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Review

Pectin in cancer therapy: A review

Wenbo Zhang (Surnames are correct)[a, *]
zhangwenbo@xxmu.edu.cn

Ping Xu[b]
13273730271@163.com

Han Zhang[a]
zhanghan317@163.com

[a]School of Life Sciences and Technology, Xinxiang Medical University, Xinxiang 453003, Henan, China

[b]School of Pharmacy, Xinxiang Medical University, Xinxiang 453003, Henan, China

* Corresponding author. Tel.: +86 373 3831928; fax: +86 373 3029887.

Pectin, a complex class of plant polysaccharides, is composed of a galacturonan backbone and neutral sugar side chains. Natural pectin is reported to prevent colon cancer as a dietary fiber (DF). To enhance its bioavailability and bioactivity, pectin was modified into bioavailable modified pectin fragments (MPs) with low molecular mass. Also, MPs had low degrees of esterification (DE) which is reported to inhibit tumor growth, induce apoptosis, suppress metastasis, and modulate immunological responses. Antitumor activity of MPs chiefly arises from intervention in ligand recognition by galectin-3 (Gal-3). In addition, pectin is a suitable vehicle for anti-cancer drug delivery systems, due to its abundant modifiable functional groups and special physicochemical properties. Here, we summarize the structural features, bio-absorption and antitumor mechanisms and the structure-activity relationship of MPs. We also offer prospects and challenges for developing pectin into nutraceuticals or drugs.

Abbreviations: AG-I, type I arabinogalactan; AG-II, type II arabinogalactan; ASGP-R, asialoglycoprotein receptor; CP, citrus pectin; CRD, carbohydrate recognition domain; CSDDS, colon-specific drug delivery system; DF, dietary fiber; DM, degree of methylation; DR, death receptor; E-Cad, E-cadherin; ECM, extracellular matrix; ERK/MAPK pathway, Extracellular signal regulated kinase/ mitogen-activated protein kinase pathway; Gal-3, galectin-3; Gal3I, galectin-3 inhibitor; GALT, gut-associated lymphoid tissue; GalpA, d-galacturonic acid; GS, substituted galacturonans; HG, homogalacturonan; HTCP, heat-treated citrus pectin; KRG, Korean red ginseng pectin; LPS, lipopolysaccharide; HUVECs, human umbilical vein endothelial cell; NK, natural killer; MCP, modified citrus pectin; Mm, molecular mass; MP, modified pectin; MUC, mucin; PSA, prostate-specific antigen; RG-I, rhamnogalacturonan-I; RG-II, rhamnogalacturonan-II; SCFA, short-chain fatty acids; TIL, tumor-infiltrating lymphocyte; TLR, Toll-like receptor; TNF-*α*, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; XGA, xylogalacturonan

Introduction

Pectin is a class of heterogeneous polysaccharides found in plant cell walls. Commercial pectin is extracted from citrus, apple, or other higher plants, and is used as a stabilizer, thickener, gelling agent, emulsifier, and drug vehicle in the food and pharmaceutical industries (Wicker et al., 2014). Pectin can be classified into natural pectin with a high molecular mass (Mm) or low Mm modified pectin according to the processing methods. Unextracted natural pectin found in fruits and vegetables is a food component, as well as a soluble dietary fiber (DF). DF is defined as a polysaccharide or resistant oligosaccharide with molecular masses ranging in the hundreds of kilo Daltons (kDas). Some commercial pectins are also designated as DF, suggesting their structures are similar to unextracted pectin. DF cannot be digested in the gastrointestinal tract; however, it can be degraded and fermented by colonic microbiota, which is helpful for reducing the risk of colon cancer (Wicker et al., 2014). Focusing on pectin structure and bioavailability, applications for this molecule in cancer therapy are summarized here and include cancer prevention and therapy with dietary pectin; antitumor activity of modified pectin; and the application of pectin as an excipient for antitumor drugs.

Annually, there are approximately 7.6 million deaths caused by tumor cases (World Health Organization, 2008). Although traditional treatments such as surgery, chemotherapy, and radiotherapy, or novel methodologies such as immunotherapy and gene therapy are constantly improving, metastasis is still the main cause for cancer-related death (Zhang, 2006; ~~Zhang, Li et al., 2013;~~ Zhang, Xu, Gao, Yan, Yang~~et al.~~, 2013). Moreover, tumor cell drug resistance complicates therapy (Leclere, Cutsem, & Michiels, 2013) and radiation therapy may cause unpredictable side effects. In contrast to chemotherapy drugs, pectin and its derivatives are non-toxic. Also, pH-modified citrus pectin (MCP), an example of MPs,

can inhibit Gal-3, a key target in metastasis. Thus, we speculate that pectin may have antitumor applications (Cobs-Rosas, Concha-Olmos, Weinstein-Oppenheimer, & Zúñiga-Hansen, 2015; Leclere et al., 2013).

Pectin structure

Pectin structure varies greatly due to its varied sources and extraction methods, but it can be classified into three types according to common features: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and substituted galacturonans (GS) (Caffall & Mohnen, 2009; Leclere et al., 2013). Typically, the percentage of HG is about 65%; RG-I is 20–35% and the rest is GS (Mohnen, 2008). HG, the backbone of pectin, is composed of d-galacturonic acids (GalpA) linked via α-1, 4 glycoside bonds (Yapo, Lerouge, Thibault, & Ralet, 2007). The smooth region from commercial CP (almost purely HG) is about 24 kDa (Thibault, Renard, Axelos, Roger, & Crepeau, 1993). According to the degree of methylation (DM) of the C-6 carboxymethyl group of GalpA, pectin can be classified into high DM pectin (HM pectin) or low DM pectin (LM pectin), both of which have different industrial applications. RG-I, the main ramified structure of pectin, is composed of a repeating core sequence: [(→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→)]n. GalpA in RG-I is usually not linked with side chains, whereas about 20–80% of the C-4 hydroxyl group of rhamnose in RG-I is linked with varied side chains. Varying by plant sources, several side chains exist, such as β-(1, 4) galactan, type I arabinogalactan (AG-I), and type II arabinogalactan (AG-II). In addition to galactan, the RG-I of commercial CPs is mainly composed of AG-I, whose backbone is composed of β-(1, 4) and β-(1, 3) galactan. l-arabinofuranose (L-Araf) is frequently linked with the terminal galactose of β-(1, 4) galactan by α-(1, 5) glycoside bond or interrupted in the backbone of galactan (Gao et al., 2012b; Hinz, Verhoef, Schols, Vincken, & Voragen, 2005). RG-II is the main GS structure found in most pectic substances, which is significantly different from RG-I. RG-II generally have A and B side chains linked with the HG backbone, and each side chain has 9 or 10 monosaccharide residues linked with at least 22 glycoside bonds. Another GS found in many higher plants, such as citrus, is xylogalacturonan (XGA), which is a branching structure, linked through a β-glycoside bond with the O-3 of GalpA in HG.

Antitumor activity of pectin

Antitumor activity of dietary pectin

Dietary pectin from citrus, apple, potato or sweet potato has antitumor activity (Bergman, Djaldetti, Salman, & Bessler, 2010; Zhang, Mu, & Zhang, 2012), although, other reports contradict these data (Heitman, Hardman, & Cameron, 1992; Jacobs & Lupton, 1986; Jacobasch et al., 2008). Pectin's purported tumor prevention may be enhanced by formulating this fiber with a chemo-protective food component, for instance, fish oil (Cho et al., 2012; Umar, Morris, Kourouma, & Sellin, 2003). The antitumor mechanisms of dietary pectin are correlated with their probiotic activity, immune-potentiation (Chen et al., 2006; Flint, Bayer, Rincon, Lamed, & White, 2008; Georgiev, Ognyanov, Yanakieva, Kussovski, & Kratchanova, 2012), tumor growth inhibition (Cheng et al., 2011), anti-mutagenic potential (Hensel & Meier, 1999), and the regulation of transformation-related microRNA/oncogenes. These antitumor mechanisms can be characterized as having effects on colonic cells and cellular immunological activity (Jeon et al., 2011).

Most antitumor studies of dietary pectin have focused on colon cancer (Cheng et al., 2011; Schmidgall & Hensel, 2002) and how its mechanisms are directly or indirectly correlated with its probiotic activity. Pectic-oligosaccharides inhibit the growth of harmful colon microbiota, while benefitting probiotics, such as Bifidobacteria spp. and Lactobacillus spp. (Avivi-Green, Polak-Charcon, Madar, & Schwartz, 2000a; Lee, Shim, Lee, Kim, Chung, et al., 2006; Lee, Shim, Lee, Kim, Yang, et al., 2006; Olano-Martin, Gibson, & Rastell, 2002). Dietary pectin is fermented in the colon into short-chain fatty acids (SCFA), such as butyrate, which can normalize gut microbiota, affect the galectin network, regulate apoptotic proteins in colonic crypts and enhance crypt colonocyte growth (Avivi-Green, Polak-Charcon, Madar, & Schwartz, 2000b; Gómez et al., 2014; Katzenmaier, André, Kopitz, & Gabius, 2014; Louis, Hold, & Flint, 2014; Rao, Chou, Simi, Ku, & Reddy, 1998). Apple pectin (AP) can decrease fecal bacterial enzyme activity (β-glucuronidase, β-glucosidase, and tryptophanase) (Ohkami et al., 1995), reducing the occurrence of colon cancer induced by carcinogens azoxymethane (AOM) or 1, 2-dimethylhydrazine (DMH) (Ohkami et al., 1995; Ohno et al., 2000). AP also scavenges free radicals (Urias-Orona et al., 2010), reduces DNA adducts (Zunft, Goldin-Lang, & Dongowski, 1997), and regulates microRNAs (miR-16, miR-19b, miR-21, miR26b, miR27b, miR-93, and miR-203). Fish oil and pectin synergistically inhibit microRNA-mediated tumor transformation in a rat model by increasing the inhibition of oncogenic proteins PTK2B, PDE4B, and TCF4 (Shah et al., 2011). Ginseng pectin (GP) PG-F2 prevents gastric transformation induced by Helicobacter pylori colonization by blocking its adhesion to gastric epidermal cells (Fowler, Thomas, Atherton, Roberts, & High, 2006; Lee, Shim, Lee, Kim, Chung, et al., 2006; Lee, Shim, Lee, Kim, Yang, et al., 2006).

Although dietary pectin is mainly active within the gastrointestinal tract, evidence suggests that pectin may augment the immune system. In traditional Chinese medicine, ginseng and other herbals are used as tonics and some active ingredients in these herbals have been extensively studied (Fan et al., 2010; Yang et al., 2013; Zhang, Li, et al., 20132; Zhang, Mu, et al., 2012). For example, Korean red ginseng pectin (KRG) can activate the NF-κB pathway to enhance macrophage function and inhibit myeloid-derived suppressor cells to enhance T cell activity (Choi et al., 2008; Jeon et al., 2011). GP inhibits the migration of L-929 cells, which helps inhibit tumor cell metastasis (Fan et al., 2010). HBE-III, an RG–II–like pectin fragment from the Korean Citrus Hallabong, significantly inhibited lung metastasis of Colon 26-M3.1 cells in a dose-dependent manner via activation of macrophages and natural killer (NK) cells (Lee et al., 2014). Pectin from Centella asiatica (L.) Urban, a traditional Chinese herbal compound may increase immunological activity of T and B cells, and this is modulated by the carboxyl and acetyl groups of this pectin (Wang, Dong, Zuo, & Fang, 2003).

Antitumor activity of MPs: preclinical investigations

Examples of antitumor MPs

Modification with chemicals (Almeida et al., 2015; Platt & Raz, 1992), heating (Cheng et al., 2011; Hao et al., 2013; Jackson et al., 2007), radiation (Kang et al., 2006) and/or enzymes (Olano-Martin et al., 20023; Zhang, Xu,et al. ~~Gao, Yan, & Yang~~, 2013) for

pectin to degrade the polymer and to decrease its DE may produce antitumor activity (Fig. 1). For example, pH-modified citrus pectin (MCP) inhibits tumor growth, angiogenesis, and metastases (Glinsky & Raz, 2009) and heat-treated citrus pectin (HTCP) can induce apoptosis of prostate cancer cells (Jackson et al., 2007). Finally, modified pectin is nontoxic (Garthoff et al., 2010). For example, heat-treated ginseng pectin (GP) inhibits the proliferation of HT-29 colon cancer cells (Cheng et al., 2011) and pectin treated with 20 kGy of γ-irradiation not only is not mutagenic, but also inhibits HT-29 and other tumor cells (Kang et al., 2006).

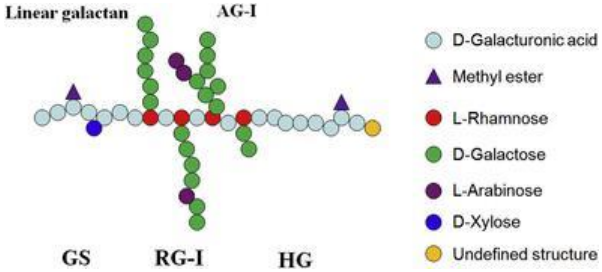

Fig. 1 Diagram of MP structure. MP has a less complex structure with shorter side-chains and a lower DE in contrast to natural pectin. Undefined structures may occur during modification. GS, such as RG-II and XGA, is thought to be significantly reduced.

Structural modification increases the bioactivity and bioavailability

MCP was prepared from CP by acid and base modification (Nangia-Makker et al., 2002; Zhang, 2006) and questions have arisen as to why CP is not anti-metastatic but MCP is. This can be explained by evidence for physico-chemical property changes due to structural modifications and how these correlate with increased bioavailability. First, pectin solubility is significantly increased due to β-elimination treatment with sodium hydroxide at 50–60 °C, which shortens the CP backbone and decreases the DE from about 80% to below 10% (Eliaz, Hotchkiss, Fishman, & Rode, 2006; Zhang, Liu, & Gao, 2010). Second, it is assumed that the so-called "pharmacophores" of pectin are enriched during β-elimination and acid hydrolysis. Found in the RG-I domain of pectin, galactans rich in terminal β-galactosides are generally regarded as pharmacophores. They can be recognized by a carbohydrate recognition domain (CRD) of Gal-3, the in vivo target of MCP and other MPs (Krall & McFeeters, 1998; Morris, Gromer, Kirby, Bongaerts, & Gunning, 2011). Glycosidic bonds linking furanoses are typically much more labile to acid than bonds linking pyranoses. Therefore, xylanan, arabinan, and some other oligosaccharides, mainly comprising furanoses in RG-I have larger acid-hydrolysis rates than galactans do. Consequently, modified pectin would have more galactoside residues than xylanan and arabinan residues. As a result, modification of harvested novel pectin fragments rich in RG-I domains and smaller HG backbones may better present β-galactosides to the CRD of Gal-3 in vivo. Moreover, molecular mass (Mm) is a key factor for MCP pharmacokinetics, which influence blood concentration, absorption, and excretion (Zhang, Gao, Shi, & Zhang, 2007). Mm of most reported MPs ranges from 3 to ~60 kDa (Gao et al., 2012b; Ramachandran et al., 2011; Zhang et al., 2007).

Bio-absorption mechanism of MP

By comparing physicochemical and pharmaceutical properties of MP and β-glucan, Maxwell and colleagues (Maxwell, Belshaw, Waldron, & Morris, 2012; Morris, Belshaw, Waldron, & Maxwell, 2013) suggested that pectin fragments can be absorbed by passive absorption or active cell capture (e.g. intestinal epithelial cells, GALT and M-cell), and then they are modified, transported, and released. Passive absorption of MPs may depend on their physicochemical properties, such as molecular charges, DE, Mm, and structure, which determine MP bioavailability. Research suggests that the molecular charge is a dominating factor for absorption. Research on the trans-membrane absorption of MCP with a Caco-2 cell two-chamber model, a common model for studying drug absorption, shows that only neutral fragments of pectin can be transported across the Caco-2 cell monolayers, whereas acidic fragments of pectin with positive charges cannot (Courts, 2013). DE is another critical factor for passive absorption of MP as supported by the fact that decreasing the DE of pectin is beneficial for in vivo activity of MCP in heavy metal detoxification and inhibition of lung metastasis (Eliaz et al., 2006; Pienta et al., 1995; Wai, AlKarkhi, & Easa, 2010). Some macro-biomolecules can undergo endocytosis via receptors: for example, β-glucan can be actively transported by macrophages via dectin-1 (Brown et al., 2002; Ozment, Goldman, Kalbfleisch, & Williams, 2012; Weigel & Yik, 2002). Several studies have been performed to study how MP can be actively transported by Gal-3 or other receptors. Studies show that some glycoproteins in the epithelial membrane can be recognized and endocytosed by Gal-3 (Gao et al., 2012a). In addition, asialoglycoprotein receptors (ASGP-Rs), which are densely populated on hepatic cell membranes, transport glycoproteins rich in terminal galactosides into liver cells. Some reports indicate that MP inhibits liver tumors (Liu, Huang, Yang, Lu, & Yu, 2008; Straube et al., 2013; Zhang et al., 2010), which suggest that ASGP-R may transport MP into liver cells. If endocytosis of MPs can be confirmed, it may improve bioavailability and bioactivity of MPs. In addition, even though they can be administered via parenteral routes (such as by injection), circumventing intestinal epithelial cell absorption, strong hydrophilic MPs are unlikely to be transported through the bio-membrane system without an active transporter. Gal-3, an MP target, has different subcellular localizations (in the nucleus, cytoplasm, or extracellular sites), where it has different functions. Identifying the site of interaction of MP with Gal-3 would be of interest although at this time subcellular localization of MPs has not been reported.

Structure-activity relationship of MP

Antitumor mechanisms of MPs are correlated with their apoptosis-inducing activity. First, MPs induce tumor cell anoikis, a type of programmed cell death induced by cell detachment from its matrix (Glinsky & Razet al., 2009; Newlaczyl & Yu, 2011). Second, Jackson (Jackson et al., 2007) reported that a base-sensitive structure in HG of HTCP induced apoptosis; whereas natural CP and MCP did not induce apoptosis. Third, MP can sensitize tumor cells to chemotherapeutic drugs. Several studies suggest

that the MP structure is correlated with apoptosis; however, results are inconsistent (Attari, Sepehri, Delphi, & Goliaei, 2009; Cheng et al., 2011; Jackson et al., 2007; Yan & Katz, 2010). In fact, the apoptosis-inducing structure of HG from HTCP was not well-defined. Unsaturated sugar residues were produced by β-elimination and these residues were correlated with NK-inducing activity of MCP (Ramachandran et al., 2011). Heat treatment also causes β-elimination producing unsaturated sugar residues. Furthermore, some rearrangements, aldonic acid, or some undefined structures may be generated during heating. Still, we are uncertain whether RG-I can induce tumor cell anoikis and what may be the relationship between apoptosis and tumor sensitization to chemotherapy drugs. Studies are needed to characterize the structures that directly induce apoptosis and anoikis.

Terminal galactose and the terminal structure of MPs are key factors for anti-tumor activity of MPs. Evidence suggests that the activity may lie in the RG-I domain (Gao et al., 2012b; Gunning, Bongaerts, & Morris, 2009). Pectins from okra and potato are rich in RG-I structures (Cheng et al., 2013; Vayssade et al., 2010), which all have antitumor activity. Experimental results from fluorescence microscopy, fluorescence-activated cell sorting (FACS) and atomic force microscopy (AFM) show that galactan from pectin fragments can bind human recombinant Gal-3 (Gunning et al., 2009). The dissociation coefficient between β-D-galactobiose and Gal-3 is 0.33 s^{-1} (Gunning, Pin, & Morris, 2013). Gao's group (2012b) prepared MCP-N, which is a neutral pectin fragment mainly composed AG-I and M-galactan by treating MCP-N with α-L-arabinofuranosidase to harvest smaller fragments (around 18 kDa) mainly composed of galactan linked via β-1,4-glycosides. Data show that M-galactan has stronger Gal-3-binding affinity than MCP-N, possibly due to more terminal galactose for M-galactan.

Galactan from RG-I does not contribute to all anti-cancer activity of MPs (Bergman et al., 2010; Cheng et al., 2013; Kang et al., 2006). Though it is galactan and not HG that can specifically interact with CRD of Gal-3, the HG backbone also contributes to this activity (Gao et al., 2013). Data from Gao's group (2012b) demonstrated that separated pectin fragments can be divided into two groups with chromatography according to GalpA: MCP-A, rich in GalpA and MCP-N, deficient in GalpA. Surprisingly, MCP-A binds Gal-3 with a stronger affinity than MCP-N. One hypothesis for HG interacting with Gal-3 is that GalpA in the pectin backbone may be helpful for maintaining the terminal galactan conformation, which is beneficial for cooperative interaction of galactans with Gal-3 (Gao et al., 2013). If there is a multivalent effect between ligands and lectin, the interaction will be strengthened (Wittmann & Pieters, 2013). Thus, HG in MP may act as a "bridge" linking these galactan ligands to facilitate a multivalent effect. In contrast, CRD of Gal-3 may recognize HG by charge–charge or charge–dipole interactions at physiological pH. CRD (uniprot/P17931) consists of 135 amino acids at the C terminal of Gal-3 (Seetharaman et al., , 1998). Analysis with the pI prediction tool (http://web.expasy.org/compute_pi/) demonstrates that CRD (pI = 9.41) has a positive charge under physiological pH, whereas HG is negatively charged owing to the carboxyl group. The recognition of Gal-3 is affected by the ambient pH (von Mach et al., 2014). On the one hand, pH influences the polarity of ligands; on the other hand, ambient charges could slightly modulate the structure of CRD. Gao et al. (2013) observed that a pectin backbone with little galactose can interact with Gal-3, and this interaction cannot be inhibited by lactose. Consequently, the MP backbone can interact with CRD, although the interaction would not be specific. Some CRD sites are related with type-C self-association (Lepur, Salomonsson, Nilsson, & Leffler, 2012). The assumed interaction between the MP acid backbone and CRD could affect the type-C self-association by charge–charge interaction, steric hindrance or other unknown factors.

Monosaccharide residues in MPs other than galactose also influence antitumor activity of the macromolecules. For example, Gao's group (2013) reported that arabinose can increase or decrease the interaction between galactan and Gal-3. In addition to affinity, specificity of animal lectin can also be affected by sugar residue composition. For example, the penultimate monosaccharide residue modulates lectin recognition specificity (Nakahara & Raz, 2008). Consequently, the terminal residue structure of carbohydrate ligands are of interest because of the abundance of galactoside-specific lectins in the human body.

Establishing a screening protocol to study the structure-activity relationship (SAR) and pharmacokinetics of MPs is necessary for optimizing a galectin-3 inhibitor (Gal3I). Gal-3 is a promising target for anti-tumor therapy, and several structurally diversified Gal3Is have been developed (Klyosov, 2012; Pieters, 2006; Zhang, 2009), which are not only drug leads but also useful tools for tumor detection. However, developing MP-based Gal3I leads has been challenged by chemical synthesis. First, drug-likeness and druggability (the potential for a compound to be used commercially as a drug) are important standards for optimizing leads and screening drug candidates. Chemically synthetic Gal3Is have well-defined structures and may have better drug-likeness and druggability than an MP-based Gal3I. MPs, even when fractionated and purified, are micro-heterogeneous because pectin is a complex and heterogeneous polysaccharide. MCP, prepared by Zhang (Platt et al.& Raz, 1992; Zhang, 2006) with commercial CP was proven to be mono-dispersed by high performance size exclusion chromatography (HPSEC) and agarose gel electrophoresis. However, nearly 1% of neutral sugars can be separated by hexadecyltrimethylammonium bromide (CTAB) precipitation. Gao's group (2012b) separated a neutral MCP polysaccharide fragment (MCP-N) with DEAE cellulose chromatography. Considering acidic and neutral fragments of pectin have different properties, a new protocol to prepare structurally consistent pectin fragments to study MP SAR is needed and this will improve MP modification methodologies. In the absence of toxicity data about chemically synthetic Gal3Is, screening plant Gal3Is, especially from food sources, will be interesting (Mossine, Glinsky, & Mawhinney, 2008; Sathisha, Jayaram, Harish Nayaka, & Dharmesh, 2007). The recognition mechanism for Gal-3 CRD to a chemically synthetic Gal3I should be also useful for establishing a new screening protocol to select novel MPs with higher bioactivity and less toxicity which would minimize poor drug-likeness and druggability for polysaccharide-derived drug candidates.

For screening Gal-3 ligands, specificity is much more important than the affinity for minimizing perturbations to normal body functions. First, there are at least 15 galectins in the human body which can bind β-galactose; hence, we cannot exclude the possibility that some MPs can be recognized by other galactose-binding lectins (Heusschen, Griffioen, & Thijssen, 2013). Second, Gal-3 is involved in many diseases, but there are few cases to study the relationship between cancer lesion restoration and those diseases. It was previously reported that MCP can protect mice from experimental kidney injuries (Kolatsi-Joannou, Price, Winyard, & Long, 2011). In this case study, MCP down-regulated the expression of Gal-3, but it did not affect the expression of Gal-1 and Gal-9. It has been recommended that the lectinomics methodology should be applied to future systematic investigations of in vivo activity of MPs to examine their mechanism of action. In addition, several C-type lectins (such as ASGP-R), cytokines (Liu et al., 2001; Salman, Bergman, Djaldetti, Orlin, & Bessler, 2008) and death receptors (Chauhan et al., 2005) may possibly interact with MP.

Antitumor mechanisms of MPs

We studied anti-tumor activity of MP in lung metastasis of prostate cancer, lung metastasis of melanoma, liver metastasis of colon cancer, breast cancer, and angiosarcoma (Johnson et al., 2007; Liu et al., 2008; Nangia-Makker et al., 2002; Platt et al.~~& Raz~~, 1992; Pienta et al., 1995). Anti-tumor mechanisms of MCP are summarized, including inhibition of tumor growth and metastasis, sensitization of tumor cells to chemotherapy drug, and immune cell regulation (Fig. 2 & Fig. 3).

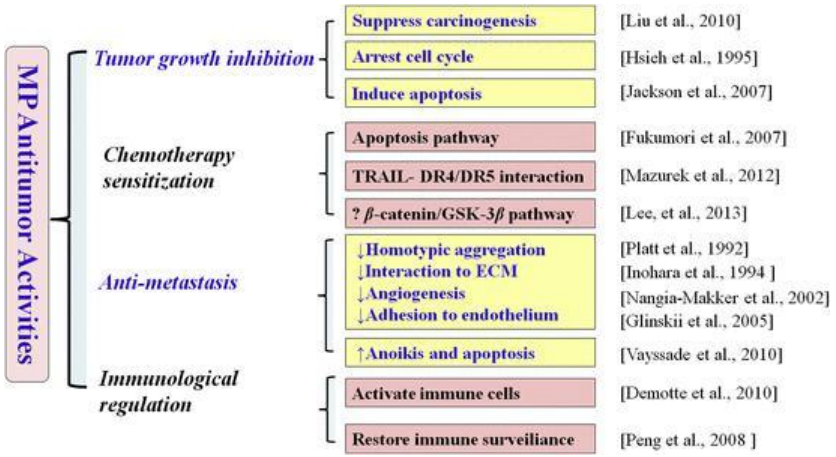

Fig. 2 MP antitumor activity. (↑denotes induction; ↓ is inhibition; ? is a hypothetical pathway) MP-induced inhibition of Gal-3 inhibits tumor growth, sensitizes tumors to chemotherapy, and regulates immune function.

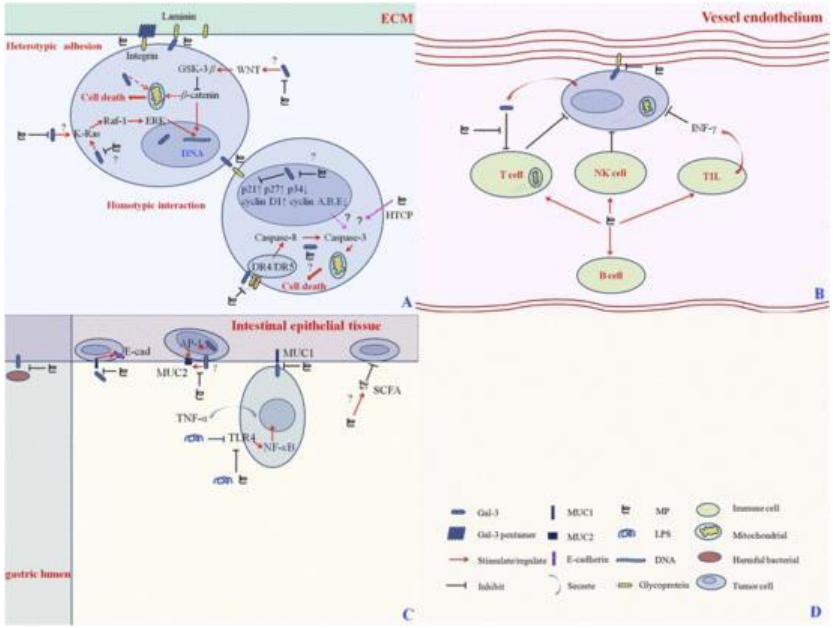

Fig. 3 Proposed MP antitumor mechanism. Panel A: MPs can inhibit tumor growth and metastasis. First, MPs retard tumor growth by suppressing survival pathways (Ras-ERK/MAPK pathway & Wnt/β-catenin pathway), arresting the cell cycle and inducing apoptosis. However, the strong hydrophilicity of MPs diminishes its accessibility to the cytoplasm and the nucleus, creating inconsistent tumor activities. Next, MPs suppress metastasis by inhibiting homotypic aggregation and heterotypic adhesion. Panel B: MPs activate immune cells (T, B, NK & TIL) while tumor cells undermine immune surveillance by secreting Gal-3 to induce T cell apoptosis. Panel C: MP can inhibit carcinogenesis by preventing the adhesion of harmful bacteria (such as H. pylori), interfering with interactions between free Gal-3 and MUC1 and blocking the TLR4/NF-κB pathway in the gastrointestinal tract. Additionally, MP can be fermented into SCFA modulating colonocytes and colon cancer cells. Panel D: Legends.

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MP-induced tumor growth inhibition Experimental data from animal models suggest that MCP can reduce solid tumor size; although, some other reports suggest that MP was not cytotoxic to tumor cells and did not inhibit growth. Whether MP can inhibit tumor cells depends on the tumor cell origin or the bio-distribution of pectic fragments (Nangia-Makker et al., 2002; Platt et al.~~& Raz~~, 1992; Zhang et al., 2010). Raz's group (Inohara & Raz, 1994; Nangia-Makker et al., 2002; Platt et al.~~& Raz~~, 1992) found that MCP reduced tumor growth rates in metastatic colon (LSLiM6) and breast cancer (MDA-MB-435) cell lines. Hayashi (Hayashi, Gillen, & Lott, 2000) studied the effect of MCP on the tumor size and weight in Balb-c mice by implanting colon-25 tumors. They reported that tumor size in the low- (0.8 g/L MCP) and high-dose groups (1.6 g/L MCP) were both significantly reduced compared to controls, at the 20th day after tumor palpation. To explain these findings, several signaling cascades involving tumor growth were described and related to carcinogenesis (Liu et al., 2010; Shah et al., 2011), tumor cell growth, and apoptosis.

MP inhibits carcinogenesis in a manner similar to dietary pectin in the colon. One possible mechanism for such inhibition is through mucin 2 (MUC2), a mucin-type glycoprotein bearing O-glycan. MUC2 is a Gal-3 ligand and its abnormal expression is correlated with colon carcinogenesis and metastasis. Another possible mechanism involves competition with the recognition of Gal-3 with the surface sugar chains of MUC2. Gal-3 also up-regulates MUC2 at the transcriptional level by activating transcription factor AP-1 (Dudas, Yunker, Sternberg, Byrd, & Bresalier, 2002; Song et al., 2005; Wong, Colombo, & Sonvico, 2011). In kidney cells, MP down-regulates Gal-3 (Kolatsi-Joannou et al., 2011). If MP also down-regulates Gal-3 in colonic epithelial cells, this may indirectly down-regulate MUC2. An additional mechanism for MPs' inhibition of colon cancer is that pectic substances suppress cancer by inhibiting inflammation. Colitis is highly correlated with colon cancer and NF-κB is important in the transformation of colitis into colon cancer by LPS. Galactan, extracted from apple pectin, can inhibit carcinogenesis via the LPS/TLR4/NF-κB pathway (Liu et al., 2010).

MCP can inhibit tumor cell growth by regulating the cell cycle. For example, MCP can inhibit JCA-1 prostate cancer cell growth and reduce the rate of incorporating [^{3}H] thymidine into DNA, which is related to down-regulation of cyclin B and p34 cdc2 (Hsieh & Wu, 1995). These results indicate that MCP inhibits growth via the early G2 cell cycle phase. Although expression cyclin A, cyclin E and p21 have not been reported to be correlated with inhibition of Gal-3 by MCP, Gal-3 did down-regulate cyclin A and cyclin E (Kim, Lin, Biliran, & Raz, 1999; Streetly et al., 2010; Yoshii et al., 2002). Moreover, Gal-3 regulates the stability of p21 (Wang et al., 2013). Overall, these data demonstrate that MCP may affect the cell cycle by inhibiting Gal-3.

The mechanism by which of MCP inhibits tumors includes inhibition of tumor survival signaling and the induction of apoptosis. For example, PectaSol-C, a commercial pH-modified citrus pectin can induce apoptosis in LNCaP and PC3 prostate tumor cells. Also, MCP inhibits the activation of the MAPK pathway (Yan et al., ~~& Katz~~, 2010). Gal-3 may mediate anti-growth activity of MPs, because it is related with the apoptosis pathway (Harazono, Nakajima, & Raz, 2014) and survival pathways, such as the MAPK and Wnt pathways (Lee, Lin, Chang, & Lo, 2013; Maxwell et al., 2012;

Song et al., 2012). MPs activities, such as the apoptosis-inducing potential of pectin fragments, are not consistent, and this has been ascribed to their different structural features (Jackson et al., 2007). Hence, an SAR analysis would help to prepare MPs with consistent activity. In contrast, tumor cell heterogeneity may be another reason for the inconsistency of MPs in inhibiting tumor growth.

Sensitization of MP to chemotherapy Some cancer cells are resistant to chemotherapy drugs and apoptosis cannot be induced and this must be overcome. MP can increase drug-resistant tumor cell apoptosis. For example, GCS-100, a commercial MCP, overcomes bortezomib resistance and enhances dexamethasone-induced apoptosis in multiple myeloma cells (Chauhan et al., 2005). Because MP significantly increased cell sensitivity to chemotherapy drugs, a protocol of combining MP and chemotherapy drugs may be beneficial (Chauhan et al., 2005; Hossein, Keshavarz, Ahmadi, & Naderi, 2013; Jiang, Eliaz, & Sliva, 2013; Lu, Wang, & Liu, 2013; Wang & Liu, 2011).

Sensitization of tumor cells to chemotherapy drugs is correlated with Gal-3 inhibition by MP. One hypothesis suggests that Gal-3 inhibitors can reverse tumor cell drug resistance due to the involvement of Gal-3 in apoptosis-resistance and maintaining drug-resistance (Fukumori, Kanayama, & Raz, 2007). Another model suggests that Gal-3 interferes with interactions between TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and its receptors DR4 and DR5, which undermine the formation of DISC (death-inducing signaling complex) (Mazurek et al., 2012). Considering that DR4 and DR5 are expressed at the cell surface, possibly MPs bind extracellular Gal-3, eliminating the interference of Gal-3 with TRAIL and DR4/DR5, thus transforming tumor cells from drug resistant to drug sensitive. However, we cannot rule out the possibility that MP enters the cytoplasm to inhibit intracellular Gal-3.

Anti-metastasis of MP The most prominent and well-studied anti-cancer activity of MP is anti-metastasis (Dange et al., 2014; Pienta et al., 1995; Platt et al.& Raz, 1992). The earliest experiment of MCP was performed by Platt and colleagues (Platt et al. & Raz, 1992) who reported that B16-F1 experimental metastasis in a mouse model was reduced significantly by injection of MCP; however, lung colonizations in the CP group increased up to three-fold. The authors suggested that MCP, but not CP, inhibited B16-F1 melanoma cell adhesion to laminin and asialofetuin-induced homotypic aggregation (Inohara et al.& Raz, 1994; Nangia-Makker et al., 2002; Platt et al.& Raz, 1992). Data from Raz's group proved that Gal-3 plays important roles in tumor embolism and anchorage-dependent growth, which are mediated by carbohydrate recognition of Gal-3 to the extracellular matrix (ECM). Gal-3 is involved in many steps of metastasis, such as angiogenesis, anoikis, and adhesion to the endothelium. Therefore, MP's anti-metastatic activity should involve induction of anoikis, inhibition of angiogenesis, and inhibition of adhesion to the endothelium.

Inhibition of Gal-3 may cause anoikis of metastatic cells. For instance, okra RG-I, a pectin fragment carrying short galactan side chains was added by Vayssade and colleagues (Vayssade et al., 2010) to 3D cultures (on poly(2-hydroxyethylmethacrylate), polyHEMA) of highly metastatic B16F10 mouse melanoma cells. B16F10 cells were induced to arrest at the G2/M phase, and this confirmed that okra RG-I may have induced anoikis. Because okra RG-I is mainly composed of pure galactan, anoikis is may be mediated by Gal-3. Both free circulating Gal-3 (Zhao et al., 2010), and cellular Gal-3 of the tumor cell can be inhibited by MP. For Gal-3 secreted or on the surface, MPs may inhibit interactions between Gal-3 and MUC1 to prevent heterotypic aggregation, resulting in anoikis. For cellular Gal-3, it is necessary to locate the site of interaction with MP first, because Gal-3 can be expressed in the cytoplasm, the nucleus, and on the cell membrane surface. It is assumed that the interaction inducing anoikis occurs at the cell surface cell, because anoikis is usually triggered by detaching anchorage-dependent cells from the surrounding ECM. Gal-3 is as an anti-apoptosis mediator and its overexpression can trigger cell cycle arrest at the G1 phase, down-regulate G1-S phase cyclin (cyclin E and cyclin A), up-regulate cyclin-dependent kinase inhibitors ($p21^{WAF1}$ and $p27^{KIP1}$) and influence mitochondrial homeostasis (Kim et al., 1999; Matarrese et al., 2000). These data confirm that anti-apoptotic activity of Gal-3 is related to both intrinsic and extrinsic apoptosis pathways (Chauhan et al., 2005). However, there is no evidence to confirm that MP is involved in the in anoikis cascade. Possibly, MP may suppress anoikis and TRAIL-R2 (DR5) may be a key mediator (Fig. 4.).

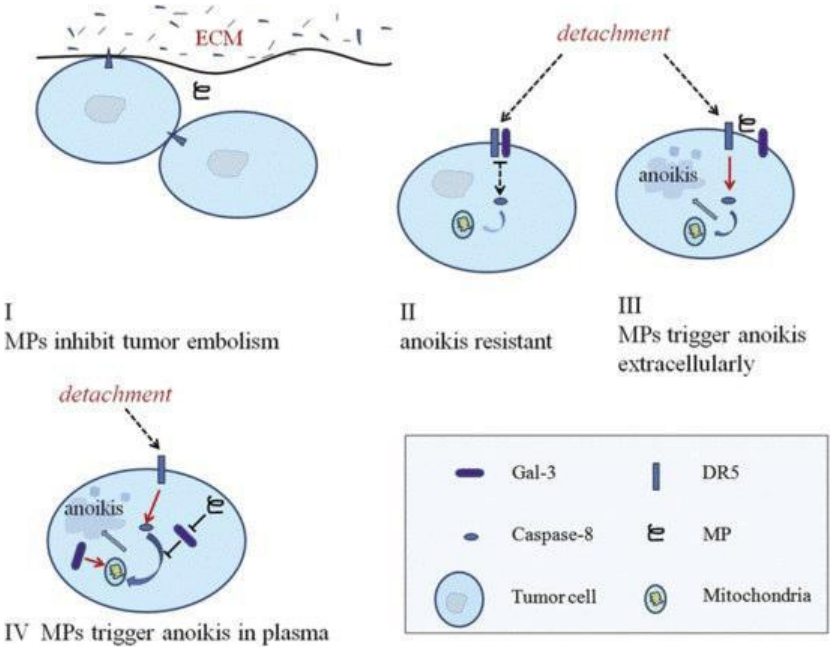

Fig. 4 Hypothetical mechanisms of MPs in suppressing anoikis. I: MPs can inhibit Gal-3 mediated tumor embolism (Glinskii et al., 2005; Glinsky, Huflejt, Glinsky, Deutscher, & Quinn, 2000; Inohara & Raz, 1994; Platt et al.~~& Raz~~, 1992). II: Ligands of Gal-3, such as DR-5 & DR-4, are involved in anoikis (Laguinge et al., 2008; Mazurek et al., 2012). These molecules can "code" detachment signals into "death signals,"~~,~~ transfer these to the cytoplasm and activate procaspase-8 or others to initiate the apoptosis cascade. Gal-3, liberated from the heterotypic and the homotypic interactions on the circulating tumor cell surface, can suppress anoikis by intervening in signal transferring mediated via DR-5. III: MPs induces anoikis by abolishing interactions between DR-5 and Gal-3 at the tumor cell surface. IV: MPs possibly triggers anoikis within the tumor cell, if MPs can permeate the membrane.

MP can inhibit angiogenesis and tumor cell adhesion to endothelial cells, which is critical for metastasis. For example, tumor size, angiogenesis, and spontaneous metastasis were reduced in mice fed MCP. MCP inhibits the adhesion of MDA-MB-435 to human umbilical vein endothelial cells (HUVECs), mediated by Gal-3 in a dose-dependent manner (Nangia-Makker et al., 2002). In contrast, in vivo metastatic deposit formation assays support the perception that mechanical entrapment is insufficient and intercellular adhesion is essential for metastatic cell arrest in distant organs. For example, the adhesion between Gal-3 and Thomsen-Friedenreich glycoantigen is necessary for precluding malignant cell lodging in target organs in the model examined (Glinskii et al., 2005). Thus, MP may inhibit tumor embolism formation, which could further induce anoikis for those detached tumor cells.

Regulation of immunological system There are two mechanisms by which MP exerts its activity on the immune cells: first, MPs are biological response modifiers (BRMs) (Radosavljevic et al., 2012); second, MPs can restore immunologic surveillance undermined by secreted Gal-3. As a BRM, MCP activates diverse immune cells. For example, MCP activates Tc and B cells in a dose-dependent manner. To analyze the role of Gal-3 in immunologic surveillance, one must address the fact that free Gal-3 in cancer patient blood is greater than normal controls (Iurisci et al., 2000. One role of circulating Gal-3, as a multifunctional molecule, is to inhibit T cell growth resulting immune cell apoptosis, which causes immune tolerance (Peng, Wang, Miyahara, Peng, & Wang, 2008; Xue et al., 2013). Thus, it is of interest to design experiments to examine whether MP can inhibit T cell apoptosis mediated by free Gal-3, which is similar to the role of TFD 100, a cod glycoprotein with high affinity to Gal-3 suppressing immune escape (Guha et al., 2013). In addition, Gal-3 ligands can correct impaired T cell function through IFN-γ secretion possibly playing an adjuvant role in a mouse model. For example, GCS-100 potentiates tumor-infiltrating lymphocytes (TIL), releasing more INF-γ (Demotte et al., 2010). Additionally, MCP activates NK cells, which cause apoptosis of K562 tumor cells (Ramachandran et al., 2011).

Clinical trials

A Phase II pilot clinical trial for MCP was performed by Guess and colleagues (Guess et al., 2003) to investigate the tolerability and effect of modified citrus pectin (Pecta-Sol) in 13 men with prostate cancer and biochemical prostate-specific antigen (PSA) failure after localized treatment. The PSA doubling time increased (P-value < 0.05) in seven (70%) of 10 men after taking MCP for 12 months compared to before taking MCP. Data show that MCP decreased prostate cancer tumor growth but subjects enrolled were fewer than those enrolled in typical Phase I clinical trials. Moreover, this report (Guess et al., 2003) provided no MCP batch number, no placebo group, and no direct information about the tumor. Consequently, more information is needed to establish a causal relationship between tumor alleviation and MCP administration.

In contrast to the study performed by Guess (Guess et al., 2003), Azémar and colleagues (Azémar, Hildenbrand, Haering, Heim, & Unger, 2007) chose a different sample of enzyme-treated MCP with DM lower than 20%. In a pilot trial to assess tolerability, clinical benefit and antitumor efficacy of MCP in 49 patients with various solid tumors in an advanced state of progression, they found that after 2 cycles of oral intake of MCP, 11/49 patients (22.5%) had stable disease and 6/29 patients (20.7%) had an

overall clinical benefit response associated with a stabilization or improvement in quality of life. Overall, MCP may be of clinical benefit and may improve quality of life for patients with far advanced solid tumors. Thus, this clinical trial encourages people to investigate the role of MCP in cancer prevention and treatment. Still, these reports did not mention structural details about the commercial enzyme-treated MCP, except DE.

Currently, EcoNugenics Inc. is recruiting volunteers for a Phase III clinical trial to study the effectiveness and safety of PectaSol-C as dietary supplement in Israel (NCT: NCT01681823). The purpose of this clinical study is to determine the effect of oral administration of PectaSol-C for improving PSA kinetics in men with biochemical relapsed prostate cancer and serial increases in PSA. Additionally, intranasal transmucosal fentanyl pectin, a pectin-based drug delivery system, for breakthrough cancer pain in radiation-induced oropharyngeal mucositis is currently under study in a clinical trial in Spain (NCT: NCT02050503). Clinical trials for MPs are not limited to cancer therapy. For example, a clinical trial sponsored by Boston Medical Center in MA (NCT: NCT01960946), is recruiting volunteers for investigating the benefits of MCP as Gal3I for patients with heart failure based on clinical hypertension and elevated Gal-3 concentrations. A safety study of GCS-100 to treat chronic kidney disease was completed by La Jolla Pharmaceutical Company (NCT: NCT01717248). These two clinical trials will also inform our understanding of the toxicology and pharmacokinetics of MCP.

Pectin applications and chemotherapeutic delivery vehicles

Pectin is an approved drug delivery vehicle. Its lack of toxicity, gelling potential, and easily modified functional groups (i.e., –COOH, –OH), allows wide application for drug delivery systems (DDS). Pectin-based DDS can be developed for enteral and parenteral administration. An enteral example is via a colon-specific drug delivery system (CSDDS) which can be delivered orally. This CSDDS has suitable bioavailability and improves patient compliance (Dev, Bali, & Pathak, 2011; Wong et al., 2011). A biphasic pectin-based drug release system was applied for colon cancer therapy, and it was well absorbed in vitro and in vivo (He, Du, Cao, Xiang, & Fan, 2008). Although there is no enough examples supporting the argument, MP should outweigh pectin in parenteral administration significantly. MP has smaller molecular mass, which make the DDS using MP more available for the body (Tang et al., 2010). For example, three types of positively-charged pectins were created by modifying carboxyl groups of galacturonic acids with three different primary amine groups and were designed as DNA carriers, which could transfer DNA into HEK293 cells (Katav et al., 2008). Such a DNA delivery system broadened pectin applications in gene therapy. For this type of DDS, pectin galactose residues contributed to foreign gene transfection. Abundant terminal galactose residues are available on RG-I side-chains, which may be recognized by ASGP-R, a lactose-binding lectin densely expressed on hepatic cell surfaces. Accordingly, pectin fragments or MCPs could be applied to liver-targeted DDS (Yu et al., 2014).

Furthermore, other types of pectin-based DDS have been designed for reducing chemotherapy drug toxicity or improving bioavailability. For example, pectin was modified and/or cross-linked with anti-cancer drugs to create a pro-drug (Tang et al., 2010), hydrogel (Takei, Sato, Ijima, & Kawakami, 2010), microgel (Puga, Lima, Mano, Concheiro, & Alvarez-Lorenzo, 2013), or other sustained drug release system. As a mucoadhesive polymer, LM pectin was applied in a fentanyl (an opioid painkiller) pectin nasal spray, which was proven to improve analgesic onset, treatment efficacy and acceptability for treating breakthrough cancer pain (Munarin, Tanzi, & Petrini, 2012). Fentanyl pectin nasal spray is currently under clinical investigation for relieving chronic cancer pain (NCT: NCT02050503).

Concluding remarks

Here, we summarize applications of pectin in cancer therapy, as a dietary fiber or as an MP, and in DDS. First, most reports suggest that dietary pectin is beneficial for treating colon cancer and its mechanisms involve preventing carcinogenesis and modulating immune cells. Because the structure of natural pectin is complex and dietary pectin content of some foods is inconsistent, anti-tumor SAR studies of pectin should be conducted to create more effective and structurally consistent nutraceuticals (McCarty & Block, 2006). Second, there are fewer challenges for developing pectin and MP as a drug vehicle for anticancer DDS. MPs are being developed into anti-tumor drugs, but concerns remain. First, screening methods for harvesting novel pectic fragments with improved affinities with their targets must be improved. Also, factors that can influence the recognition of targets with MPs must be confirmed and the ability of MPs to directly/indirectly influence cytokine functions must be defined (Dennis, Lau, Demetriou, & Nabi, 2009). Translating complex polysaccharide drug hits into drug candidates is challenging due to structural micro-heterogeneity. Maintaining structural consistency in scalable processes is another challenge. Thus, how these factors influence structural features and the bio-availabilities of MPs must be resolved, such as pectin polydispersity in the manufacturing processes, as well as microbiota degradation and formulation processes. Pectin and its derivatives are generally regarded as safe but more testing would be beneficial. The human body is reported to produce anti-rhamnose antibodies (Jia, 2010; Pazur, Erikson, Tay, & Allen, 1983). If MP is administered frequently, anti-rhamnose antibody may eliminate MP bearing terminal rhamnose. Possibly, detectable tags can be conjugated with MP, widening the range of MP applications. For example, if fluorescent tags or quantum dots could be conjugated to MP, this new molecule could be used to detect/track circulating Gal-3, circulating tumor cells, and micro-metastases. Finally, two fundamental issues require additional study for promoting applications of pectin in cancer therapy: detailed roles of Gal-3 in cancer, MP per se, especially antitumor SARs, and pharmacokinetic/pharmacodynamic behaviors.

Uncited reference

Jia, 2010, Streetly et al., 2010.

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Highlights

- Overview of the application and anti-cancer mechanisms of dietary pectin.
- Structure-activity relationships of modified pectin are discussed.
- Anti-cancer mechanisms of modified pectin are analyzed.
- Application of pectin in anti-cancer drug as a drug vehicle.

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