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### **Can dietary flavonoids be useful in the personalized treatment of colorectal cancer?**

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#### **Abstract**

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Activating mutations in the oncogenes KRAS, BRAF and PI3K define molecular colorectal cancer (CRC) subtypes because they play key roles in promoting CRC development and in determining the efficacy of chemotherapeutic agents such as 5-fluorouracil and anti-EGFR monoclonal antibodies. Survival of patients with cancers displaying these molecular profiles is low. Given the limited efficacy of therapeutic strategies for CRC presenting mutational activations in MAPK and/or PI3K pathways, developing combination therapies with natural flavonoids or other phytochemicals with demonstrated effects on these pathways (and little or no toxic effects) may constitute a valuable path forward.

Much has been published on the anticancer effects of dietary phytochemicals. However, even an exhaustive characterization of potential beneficial effects produced by *in vitro* studies cannot be extrapolated to effects in humans. So far the available data constitute a good starting point. Published results show quercetin and curcumin as possibly the best candidates to be further explored in the context of adjuvant colorectal cancer therapy either as part of dietary prescriptions or as purified compounds in combination regimens with the drugs currently used in CRC treatment.

Clinical trial data is still largely missing and is urgently needed to verify relevant effects and for the development of more personalized treatment approaches.

**Key Words:** colorectal cancer; personalized treatment; quercetin; curcumin; KRAS; BRAF

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**Core Tip:** Given the limited efficacy of therapeutic strategies for CRC presenting mutational activations in MAPK and/or PI3K pathways, developing combination therapies with natural flavonoids or other phytochemicals with demonstrated effects on these pathways (and little or no toxic effects) may constitute a valuable path forward. Published results show quercetin and curcumin as possibly the best candidates to be further explored in the context of adjuvant colorectal cancer therapy either as part of dietary prescriptions or as purified compounds in combination regimens with the drugs currently used in CRC treatment. Clinical trial data is still largely missing and is urgently needed to verify relevant effects and for the development of more personalized treatment approaches.

## **INTRODUCTION**

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### **Background**

Colorectal cancer (CRC) is the fourth most common cause of cancer related death worldwide and accounts for ca. 9% and 10% of all new cancer diagnoses per year in women and men, respectively<sup>[1]</sup>.

Wide variation in geographical distribution of CRC exist with incidence rates in Australia and New Zealand being 10 fold higher than in Western Africa<sup>[1]</sup>. Highest mortality rates are, however, registered in Central and Eastern Europe<sup>[1]</sup>. Both CRC incidence and mortality are associated with higher income countries with westernized lifestyles and CRC rates are expected to increase by about 60% over the next 15 years<sup>[1]</sup>. Lifestyle and dietary choices play an important role in CRC development. Smoking and excessive alcohol consumption also increase CRC risk<sup>[1]</sup>.

There is strong evidence that being physically inactive, overweight or obese, and consuming red or processed meat increases the risk of developing CRC. There is also moderate evidence that low consumption of non-starchy vegetables and fruits might increase the risk of CRC. On the other hand, consuming foods containing dietary fiber decreases CRC risk<sup>[1]</sup>.

The relevance of lifestyle and dietary choices to CRC risk are reflected in the fact that only 5-10% of CRC cases have a known hereditary cause. These hereditary types of CRC are classified in Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC or Lynch syndrome) and are attributed to germline mutation in the APC gene or in DNA repair genes, respectively. A further 20% of cases have a family history of CRC (but no known germline mutations) and the remaining 70% of CRC cases are sporadic (caused by gene defects that are not germline mutations)<sup>[2,3]</sup>. In addition to dietary and lifestyle factors, patients with irritable bowel disease syndrome are at significantly higher risk of developing CRC<sup>[3]</sup>.

CRC progression takes place over many years through a series of stages that go from lesions in single cells of the epithelium, to benign tumors to malignant invasive carcinomas. The evolution from a precursor lesion to CRC is estimated to take 10-15 years.

Accumulation of mutations due to loss of genomic stability is an important driver CRC development. Chromosomal instability (CIN) or a mutator phenotype involving DNA repair defects or aberrant DNA methylation have been identified as major mechanisms in CRC development<sup>[2,3]</sup>.

Chromosomal instability may cause loss of a wild-type copy or inactivating mutations in tumor suppressor genes such as the APC or TP53<sup>[2,3]</sup>. APC mutations are the most common early mutations in CRC, present in up to 85% of cases. APC germline mutations cause Familial Adenomatous Polyposis (FAP) and APC somatic mutations or deletions are found in most sporadic (nonhereditary) cases of CRC. TP53 mutations are the second most common in CRC, present in up to 55% of cases, and inactivate the p53 pathway which compromises cell-cycle arrest and cell death pathways and also has

strong implications on decreased responsiveness to therapy [2,3]. Other relevant and frequently mutated genes in CRC are the oncogenes KRAS and BRAF which activate proliferative and anti-apoptotic pathways[2,3].

### **Diet and CRC risk**

Factors that prevent DNA lesions, induce DNA repair at the cancer initiation stages or that modulate the rate of tumor growth and invasiveness have potential in cancer preventive strategies. Many food constituents have been described that, due to their role as antioxidants or to their effects on the activity of genotoxic metabolizing enzymes, exert cancer preventing effects. Multiple reports have been published on the anticancer potential of plant foods and individual phytochemicals. Molecular targets for dietary constituents that may be responsible for their cancer chemopreventive effects have been identified. This has been the subject of intense research and major results compiled in several recent reviews[4,5,6,7]. Green tea, cruciferous vegetables, red grapes, turmeric, garlic, soybean, apples and citrus fruits are examples of plant foods with established anti-cancer properties[4].

In spite of the recognized health benefits of the intake of dietary fruits and vegetables, the WCRF/AICR report<sup>[1]</sup> does not classify the evidence gathered from human studies as being convincingly strong with regard to reduced CRC risk. It is possible that the explanation for this is that effects vary according to CRC molecular type and that this precludes generalized conclusions.

In a large pooled analysis by Hikada *et al* [8] of associations by CRC molecular type, the authors found that higher fruit intake associated with lower risk of BRAF mutated CRC and the traditional adenoma-colorectal cancer pathway<sup>[8]</sup>. However, most other epidemiologic studies investigating the association of different molecular CRC subtypes and intake of fruits, vegetables and fiber ingestion have been inconclusive.

With regard to the individual food chemical constituents responsible for the beneficial effects much attention has been given to polyphenols, and in particular to flavonoids,

due in part to their strong anti-oxidant activity, repeatedly demonstrated in *in vitro* and animal studies.

Fruits and vegetables are rich sources of flavonoids and other polyphenols (Table 1). These compounds, flavonoids in particular, are potent antioxidants, have anti-inflammatory properties and have been extensively studied with regard to their potential as anticancer dietary constituents<sup>[4,5,7]</sup>.

The effects of human dietary intake of flavonoids on decreased risk of CRC have been subject of much fewer reports. Procyanidins (oligomeric forms of catechin and epicatechin) and isoflavones ingestion was found by He and Sun <sup>[9]</sup> in population studies to decreased CRC risk, while total flavonoid intake was not found to associate with decreases in CRC risk<sup>[9]</sup>.

In another report, Chang and collaborators <sup>[6]</sup> found that high intake of flavonols, flavones and anthcyanidins may decrease CRC risk. However, Chang *et al* <sup>[10]</sup> corroborated that total flavonoid intake did not associate with CRC risk nor did total flavanones or flavan-3-ols. High variability between the studies in this meta-analysis precluded any further conclusions<sup>[6]</sup>.

Previously, Djuric *et al* <sup>[10]</sup>, found that quercetin decreased risk of proximal CRC but lost its effect if tea consumption was high. Importantly, the authors found that <sup>2</sup> increased intake of quercetin was associated with increased risk of distal colon cancer when total fruit intake was low<sup>[10]</sup>.

On the other hand APC mutations were found to be associated with alcohol and processed meat consumption<sup>[11]</sup>.

### **CRC treatment**

Endoscopic or surgical lesion removal is the best curative strategy for CRC <sup>[3]</sup>. In order to increase treatment efficacy, adjuvant fluoropyrimidine based (5- fluorouracil (5-FU) or capecitabine) chemotherapy combined with oxaliplatin and/or irinotecan is used<sup>[3]</sup>.

Another group of drugs known as “biologics” are now also used in the treatment of CRC. These are monoclonal antibodies such as bevacizumab, an anti-VEGF antibody

that targets angiogenesis, or cetuximab and panitumumab, anti-EGFR antibodies that target proliferative signals mediated by epithelial growth factor receptor<sup>[3,12]</sup> (Figure 1).

<sup>6</sup> FOLFOX (leucovorin, 5-FU, and oxaliplatin) or FOLFIRI (leucovorin, 5-FU, and irinotecan), frequently combined with EGFR or VEGFR inhibitors are the most commonly utilized chemotherapeutic regimes in CRC treatment <sup>[12]</sup>.

Under normal conditions, upon ligand binding to EGFR or VEGFR, both the MAPKinase (RAS/RAF/MEK/ERK) and the PI3K/AKT/mTOR phosphorylation cascades will be activated, leading to cell proliferation and survival <sup>[3,14]</sup>. Although EGFR is overexpressed in 60-80% of CRC tumors, (which makes it a good anticancer target) favoring the proliferative and antiapoptotic activity of the MAPK and PI3K pathways<sup>[16]</sup>, efficacy of anti-EGFR drugs (and to a smaller extent also anti-VEGFR) decreases dramatically in the presence of KRAS and particularly BRAF mutations<sup>[3,12,14]</sup> due to their overriding downstream effects on the signaling pathways. (Figure 1).

Mutations in the oncogene KRAS occur in 37% of CRCs and constitutively activate proliferative and anti-apoptotic pathways such as the mitogen-activated protein kinase (MAPK) and the PI3K pathway. Mutations in the oncogene BRAF occur in 13% of CRCs activate the MAP kinase pathway downstream of KRAS but not the PI3K pathway<sup>[2,13]</sup> KRAS mutations do not co-occur with BRAF mutations in CRC<sup>[14,15]</sup>. Activating mutations of PI3K are present in 15-20% of CRC and loss of PTEN, an inhibitor of the PI3K pathway, also contribute to increased activity of the PI3K/AKT pathway<sup>[3,14]</sup>.

Therefore, mutations in the key players KRAS, BRAF and PI3K, promote CRC development, define molecular subtypes and are relevant for the efficacy of chemotherapeutic agents such as anti-EGFR monoclonal antibodies<sup>[2,3,12,13,16]</sup>.

In addition, targeted anti-EGFR therapy with cetuximab or panitumumab, often leads to secondary resistance through selection of resistant cells even in patients wild type for EGFR, KRAS and BRAF<sup>[14,16]</sup>. More recently, regorafenib has shown to be of use as third line treatment for KRAS and BRAF mutated tumors<sup>[15,18]</sup>. (Figure 1).

Genetic profiling to determine the presence of EGFR, KRAS or BRAF mutation is, therefore, important to predict treatment outcome although other unknown factors play



important roles in a patient's response to therapy<sup>[16]</sup>. Targeted chemotherapy tailored to improve patient's response with minimal side effects remains a goal but much work remains still to be done in order to achieve it since the 5 year survival rate of CRC patients is still less than 15% for those diagnosed in advanced stages of the disease with metastatic CRC<sup>[16]</sup>.

Therapeutic strategies to treat tumors with mutations in these key genes remain limited and improving treatment efficacy for these patients constitutes a pressing clinical need. Despite this, dietary and other plant derived phytochemicals remain an untapped resource, despite the large body of evidence of their acting on the relevant molecular targets.

### **Chemotherapeutic potential of dietary phytochemicals**

Several compounds have been shown to target the MAPK and/or the PI3K pathways. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol also present in chocolate. EGCG has been shown to inhibit EGFR signaling and ERK1/1 (MAPK pathway) and PI3K/AKT activation, with effects on cell proliferation and survival<sup>[16,19]</sup>.

Curcumin, another natural polyphenol present in turmeric, has also been shown to decrease EGFR gene expression thereby decreasing ERK mediated signaling and gene expression<sup>[16]</sup>. In addition, curcumin suppressed the PI3K/Akt pathway *in vitro* with induction of apoptosis<sup>[20,21]</sup> and decreased NFkB activation by traditional chemotherapeutic drugs thereby contributing to overcome treatment resistance<sup>[22]</sup>. Inhibitory effects on PI3K/Akt and NFkB seem also to be involved in sensitization to treatments with 5-FU and Capacetabine<sup>[23,24]</sup>.

In other studies, curcumin was shown to produce a similar effect to that of the MEK inhibitor U0126 and to synergistically enhance the efficacy of the multi kinase inhibitor regorafenib in HCT116 cells <sup>[15,25]</sup>. New delivery formulations that address curcumin's low bioavailability look promising and will increase the compound's clinical use <sup>[22]</sup>.

Quercetin, is a flavonoid particularly abundant in onions, apples and broccoli. The health benefits of quercetin have been widely reported and include anti-oxidant, anti-



inflammatory and cancer chemopreventive activity. Recent reviews of quercetin's cancer chemopreventive mechanisms have been published by Rather and Bhagat<sup>[26]</sup> and Kashyap<sup>[27]</sup>. These include scavenging of reactive oxygen species and induction of antioxidant defenses, modulation of signaling pathways resulting in decreased cell proliferation and increased apoptotic cell death, cytochrome P450 (CYP) enzyme activity modulation, induction of Nrf2-mediated phase II enzymes, and inhibitory effects on inflammatory markers such as iNOS, COX2, IL-6 and TNF-alpha among others. Quercetin has also been shown to decrease signaling through the MAPK and PI3K pathways as well as improve the response to chemotherapeutic drugs<sup>[26,27,28,29,30]</sup>.

In studies with human CRC derived cell lines harboring either KRAS or BRAF mutations, it is possible to test the impact of individual compounds (or complex plant extracts) on the activity of pathways caused by that particular mutation thereby identifying the subset of patients that would benefit the most from strategies involving that particular flavonoid.

Quercetin (Q) and luteolin (L) are two of the best studied flavonoids and also two of the most abundant in plant foods. They have demonstrated anti CRC activity in numerous *in vitro* and *in vivo* studies<sup>[29]</sup>.

In a study using the human CRC cells lines HCT15, (harboring a KRAS mutation and wild type BRAF) and CO115 (harboring a BRAF mutation and wild type KRAS) the authors Xavier *et al*<sup>[28]</sup> showed that both Q and L decreased MAPK kinase activity in HCT15 but not in CO115 cells. It seems, therefore, that the BRAF mutation present in CO115 overrides the inhibitory effects of Q and L on MAPK pathway activity. In addition, Q and L decreased KRAS total protein expression but had no effect on total BRAF expression levels.

This is an indication that Q and L could benefit MAPK dependent therapeutic effects in KRAS mutated but not be as effective in BRAF mutated tumors<sup>[28]</sup>.

Quercetin and several of its analogs are also known inhibitors of PI3K activity<sup>[26,27,28,29,30]</sup>.

Crosstalk between MAPKinase and PI3K/AKT suggests that levels of PI3K pathway activity may also be affected in KRAS mutated cells and contribute to the overall effects on cell proliferation and apoptotic cell death. However, PI3K activity may also be regulated independently of its upstream regulator KRAS.

Interesting in the Xavier *et al* study<sup>[28]</sup> was the fact that the specific MEK inhibitor PD-98059 (MEK a downstream link of both KRAS and BRAF in the MAPK pathway) significantly reduced phosphor-ERK levels but did not significantly decrease cell proliferation or induce apoptotic cell death<sup>[28]</sup>. Also wortmanin, a reference PI3K inhibitor, decreased phosphor AKT levels, an indication of pathway inhibition, without producing significant effects on proliferation or apoptotic cell death. Of relevance here, in the comparison between the effects of the reference compounds PD-98059 and wortmanin and Q or L, is the fact that the flavonoids had significant effects on cell proliferation and cell death, a clear indication that the multitarget nature of the flavonoids' actions was beneficial and not detrimental to the overall effect<sup>[28]</sup>.

In another study by Yang and collaborators<sup>[31]</sup>, quercetin induced apoptosis in KRAS mutated cells in a c-Jun N-terminal kinase (JNK) activation dependent way. Both G13D and G12V mutation of KRAS rendered CRC cell more sensitive to quercetin than KRAS wild-type cells<sup>[31]</sup>.

### **Combination of flavonoids plus chemotherapeutic agents**

Studies where flavonoids are tested in combination with chemotherapeutical drugs also demonstrate possible enhancement of therapeutic effects and consequent benefits of co-administration regimens. The majority of studies involving the role of phytochemicals in combination with chemotherapeutic drugs, use 5-FU and address the involvement of p53 in the response, due to the key role of p53 in apoptosis induction and treatment sensitivity<sup>[17,30, 32,33,34,35,36,37,38,39]</sup>. Tumor progression and low treatment efficacy is strongly dependent on successful evasion of cell death due to mutations in TP53<sup>[17]</sup>, present in 35-55% of CRC cases.

In addition to p53 status, CRC displaying DNA mismatch repair defects and microsatellite instability (MSI) show lower sensitivity to 5-FU and poorer treatment outcome. In this scenario of increased treatment resistance, quercetin (Q) was tested and the dependence on p53 status for the cell's response to 5-FU determined<sup>[30]</sup>. Q significantly increased apoptotic cell death in response to 5-FU in p53 wild type CO115 and HCT116 comparatively to effects on HCT15 p53 mutated cells. P53 silencing in CO115 completely abrogated the apoptotic effects of Q + 5-FU. This p53 dependence was further corroborated in the isogenic HCT116 p53<sup>-/-</sup> cells, where both the effects of the drug alone and of the combination Q + 5-FU were lost. This constituted a clear indication that p53 wild type was required for Q enhancement of 5-FU apoptotic effects and suggested that tumors displaying wild-type p53 are more likely to benefit from combination treatment of 5-FU with Q than tumors harboring inactivating mutations of p53<sup>[30]</sup>.

Resveratrol, on the other hand, showed to increase <sup>5</sup>5-FU induced apoptosis in a p53 independent manner in both HCT116 p53 wild type and p53 deficient cells. Resveratrol also showed to decrease MAPK and PI3K/AKT signaling and to upregulate miR-34a expression, in a new mechanism of inhibition of HCT116 proliferation induced by the combination of resveratrol + 5-FU<sup>[40]</sup>. Upregulated miR-34a expression was also involved in chemosensitization of HCT116 and HT-29 to oxaliplatin by resveratrol<sup>[41]</sup>.

Combination exposures to FOLFOX and curcumin showed reduction of tumor explant cell survival due to enhanced antiproliferative effects<sup>[42,43]</sup>. Information on the anti-CRC effects of curcumin including clinical trials involving curcumin are the subject of a recent publication<sup>[22]</sup>. Molecular targets for curcumin include MAPK and PI3K pathways and also decreases in chemotherapy induction of NFkB and antiapoptotic gene expression.

Yue and colleagues<sup>[44]</sup> showed that turmeric extract in combination with bevacizumab produced comparable effects to bevacizumab plus FOLFOX with the advantage that it increased survival of tumor bearing mice. In another study<sup>[45]</sup>, the combination of

curcumin with the multi kinase inhibitor regorafenib produced an increase in cell death in KRAS mutated CRC cells and not in KRAS wild-type.

With regard to clinical trials<sup>[22]</sup>, most involve curcumin and address the pharmacokinetic profile and toxicity of curcumin formulations. Some trials test curcumin in combination with drugs such as 5-FU or irinotecan and will provide useful information, even if enrolment is low and patients are most likely not stratified by tumor molecular subtype.

Much remains to be done with regard to the effects on patients of phytochemicals whether isolated or in combination with chemotherapeutical drugs.

### **Concluding remarks and future perspectives**

Although a diet rich in fruits and vegetables is generally beneficial with regard to CRC, variations in response among individuals to similar dietary choices strongly suggest that there may be interactions of food constituents with particular molecular targets that will benefit patients differently according to their tumor's molecular signature.

The capacity to inhibit proliferation and induce apoptosis through effects on molecular targets of the MAPK and/or the PI3K/AKT pathways makes flavonoids potentially strong allies if used as adjuvants in combination treatment improving therapeutic efficacy and patient survival. However, in order to take full advantage of the anticancer potential of these natural compounds, a detailed and systematic characterization of the compound's mechanisms of action is required. Although much has been published on the anticancer effects of dietary phytochemicals, such an exhaustive characterization of potential beneficial and adverse effects in the setting of cancer treatment in humans is still lacking. Results from *in vitro* studies cannot be extrapolated to effects in humans. Also, care should be taken that some flavonoids' and other phytochemicals' potential to prevent DNA damage or induce repair of the lesions produced by the therapeutical agent do not hinder treatment efficacy.

Clinical trials are needed to verify relevant effects and those remain insufficient in number and in patient enrollment. Also, patients are generally not selected on the basis

of their tumors' molecular profiles which would be a requirement for a more personalized treatment approach.

Moreover, efforts should be concerted towards establishing nutritional guidelines that prescribe the ingestion of the right plant food sources or of individual flavonoids as part of nutraceutical formulations in the several phases of CRC progression and treatment. Much work remains to be done not least the introduction of standardization and regulatory systems as well as extensive testing through clinical trials of possible toxicity of the new formulations.

Reduced bioavailability often mean that polyphenols have limited efficacy in *in vivo* studies. However, these issues can today be addressed by nanoencapsulation which may increase bioavailability and increase circulating concentrations or enable delivery into the colon and cause direct exposure of colonocytes to the active compounds limiting possible systemic side effects.

## **CONCLUSION**

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