

# **A New Approach to Metastatic Cancer Prevention: Modified Citrus Pectin (MCP), A Unique Pectin that Blocks Cell Surface Lectins**

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## **Abstract**

Citrus pectin (CP) is a commercially available, water-soluble fiber with proven health benefits. The branching polysaccharide structure of CP can be altered to produce a lower molecular weight, galactose-rich, modified citrus pectin (MCP) which has unique properties. Specifically, MCP, but not CP, might help retard cancer metastasis by combining with an array of galactose-specific proteins on the cancer cell surface called galectins (for galactose-specific lectins). As with many human cancer cell lines that have been studied, the potentially metastatic B16-F1 (mouse melanoma) and MLL (rat prostate) cells carry galectins, cell surface proteins that bind to galactose on neighboring cancer cells and oligosaccharides on the host cell surface. MCP inhibits metastasis by the cells in the mouse and the rat, respectively. Unlike the much larger CP polysaccharide, galactose-rich MCP may be small enough to access and bind tightly with galectins on the cancer cell surface, saturating the galactose binding sites of the cancer cell lectins, and thereby inhibiting both aggregation of tumor cells and adhesion to normal cells. Thus deprived of adhesion, the cancer cells fail to metastasize. Undeniably, important gaps still exist in the current understanding of MCP's clinical efficacy and its mode(s) of action. But MCP's apparent safety and proven anti-metastatic action, and the lack of proven therapies against metastasis, together may justify its inclusion into comprehensive orthomolecular anticancer regimens.

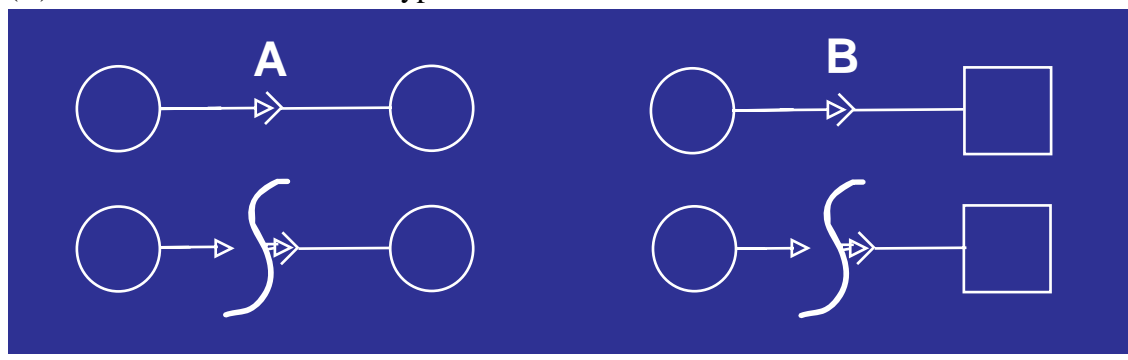
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## **Citrus Pectin (CP) and Modified Citrus Pectin (MCP)**

Pectin is a water-soluble fiber that is present in the cell walls of all plant tissues, where it functions as a cementing material to hold adjacent cells together. As a human dietary supplement, pectin prepared from citrus (CP) offers multiple health benefits.<sup>1-3</sup> Results from preliminary animal studies indicated that the biological effects of pectin could be altered by modifying its methyl group distribution patterns. More recent evidence indicates that a more extensively modified citrus pectin (Modified Citrus Pectin, MCP) may be capable of retarding cancer cell metastasis. MCP may specifically combine with, and block, lectin molecules on the cell surface that mediate metastasis. These findings and their implications for human cancer management are the subject of this review.

**FIGURE 1.**

Cell-to-cell interactions mediated by lectins. (A) Between cells of the same type; (B) between cells of different types.



Chemically, pectin is the collective name for a group of heterogeneous, high molecular weight, branched polygalacturonic acid polysaccharides. The galacturonic acid makes up the bulk of a central chain, which is composed of partially esterified D-anhydrogalacturonic acid (AGA) units, linked through alpha (1—4) glycosidic bonds and interrupted in places by (1—2) linked L-rhamnose sugars. The side chains are composed mostly of the sugars D-galactose, L-arabinose, D-glucose, and D-xylose. Typically, some of the hydroxyl groups of the sugars are methoxylated, and the most common pectin as isolated from citrus (citrus pectin, CP) contains up to 10% methyl ester groups.

Commercially available CP is extensively branched and averages 70-100 kDa. MCP was first produced by the relatively crude technique of high-pH treatment to degrade the main galacturonic acid chains followed by low-pH treatment to partially degrade the carbohydrates. These steps result in more or less nonbranched, smaller carbohydrate chains averaging 10 kDa.<sup>4</sup> More elegant techniques (e.g., U. S. Patent 5,498,702) have since been developed that more effectively standardize the product.

### Modified Citrus Pectin and Experimental Metastasis

MCP produces markedly different biological activity than does CP, since MCP contains relatively more sugar groups able to specifically block the surface carbohydrate binding proteins known as lectins. Galactose-binding lectins (galectins) are thought to give cancer cells much of their metastatic potential. Figure 1 illustrates various types of cell-to-cell interactions mediated by lectins.

Metastasis, the spread of cancer throughout the body, has been described eloquently<sup>5</sup> as the “apotheosis of neoplastic transformation.” Whatever one’s philosophical perspective on this pathophysiologic process, metastasis is undeniably one of the most life-threatening aspects of cancer. At this time, very few effective therapeutic tools against metastasis exist. Preliminary research findings suggest MCP does have value for this application, especially since animal studies suggest that there may be no acute toxic effects in humans.

Research aimed at elucidating the mechanisms of metastasis has been ongoing for more than a century, and this process (or family of processes) has been well studied in various animal and “*in vitro*” experimental models.

**Metastasis proceeds in stages:**

- A malignant tumor develops somewhere in the body;
- At some point in the tumor's development, groups of cells break away (called detachment);
- These detached tumor cells invade through the surrounding solid tissue to reach body cavities, the lymphatics, or the blood stream;
- Cells are transported by blood or lymph to distant tissues;
- Cells arrest on the distant tissues, invade, and proliferate.

The essence of metastasis is the transfer of cells from one location within the body to another. With respect to how groups of malignant cells become translocated, two schools of thought exist, both of which appear to have validity to clinical metastasis.<sup>5,6</sup> One is the "anatomical-mechanical" model, which maintains that translocation has to do more with physical events that "trap" cells in certain locations. The other is the "seed-soil" model, which propounds that metastatic tumor cells take root in another tissue only as they are allowed by favorable conditions for attachment and proliferation at that location. The current consensus position is that these suggested mechanisms are not mutually exclusive. Both are seen clinically, and in the very same tumor system some malignant cells can metastasize mainly by "anatomical-mechanical" and others by "seed-soil" mechanisms.<sup>6</sup>

The life-threatening nature of metastasis dictates that virtually all the studies on its mechanisms must be done in animal models rather than in human subjects. However, animal models can at best offer only clues to understanding human disease. In the case of metastasis, the usual limitations of animal models are further complicated by the profound changes that tumor cell lines undergo when maintained in culture. For animal cancer models to have clinical relevance, they must first

be carefully evaluated and refined. In this context, one of the better animal models for the study of metastasis is the mouse B16-F1 melanoma, which is frequently used to study metastasis, in part because it is highly metastatic in predictable patterns.

Following injection into the mouse tail vein, the B16-F1 melanoma cells consistently metastasize to the lung. Platt and Raz,<sup>7</sup> to compare the anti-metastatic potential of CP to MCP, used this system in two experiments with the C57BL/6 mouse. The first utilized 53 mice in 5 groups, and the second, 125 mice in 3 groups. After 17 days, the mice were autopsied and the tumor colonies in the lungs were counted. The results were clear-cut: CP failed to block metastasis, whereas MCP blocked metastasis in the concentration ranges of 0.05-0.50% contained within the 0.2 cc used for injection.

This study successfully demonstrated the anti-metastatic potential of MCP. However, the one-time administration of cancer cells into the circulation of the mouse is not likely to be representative of the situation in the human cancer patient. Such a patient is likely to have one or more primary tumors that seed off a significant number of cells on many occasions over a prolonged period of time. Results from more experiments with MCP, this time done with rats, do give further support for a clinical anti-metastatic action of MCP.

Raz and his collaborators<sup>8</sup> have also studied metastasis in the Dunning rat prostate model. Dunning developed this metastasis model from a spontaneously occurring prostate adenocarcinoma found in the male Copenhagen inbred rat. Selection in culture from the primary tumor led to an aggressively cancerous subline called MAT-LyLu (here called MLL). Injection of 1 million MLL cells into the male rat predictably leads to death

within 27 days. Metastasis begins at 10-12 days after the inoculation. If the primary tumor is removed by limb amputation before this point, the animals do not die; if amputation is performed after day 12 the animals die of lung and lymph node metastases by day 40.

Pienta, Raz, and their collaborators<sup>8</sup> used this MLL-Copenhagen rat model to evaluate the efficacy of orally administered MCP against metastasis. On day 4 after injection with the MLL cells, groups were put on drinking water containing 0.00%, 0.01%, 0.10%, or 1.00% MCP. On day 14 the tumor-bearing hind limbs were amputated under anesthesia, and the tumors weighed. At day 30 the animals were sacrificed and the numbers of MLL secondary tumors counted.

Orally administered MCP did not affect primary tumor growth in the rats. However, MCP did inhibit metastases. In the 2 control groups (0.00%, 0.01% MCP), 15 of the 16 rats had lung metastases (the 16th had only lymph node metastases). Of the groups of rats receiving 0.10% and 1.0% of MCP in their water, roughly half in each group had no metastases. In addition, the 1.0% MCP group had markedly fewer metastases in the lungs ( $P < .001$ ) and lymph nodes ( $P < .01$ ) when compared to controls.

The effective concentrations of MCP fell within the range of the earlier mouse experiment, in which MCP reduced the metastatic viability of the B16-F1 melanoma cells when mixed together with them prior to injection. Pienta et al<sup>8</sup> note that “the concentrations of modified citrus pectin that inhibit metastasis *in vivo* are similar to the concentrations of modified citrus pectin that inhibit adhesion and colony formation *in vitro*. This may reflect that concentrations in the bloodstream may need to be similar to those observed “in vitro.” To better critique these ex-

perimental findings in the context of clinical metastatic cancer, it is necessary to explore the cell-level mechanisms of the biological action of MCP.

### The Biology of “Fit”: Cell Surface Lectins and Metastasis

Both the “anatomical-mechanical” and “seed-soil” hypotheses for cancer cell metastasis require that cancer cells first must adhere to a new tissue into which they have been transported, then they must stabilize and proliferate to establish new tumor foci.<sup>6</sup> For adherence to occur, the cancer cells must have some capacity to specifically recognize a particular type of host tissue. Specific molecules are located on the cancer cells’ surfaces to ensure these functions. Although the basic processes of cell-to-cell recognition appear to be the same in normal and abnormal cells, the cancerous cells seem to have their own specific surface patterns that favor specific recognition of host tissues compatible with their survival.<sup>6,9-13</sup>

Cell-to-cell recognition occurs by way of molecules that interact following the principles of “lock-and-key” correspondence, or simply “fit.” This concept was first championed by Emil Fischer almost a century ago, to account for the specific interactions between enzymes and their substrates in solution. Later, it was extended by Ehrlich, then by Lillie, to the interactions between cells in the solid phase.<sup>9</sup> It is now known that a type of “fit” between cell surface recognition molecules underlies the preferential associations between metastasizing cancer cells and their target organs.<sup>10-13</sup>

The modern concept of “fit” in cell-to-cell adhesion involves two major components of the cell surface: a carbohydrate-rich protein (glycoprotein) and a carbohydrate-poor

protein (lectin).<sup>9</sup> Details of the recognition phenomenon still remain to be elucidated, but it appears to be dependent upon the characteristic surface patterning of thousands of glycoprotein and lectin molecules carried by each cell. As one cell comes into contact with another, the information inherent in the patterns of the glycoprotein sugars can be “recognized” by lectins on the other cell. The multiple “keys” on the two cells can then fit into the multiple “locks” of both, and recognition occurs (see Figure 1). Structural and functional changes are then triggered in both cells. The two cells have a number of options for further association: they can proceed into a more stable, closer association; they can move away from each other; or, (as in the case of an immune cell coming in contact with a cancer cell or a bacterium) they can begin to conduct hostile actions toward each other. Interestingly, the “homing” of lymphocytes to lymph node endothelium is a process closely related to metastasis.<sup>14</sup>

Malignant cells capable of metastasis tend to differ in their surface glycoprotein and lectin distribution patterns from normal cells.<sup>15,16</sup> Two pro-metastatic lectins have been identified that have molecular weights of about 14,500 and 34,000 kDa, respectively. Also, tumor cells with higher metastatic potential tend to carry more of these lectins on their surfaces,<sup>10-13,17</sup> although there may be exceptions to this trend.<sup>18,19</sup> These differences appear to be the basis for the efficacy of MCP in blocking metastasis.<sup>11</sup>

There is a consistent correlation between the capacities of B16-F1 cells to aggregate and their potential to metastasize *in vivo*—the greater the aggregability, the greater the capacity to metastasize.<sup>20</sup> In their study, Platt and Raz<sup>7</sup> found that CP increased the aggregation of the cells. This could help explain why data from the coinjection experiment indicated CP may have increased metastasis over

controls. In contrast, MCP blocks cancer cell aggregation and reduces metastatic potential.

In these B16-F1 aggregation experiments, MCP almost completely blocked cell aggregation.<sup>7</sup> This outcome is consistent with MCP’s almost complete inhibition of metastasis into the mouse lung.<sup>8</sup> Furthermore, Pienta et al<sup>8</sup> measured the capacity of MLL cells to adhere to rat aortic endothelial cells, an assay which simulates metastasis via blood circulation.<sup>21</sup> MCP proved to be a potent inhibitor of MLL adhesion.

Pienta et al<sup>8</sup> also cultured MLL cells in a semi-solid medium of agarose to simulate the cells’ ability to proliferate and establish new metastatic foci. Here also MCP blocked malignant cell colony formation, an important stage of the metastatic process, at concentrations of 0.01-1.00%. The investigators searched the MLL cell surfaces for a particular lectin that targets galactose residues, known as gal-lectin or galectin, and confirmed its presence. Similarly, the B16-F1 melanoma cells also were found to carry galectins on their surfaces.<sup>22</sup> To further confirm the relevance of these experimental findings to human prostate cancer, Pienta et al<sup>8</sup> also looked for galectin in human prostate cancer tissue biopsies specimens. They found galectin in copious quantities in this variety of human cancer specimens.

### **Conclusion: MCP’s Promise and Limitations**

Commercially available citrus pectin (CP) can be structurally altered using a series of pH changes to separate the large, branching, tree-like pectin molecule into smaller and more linear pieces that average 10,000 kDa. This modified citrus pectin consists of small polysaccharides rich in galactose residues. The potentially metastatic B16-F1 melanoma, MLL, and numerous other neoplastic lines



carry galectins -- cell surface proteins that seek out galactose. MCP blocks metastasis of these cells in both mouse and rat models. Unlike the much larger CP polysaccharide, the galactose-rich MCP may be small enough to access and bind tightly with galectins on the cancer cell surfaces, thus blocking their access to the galactose of the host cell surfaces or the intercellular matrix. Deprived of a close enough approach to these surfaces, the cancer cells fail to adhere or proliferate and thus fail to metastasize.

The significant reduction of experimental lung metastasis by the coinjection of MCP with B16-F1 melanoma cell is not unique, since other substances are known to block cancer cell metastasis in this system. Simple sugars like galactose and lactose can block pro-metastatic lectins and interfere with metastasis *in vivo*,<sup>23,24</sup> but these are unlikely to be suitable for chronic oral intake due to the likelihood of development of gastric intolerance and the rapid metabolism of simple carbohydrates. However, in another animal model more representative of clinical metastasis, MCP did largely prevent metastasis when given orally with the drinking water, beginning 4 days after inoculation of the cancer. As a soluble fiber, MCP poses minimal risk of triggering intolerance. MCP still has not been put to the major challenge that the clinician must constantly face, namely to prevent metastases. But MCP is deserving of further research to assess just this capacity, for several reasons:

- Currently, few other promising anti-metastatic interventions are available;
- MCP inhibits metastasis in 2 animal models;
- Although unproven against established metastases, MCP could provide benefit to the cancer patient solely by blocking further metastasis;
- The mechanism of action of MCP is

rationally understood;

- MCP has been found to be effective with both oral and intravenous administration; and
- MCP is unlikely to be toxic, even at high levels of intake.

Due to the heterogeneity of human cancers and the variability and complexity of their metastatic patterns it is unlikely that a single agent will be discovered that prevents all metastasis. However, in a recent presentation to the American Association for Cancer Research, Naik, Pienta, Raz, and others<sup>25</sup> reported that MCP blocked the adhesion to blood vessel endothelia of 5 different human cancer cell lines: human prostate adenocarcinoma PC-3, human breast carcinoma MCF-7 and T-47D, human melanoma A-375, and human laryngeal epidermoid carcinoma HEp-2.

Decades of intense study into the mechanisms of metastasis have finally begun to spawn rational approaches to its clinical management. Whether MCP will ever attain the status of a major breakthrough for the management of metastatic cancer awaits the necessary clinical trials. Nonetheless, from its record to date, MCP should be a prime candidate for further clinical investigation.

It would be counterproductive to deny that important gaps exist in the current understanding of MCP, its clinical efficacy and its mode(s) of action. But MCP's apparent safety and proven anti-metastatic action, and the lack of proven therapies against metastasis, would justify its inclusion into comprehensive orthomolecular anticancer regimens.

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