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Minireview

Modified citrus pectin anti-metastatic properties: one bullet, multiple targetsVladislav V. Glinsky^{a,b,*}, Avraham Raz^{c,*}^a Harry S. Truman Memorial Veterans' Hospital, Columbia, MO 65201, USA^b Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, MO 65212, USA^c Tumor Progression and Metastasis Program, Karmanos Cancer Institute, Wayne State University, Detroit, MI 48201, USA

ARTICLE INFO

Article history:

Received 31 July 2008

Accepted 19 August 2008

Available online 26 September 2008

Keywords:

Modified citrus pectin

Cancer metastasis

Galectin-3

Adhesion

Apoptosis

ABSTRACT

In this minireview, we examine the ability of modified citrus pectin (MCP), a complex water soluble indigestible polysaccharide obtained from the peel and pulp of citrus fruits and modified by means of high pH and temperature treatment, to affect numerous rate-limiting steps in cancer metastasis. The anti-adhesive properties of MCP as well as its potential for increasing apoptotic responses of tumor cells to chemotherapy by inhibiting galectin-3 anti-apoptotic function are discussed in the light of a potential use of this carbohydrate-based substance in the treatment of multiple human malignancies.

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Metastasis, a spread of cancer from the site of a primary tumor growth to distant organs and tissues, which causes most of cancer-related morbidity and mortality, is by far the biggest clinical challenge associated with cancer. In the search of naturally occurring substances that could be useful in controlling and treating cancer metastasis, modified citrus pectin (MCP), a complex water soluble indigestible polysaccharide obtained from the peel and pulp of citrus fruits and modified by means of high pH and temperature treatment,¹ has emerged as one of the most promising anti-metastatic drugs. Ever since first reports indicating that MCP is capable of inhibiting melanoma¹ and prostate carcinoma² experimental metastasis appeared in the literature, this carbohydrate-based compound sparked significant attention among cancer research community. Since then, MCP has been shown to be effective either in vitro or in vivo, or both, against prostate carcinoma,^{2–4} colon carcinoma,^{5,6} breast carcinoma,^{4,6,7} melanoma,^{1,8} multiple myeloma,⁹ and hemangiosarcoma.¹⁰ So how does this nontoxic naturally occurring carbohydrate substance affects metastatic dissemination of various malignancies? MCP is rich in β -galactose,¹ and the main established mechanism of action for MCP is by antagonizing a β -galactoside binding protein galectin-3 (Gal-3).^{1–10} Thus, to understand better how MCP acts upon metastatic cancer spread we will follow neoplastic cells as they proceed through the metastatic cascade and discuss how MCP could affect critical rate-limiting steps in this process by inhibiting Gal-3 and Gal-3-mediated (i.e., β -galactoside-mediated) interactions.

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MCP effects on different steps in metastasis**Anoikis, galectin-3, and MCP**

Following the escape from primary tumor and intravasation, the first task that blood-borne neoplastic cells encounter is to survive the apoptosis associated with the loss of anchorage (anoikis) and a journey through the circulation. Galectin-3 has been shown to protect cancer cells from anoikis^{11,12} by regulating their transition through the cell cycle, that is, by inducing a cell cycle arrest at an anoikis-insensitive point (late G1 phase).¹¹ This effect was associated with the induction of cyclin D1 (an early G1 cyclin)¹¹ and downregulation of cyclin E and cyclin A (G1-S cyclins) levels,¹¹ as well as with up-regulation of p21(WAF1/CIP1) and p27KIP1.¹¹ Earlier work by Hsieh and Wu demonstrated that MCP may effect cell cycle regulation in human prostatic JCA-1 cells by downregulating cyclin B and cdc2.¹³ It is likely that MCP-induced cyclin B and cdc2 downregulation may result in the accumulation of cancer cells in G2/M and subsequent apoptosis induction. Therefore, it is conceivable that MCP may reduce Gal-3 anti-anoikis effect. However, at present, there is no direct experimental evidence of MCP effect on cancer cell anoikis, and further studies are necessary to investigate whether MCP can increase metastatic cell susceptibility to this form of apoptosis.

MCP effect on metastatic cell arrest in target organs

The next rate-limiting step in cancer metastasis is associated with tumor cell arrest in distant organ microvasculature. The role for Gal-3 in mediating metastatic cell adhesion to the endothelium is well established.^{14–18} Further, it appears that in vitro and in vivo

Gal-3 interactions with cancer-associated Thomsen–Friedenreich glycoantigen mediate both the initial adhesion of cancer cells to the vascular wall and subsequent tumor cell homotypic aggregation at the site of primary attachment to the endothelium.¹⁷ Thus, the anti-adhesive properties of MCP were perhaps the most and the best studied aspects of its anti-metastatic effects. From the earliest works,^{1,2,8} it was noted that anti-metastatic effect of MCP on mouse B16 melanoma^{1,8} and rat MAT-LyLu prostate cancer cells is linked to the ability of MCP for inhibiting both tumor cell adhesion to the endothelium² and their homotypic aggregation.^{1,8} In the later study,¹⁴ Lehr and Pienta demonstrated that in the panel of 11 anti-adhesion agents tested, MCP was the most potent inhibitor of human prostate cancer cell preferential adhesion to bone marrow endothelium *in vitro*. Similarly, a dose-dependent inhibition of MDA-MB-435 cells to human endothelial cells *in vitro* was demonstrated.⁶ And finally, in the most recent study, we showed that MCP is capable of inhibiting the *in vivo* formation of metastatic deposits of human breast and prostate (Fig. 1) carcinoma cells in lungs and bones by >90%.⁴ Thus, MCP is an efficient inhibitor of tumor cell adhesion to the endothelium and cancer cell homotypic aggregation involved in the initial metastatic cell arrest in distant organs and in the formation of intravascular metastatic deposits.

MCP effect on cancer cell invasion

After tumor cells lodge in target organ microvessels, they can either proliferate intravascularly, until the metastatic tumor outgrow the blood vessel and invade distant organ parenchyma,¹⁹ or extravasate before initiating a secondary tumor growth. The process of extravasation depends greatly on cancer cell invasive propensity. It involves series of tumor cell interactions with extracellular matrix (ECM) proteins associated with the basement membrane and target organ stroma. In this regard, the ability of MCP to inhibit efficiently Gal-3-mediated tumor cell interactions with ECM proteins such as laminin was reported.⁸ Further, citrus pectin polysaccharides were shown to inhibit in a dose-dependent manner the invasion through matrigel of human endothelial cells,⁶ of MDA-MB-231 human metastatic breast carcinoma cells,⁷ and human buccal metastatic cells.⁷ Based on this result, it is conceivable that *in vivo* effects of MCP on experimental metastasis of various malignancies involve inhibition of tumor cell invasion.

Effect of MCP on survival of early metastatic colonies

Following the initial arrest in distant organs and extravasation, the vast majority of cancer cells die due to apoptosis induced by various factors, and only a few of them (<2%) survive and give rise to micrometastases.²⁰ Therefore, clonogenic survival of early metastatic colonies is one of the most important rate-limiting steps determining the efficiency of the metastatic process. The main molecular target of MCP, galectin-3, is an important regulator of cancer cell apoptosis.^{21–26} Several fairly recent review articles examine in great detail how Gal-3 protects cancer cells from various forms of apoptosis.^{25–27} Importantly, as Gal-3 exerts its anti-apoptotic effects by functioning upon major (i.e., mitochondrial) apoptosis pathways,^{25–27} it could play a significant role in metastatic cancer cell clonogenic survival. It has been proposed that Gal-3 anti-apoptotic function could be targeted by MCP.²⁷ Therefore, inhibiting Gal-3 by MCP may result in a reduced clonogenic survival of cancer cells. Indeed, our recent results¹⁰ demonstrate that MCP inhibits efficiently clonogenic survival of hemangiosarcoma cells in a dose-dependent manner (Fig. 2), and this inhibition is associated with an increase in tumor cell apoptosis.¹⁰ Thus, clonogenic survival of early metastatic colonies represent yet another therapeutic target for MCP.

MCP effect on angiogenesis

As micrometastases evolve into clinically relevant secondary tumors they become critically dependent on the development of new blood vessels occurring through the process of angiogenesis. Galectin-3 has been shown to be intimately involved in endothelial cell morphogenesis and angiogenesis.^{28–31} The ability of Gal-3 to act as a chemoattractant for endothelial cells and induce endothelial cell motility, invasion through matrigel and capillary tube formation, thus functioning as a potent angiogenic factor was demonstrated.^{6,28} Hence, the ability of MCP to inhibit Gal-3 angiogenic activity was thought and successfully confirmed.²⁸ MCP blocked chemotaxis of human endothelial cells toward galectin-3 in a dose-dependent manner, reducing it by 68% at 0.005% ($P < .001$) and inhibiting it completely at 0.1% ($P < .001$).²⁸ MCP also inhibited *in vitro* capillary tube formation by endothelial cells in a dose-dependent manner.²⁸ Furthermore, angiogenesis and spontaneous metastasis *in vivo* were statistically significantly reduced in

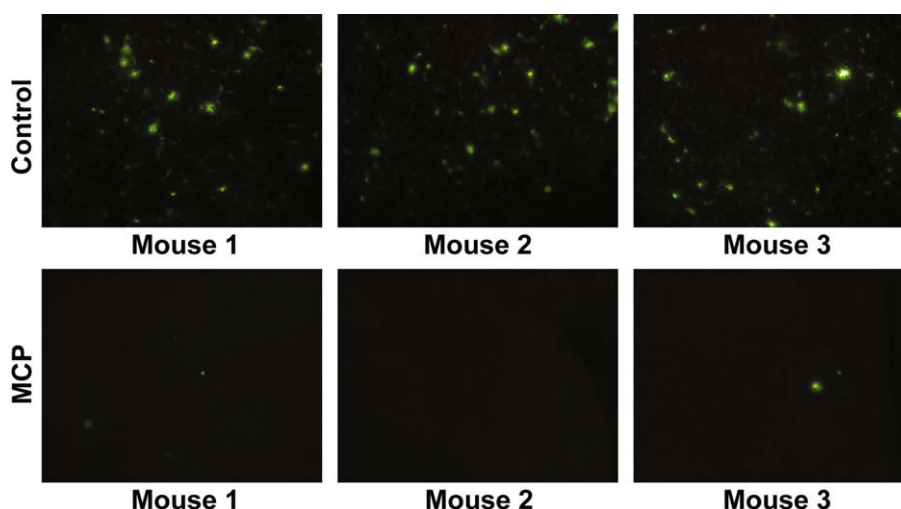


Figure 1. The effect of MCP on *in vivo* metastatic deposit formation of DU-145 human metastatic prostate carcinoma cells in mice. Six-week-old male HsdIcr:Ha(ICR)-scid mice were injected intravenously (into a lateral tail vein) with 1×10^6 of fluorescently labeled cancer cells in 200 μ l of complete RPMI-1640 medium (untreated control, top panel), or complete RPMI-1640 medium supplemented with 0.25% (w/v final concentration) of MCP (bottom panel). Three hours post injection, the animals were euthanized, lungs were removed and examined by epifluorescent microscopy.

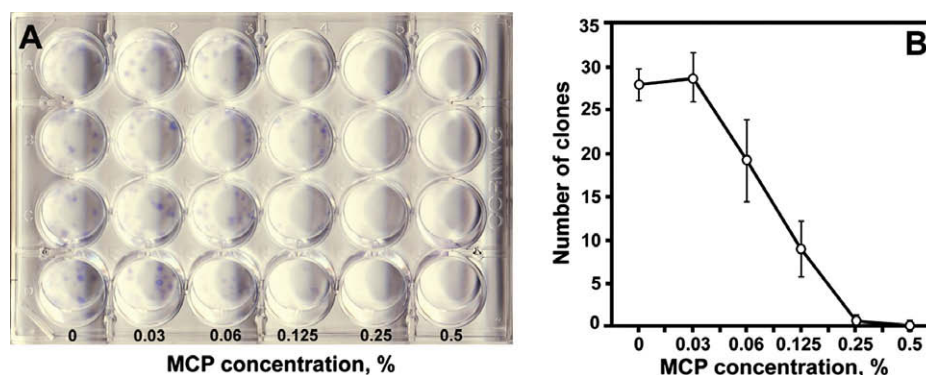


Figure 2. The effect of MCP on clonogenic survival and growth of SVR hemangiosarcoma cells. SVR cells were plated at low density (200 cell/well) in 24-well plates in the presence of increasing concentrations of MCP (from 0% to 0.5%). Seven days later, the colonies ≥ 15 cells were scored. Note dose-dependent inhibition of clonogenic survival and growth of SVR hemangiosarcoma cells by MCP. Reproduced with permission from Ref. 10.

tumor bearing mice fed MCP.²⁸ As anti-angiogenic therapy is viewed currently as one of the most promising and important aspects of cancer therapy, the ability of MCP to inhibit tumor-associated angiogenesis is an important property of this potential anti-metastatic drug.

MCP effect on cancer cell resistance to chemotherapy

The vast majority of currently used anti-neoplastic drugs act by inducing tumor cell apoptosis via the intrinsic (mitochondrial) apoptosis pathway.³² It appears that Gal-3, an important regulator of cancer cell apoptosis, suppresses mitochondrial apoptosis pathway.^{12,21,22,33,34} Consequently, Gal-3 was shown to regulate directly sensitivity of cancer cells to various chemotherapeutic agents such as cisplatin,^{22,34,35} staurosporine,²² etoposide,³⁴ bortezomib,⁹ dexamethasone,⁹ and doxorubicin.¹⁰ Thus MCP, as Gal-3 inhibitor, may hold a potential of changing dramatically cancer cell sensitivity to cytotoxic drugs by suppressing Gal-3 anti-apoptotic effect upon mitochondrial apoptosis pathway. If this is true, then it would have tremendous implications not only toward treating and controlling tumor metastasis, but also toward cancer therapy in general. To date, it was demonstrated that inhibition of Gal-3 anti-apoptotic function by MCP was sufficient to reverse multiple myeloma cell resistance to bortezomib and enhance their response to apoptosis induced by dexamethasone.⁹ In our recent study, treatment of hemangiosarcoma cells with MCP increased dramatically their sensitivity to doxorubicin-induced apoptosis, causing a 10.7-fold reduction of doxorubicin IC_{50} in vitro (from 0.0075 μ g/mL to 0.0007 μ g/mL).¹⁰ These results suggest strongly that addition of MCP to therapeutic regimens for treating Gal-3 expressing malignancies could potentially improve the effect of chemotherapy.

On a separate note, it is interesting that at least in two recent studies, in addition to enhancing apoptosis induced by cytotoxic drugs, the ability of MCP itself to induce apoptosis in cancer cells was reported.^{9,36} Interestingly, it appears that the induction of apoptosis by MCP in multiple myeloma cells proceeds through a caspase-8-to-caspase-3 signaling cascade occurring, however, in the absence of significant changes in mitochondrial membrane potential.⁹ An interesting study investigating the effect of several forms of citrus pectin on apoptosis induction in human prostate cancer cells was reported recently.³⁶ The authors reported that commercially available fractionated pectin powder (FPP) induced apoptosis approximately 40-fold above non-treated cells in LNCaP and C4-2 prostate cancer cells. In contrast, citrus pectin (CP) and the pH-modified CP marketed as PectaSol had little-to-none apoptotic activity. While glycosyl residue composition and linkage

analyses revealed no significant differences among these pectins, mild base treatment to remove ester linkages destroyed FPP's apoptotic activity, whereas heat treatment of CP led to the induction of significant levels of apoptosis comparable to that of FPP.³⁶ Based on these results, the authors concluded that specific structural elements within citrus pectin are responsible for the apoptotic activity, and that this structure can be generated, or enriched for, by heat treatment of citrus pectin.³⁶ Based on this study, it appears that pH treatment is not important for generating apoptosis inducing forms of citrus pectin. However, earlier studies demonstrated that pH modification is critical for anti-adhesive properties of MCP.^{1,2,8} Thus, it is possible that a combination of pH and temperature treatment used in preparing MCP^{1,2} is an optimal combination for generating pectic polysaccharides with both anti-adhesive and apoptosis inducing properties.

Conclusions

Due to its anti-adhesive, apoptosis-promoting, and apoptosis-inducing properties, it appears that MCP is capable of targeting multiple critical rate-limiting steps involved in cancer metastasis (Fig. 3). In addition, by inhibiting Gal-3 anti-apoptotic function and enhancing apoptosis induced by cytotoxic drugs, it holds the potential to increase dramatically the efficiency of a conventional chemotherapy. The progression of this promising anti-cancer agent into clinical practice, hampered by various factors, was rather slow. Nevertheless, limited clinical studies performed to date demonstrated that MCP significantly increased prostate specific antigen doubling time in patients with recurrent prostate cancer,³⁸ thus confirming its potential usefulness in treating prostatic neoplasia. As the potential and the necessity of developing MCP-based

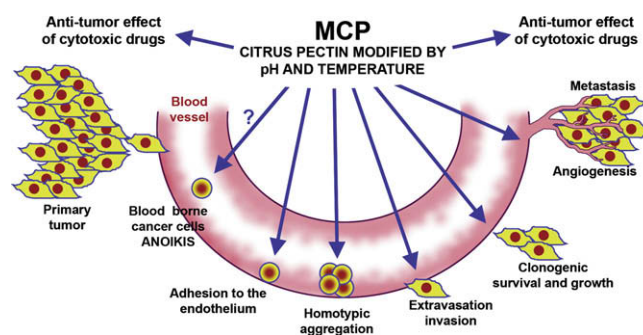


Figure 3. A schematic representation of critical rate-limiting steps in cancer metastasis, which could be efficiently targeted by MCP.

pharmaceuticals and nutraceuticals is becoming more and more commonly recognized,^{27,36,37} the addition of MCP to armamentarium of anti-cancer drugs holds the promise of improving treatment of multiple human malignancies.

Acknowledgments

V.V.G. is supported by the funds from the VA Biomedical Laboratory Research and Development Service. A.R. is supported by the NIH Grant R37CA46129.

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