathway is the most recent addition to the CRC etiologies [97]. While CpG islands found in mammalian gene promoters are generally devoid of methylation and result in constitutive transcription of the accompanying genes [98], widespread methylation of CpG islands occurs in CIMP, resulting in gene silencing [97]. Silencing of tumor suppressor genes has been hypothesized as a hallmark in emergence of CRC.

The extension of methylation of specific genes can further distinguish between CIMP-high and CIMP-low specific loci [99]. Additionally, a CIMP-low region is associated with wild-type BRAF and KRAS mutations and occurs more in men than in women [99]; by contrast, the CIMP-high locus is associated with wild-type KRAS and BRAF mutations, occurring more frequently in women than in men, and is commonly associated with MSI [99,100]. The CIMP-high pathway can give rise to the MSI pathway by AXIN2 methylation [83]. At least four loci can serve as sensitive and specific markers for CIMP-high, these include RUNX3, CACNA1G, IGF2, and MLH1 [101]. The probability that none of these four genes are methylated in CIMP-high is less than 1% [101].

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