Commentary

Cancer Prevention Research

Chitin or Chitin-Like Glycans as Targets for Late-Term Cancer Chemoprevention

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Abstract

A consistent observation in studies of carcinogenesis is that some glycans are expressed differently in cancer cells than in normal cells. A well-known example is the aberrant β1–6 *N*-acetyl-D-glucosamine branching associated with metastasis and poor prognosis in many cancers. This commentary proposes that, although not found in normal mammalian cells, a chitin (β-1,4-linked *N*-acetyl-D-glucosamine) or a chitin-like polysaccharide (e.g., hyaluronan) may exist as a cancer-associated glycan, which can be targeted by the novel pyrimidine nucleotide derivative SP-1015 (designed as a chitin synthase inhibitor). Preliminary chemoprevention data of our group showed SP-1015 in the diet can inhibit benzo(a)pyrene-induced neoplasia in the forestomach of female A/J mice, and, of importance, this activity occurred at late stages in carcinogenesis. While no effect was seen in the murine lung, this may be due to the low bioavailability of the compound. A different route of administration (e.g. inhalation of an aerosol) may have potential to inhibit pulmonary carcinogenesis. We hypothesize that inhibitors of chitin or chitin-like glycan formation may be effective chemopreventive agents and suggest that further work is needed to study these novel targets for cancer prevention. *Cancer Prev Res; 3(12); 1519–22. ©2010 AACR.*

Glycans, Chitins, and Carcinogenesis

A consistent observation in studies of carcinogenesis is that expression of glycans is different in cancer cells than in normal cells. Glycans—oligosaccharides or polysaccharides often attached to proteins or lipids—are found on the surfaces of all eukaryotic cells and in the extracellular matrix and some organelles. Glycans are formed in the process of glycosylation through the creation of glycosidic linkages, the bonds between carbohydrate units and other molecules. During glycosylation in the Golgi apparatus, glycotransferases (enzymes which form glycosidic linkages) and glycosidases (enzymes which cleave glycosidic linkages) often exhibit changed levels of expression in cancer cells compared with their normal cell counterparts. The resulting aberrant glycosylation leads to changes in the core glycan structure, such as altered branching and increased glycan size, and can lead to other changes in chemical composition associated with carcinogenesis. Thus, over- or underexpression of particular glycans, generally structurally modified from their normal-cell counterparts, is characteristic of cancer cells (reviewed in refs. 1–5).

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Perhaps the most-widely studied example of an aberrant structure associated with cancer cell surfaces is the occurrence of β1–6 branching on *N*-glycans between *N*-acetyl-D-glucosamine (GlcNAc) and mannose (Man). This branching is caused by the activity of the glycotransferase *N*-acetlyglucosaminyltransferase-V (GnT-V), which catalyzes the addition of GlcNAc to a particular Man residue on an asparagine-linked glycan, forming the branching structure GlcNAcβ1–6–Manα1–6Manβ1 (Fig. 1; refs. 1–5). The additional branch provides an additional substrate for glycosylation, so the glycan increases in size and structural complexity and differs in conformation from other *N*-glycans. The presence or increased appearance of β1–6 GlcNAc branching occurs in carcinogenesis in many cancers (2). Several *in vivo* studies have shown that the expression of GnT-V and the presence of β1–6 GlcNAc branching play a functional role in carcinogenesis and are associated with metastasis and poor prognosis at least in cancers of the breast (3–9) and colon-rectum (3), and melanoma (4). Studies involving attempts to inhibit the formation of β1–6 branching have shown therapeutic effects in murine and human cancer cells (5, 6).

Chitin is a linear long-chain polysaccharide consisting of β-1,4-linked GlcNAc and is a glycan that, although not found in normal mammalian cells, is highly abundant in nature. It is a major structural component in many organisms including the cell walls of fungi, cyst walls of many protozoans, exoskeletons of arthropods such as crustaceans and insects, and the radulas and beaks of mollusks. Three crystal polymorphisms of chitin have been described and differ in the orientation of their "piles" of parallel chains: Alpha-chitin (the most common) is antiparallel; beta-chitin

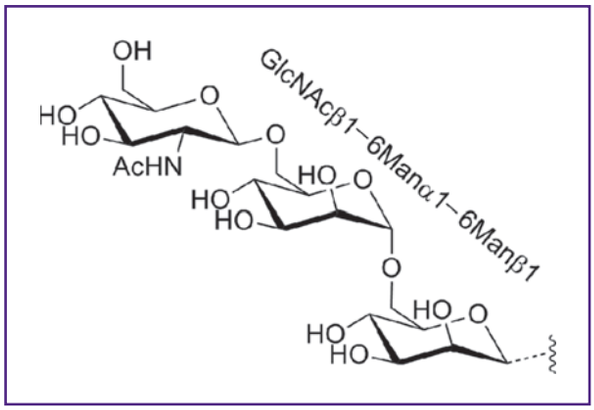

Fig. 1. The β1–6 *N*-acetyl-D-glucosamine (GlcNAc)-branching structure of *N*-glycans, synthesized by the activity of glycotransferase *N*-acetylglucosaminyltransferase-V (GnT-V), which is highly expressed in many cancer cells.

is parallel; and gamma-chitin has 2 sheets parallel with a third antiparallel (7).

Biosynthesis of chitin is performed by chitin synthases, a specific class of glycosyltransferase that catalyzes the polymerization of GlcNAc (Fig. 2A). Hydrolysis/biodegradation of chitin chains is performed by glycosidases called chitinases. Both chitin synthases and chitinases are required for the growth of many pathogens, especially fungi, and pharmacologic applications often utilize inhibitors of chitin synthases (e.g., the nikkomycins and polyoxins) to target biosynthesis of chitin. Such compounds are thought to inhibit chitin synthase competitively by mimicking the substrate uridine diphosphate–GlcNAc or its transition state (8).

Although no mammalian chitin synthases have been reported, chitinases are produced by mammalian macrophages in what is thought to be a defense mechanism against chitin-containing pathogens (9). In an intriguing 2005 study, administration of bacterial chitinases produced breast cancer regression *in vitro* and *in vivo*; human tumor cells were selectively lysed by chitinase in culture and in mouse xenografts (10). Additionally, a plant-derived chitinase which has been cloned and expressed in bacteria has shown strong cytotoxicity against human cell lines of lymphoma and choriocarcinoma (11). Other recent studies have shown that chitin-like polysaccharides are present in human tissues associated with Alzheimer's disease, indicating that, at least under certain circumstances, mammalian cells have the ability to synthesize aberrant chitin or chitin-like polysaccharides (12, 13).

Consistent with findings of these types, we propose the possible existence of a "cancer chitin," a chitin or chitin-like glycan associated with cancer cells, which can be targeted by an inhibitor of chitin synthase for the chemoprevention of carcinogenesis. A plausible candidate for such a chitin-like cancer glycan is hyaluronan, a linear glycosaminoglycan found in the extracellular matrix of most mammalian tissues. Hyaluronan is a polymer of the disaccharides D-glucoronic acid and GlcNAc, linked together via alternating β-1,4 and β-1,3 glycosidic bonds (Fig. 2B), and its synthesis is catalyzed by hyaluronan synthases. Several recent reviews summarize the multiple functional roles of hyaluronan in cancer initiation and progression and its potential as a therapeutic target (14–16).

Novel Studies of Chitin Synthesis Inhibition

The pyrimidine nucleotide derivative SP-1015 was originally developed by Dr. Steven Patterson and colleagues as a chitin synthase inhibitor for the treatment of *giardiasis* and *Entamoeba* infections. Although there is a lack of direct evidence that the mechanism of action of SP-1015 is to inhibit chitin synthase, assays performed by Drs. Patterson and Christine Dykstra demonstrate biological activity that suggests this function. First, SP-1015 was found to be modestly active in screening for activity against the growth of the fungus *Candida albicans* according to the method described by Del Poeta et al. (17). Although the compound poorly inhibited fungal growth [minimum inhibitory concentration (MIC) greater than 100 μmol/L; unpublished data of Drs. Patterson and Dykstra], fungal cells grown in the presence of SP-1015 showed an irregular filamentous shape consistent with

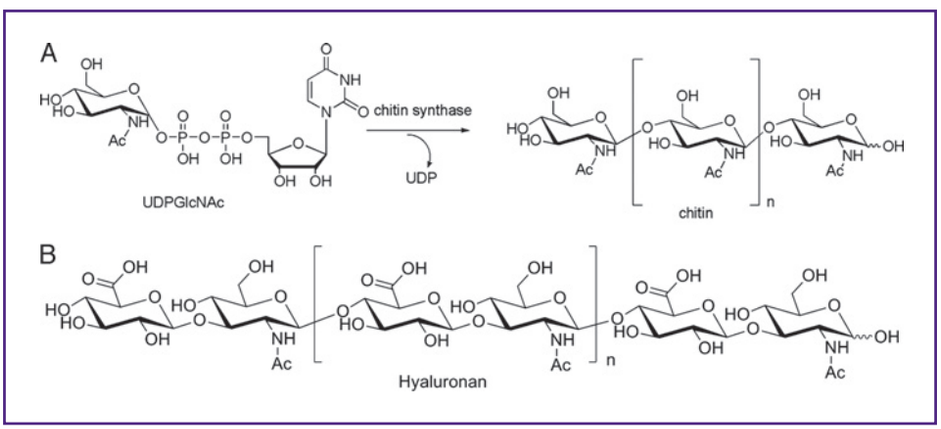

Fig. 2. The structure of chitin [a polymer of β-1,4-linked *N*-acetyl-D-glucosamine (GlcNAc) subunits] and its synthesis is shown in panel A. The structure of hyaluronan is shown in panel B.

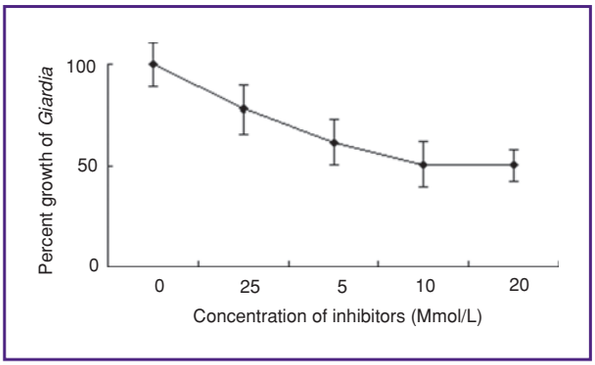

Fig. 3. The activity of SP-1015 against *Giardia lambia*. $IC_{50} = 10$ μm.

the disruption of cell-wall synthesis. This shape is similar to the change in morphology exhibited by *C. albicans* when grown in the presence of the known chitin synthase inhibitor polyoxin D (18). Second, SP-1015 inhibited the growth of cysts formed by the protozoal intestinal parasite *Giardia lambia* with a half MIC (IC_{50}) of 10 μmol/L (Fig. 3; unpublished data), presumably by inhibiting the biosynthesis of chitin-like poly(*N*-acetylgalactosamine), which composes the *G. lambia* cyst cell wall.

Our group has conducted 2 studies of SP-1015 as a dietary chemopreventive agent in carcinogen-induced neoplasia of the lung and forestomach in female A/J mice (unpublished data). In the first study, 15-week-old mice were treated with benzo(a)pyrene and began oral feeding with SP-1015 (dose equivalent of 12 mg/kg body weight) 10 weeks after carcinogen cessation. The diet additions continued for 7 weeks, when the experiment was terminated. Lungs and forestomachs were observed macroscopically to obtain tumor counts, and forestomach tumor surface area was measured as a percentage of total forestomach surface area. The second study followed up on the first and examined the response to the timing of SP-1015 administration. This study involved the following 5 groups of mice: 1 control group that received no SP-1015; 2 experimental groups, one that began SP-1015 diet feeding 9 weeks after the last carcinogen dose (the 9-week group) and one that began SP-1015 13 weeks after the last carcinogen dose (the 13-week group); and 2 baseline groups, which were sacrificed at 9 weeks or 13 weeks after the last carcinogen dose to establish the existence of tumor growth at the time the corresponding experimental group began SP-1015. All control and experimental-group mice were sacrificed at 17 weeks after the last dose of carcinogen, at which time the 9-week group had received 8 weeks worth of diet additions and the 13-week group had received 4 weeks worth of diet additions.

In both studies, there were no signs of toxicity and no change in the number of lung tumors. Statistically significant changes, however, were found in regard to forestomach tumors. In the first study, the average number of tumors per forestomach was reduced by 35%, from 6.6 in the control group to 4.3 in the experimental group ($P < 0.05$). In the second study, although the number of forestomach tumors was not affected, there was a trend toward smaller such tumors and a significant reduction in the area of forestomach covered by tumor. The treatment groups showed a notable but statistically nonsignificant decrease in the average area of an individual tumor—3.6 square mm in the 9-week group and 3.9 square mm in the 13-week group, compared with 6.7 square mm in the control group. The average percentage of forestomach tissue covered by tumor tissue was reduced, compared with controls (21% coverage), by a significant 55% in the 13-week group (to 9.4% coverage; $P < 0.05$) and marginally significantly in the 9-week group (to 9.9% coverage; $P = 0.05$).

Therefore, a signal of SP-1015 activity in inhibiting forestomach tumors was picked up in both studies, although the results of the first study were not perfectly replicated in the second one. We are unable to account for the 2 different ways in which inhibition presented itself in the forestomach. Furthermore, no significant effects were seen on the number of pulmonary tumors, which we hypothesize is a result of low bioavailability of oral SP-1015. As a pyrimidine nucleotide derivative, SP-1015 has a structure that would predict poor absorption via the oral route (19). Follow-up studies that administer SP-1015 directly into the lung via aerosol inhalation will allow the potential of SP-1015 to inhibit pulmonary carcinogenesis to be investigated more thoroughly.

Of added interest, because SP-1015 showed activity whether it was added to the diet beginning at 9, 10, or 13 weeks postcarcinogen, it appeared to be effective at a late stage in the carcinogenesis process. Identifying compounds with the potential for late-term chemoprevention is particularly important and valuable because the time period required for administering the agents is decreased. The chemopreventive effectiveness of SP-1015 at a late stage of carcinogenesis is highlighted in the second study by the effects of SP-1015 begun at 13 weeks versus at 9 weeks postcarcinogen. The inhibition in forestomach tumor coverage exhibited by the 13-week group (from 21% to 9.4%) was not significantly different than that demonstrated by the 9-week group (from 21% to 9.9%), even though the 9-week group began SP-1015 feeding with substantially less forestomach tumor coverage (1.8% versus 5.5%) and continued experimental feeding for twice as long (8 weeks versus 4 weeks) as the 13-week group.

Conclusions

In this commentary, we have addressed the potential of cancer-associated glycans as targets for chemoprevention. In particular, we have proposed the possibility of a chitin or chitin-like molecule (e.g., hyaluronan) associated with cancer cells, which might serve as a target of the pyrimidine nucleotide derivative SP-1015, a putative chitin synthase inhibitor. Our preliminary chemoprevention studies support the potential of this approach by demonstrating the effects of adding SP-1015 to the diet on carcinogenesis of the mouse forestomach, even at a late stage in the process. We suggest that further consideration be given to studies targeting the formation

of cancer-associated chitins and chitin-like molecules for the prevention of cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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