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Review

Pectin in cancer therapy: A review

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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; Gal*p*A, 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 (Gal*p*A) 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 Gal*p*A, 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-Gal*p*A-(1→2)-*α*-L-Rha*p*-(1→)]*n*. Gal*p*A 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-Ara*f*) 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 Gal*p*A 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*., 2013~~2~~; Zhang, Mu, *et al*., 2012). For example, Korean red ginseng pectin (KRG) can activate the NF-*κ*B 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*., 2002~~3~~; 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).

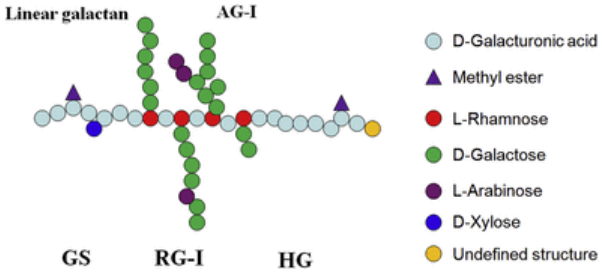

Fig. 1 Diagram of MP structure. MP has a less complex structure with shorter side-chains and a lower DE in contrast to natural pectin. Undefined structures may occur during modification. GS, such as RG-II and XGA, is thought to be significantly reduced.

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 Gal*p*A: MCP-A, rich in Gal*p*A and MCP-N, deficient in Gal*p*A. Surprisingly, MCP-A binds Gal-3 with a stronger affinity than MCP-N. One hypothesis for HG interacting with Gal-3 is that Gal*p*A 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).

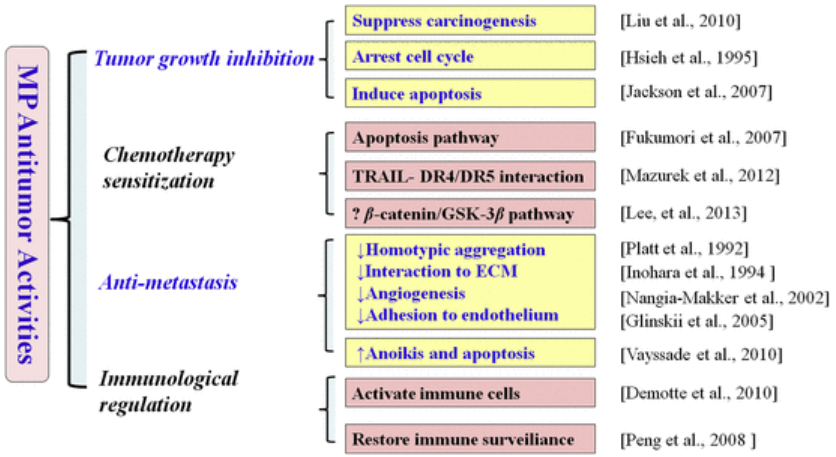

Fig. 2 MP antitumor activity. (↑denotes induction; ↓ is inhibition; ? is a hypothetical pathway) MP-induced inhibition of Gal-3 inhibits tumor growth, sensitizes tumors to chemotherapy, and regulates immune function.

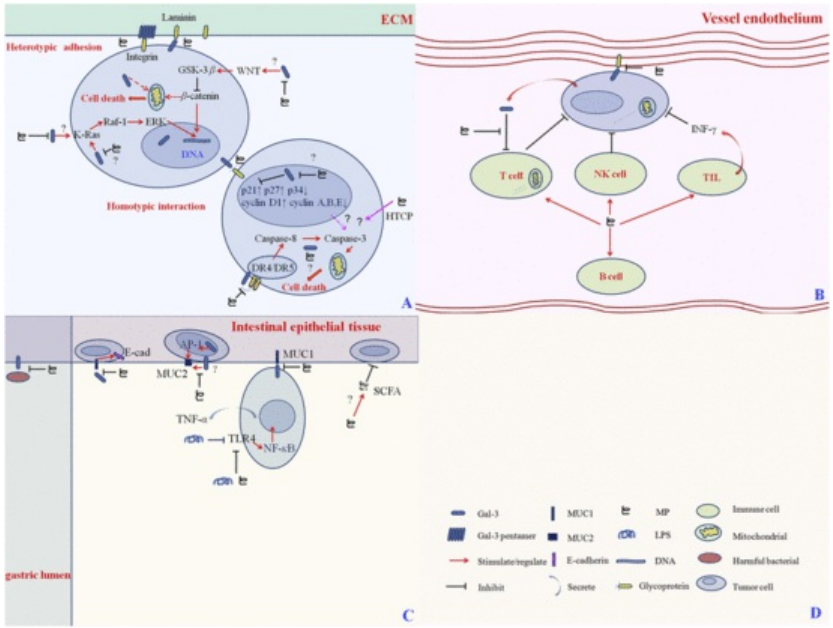

Fig. 3 Proposed MP antitumor mechanism. Panel A: MPs can inhibit tumor growth and metastasis. First, MPs retard tumor growth by suppressing survival pathways (Ras-ERK/MAPK pathway & Wnt/β-catenin pathway), arresting the cell cycle and inducing apoptosis. However, the strong hydrophilicity of MPs diminishes 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.

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.).

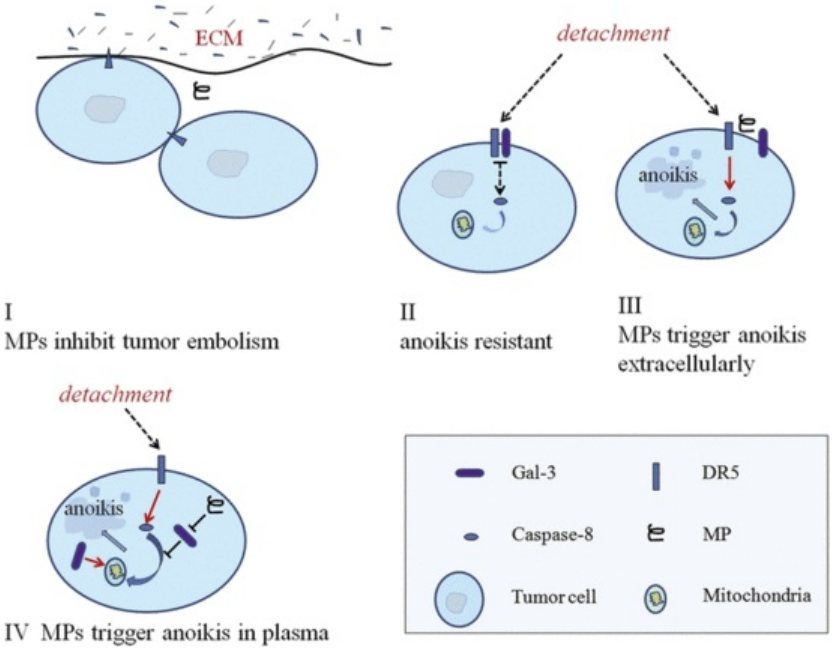

Fig. 4 Hypothetical mechanisms of MPs in suppressing anoikis. I: MPs can inhibit Gal-3 mediated tumor embolism (Glinskii *et al.*, 2005; Glinsky, Huflejt, Glinsky, Deutscher, & Quinn, 2000; Inohara & Raz, 1994; Platt et al.~~& Raz~~, 1992). II: Ligands of Gal-3, such as DR-5 & DR-4, are involved in anoikis (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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References

Almeida E.A., Facchi S.P., Martins A.F., Nocchi S., Schuquel I.T., Nakamura C.V., et al., Synthesis and characterization of pectin derivative with antitumor property against Caco-2 colon cancer cells, *Carbohydrate Polymers* **115**, 2015, 139–145.

Attari F., Sepehri H., Delphi L. and Goliaei B., Apoptotic and necrotic effects of pectic acid on rat pituitary GH3/B6 tumor cells, *Iranian Biomedical Journal* **13** (4), 2009, 229–236.

Avivi-Green C., Polak-Charcon S., Madar Z. and Schwartz B., Apoptosis cascade proteins are regulated *in vivo* by high intracolonic butyrate concentration: correlation with colon cancer inhibition, *Oncology Research* **12** (2), 2000a, 83–95.

Avivi-Green C., Polak-Charcon S., Madar Z. and Schwartz B., Dietary regulation and localization of apoptosis cascade proteins in the colonic crypt, *Journal of Cellular Biochemistry* **77** (1), 2000b, 18–29.

Azémar M., Hildenbrand B., Haering B., Heim M.E. and Unger C., Clinical benefit in patients with advanced solid tumors treated with modified citrus Pectin: a prospective pilot study, *Clinical Medicine: Oncology* **1**, 2007, 73–80.

Bergman M., Djaldetti D., Salman H. and Bessler H., Effect of citrus pectin on malignant cell proliferation, *Biomedicine & Pharmacotherapy* **64** (1), 2010, 44–47.

Brown G.D., Taylor P.R., Reid D.M., Willment J.A., Williams D.L., Martinez-Pomares L., et al., Dectin-1 is a major beta-glucan receptor on macrophages, *Journal of Experimental Medicine* **196** (3), 2002, 407–412.

Caffall K.H. and Mohnen D., The structure, function, and biosynthesis of plant cell wall pectic polysaccharides, *Carbohydrate Research* **344** (14), 2009, 1879–1900.

Chauhan D., Li G., Podar K., Hideshima T., Neri P., He D., et al., A novel carbohydrate-based therapeutic GCS-100 overcomes bortezomib resistance and enhances dexamethasone-induced apoptosis in multiple myeloma cells, *Cancer Research* **65** (18), 2005, 8350–8358.

Cheng H., Li S., Fan Y., Gao X., Hao M., Wang J., et al., Comparative studies of the antiproliferative effects of ginseng polysaccharides on HT-29 human colon cancer cells, *Medical Oncology* **28** (1), 2011, 175–181.

Cheng H., Zhang Z., Leng J., Liu D., Hao M., Gao X., et al., The inhibitory effects and mechanisms of rhamnogalacturonan I pectin from potato on HT-29 colon cancer cell proliferation and cell cycle progression. International Journal of Food Science & Nutrition, *2013* ,**64** (1), 2013, 36–43.

Chen C.H., Sheu M.T., Chen T.F., Wang Y.C., Hou W.C., Liu D.Z., et al., Suppression of endotoxin-induced proinflammatory responses by citrus pectin through blocking LPS signaling pathways, *Biochemical Pharmacology* **72** (8), 2006, 1001–1009.

Choi H.S., Kim K.H., Sohn E., Park J.D., Kim B.O., Moon E.Y., et al., Red ginseng acidic polysaccharide (RGAP) in combination with IFN-gamma results in enhanced macrophage function through activation of the NF-kappaB pathway, *Bioscience Biotechnology & Biochemistry* **72** (7), 2008, 1817–1825.

Cho Y., Turner N.D., Davidson L.A., Chapkin R.S., Carroll R.J. and Lupton J.R., A chemoprotective fish oil/pectin diet enhances apoptosis via Bcl-2 promoter methylation in rat azoxymethane-induced carcinomas, *Experimental Biology & Medicine (Maywood, N.J.)* **237** (12), 2012, 1387–1393.

Cobs-Rosas M., Concha-Olmos J., Weinstein-Oppenheimer C. and Zúñiga-Hansen M.E., Assessment of antiproliferative activity of pectic substances obtained by different extraction methods from rapeseed cake on cancer cell lines, *Carbohydrate Polymers* **117**, 2015, 923–932.

Courts F.L., Profiling of modified citrus pectin oligosaccharide transport across Caco-2 cell monolayers, *PharmaNutrition* **1** (1), 2013, 22–31.

Dange M.C., Srinivasan N., More S.K., Bane S.M., Upadhya A., Ingle A.D., et al., Galectin-3 expressed on different lung compartments promotes organ specific metastasis by facilitating arrest, extravasation and organ colonization via high affinity ligands on melanoma cells, *Clinical & Experimental Metastasis* **31** (6), 2014, 661–673.

Demotte N., Wieërs G., van der Smissen P., Moser M., Schmidt C., Thielemans K., et al., A galectin-3 ligand corrects the impaired function of human CD4 and CD8 tumor-infiltrating lymphocytes and favors tumor rejection in mice, *Cancer Research* **70** (19), 2010, 7476–7488.

Dennis J.W., Lau K.S., Demetriou M. and Nabi I.R., Adaptive regulation at the cell surface by N-glycosylation, *Traffic* **10** (11), 2009, 1569–1578.

Dev R.K., Bali V. and Pathak K., Novel microbially triggered colon specific delivery system of 5-fluorouracil: statistical optimization, in vitro, in vivo, cytotoxic and stability assessment, *International Journal of Pharmaceutics* **411** (1–2), 2011,

142–151.

Dudas S.P., Yunker C.K., Sternberg L.R., Byrd J.C. and Bresalier R.S., Expression of human intestinal mucin is modulated by the beta-galactoside binding protein galectin-3 in colon cancer, *Gastroenterology* **123** (3), 2002, 817–826.

Eliaz I., Hotchkiss A.T., Fishman M.L. and Rode D., The effect of modified citrus pectin on urinary excretion of toxic elements, *Phytotherapy Research* **20** (10), 2006, 859–864.

Fan Y., Cheng H., Liu D., Zhang X., Wang B., Sun L., et al., The inhibitory effect of ginseng pectin on L-929 cell migration, *Archives of Pharmacal Research* **33** (5), 2010, 681–689.

Flint H.J., Bayer E.A., Rincon M.T., Lamed R. and White B.A., Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis, *Nature Reviews Microbiology* **6** (2), 2008, 121–131.

Fowler M., Thomas R.J., Atherton J., Roberts I.S. and High N.J., Galectin-3 binds to Helicobacter pylori O-antigen: it is upregulated and rapidly secreted by gastric epithelial cells in response to H. pylori adhesion, *Cellular Microbiology* **8** (1), 2006, 44–54.

Fukumori T., Kanayama H.O. and Raz A., The role of galectin-3 in cancer drug resistance, *Drug Resistance Updates* **10** (3), 2007, 101–108.

Gao X., Liu D., Fan Y., Li X., Xue H., Ma Y., et al., The two endocytic pathways mediated by the carbohydrate recognition domain and regulated by the collagen-like domain of galectin-3 in vascular endothelial cells, *PLoS One* **7** (12), 2012a, e52430.

Gao X., Zhi Y., Sun L., Peng X., Zhang T., Xue H., et al., The inhibitory effects of a rhamnogalacturonan I (RG-I) domain from ginseng pectin on galectin-3 and its structure-activity relationship, *Journal of Biological Chemistry* **288** (47), 2013, 33953–33965.

Gao X., Zhi Y., Zhang T., Xue H., Wang X., Foday A.D., et al., Analysis of the neutral polysaccharide fraction of MCP and its inhibitory activity on galectin-3, *Glycoconjugate Journal* **29** (4), 2012b, 159–165.

Garthoff J.A., Heemskerk S., Hempenius R.A., Lina B.A., Krul C.A., Koeman J.H., et al., Safety evaluation of pectin-derived acidic oligosaccharides (pAOS): genotoxicity and sub-chronic studies, *Regulatory Toxicology & Pharmacology* **57** (1), 2010, 31–42.

Georgiev Y., Ognyanov M., Yanakieva I., Kussovski V. and Kratchanova M., Isolation, characterization and modification of citrus pectins, *Journal of Bioscience & Bioengineering* **1** (3), 2012, 223–233.

Glinskii O.V., Huxley V.H., Glinsky G.V., Pienta K.J., Raz A. and Glinsky V.V., Mechanical entrapment is insufficient and intercellular adhesion is essential for metastatic cell arrest in distant organs, *Neoplasia* **7** (5), 2005, 522–527.

Glinsky V.V., Huflejt M.E., Glinsky G.V., Deutscher S.L. and Quinn T.P., Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on beta-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells, *Cancer Research* **60** (10), 2000, 2584–2588.

Glinsky V.V. and Raz A., Modified citrus pectin anti-metastatic properties: one bullet, multiple targets, *Carbohydrate Research* **344** (14), 2009, 1788–1791.

Gómez B., Gullón B., Remoroza C., Schols H.A., Parajó J.C. and Alonso J.L., Purification, characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes, *Journal of Agricultural and Food Chemistry* **62** (40), 2014, 9769–9782.

Guess B.W., Scholz M.C., Strum S.B., Lam R.Y., Johnson H.J. and Jennrich R.I., Modified citrus pectin (MCP) increases the prostate-specific antigen doubling time in men with prostate cancer: a phase II pilot study, *Prostate Cancer & Prostatic Diseases* **6** (4), 2003, 301–304.

Guha P., Kaptan E., Bandyopadhyaya G., Kaczanowska S., Davila E., Thompson K., et al., Cod glycopeptide with picomolar affinity to galectin-3 suppresses T-cell apoptosis and prostate cancer metastasis, *Proceedings of the National Academy of Sciences, U S A* **110** (13), 2013, 5052–5057.

Gunning A.P., Bongaerts R.J. and Morris V.J., Recognition of galactan components of pectin by galectin-3, *FASEB Journal* **23** (2), 2009, 415–424.

Gunning A.P., Pin C. and Morris V.J., Galectin 3–β-galactobiose interactions, *Carbohydrate Polymers* **92** (1), 2013, 529–533.

Hao M., Yuan X., Cheng H., Xue H., Zhang T., Zhou Y., et al., Comparative studies on the anti-tumor activities of high temperature- and pH-modified citrus pectins, *Food & Function* **4** (6), 2013, 960–971.

Harazono Y., Nakajima K. and Raz A., Why anti-Bcl-2 clinical trials fail: a solution, *Cancer & Metastasis Reviews* **33** (1), 2014, 285–294.

Hayashi A., Gillen A.C. and Lott J.R., Effects of daily oral administration of quercetin chalcone and modified citrus pectin on implanted colon-25 tumor growth in Balb-c mice, *Alternative Medicine Review* **5** (6), 2000, 546–552.

He W., Du Q., Cao D.Y., Xiang B. and Fan L.F., Study on colon-specific pectin/ethylcellulose film-coated 5-fluorouracil pellets in rats, *International Journal of Pharmaceutics* **348** (1–2), 2008, 35–45.

Heitman D.W., Hardman W.E. and Cameron I.L., Dietary supplementation with pectin and guar gum on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats, *Carcinogenesis* **13** (5), 1992, 815–818.

Hensel A. and Meier K., Pectins and xyloglucans exhibit antimutagenic activities against nitroaromatic compounds, *Planta Medica* **65** (5), 1999, 395–399.

Heusschen R., Griffioen A.W. and Thijssen V.L., Galectin-9 in tumor biology: a jack of multiple trades, *Biochimica et Biophysica Acta* **1836** (1), 2013, 177–185.

Hinz S.W., Verhoef R., Schols H.A., Vincken J.P. and Voragen A.G., Type I arabinogalactan contains *β*-D-Galp-(1-->3)-*β*-D-Gal*p* structural elements, *Carbohydrate Research* **340** (13), 2005, 2135–2143.

Hossein G., Keshavarz M., Ahmadi S. and Naderi N., Synergistic effects of PectaSol-c modified citrus pectin an inhibitor of Galectin-3 and Paclitaxel on apoptosis of human SKOV-3 Ovarian Cancer cells, *Asian Pacific Journal of Cancer Prevention* **14** (12), 2013, 7561–7568.

Hsieh T. and Wu J.M., Changes in cell growth, cyclin/kinase, endogenous phosphoproteins and nm23 gene expression in human prostatic JCA-1 cells treated with modified citrus pectin, *Biochemistry & Molecular Biology International* **37** (5), 1995, 833–841.

Inohara H. and Raz A., Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions, *Glycoconjugate Journal* **11** (6), 1994, 527–532.

Iurisci I., Tinari N., Natoli C., Angelucci D., Cianchetti E. and Iacobelli S., Concentrations of galectin-3 in the sera of normal controls and cancer patients, *Clinical Cancer Research* **6** (4), 2000, 1389–1393.

Jackson C.L., Dreaden T.M., Theobald L.K., Tran N.M., Beal T.L., Eid M., et al., Pectin induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure, *Glycobiology* **17** (8), 2007, 805–819.

Jacobasch G., Dongowski G., Florian S., Müller-Schmehl K., Raab B. and Schmiedl D., Pectin does not inhibit intestinal carcinogenesis in APC-deficient Min/+ mice, *Journal of Agricultural and Food Chemistry* **56** (4), 2008, 1501–1510.

Jacobs L.R. and Lupton J.R., Relationship between colonic luminal pH, cell proliferation and colon carcinogenesis in 1,2-dimethylhydrazine treated rats fed high fiber diets, *Cancer Research* **46** (4 Pt 1), 1986, 1727–1734.

Jeon C., Kang S., Park S., Lim K., Hwang K.W. and Min H., T cell stimulatory effects of Korean red ginseng through modulation of myeloid-derived suppressor cells, *Journal of Ginseng Research* **35** (4), 2011, 462–470.

Jia X., The use of natural anti-Rhamnose antibody to enhance recognization and killing of tumor cells, M.S. thesis, 2010, Shandong University; Jinan.

Jiang J., Eliaz I. and Sliva D., Synergistic and additive effects of modified citrus Pectin with two polybotanical compounds, in the suppression of invasive behavior of human breast and prostate cancer cells, *Integrative Cancer Therapies* **12** (2), 2013, 145–152.

Johnson K.D., Glinskii O.V., Mossine V.V., Turk J.R., Mawhinney T.P., Anthony D.C., et al., Galectin-3 as a potential therapeutic target in tumors arising from malignant endothelia, *Neoplasia* **9** (8), 2007, 662–670.

Kang H.J., Jo C., Kwon J.H., Son J.H., An B.J. and Byun M.W., Antioxidant and cancer cell proliferation inhibition effect of citrus pectin-oligosaccharide prepared by irradiation, *Journal of Medicinal Food* **9** (3), 2006, 313–320.

Katav T., Liu L., Traitel T., Goldbart R., Wolfson M. and Kost J., Modified pectin-based carrier for gene delivery: cellular barriers in gene delivery course, *Journal of Controlled Release* **130** (2), 2008, 183–191.

Katzenmaier E.M., André S., Kopitz J. and Gabius H.J., Impact of sodium butyrate on the network of adhesion/growth-regulatory galectins in human colon cancer, *in vitro. Anticancer Research* **34** (10), 2014, 5429–5438.

Kim H.R., Lin H.M., Biliran H. and Raz A., Cell cycle arrest and inhibition of anoikis by galectin-3 in human breast epithelial cells, *Cancer Research* **59** (16), 1999, 4148–4154.

Klyosov A., Galectin-targeted drug design, In: Klyosov A., (Ed), *Glycobiology & drug design. ACS symposium series,* 2012, American Chemical Society; Washington, DC, 25–66.

Kolatsi-Joannou M., Price K.L., Winyard P.J. and Long D.A., Modified citrus pectin reduces galectin-3 expression and disease severity in experimental acute kidney injury, *PLoS One* **6** (4), 2011, e18683.

Krall S.M. and McFeeters R.F., Pectin hydrolysis: effect of temperature, degree of methylation, pH, and calcium on hydrolysis rates, *Journal of Agricultural & Food Chemistry* **46** (4), 1998, 1311–1315.

Laguinge L.M., Samara R.N., Wang W., El-Deiry W.S., Corner G., Augenlicht L., et al., DR5 receptor mediates anoikis in human colorectal carcinoma cell lines, *Cancer Research* **68** (3), 2008, 909–917.

Leclere L., Cutsem P.V. and Michiels C., Anti-cancer activities of pH- or heat-modified pectin, *Frontiers in Pharmacology* **4** (128), 2013, 1–8, eCollection.

Lee Y.K., Lin T.H., Chang C.F. and Lo Y.L., Galectin-3 silencing inhibits epirubicin-induced ATP binding cassette transporters and activates the mitochondrial apoptosis pathway via β-catenin/GSK-3β modulation in colorectal carcinoma, *PLoS One* **8** (11), 2013, e82478.

Lee E.H., Park H.R., Shin M.S., Cho S.Y., Choi H.J. and Shin K.S., Antitumor metastasis activity of pectic polysaccharide purified from the peels of Korean *citrus* Hallabong, *Carbohydrate Polymers* **111**, 2014, 72–79.

Lee J.H., Shim J.S., Lee J.S., Kim M.K., Chung M.S. and Kim K.H., Pectin-like acidic polysaccharide from Panax ginseng with selective antiadhesive activity against pathogenic bacteria, *Carbohydrate Research* **341** (9), 2006, 1154–1163.

Lee J.H., Shim J.S., Lee J.S., Kim J.K., Yang I.S., Chung M.S., et al., Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*), *Journal of Agricultural & Food Chemistry* **54** (23), 2006, 8717–8723.

Lepur A., Salomonsson E., Nilsson U.J. and Leffler H., Ligand induced galectin-3 protein self-association, *Journal of Biological Chemistry* **287** (26), 2012, 21751–21756.

Liu Y., Ahmad H., Luo Y., Gardiner D.T., Gunasekera R.S., McKeehan W.L., et al., Citrus pectin: characterization and inhibitory effect on fibroblast growth factor-receptor interaction, *Journal of Agricultural & Food Chemistry* **49** (6), 2001, 3051–3057.

Liu H.Y., Huang Z.L., Yang G.H., Lu W.Q. and Yu N.R., Inhibitory effect of modified citrus pectin on liver metastases in a mouse colon cancer model, *World Journal of Gastroenterology* **14** (48), 2008, 7386–7391.

Liu L., Li Y.H., Niu Y.B., Sun Y., Guo Z.J., Li Q., et al., An apple oligogalactan prevents against inflammation and carcinogenesis by targeting LPS/TLR4/NF-κB pathway in a mouse model of colitis-associated colon cancer, *Carcinogenesis* **31** (10), 2010, 1822–1832.

Louis P., Hold G.L. and Flint H.J., The gut microbiota, bacterial metabolites and colorectal cancer, *Nature Reviews Microbiology* **12** (10), 2014, 661–672.

Lu W.Q., Wang F. and Liu H.Y., Influence of oxaliplatin combined with LCP on proliferation and apoptosis of colon cancer cell, *Chinese Journal of Gastrointestinal Surgery* **16** (1), 2013, 84–89.

von Mach T., Carlsson M.C., Straube T., Nilsson U., Leffler H. and Jacob R., Ligand binding and complex formation of galectin-3 is modulated by pH variations, *Biochemical Journal* **457** (1), 2014, 107–115.

Matarrese P., Tinari N., Semeraro M.L., Natoli C., Iacobelli S. and Malorni W., Galectin-3 overexpression protects from cell damage and death by influencing mitochondrial homeostasis, *FEBS Letter* **473** (3), 2000, 311–315.

Maxwell E.G., Belshaw N.J., Waldron K.W. and Morris V.J., Pectin - an emerging new bioactive food polysaccharide, *Trends in Food Science & Technology* **24** (2), 2012, 64–73.

Mazurek N., Byrd J.C., Sun Y., Hafley M., Ramirez K., Burks J., et al., Cell-surface galectin-3 confers resistance to TRAIL by impeding trafficking of death receptors in metastatic colon adenocarcinoma cells, *Cell Death & Differentiation* **19** (3), 2012, 523–533.

McCarty M.F. and Block K.I., Toward a core nutraceutical program for cancer management, *Integrative Cancer Therapies* **5** (2), 2006, 150–171.

Mohnen D., Pectin structure and biosynthesis, *Current Opinion in Plant Biology* **11** (3), 2008, 266–277.

Morris V.J., Belshaw N.J., Waldron K.W. and Maxwell E.G., The bioactivity of modified pectin fragments, *Bioactive Carbohydrates & Dietary Fibre* **1** (1), 2013, 21–37.

Morris V.J., Gromer A., Kirby A.R., Bongaerts R.J.M. and Gunning A.P., Using AFM and force spectroscopy to determine pectin structure and (bio) functionality, *Food Hydrocolloids* **25** (2), 2011, 230–237.

Mossine V.V., Glinsky V.V. and Mawhinney T.P., Food-related carbohydrate ligands for galectins, In: Klyosov A.A., Witczak Z.J. and Platt D., (Eds.), *Galectin,* 2008, John Wiley; New Jersey, 235–270.

Munarin F., Tanzi M.C. and Petrini P., Advances in biomedical applications of pectin gels, *International Journal of Biological Macromolecules* **51** (4), 2012, 681–689.

Nakahara S. and Raz A., Biological modulation by lectins and their ligands in tumor progression and metastasis, *Anti-Cancer Agents in Medicinal Chemistry* **8** (1), 2008, 22–36.

Nangia-Makker P., Hogan V., Honjo Y., Baccarini S., Tait L., Bresalier R., et al., Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin, *Journal of the National Cancer Institute* **94** (24), 2002, 1854–1862.

Newlaczyl A.U. and Yu L.G., Galectin-3-A jack-of-all-trades in cancer, *Cancer Letters* **313** (2), 2011, 123–128.

Ohkami H., Tazawa K., Yamashita I., Shimizu T., Murai K., Kobashi K., et al., Effects of apple pectin on fecal bacterial enzymes in azoxymethane-induced rat colon carcinogenesis, *Japanese Journal of Cancer Research* **86** (6), 1995, 523–529.

Ohno K., Narushima S., Takeuchi S., Itoh K., Mitsuoka T., Nakayama H., et al., Inhibitory effect of apple pectin and culture condensate of *Bifidobacterium longum* on colorectal tumors induced by 1,2-dimethylhydrazine in transgenic mice harboring human prototype c-Ha-ras genes, *Experimental Animals* **49** (4), 2000, 305–307.

Olano-Martin E., Gibson G.R. and Rastell R.A., Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides, *Journal of Applied Microbiology* **93**, 2002, 505–511.

Ozment T.R., Goldman M.P., Kalbfleisch J.H. and Williams D.L., Soluble glucan is internalized and trafficked to the Golgi apparatus in macrophages via a clathrin-mediated, lipid raft-regulated mechanism, *Journal of Pharmacology & Experimental Therapeutics* **342** (3), 2012, 808–815.

Pazur J.H., Erikson M.S., Tay M.E. and Allen P.Z., Isomeric, anti-rhamnose antibodies having specificity for rhamnose-containing, streptococcal heteroglycans, *Carbohydrate Research* **124** (2), 1983, 253–263.

Peng W., Wang H.Y., Miyahara Y., Peng G. and Wang R.F., Tumor-associated galectin-3 modulates the function of tumor-reactive T cells, *Cancer Research* **68** (17), 2008, 7228–7236.

Pienta K.J., Naik H., Akhtar A., Yamazaki K., Replogle T.S., Lehr J., et al., Inhibition of spontaneous metastasis in a rat prostate cancer model by oral administration of modified citrus pectin, *Journal of the National Cancer Institute* **87** (5), 1995, 348–353.

Pieters R.J., Inhibition and detection of galectins, *Chembiochem* **7** (5), 2006, 721–728.

Platt D. and Raz A., Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin, *Journal of the National Cancer Institute* **84** (6), 1992, 438–442.

Puga A.M., Lima A.C., Mano J.F., Concheiro A. and Alvarez-Lorenzo C., Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation, *Carbohydrate Polymers* **98** (1), 2013, 331–340.

Radosavljevic G., Volarevic V., Jovanovic I., Milovanovic M., Pejnovic N., Arsenijevic N., et al., The roles of Galectin-3 in autoimmunity and tumor progression, *Immunologic Research* **52** (1–2), 2012, 100–110.

Ramachandran C., Wilk B.J., Hotchkiss A., Chau H., Eliaz I. and Melnick S.J., Activation of human T-helper/inducer cell, T-cytotoxic cell, B-cell, and natural killer (NK)-cells and induction of natural killer cell activity against K562 chronic myeloid leukemia cells with modified citrus pectin, *BMC Complementary & Alternative Medicine* **11**, 2011, 59–68.

Rao C.V., Chou D., Simi B., Ku H. and Reddy B.S., Prevention of colonic aberrant crypt foci and modulation of large bowel microbial activity by dietary coffee fiber, inulin and pectin, *Carcinogenesis* **19** (10), 1998, 1815–1819.

Salman H., Bergman M., Djaldetti M., Orlin J. and Bessler H., Citrus pectin affects cytokine production by human peripheral blood mononuclear cells, *Biomedicine & Pharmacotherapy* **62** (9), 2008, 579–582.

Sathisha U.V., Jayaram S., Harish Nayaka M.A. and Dharmesh S.M., Inhibition of galectin-3 mediated cellular interactions by pectic polysaccharides from dietary sources, *Glycoconjugate Journal* **24** (8), 2007, 497–507.

Schmidgall J. and Hensel A., Bioadhesive properties of polygalacturonides against colonic epithelial membranes, *International Journal of Biological Macromolecules* **30** (5), 2002, 217–225Seetharaman, J., Kanigsberg, A., Slaaby, R., Leffler, H., Barondes, S.H. and Rini, J.M., X-ray crystal structure of the human galectin-3 carbohydrate recognition domain at 2.1-A resolution, *Journal of Biological Chemistry* **273** (21), 1998, 13047-13052..

Shah M.S., Schwartz S.L., Zhao C., Davidson L.A., Zhou B., Lupton J.R., et al., Integrated microRNA and mRNA expression profiling in a rat colon carcinogenesis model: effect of a chemo-protective diet, *Physiological Genomics* **43** (10), 2011, 640–654.

Song S., Byrd J.C., Mazurek N., Liu K., Koo J.S. and Bresalier R.S., Galectin-3 modulates MUC2 mucin expression in human colon cancer cells at the level of transcription via AP-1activation, *Gastroenterology* **129** (5), 2005, 1581–1591.

Song S., Ji B., Ramachandran V., Wang H., Hafley M., Logsdon C., et al., Overexpressed galectin-3 in pancreatic cancer induces cell proliferation and invasion by binding Ras and activating Ras signaling, *PLoS One* **7** (8), 2012, e42699.

Straube T., von Mach T., Hönig E., Greb C., Schneider D. and Jacob R., pH-dependent recycling of galectin-3 at the apical membrane of epithelial cells, *Traffic* **14** (9), 2013, 1014–1027.

Streetly M.J., Maharaj L., Joel S., Schey S.A., Gribben J.G. and Cotter F.E., GCS-100, a novel galectin-3 antagonist, modulates MCL-1, NOXA, and cell cycle to induce myeloma cell death, *Blood* **115** (19), 2010, 3939–3948.

Takei T., Sato M., Ijima H. and Kawakami K., In situ gellable oxidized citrus pectin for localized delivery of anticancer drugs and prevention of homotypic cancer cell aggregation, *Biomacromolecules* **11** (12), 2010, 3525–3530.

Tang X.H., Xie P., Ding Y., Chu L.Y., Hou J.P., Yang J.L., et al., Synthesis, characterization, and in vitro and in vivo evaluation of a novel pectin-adriamycin conjugate, *Bioorganic & Medicinal Chemistry* **18** (4), 2010, 1599–1609.

Thibault J.-F., Renard C.M.G.C., Axelos M.A.V., Roger P. and Crepeau M.-J., Studies of the length of homogalacturonic regions in pectins by acid hydrolysis, *Carbohydrate Research* **238**, 1993, 271–286.

Umar S., Morris A.P., Kourouma F. and Sellin J.H., Dietary pectin and calcium inhibit colonic proliferation in vivo by differing mechanisms, *Cell Proliferation* **36** (6), 2003, 361–375.

Urias-Orona V., Huerta-Oros J., Carvajal-Millán E., Lizardi-Mendoza J., Rascón-Chu A. and Gardea A.A., Component analysis and free radicals scavenging activity of *Cicer arietinum* L. husk pectin, *Molecules* **15** (10), 2010, 6948–6955.

Vayssade M., Sengkhamparn N., Verhoef R., Delaigue C., Goundiam O., Vigneron P., et al., Antiproliferative and proapoptotic actions of okra pectin on B16F10 melanoma cells, *Phytotherapy Research* **24** (7), 2010, 982–989.

Wai W.W., AlKarkhi A.F.M. and Easa A.M., Comparing biosorbent ability of modified citrus and durian rind pectin, *Carbohydrate Polymers* **79** (3), 2010, 584–589.

Wang Y., Balan V., Kho D., Hogan V., Nangia-Makker P. and Raz A., Galectin-3 regulates p21 stability in human prostate cancer cells, *Oncogene* **32** (42), 2013, 5058–5065.

Wang X.S., Dong Q., Zuo J.P. and Fang J.N., Immunological activities and structure of pectin from, *Centella asiatica. Carbohydrate Research* **338** (22), 2003, 2393–2402.

Wang F. and Liu H.Y., Inhibitory effect of combined treatment with oxaliplatin and low molecular weight citrus pectin on proliferation of HCT116 cells and its mechanism, *Chines Journal of General Surgery* **20** (10), 2011, 1053–1057.

Weigel P.H. and Yik J.H., Glycans as endocytosis signals: the cases of the asialoglycoprotein and hyaluronan/chondroitin sulfate receptors, *Biochimica et Biophysica Acta* **1572** (2–3), 2002, 341–363.

Wicker L., Kim Y., Kim M.J., Thirkield B., Lin Z. and Jung J., Pectin as a bioactive polysaccharide – extracting tailored function from less, *Food Hydrocolloids* **42** (2), 2014, 251–259.~~Wittmann,V., & Pieters, R.J., Bridging lectin binding sites by multivalent carbohydrates, *Chemical Society Reviews* **42**(10), 2013, 4492-4503.~~Wittmann,V., and Pieters, R.J., Bridging lectin binding sites by multivalent carbohydrates, *Chemical Society Reviews* **42**(10), 2013, 4492-4503.

Wong T.W., Colombo G. and Sonvico F., Pectin matrix as oral drug delivery vehicle for colon cancer treatment, *AAPS PharmSciTech* **12** (1), 2011, 201–214.

Xue J., Gao X., Fu C., Cong Z., Jiang H., Wang W., et al., Regulation of galectin-3-induced apoptosis of Jurkat cells by both O-glycans and N-glycans on CD45, *FEBS Letter* **587** (24), 2013, 3986–3994.

Yang C., Gou Y., Chen J., An J., Chen W. and Hu F., Structural characterization and antitumor activity of a pectic polysaccharide from, *Codonopsis pilosula. Carbohydrate Polymers* **98** (1), 2013, 886–895.

Yan J. and Katz A., PectaSol-C modified citrus pectin induces apoptosis and inhibition of proliferation in human and mouse androgendependent and -independent prostate cancer cells, *Integrative Cancer Therapies* **9** (2), 2010, 197–203.

Yapo B.M., Lerouge P., Thibault J.-F. and Ralet M.-C., Pectins from citrus peel cell walls contain homogalacturonans homogenous with respect to molar mass, rhamnogalacturonan I and rhamnogalacturonan II, *Carbohydrate Polymers* **69** (3), 2007, 426–435.

Yoshii T., Fukumori T., Honjo Y., Inohara H., Kim H.R. and Raz A., Galectin-3 phosphorylation is required for its anti-apoptotic function and cell cycle arrest, *Journal of Biological Chemistry* **277** (9), 2002, 6852–6857.

Yu C.Y., Wang Y.M., Li N.M., Liu G.S., Yang S., Tang G.T., et al., In vitro and in vivo evaluation of pectin-based nanoparticles for hepatocellular carcinoma drug chemotherapy, *Molecular Pharmaceutics* **11** (2), 2014, 638–644.

Zhang W.B., MCP polysaccharide: Preparation, analysis and its anti-tumor activity, Ph. D. thesis2006, Tianjin University; Tianjin.

Zhang W.B., Progress in galectin-3 and its inhibitor, *Chinese Pharmaceutical Journal* **44** (3), 2009, 165–169.

Zhang D., Li Y.H., Mi M., Jiang F.L., Yue Z.G., Sun Y., et al., Modified apple polysaccharides suppress the migration and invasion of colorectal cancer cells induced by lipopolysaccharide, *Nutrition Research* **33** (10), 2013, 839–848.

Zhang W.B., Gao L., Shi X.F. and Zhang Q.L., Determination of the molecular mass of modified citrus pectin using high performance size exclusion chromatography, *Chinese Journal of Chromatography* **25** (5), 2007, 711–714.

Zhang W.B., Liu C.Z. and Gao L., Modified citrus pectin: preparation, characterization and anti-cancer activities, *Chemical Journal of Chinese Universities* **31** (5), 2010, 964–969.

Zhang W.B., Xu P., Gao L., Yan Q.H. and Yang W.M., Preparation and antitumor application of modified pectin with high bioavailability, 2013, Chinese patent, ZL. 201110200381.4.

Zhang X., Li S., Sun L., Ji L., Zhu J., Fan Y., et al., Further analysis of the structure and immunological activity of an RG-I type pectin from, *Panax ginseng. Carbohydrate Polymers* **89** (2), 2012, 519–525.

Zhang Y.Y., Mu T.T. and Zhang M., Effects of modified sweet potato pectins on the proliferation of Cancer cells, *Scientia Agricultura Sinica* **45** (9), 2012, 1798–1806.

Zhao Q., Barclay M., Hilkens J., Guo X., Barrow H., Rhodes J.M., et al., Interaction between circulating galectin-3 and cancer-associated MUC1 enhances tumour cell homotypic aggregation and prevents anoikis, *Molecular Cancer* **9**, 2010, 154–166.

Zunft H.J., Goldin-Lang P. and Dongowski G., DNA adducts in tissues of germ-free and conventional rats fed high- and low-esterified pectins, *Cancer Letters* **114** (1–2), 1997, 43–46.

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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