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Minireview

Flavonoids: Potential Wnt/beta-catenin signaling modulators in cancer

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ABSTRACT

Flavonoids are polyphenolic compounds found throughout the plant kingdom. They occur in every organ but are usually concentrated in leaves and flowers. During the last two decades, *in vitro* and *in vivo* studies demonstrated that flavonoids have inhibitory effects on human diseases through targeting of multiple cellular signaling components. Wnt/β-catenin signaling regulates proliferation, differentiation and fate specification in developmental stages and controls tissue homeostasis in adult life. For these reasons, this pathway has received great attention in the last years as potential pathway involved in distinct Human pathologies. In this review we discuss the emerging potential mechanisms for flavonoids on Wnt/β-catenin signaling in cancer and possible investigation strategies to understand flavonoids mode of action on this signaling pathway.

Contents

Introduction

The word flavonoid has its root in the Latin word *flavus* meaning yellow. In fact, flower, fruit and leaves color accounts for the pigment containing abundant amounts of flavonoids. Flavonoids form a very large group of natural products characteristically containing a C6–C3–C6 skeleton structure (Fig. 1). Different plant families have characteristic patterns of flavonoids and their conjugates which play important biochemical and physiological roles in various cell types or plant organs where they accumulate inside the cells or in the surface. Flavonoids differ in the saturation of the heteroatomic ring C, and in the overall hydroxylation patterns (Fig. 1). They may be modified by hydroxylation, methoxylation, or *O*-glycosylation of hydroxyl groups as well as *C*-glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core (Fig. 1). Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes: the flavonoids (2-phenylbenzopyrans), isoflavonoids (3-benzopyrans), the neoflavonoids (4-benzopyrans), and chalcones (Fig. 1) (Marais et al., 2007).

The multiplicity of possible modifications of flavonoids resulted in more than 6000 different compounds from this class that was known since the end of last century and this number continues to increase (Harborne and Williams, 2000). Since flavonoids are abundantly

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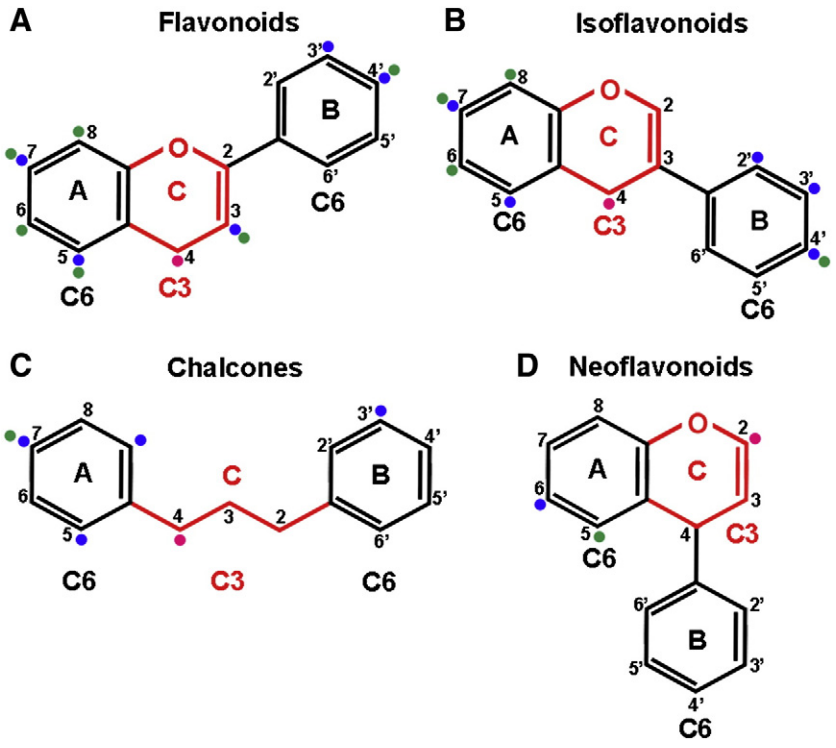

Fig. 1. Structure drawing of the distinct four skeleton cores of Flavonoid classes and its possible modification sites. The chemical structures of this class of compounds are based on a C6–C3–C6 skeleton and in the saturation of the heteroatomic ring C. It can be divided into four main classes: (A) Flavonoids; (B) Isoflavonoids; (C) Chalcones; and (D) Neoflavonoids. Blue circles indicate most frequent hydroxylation sites; green circles indicate most frequent *C*- and/or *O*-glycosylation sites and pink circles indicate most frequent carboxylation sites.

found in the plant Kingdom, they are consequently present in many animals diet, including Humans. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative (Fritz et al., 2003; Manach et al., 2004).

Flavonoids: friend or foe?

Natural products, including flavonoids, have been used worldwide as traditional medicines for thousands of years to prevent and treat various forms of diseases, including cancer. Several studies have shown that flavonoids effects on human diseases are mediated by targeting multiple cellular signaling pathways (Sarkar et al., 2009). The intake of flavonoids is associated with many beneficial effects, such as anti-oxidative, antiviral, anti-inflammatory, hepatoprotective active, prevention of cardiovascular diseases and anti-tumoral effect (Sies, 2010). In the last decade studies have attempted to understand the molecular mechanism involved in flavonoids action. However, not all flavonoids and their actions are necessarily beneficial. The dual role of this substance by producing either toxic or beneficial effects seems to depend on doses and/or the experimental cell type (Hodek et al., 2002). Some flavonoids available in the diet can cause genetic damage and contribute to the cancer development (Vanhees et al., 2011). The maternal exposure to the flavonol, Quercetin and to the isoflavone Genistein during pregnancy increases the risk of infant leukemia due to the inhibition of the DNA topoisomerase II (DNAt2) enzyme, highly expressed during embryonic development (Vanhees et al., 2011; Spector et al., 2005; Ross, 2000, 1998). The chromosomal damage caused by Quercetin can also be related to papillomavirus oncogenic cell transformation (Beniston et al., 2001). Naringenin presents high teratogenic index on tests performed in frog embryos (Pérez-Coll and Herkovits., 2004), which reflects the teratogenic hazard of this compound. This substance exerts malformations of the neural tube closure, developmental retardation and high lethality (Pérez-Coll and Herkovits., 2004). In summary, the biological properties of flavonoids have gained much attention and their beneficial or harmful health effects do not only depend on the structure of the compound, but the life period when those substances are consumed.

A considerable number of reports have shown that the consumption of fruits, vegetables and beverages, like wine and green tea, is associated with lower risks of tumor development (McCullough and Giovannucci, 2004). This effect is continuously associated with the abundant flavonoid content of those foods (Surh, 2003; Smith-Warner et al., 2003; Kac et al., 2008; Yang et al., 2009a,b). Given this importance, in the last 50 years, substantial effort has been made to understand the molecular mechanism whereby flavonoids act in cancer. The first reports are dated from the 60s and then several studies show that many flavonoids can control different types of cancer in variable doses and period of treatment targeting in different cell processes (Sokoloff et al., 1951).

Cancer targeting by flavonoids

Cancer is a highly heterogeneous pathology related to defects in regulatory circuits that govern cell homeostasis including cell death, proliferation, differentiation and migration (Hanahan and Weinberg, 2000; Kreeger and Lauffenburger, 2010). Flavonoids can affect the overall process of carcinogenesis by several mechanisms, including antioxidant activities (Duthie and Dobson, 1999), the scavenging effect on activated mutagens and carcinogens (Williamson et al., 1998; Calomme et al., 1996), interaction with proteins that control cell cycle progression depending on p53 (Plaumann et al., 1996), apoptosis induction by activation of caspase-9 and caspase-3 (Ren et al., 2003), and general inhibitors of cytokine-induced gene expression (Gerritsen, 1998) (Table 1). For instance, EGCG, the major catechin in tea, has been largely studied compared to other tea compounds in many epidemiological studies (Yang and Landau, 2000). EGCG induces a pronounced and specific growth-inhibitory effect on breast cancer cells, but not on their normal counterparts (Chen et al., 1998). EGCG has been

reported to control tumor growth of different types of cancer, such as prostatic cancer, gastric cancer, colon cancer, lung cancer and leukemia (Brusselmans et al., 2003; Hibasami et al., 1996; Horie et al., 2005; Chen et al., 2003). In addition, many *in vitro* studies show that EGCG is associated with other anticancer benefits, like inhibition of migration and invasion and induction of apoptosis in mammary cancer cell lines (Punathil et al., 2008). Genistein – a soy-derived isoflavone – is believed to contribute to the putative breast and prostate-cancer-preventive activity of soy. Because of their structural similarity to estradiol and their binding to estrogen receptors (Shao et al., 2000), these isoflavones can inhibit growth of prostate and breast cancer cells, which is intrinsically related to the levels of estrogens in the tumor tissue (Cappelletti et al., 2006). In addition, Genistein is also related to modulation of cell cycle (Mukherjee et al., 2010), induction of apoptosis (Kim et al., 2010a,b), as well as antioxidant, anti-inflammatory (Sarkar and Li, 2002), anti-invasive effects (Pavese et al., 2010), and anti-angiogenesis effects *in vitro* in other cancer cell types (Fotsis et al., 1995; Halliwell, 2008; Peterson, 1995) (Table 1). Silibinin is an antioxidant flavonoid found in *Silybum marianum* that inhibits tumor growth and metastasis in several tumor cell lines and rodent models (Deep and Agarwal, 2010). In addition, flavonoids like Baicalin and Baicalein which come from *Scutellaria baicalensis*, an herb traditionally used in China for treatment of many diseases have been described as potent anti-cancer compounds (Yano et al., 1994; Zhang et al., 2003). Several reports show that these flavonoids have cytotoxic effects in tumor cell lines derived from prostate (Pidgeon et al., 2002; Chan et al., 2000), leukemia, (Roy et al., 2007; Shieh et al., 2006) colon and lung cancers (Kunts et al., 1999). Most importantly, they show low toxicity to normal cells (Ma et al., 2007). Another well studied flavonoid is Kaempferol, a flavonol with abundant distribution in some vegetables like caper, kale cress, and broccoli (Scalbert and Williamson, 2000) and also present in green tea (Yang et al., 2009a,b). Kaempferol presents important roles in apoptosis induction (Huang et al., 2010), inhibition of cell migration (Labbé et al., 2009), antioxidant (Macpherson and Matthews, 2010) and

Table 1
A list of flavonoids and their actions in different tumor types.

Flavonoids	Plant	Action	Tumor	References
EGCG	Green tea	Cell cycle arrest	Breast	Liang et al. 1999
	(Camellia sinensis)	Induces apoptosis	Glioblastoma	Yokoyama et al. 2001
		Reduces cell adesion and migration	Leukemia	Das et al. 2010
		Inhibits cell invasion and metastasis	Prostate	Hibasami et al. 1996
		Inhibits angiogenesis	Lung	Demeule et al. 2000
			Colon	Tang et al. 2010
				Taniguchi et al. 1992
				Yang et al. 2005
				McLarty et al. 2009
				Jung et al. 2001
GENISTEIN	Soy	Cell cycle arrest	Ovarian	Valachovicova et al. 2004
	Tea	Antiinflamatory activity	Intestine	Ouyang et al. 2009
		Induces apoptosis	Breast	Ruiz and Hailer 2006
		Inhibits cell adesion and migration	Prostate	Lara et al. 2007
		Inhibits cell invasion and metastasis		
QUERCTIN	Apple	Antioxidant activity	Hepatoma	Alia et al. 2006
	Grape	Cell cycle arrest	Lung	Robaszkiewicz et al. 2007
	Lemon	Induces apoptosis and necrosis	Leukemia	Kang and Liang, 1997
	Tomato	Inhibits cell migration and invasion	Glioma	Braganhol et al. 2006
	Onion	Inhibits angiogenesis	Colon	Hosokawa et al. 1990
	Honey	Induces cell differentiation	Prostate	Czokay et al. 1997
		Antiinflamatory activity		Tang et al. 2010
				Anso et al. 2010
				Turner et al. 2009
ISOQUERCITRIN	Onion	Reduces proliferation	Glioblastoma	Amado et al. 2009
	Buckwheat	Antioxidant activity	Liver	Yokohira et al. 2008
	Hyptis fasciculata			Silva et al. 2009
KAEMPFEROL	Kale cress	Cell cycle arrest	Leukemia	Bestwick et al. 2007
	Broccoli	Induces apoptosis	Lung	Leung et al. 2007a,b
	Green tea	Antioxidant activity	Ovarian	Luo et al. 2009
	Honey	Inhibits angioenesis		Labbé et al. 2009
	Mango	Inhibits cell migration		
	Caper			
ISORHAMNETIN	Sea buckthorn	Induces apoptosis	Colorectal	Jaramillo et al. 2010
	Nelumbo nucifera	Induces necrosis	Esophagus	Ma et al. 2007
		Inhibitor of angiogenesis	Lung	Lee et al. 2008
			Liver	Hasebe et al. 2003
SILIBININ	*Silybum marianum*	Cell cycle arrest	Colorectal	Agarwal et al. 2003
		Induces apoptosis	Prostate	Zi and Agarwal 1999
		Induces cell differentiation	Lung	Mateen et al. 2010
		Reduces invasion	Liver	Ramakrishnan et al. 2009
		Inhibitor of angiogenesis	Oral cavity	Chen et al. 2006
				Singh et al. 2008
BAICALEIN	*Scutelaria baicalensis*	Cell cycle arrest	Breast	Pidgeon et al. 2002
		Induces apoptosis	Prostate	Roy et al. 2007
		Supresses adesion and migration	Leukemia	Kunts et al. 1999
			Colon	Pidgeon et al. 2002
			Lung	Leung et al. 2007a,b
			Prostate	Wang et al. 2010
BAICALIN	*Scutelaria baicalensis*	Inhibits metastasis	Leukemia	Shieh et al. 2006
		Cell cycle arrest	Prostate	Himeji et al. 2007
		Induces apoptosis	Lung	Chan et al. 2000
				Du et al. 2010

antiangiogenic effects (Luo et al., 2009) (Table 1). Quercetin, another flavonol abundantly found in human diet, has been described as anticancer and is one of the most studied phenolic compound (Chen et al., 2010). *In vitro* and *in vivo* studies have shown that quercetin exerts a dose-dependent inhibitory effect on cell growth (Yang et al., 2009a,b; Ferry et al., 1996; Amado et al., 2009) in numerous types of cancer, such as breast cancer (Singhal et al., 1995; Choi et al., 2001), leukemia (Larocca et al., 1995; Russo et al., 2007), colon cancer (Kim et al., 2005; Kalra et al., 2007), hepatoma (Granado-Serrano et al., 2006), ovarian cancer (Gates et al., 2009; Scambia et al., 1990), oral cancer (El Attar and Virji, 1999) and lung cancer (Nguyen et al., 2004) (Table 1). Quercetin effects on tumor cells are related with inhibition of cell division by interference with cell cycle components, like cyclinD1, and induction of apoptosis and necrosis (Chen et al., 2010). Besides, it is also related to antioxidant effects and inhibition of cell migration and invasion. Quercetin glycosides, like Quercitrin and Isoquercitrin, also has important anticancer effects. Recently, our group showed that treatment of Isoquercitrin isolated from *Hyptis fasciculata*, a plant found in the Atlantic coast of South American, inhibits significantly proliferation in glioblastoma cells with marked reduction of cyclin D1 levels and an increase in p27 levels, both important cell cycle components (Amado et al., 2009) (Table 1). In the last years, Isorhamnetin, a flavonol that is metabolically derived from Quercetin and shares similar chemical structure with Kaempferol (Manach et al., 1998), has also been associated with tumor growth control because its effects on apoptosis and necrosis (Jaramillo et al., 2010; Ma et al., 2007), as well as in cell cycle arrest (Ma et al., 2007). Altogether, these examples depict the hits and leads for flavonoid functions in cancer inhibition, which indicate their potential as anticancer drugs.

According to FDA (Food and Drug administration) nearly thirty flavonoid trials are in course and have advanced up to phases II and III with tests in volunteer people (www.clinicaltrial.gov).The majority of flavonoids clinical trials are still under test on phase I or II. For instance, EGCG and Genistein, are the main flavonoid target for trials in distinct phases (Tsao et al., 2009; Bettuzzi et al., 2006). Silibinin has been matter of eight advanced clinical trials in phases II or III showing its importance in control of human cancers *in vivo* (Hoh et al., 2006; Flaig et al., 2007; Deep and Agarwal, 2010). However, none of these trials have reached FDA approval to be used as medicine for human cancer treatment.

Flavonoids proposed mechanism of action on cancer signaling pathways

During the past three decades, there has been substantial progress in identifying the biochemical events that are associated with the multistage process of carcinogenesis in which distinct molecular and cellular alterations occur (Reya et al., 2001; Visvader, 2011). Many alterations described during carcinogenesis are associated with cell signaling pathways that regulate cell proliferation, death and differentiation (Hanahan and Weinberg, 2000). Often, activators, repressors, components or even target genes of different pathways are found altered in numerous human cancers. Therefore, agents that modulate these pathways have great potential for chemoprevention and can be useful in cancer therapy, either alone or in combination with conventional methods (Sarkar et al., 2009). In the last years, many investigators have focused on elucidating the molecular mechanisms and identifying the targets of these natural products in different cell pathways (Fig. 2). Indeed, it has been elucidated many flavonoid targets on pathways strongly related to tumorigenesis and tumor progression. For instance, flavonoids like Genistein, Tangeretin, EGCG and Fisetin can modulate different components of the NF-κB pathway and thus inhibit translocation of this factor to the nucleus and activation of target genes (Jaiswal et al., 2002). This pathway plays important roles in control of cell growth and apoptosis and is related with many types of cancer (Yan et al., 2005). Akt and MAPK pathways play critical roles in mammalian cell survival signaling and have been shown to be activated in various cancers (Chang et al., 2003). Akt and MAPK pathways are also targets for flavonoids like EGCG, Kaempferol, Tangeretin, Quercetin and Genistein (Sarkar et al., 2009) (Fig. 2). In addition, it is known that flavonoid modulation on a pathway component can induce effects on other signaling pathways, because of a cross-link between these pathways, which may promote an amplification of flavonoid action (Fig. 2). For example, modulation of Akt by quercetin and EGCG and modulation of MAPK by Genistein promotes indirect effects on NF-κB pathway, enhancing the antitumor effects of these natural products (Gadgeel et al., 2009). Another important signaling pathway involved in cancer is Notch pathway. When Notch signaling is abnormally activated, an increasing on proliferation of cancer cells is observed (Wang et al., 2006b). Genistein and Quercetin can decrease levels of Notch1 and Notch2 protein, respectively, and then decrease the proliferation rates of cancer cell lines (Wang et al., 2006a) (Fig. 2). Similarly, these flavonoids also inhibit, indirectly, the NF-κB pathway across modulation on Notch signaling (Fig. 2).

Wnt signaling plays a central role in many processes during development and diseases (Logan and Nusse, 2004). Wnt signaling can be broadly categorized as canonical or noncanonical pathways (Veeman et al., 2003). Noncanonical Wnt signaling pathway acts in a β-catenin independent manner (Semenov et al., 2007). The noncanonical Wnt signaling has been paradoxically implicated in tumorigenesis (McDonald and Silver, 2009). Ectopic expression of Wnt5a, which is noncanonical, in uroepithelial cancer reverted tumorigenesis (Olson et al., 1997). Conversely, recent studies have reported that Wnt5a may also enhance motility of malignant cells and tumor invasion such as in breast cancer, melanoma, and gastric cancer (Kurayoshi et al., 2006; Pukrop et al., 2006). Few reports have addressed the relation between flavonoids and noncanonical Wnt pathway. For instance, Su and colleagues demonstrated that genistein inhibited Wnt5a expression in rat mammary gland tumor cells (Su et al., 2007). This treatment increased the secreted Wnt inhibitor (sFRP2), but did not change β-catening levels indicating non-canonical effect of Genistein on mammary tumor.

In recent years, much progress has been made on understanding Wnt/β-catenin signaling in respect to cancer development and their possible modulators, which could be useful in cancer prevention and therapy. In this regard, small molecules synthesized or from natural origin, like flavonoids, have been identified as potential modulators of Wnt/β-catenin signaling pathway (Sarkar et al., 2009; Thorne et al., 2010).

Targeting Wnt/β-catenin with flavonoids

The canonical Wnt pathway has a protein β-catenin as a central component. In the absence of Wnt ligands, β-catenin is phosphorylated by a complex of proteins including axin, adenomatous polyposis coli (APC), glycogen synthase kinase (GSK)3-β, and casein kinase 1 (CK1). Phosphorylated β-catenin, in the amino terminal region, is recognized by β-TrCP, an F-box component of the E3 ubiquitin ligase complex, which promotes β-catenin ubiquitination and degradation by the ubiquitin-proteasome system (Zhang et al., 2010; MacDonald et al., 2009) (Fig. 3). Binding of Wnt ligands to their receptors, LRP6/5 and frizzled, leads to the activation and recruitment of the adaptor protein disheveled (Dsh), and recruitment of Axin complex to receptor complex. These events reduce β-catenin phosphorylation and its subsequent degradation. Stabilized cytoplasmic β-catenin is able to translocate to the nucleus where it binds to members of the T-cell factor/lymphoid-enhancing factor family of transcription factors (MacDonald et al., 2009, 2007) and activates Wnt target genes expression (Fig. 3).

The Wnt pathway plays many important roles in controlling embryonic axis formation, cell fate, proliferation, migration, tissue architecture, and organogenesis during development and play homeostatic roles in adult life. Therefore, the increasing interest of the scientific community, over the last decade, in the Wnt-dependent signaling

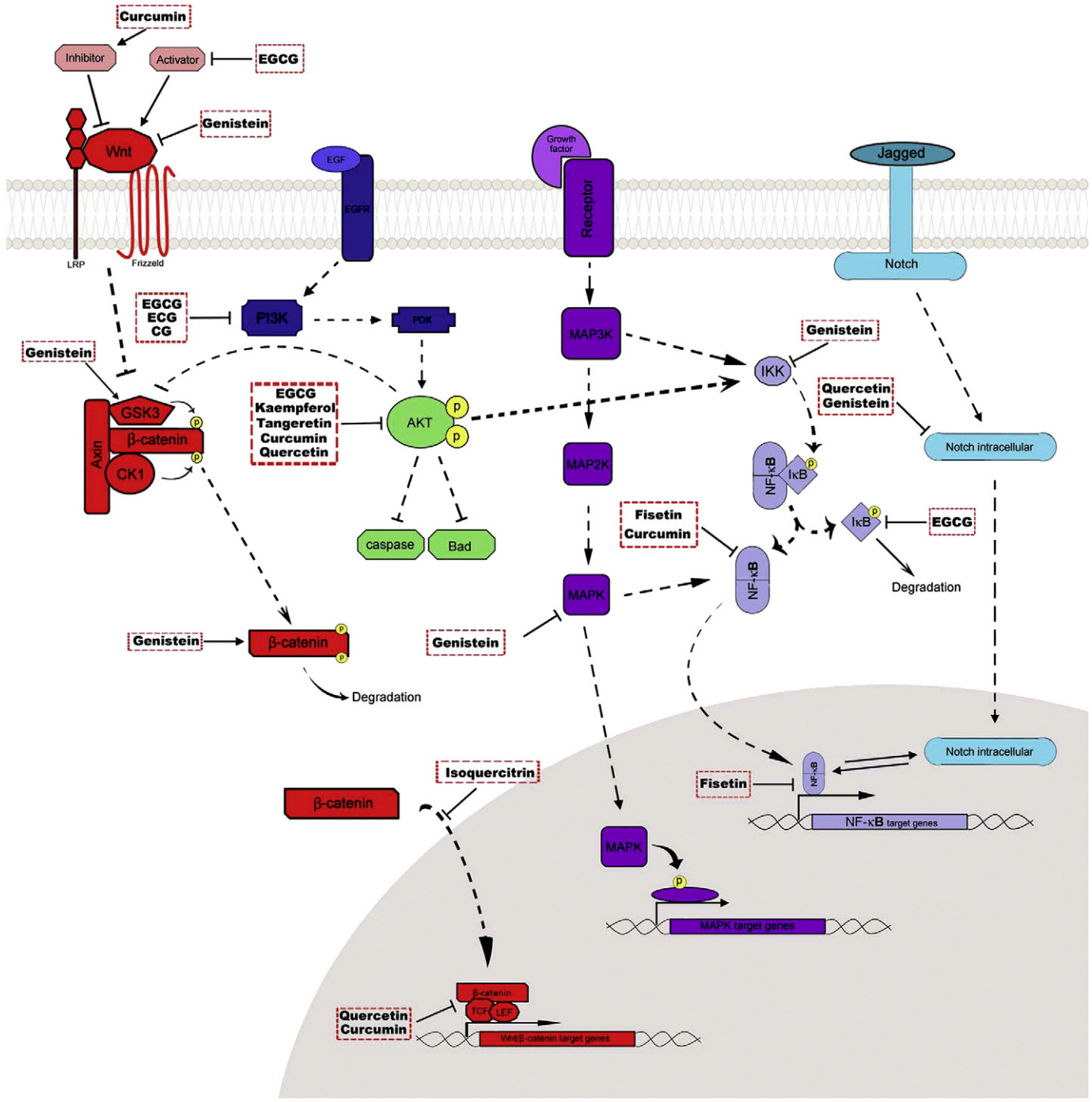

Fig. 2. Cellular signaling pathways altered by flavonoids. The scheme depicts distinct signaling pathways main components and where flavonoids have been proposed to act. The Wnt/β-catenin pathway components in shown in red, the EGF/AKT pathway in dark blue, the AKT in green, the MAPK in purple, the NF-κB in light purple and Notch pathway in light blue color. Flavonoids appear in dashed boxes.(→) Indicates flavonoid activation and (⊣) indicates inhibition.

pathways is supported by the documented importance of these pathways in a broad range of physiological conditions and disease states (Magdesian et al., 2008; Mermelstein et al., 2007; Janssens et al., 2006; Zhang et al., 2010). For instance, it has been shown that inappropriate regulation and activation of these pathways is associated with several pathological disorders including cancer (Thiago et al., 2010; MacDonald et al., 2009), retinopathy (Toomes et al., 2004), tetra-amelia (Niemann et al., 2004) and bone and cartilage disease such as arthritis (Loughlin et al., 2004). In addition, several components of the Wnt-dependent signaling pathways appear to play important roles in neurodegeneration such as Alzheimer's disease (Cerpa et al., 2009; Magdesian et al., 2008), schizophrenia (Miyaoka et al., 1999), and bipolar disorder (Gould and Manji, 2002) and in the emerging field of stem cell research (Willert et al., 2003). Due to central role in Wnt signaling, mutation of the Wnt pathway components have been associated with many disease. This is the case of colorectal cancer, where 60% of these tumors contain mutation or show abnormalities in components of the Wnt/β-catenin signaling pathway (Segditsas and Tomlinson, 2006). Besides colorectal cancer, Wnt pathway has been implicated in melanoma, hepatocellular carcinoma, gastric carcinoma,

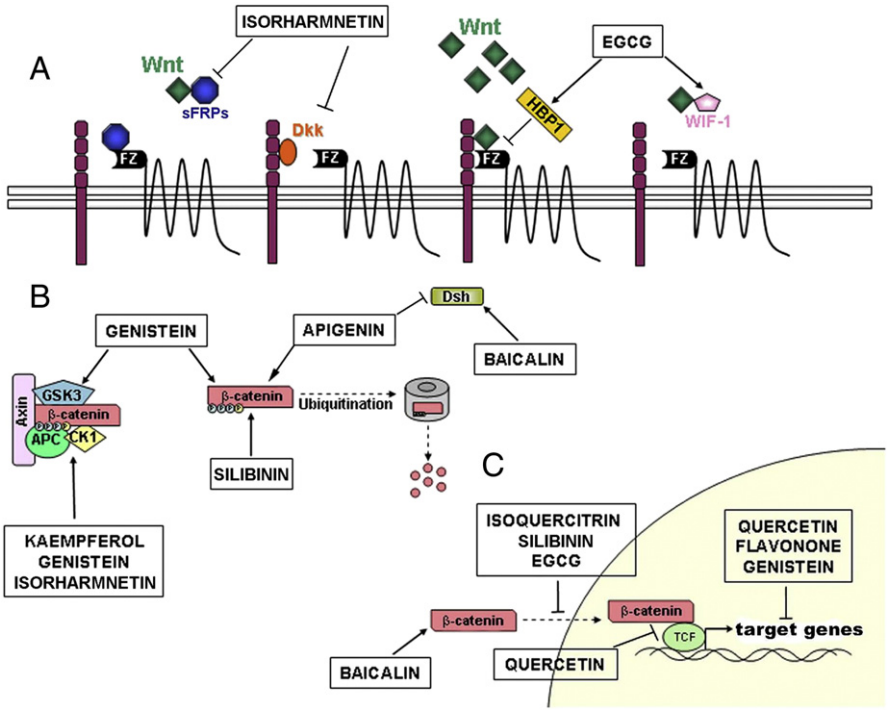

Fig. 3. Flavonoids regulate different components of Wnt/β-catenin pathway. (A) Upper panel shows flavonoids interaction with extracellular inhibitors of Wnt/β-catenin signaling sFRP1, DKK, HBP1 and WIF1. (B) Intracellularly, flavonoids interact with the degradation complex formed by GSK3, Axin, APC and CK1 as well as with phosphrylated β-catenin. (C) Flavonoids affects β-catenin nuclear translocation, β-catenin/TCF association and Wnt/β-catenin target genes.

Glioblastoma, Leukemia, Breast cancer and others. In the last 10 years, several studies have reported that the anti-tumor effect promoted by flavonoids is related to their ability to modulate the Wnt pathway (Sarkar et al., 2009). Moreover, the effects promoted by flavonoids is detected in different levels of the signaling pathway, from cell receptors to the association between β-catenin and TCF (Fig. 3), and control of the methylation of important pathway inhibitors.

Apigenin was the first described flavonoid as regulator of the Wnt pathway because of its selective inhibition of CK2 (casein Kinase II). In breast cancer cells, 40 μM of Apigenin reduces the levels of β-catenin and Dsh proteins and accelerates the degradation of β-catenin in the first two hours of treatment promoting cell cycle arrest in breast cancer cells (Song et al., 2000; Landesman-Bollag et al., 2001). Wnt signaling has been found to be inhibited by EGCG in a dose dependent manner in breast cancer, lung cancer, colon cancer and in normal cells where Wnt signaling was super-activated (Kim et al., 2006; Dashwood et al., 2002; Pahlke et al., 2006; Gao et al., 2009; Mount et al., 2006). The effect of EGCG is not direct in elements of Wnt/β-catenin, but in other proteins that regulate this pathway. In breast cancers, EGCG treatment at 25 to 100 μM induced HBP1 transcriptional repressor levels through an increase in HBP1 mRNA stability which is a suppressor of Wnt signaling. EGCG reduced both breast cancer cell proliferation and invasiveness through the induction of HBP1 and the subsequent inhibition of Wnt signaling. Consistently, the HBP1 knockdown lines had reduced sensitivity to EGCG in the suppression of Wnt signaling and of a target (Kim et al., 2006). In lung cancer cells, EGCG promotes demethylation of WIF-1 (Wnt inhibitory factor 1) (Fig. 3). WIF-1 is a Wnt antagonist that inhibits Wnt signaling by direct binding to Wnt molecules. WIF-1 is silenced when it is hypermethylation in lung cancers (Mazieres et al., 2004; Yang et al., 2009a,b). However when these cells are treated with EGCG at 0 to 50 μM for 72 h, methylation levels in WIF-1 reduce from 77.6% to 27.6% (Gao et al., 2009). Wnt specific reporter activities were significantly inhibited in Hek293 cells transfected with β-catenin and treated with EGCG for 48 h at 0 to 25 μM. In addition, total extract of white and green tea (where the major compounds are catechins) also inhibited Wnt signaling (Dashwood et al., 2002). This treatment reduced tumor multiplicity in the $Apc^{Min/+}$ mouse, a widely used model for intestinal tumorigenesis by inhibiting the translocation of Wnt mediator β-catenin to the nucleus (Ju et al., 2005; Bose et al., 2007) (Fig. 3).

While the Wnt inhibition by EGCG is indirect, the effect of Quercetin controls the Wnt pathway directly by affecting components of the pathway in several types of cells. In 2005, Park and co-workers showed that Quercetin interferes with the binding of Tcf complexes to DNA in colon cancer cells (Park et al., 2005a,b). SW480 cells (colon cancer cells) treated with 50 μM Quercetin for 24 h decreased the amount of β-catenin/Tcf complex (Park et al., 2005a,b). Consistently, another report showed that Quercetin inhibits expression of cyclin D1 and survivin as well as the Wnt/β-catenin signaling pathway (Shan et al., 2009) (Fig. 3). Quercetin is a growth suppressor for several leukemia and lymphoma cells acting through Wnt pathway inhibition (Kawahara et al., 2009). Currently, Quercetin has been considered a Wnt pathway inhibitor, being used in similar studies as negative control for modulation of this pathway (Gelebart et al., 2008; Kawahara et al., 2009; Wallace et al., 2010).

Recently, Fisetin was pointed as an inhibitor of Wnt/β-catening signaling (Syed et al., 2011). Fisetin-treated melanoma cells resulted in decreased cell viability with G1-phase arrest and disruption of Wnt/β-catenin signaling. This effect was accompanied by a decrease in the expression of Wnt protein and its co-receptors, as well as by a parallel increase in the expression of endogenous Wnt inhibitors. Fisetin-treated cells showed increased cytosolic levels of Axin and β-TrCP and decreased phosphorylation of glycogen synthase kinase 3β associated with decreased β-catenin stabilization. Fisetin-mediated interference with the functional cooperation between β-catenin and T-cell factor (TCF)-2 resulted in the downregulation of positively regulated TCF targets, such as c-myc, Brn-2, and Mitf (Syed et al., 2011).

Our group showed that Isoquercitrin (quercetin 3-O-β-D-glucopyranoside), a glycosylated derivative of Quercetin, inhibits

Glioblastoma (Gbm) cells proliferation through mechanisms related to control Wnt/β-catenin pathway. This work showed that 23% of β-catenin staining in untreated Gbm cells was found in the nuclei. However when 100 μM of Isoquercitrin was added to Gbm cell culture, nuclear β-catenin staining was dramatically decreased to 4%, while non-nuclear staining increased to 77%, supporting that isoquercitrin treatment alters the distribution of β-catenin in Gbm cells. These findings are consistent with a reduction in Wnt/β-catenin signaling activity (Amado et al., 2009) (Fig. 3).

Genistein treatment of prostate cancer cells upregulates GSK-3β expression and enhances GSK-3β association with β-catenin, leading to β-catenin phosphorylation and degradation. As consequence Genistein inhibits prostate cancer (Li et al., 2008). Genistein also suppressed β-catenin/Tcf trancriptional activity in SW480 cells (colorectal carcinoma cells) in a dose-dependent manner (Park and Choi, 2010). Genistein affect the upstream components of the β-catenin/Tcf pathway by suppression of GSK-3β and Akt phosphorylation (Park and Choi, 2010). Genistein also modulates Wnt pathway in other cell types. For instance, in mesenchymal stromal cell isolated from human umbilical cord, Genistein reduces cell proliferation through the recruitment of β-catenin to membrane and reducing cytosolic β-catenin. In addition, Genistein reduces the protein and mRNA levels of cyclin D1 (Shieh et al., 2010) (Fig. 3).

In gastric cancer the Flavanone controls cell proliferation by modulation Wnt signaling on transcriptional levels (Park et al., 2005b). Flavanone is able to reduce transcriptional activity, but not interferes with β-catenin levels, distribution or association with Tcf (Park et al., 2005b). It remains to be clarified the mechanism by which Flavonone at TCF activity.

Recently, Park and Choi showed that the binding of Tcf complexes with its specific DNA binding sites was suppressed by four flavonoids, Kaempferol, Isorharmnetin, Genistein and Baicalein in colorectal cancer through distinct mechanisms (Park and Choi, 2010). The effect of Kaempferol, Baicalein and Isorharmnetin is related to upstream regulators of the β-catenin/Tcf pathway other than GSK3, while Genistein affects the Wnt pathway by suppression of GSK3 β (Park and Choi, 2010) (Fig. 3).

It is intriguing that the effects of flavonoids have been found in specific cell lines rather than in every cell types. For instance, the flavonoid Silibinin controls the proliferation of colon tumor cells, only in cell lines where the Wnt pathway is found to be altered. In SW480 (colorectal cancer line where Wnt pathway is altered), Silibinin treatment inhibited cell growth, induced cell death, and decreased nuclear and cytoplasmic levels of β-catenin. However, in HCT116 cells (colorectal cancer wild type for Wnt signals), Silibinin have no effect, suggesting its selective effects on the Wnt/β-catenin pathway (Kaur et al., 2010).

Besides the effect on tumor cells, flavonoids may act on other cell types and may also function as activators of the Wnt pathway. For instance, the flavonoids Genistein, Daidzein, Isorharmnetin and Baicalin, the glucuronide of Baicalein, have been described to act in human adipose tissue-derived stem cells (hMASCs). These flavonoids act by activating the Wnt pathway and thereby inhibit the differentiation of adipose stem cells into adipocytes. Baicalin maintains β-catenin and Disheveled levels during adipogenesis, Isorharmnetin down-regulates the mRNA levels of Frizzled-related protein-1 and Dickkopf-1 (specific inhibitors of the Wnt pathway) (Lee et al., 2010b,a). In addition, Genistein treatment induces higher Wnt-3 and β-catenin mRNA levels, while Daidzein increased expression of β-catenin at the protein level (Kim et al., 2010b).

Conclusion

Despite sharing common general chemical structure, flavonoids present multiple functions in different tumor cells reflecting actions in diverse signaling pathways. Here we reviewed that different flavonoids interact with different components of the Wnt/β-catenin pathway modulating signaling and tumor growth. These observations strongly suggest that structural specificity may be a key for understanding flavonoid mode of action. However, it remains to be addressed what specific structural group, such as hydroxyl, methyl or glycosyl, should be added to the phenylbenzopyrano (C6–C3–C6) core to improve affinity and specificity for Wnt signaling components. More importantly, modifications on these structures may shed light in the mechanisms by which flavonoids impair cancer growth. A good chemical strategy was the recent discovery of the N-substitution of the diphenylsulfonyl sulfonamide 1 (Moore et al., 2009, 2010). This piperidinyl was identified as an inhibitor of sFRP1 (secreted Frizzled-Related Protein1) binding to Wnt ligands, therefore promoting Wnt signaling. To improve the compound effect in the Wnt signaling as well as to identify the structure region modulating Wnt/sFRP1 binding, the authors performed structure–activity relationship in the sulfone portion. As result, they found isosteric derivatives that improved binding and potency of this molecule on Wnt signaling (Moore et al., 2009, 2010). This strategy points toward a promising future in the study of small molecules, such as flavonoids, with biological potential.

Six decades ago, first studies pointed the flavonoids as potential molecules in cancer growth therapy. In last 10 years, several groups showed that the biological effect of flavonoids is linked with their ability to modulate signaling pathways. Nowadays, the central issue in flavonoids investigation is to uncover structure *versus* biological effect. These studies will allow a large improvement about the molecular mechanism that regulates the action of flavonoids, and will also provide better understanding of the Wnt signaling functioning in healthy and pathological conditions. Together these observations show the importance of flavonoids as potent modulators of Wnt signaling and highlight their potential as agents in fighting and preventing cancer, particularly where conventional therapeutic is still ineffective.

Conflict of interest

The authors declare that are no conflicts of interest.

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