Abstract
There is emerging evidence that inflammation may lead to prostate cancer development. Although inflammation is an essential response to injury or infection, chronic inflammation is harmful and causes tissue damage. Increasing evidence suggests that inflammation leads to the development of epithelial cancers; however, studies on inflammation-targeted genes that might contribute to the development of cancer are at the beginning stage. Here, we describe macrophage inhibitory cytokine-1, which provides a potential link between inflammation and prostate cancer. Understanding the regulation of macrophage inhibitory cytokine-1 in response to inflammation may have potential for novel therapeutic strategies. [Cancer Res 2009;69(1):2–5]

Introduction
Prostate cancer is the most common noncutaneous malignancy in men in Western countries, accounting for one-third of all male cancer diagnoses and 9% of all deaths due to cancer. Surgery, radiation, and chemotherapy are the most common options for localized prostate cancer treatment; however, recurrence or metastasis eventually lead to death for ~30,000 patients every year. Because the average life expectancy is continuously rising, it is estimated that there will be a 2.5-fold increase in the incidence of prostate cancer by the year 2050.

Although cellular, molecular, or genetic studies in tumor cell lines have provided supporting evidence associated with cancer growth and metastasis, they cannot duplicate the in vivo development of metastatic lesions without the supporting tumor microenvironment. Cancer in the prostate is associated with increasing age, which simultaneously increases the susceptibility of prostate tissue to injury or to the chance of infection leading to an inflammatory response. It has long been suggested that the extent of inflammation plays a crucial role in the development of the tumor microenvironment. Such inflammation might trigger an influx of various innate immune cells potentially contributing to a protumor environment. Genetic and epigenetic modulations have been linked to the inflammation seen in prostate cancer. However, studies to analyze the direct effect of inflammation on prostate cancer are limited due to a lack of suitable animal models and defined inflammation-associated molecules. In this review, we discuss the potential interplay between inflammation and prostate-derived factor [PDF/macrophage inhibitory cytokine (MIC-1)] that has recently been established as contributing to prostate tumor progression.

MIC-1 and Macrophages
MIC-1 is a member of the transforming growth factor-β superfamily and was first isolated based on its increased mRNA expression associated with macrophages. In monocytes, MIC-1 expression is up-regulated by interleukin (IL)-1β, phorbol myristate acetate, tumor necrosis factor-α, IL-2, and macrophage colony-stimulating factor. Macrophages are one of the sentinel cells of the innate immune system and have a significant influence on the overall development of the body’s immune response. One of the main functions of macrophages is to provide a defense mechanism against cancer cells in which the destruction of tumor cells via macrophages involves cell contact–dependent and cell contact–independent mechanisms. On the other hand, macrophages also contribute significantly to the tumor microenvironment by secreting a wide array of biologically active molecules that participate in tumor cell migration and metastasis. The presence of MIC-1 in the tumor microenvironment can inhibit the secretion of tumor necrosis factor-α by activated macrophages, therefore, reducing the tumor killing (functional) activity of macrophages.

MIC-1 and Prostate Cancer
Identification of molecules associated with carcinogenesis or tumor growth and metastasis has been critical to developing potential therapeutic interventions. Gene microarray studies have provided a unique opportunity to identify molecules associated with the development and progression of various cancers. Functional analysis of such identified genes has greatly improved our potential for therapeutic interventions. Using a progressive in vitro cell culture model of a human androgen-sensitive prostate cancer cell line (LNCaP-C33), cDNA microarray studies revealed an up-regulation of MIC-1 in the cells of later passages (LNCaP-C81; androgen-independent; ref. 11). In continuous cultures, these LNCaP cells (C33 and C81) maintained a similar genetic profile, displayed useful characteristics such as aggressive cell growth (both in vitro and in vivo), higher levels of prostate-specific antigen (PSA) secretion, and produced a similar level of functional androgen receptor protein as seen in patients with advanced prostate cancer.

Although expressed in a variety of cells including breast, gastric, and colorectal cancer cells, MIC-1 has drawn significant...
attention due to its increased association with high-grade prostate tumors. Protein profiling on microdissected samples of matched normal prostate tissue, high-grade prostatic intraepithelial neoplasia, and prostate cancer revealed MIC-1 expression in high-grade prostatic intraepithelial neoplasia and in cancer cells but not in normal cells (13), hence, implicating a potential role for MIC-1 in the pathogenesis of prostate cancer. Studies in human prostate cancer cell lines (such as LNCaP, PC3, MDAPCa2b, and DU145) revealed MIC-1 expression restricted to the cell lines expressing the androgen receptor (LNCaP and MDAPCa2b; refs. 14, 15).

MIC-1 is a secretory protein and elevated serum MIC-1 levels are associated with a number of disease conditions (16–18). Specifically in the prostate, increasing serum MIC-1 levels are associated with the progression of metastatic prostate cancer. It is well established that secreted levels of PSA correlate with prostate cancer cell proliferation. Similarly, forced expression of MIC-1 induces LNCaP cell proliferation as well as secretion of PSA (14), demonstrating a correlation between the increased expression of MIC-1 and PSA with aggressive growth in prostate cancer cells. Studies have demonstrated that serum MIC-1, as a diagnostic marker in combination with PSA, show an improved specificity for prostate cancer detection and is a predictor of bone metastasis (19, 20). Thus, a combination of markers including MIC-1 may help in improving the specificity of PSA and help reduce unnecessary biopsies (19, 20).

### Immunobiology of Prostate and Activation of MIC-1

The pathogenesis of prostate cancer is believed to involve environmental, hereditary, as well as possible other unknown factors. Exposure to environmental factors or infectious agents might trigger the inflammatory states that may account for up to 20% of all human cancers (21). Inflammation is a localized and highly regulated host response to fight infection and promote wound healing. Tumor necrosis factor and other proinflammatory cytokines are important mediators of inflammation produced by activated macrophages and other immune cells (3). The prostate is populated by a small number of T cells, B cells, macrophages, and mast cells, and T cells can be seen as early as 12 weeks of gestation (22). Although the causes of prostatic inflammation are not always clear, there are various potential events, i.e., direct infection, urine reflux, dietary factors, or hormonal imbalance with age that may provoke such a reaction (3). Most adult prostate tissues contain increased inflammatory cells depending on the extent and type of inflammation. This results in an increased pool of cytokines and growth factors, including IL-1, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, and transforming growth factor-β and IFN-γ in the prostate tissue (3, 22). Controlled regulation of the inflammatory response is not harmful, however, it is of particular interest that cytokines such as IL-4, IL-10, and IL-13 significantly influence the biological behavior of monocytes/macrophages and result in a microenvironment that favors tumor growth (23). In recent years, it has been projected that inflammation and a proinflammatory microenvironment make important contributions to tumor development. Interestingly, MIC-1 has been associated with an inflammatory-related pathway, and is postulated as a biomarker for p53 pathway activation, which is a key responder to inflammatory stress (24).

When comparing matched prostate cancer and normal prostate specimens, most studies describe an increased expression of MIC-1 in cancer and nondetectable levels in normal tissue. Gene expression analysis between normal peripheral zones and transition zones of the specimens obtained from patients with prostate cancer revealed a preferential expression of MIC-1 in the peripheral zone (predominant site of tumor occurrence) compared with the transition zone (site of benign prostatic hyperplasia; ref. 25). Paralkar and colleagues also reported the expression of MIC-1 in adult human prostate tissues (26). Indeed, this is an indication that MIC-1 expression may be found in normal adult prostate due to an inflammatory response. An induction in MIC-1 expression has also been reported in kidney, lung, and liver tissue due to injury following surgical, chemical, or heat shock methods (27). Further supporting this theory are our studies using prostate tissues from 4-week-old PSA-transgenic mice in which the expression of MIC-1 was very low to nondetectable, whereas its expression increased in the adult (10–16 months) mouse prostate. It is likely that induction of MIC-1 is an early response due to inflammation, infection, or injury in the prostate for cell growth advantage leading to an environment in favor of prostate cancer development. Because innate immune cells are the first responders in any infection, macrophages (the key components of an inflammatory response) may play a key role in regulating the level of MIC-1 in the prostate. Therefore, it is important to understand the contribution and regulation of MIC-1 involving macrophages in prostate carcinogenesis.

### Putative Function of MIC-1

The role of MIC-1 has been implicated directly with cancer, in which both antiapoptotic and proapoptotic effects have been described in a variety of tumor cell types. Experimental studies using human prostate LNCaP cells support the role of MIC-1 in cell proliferation. Overexpression of MIC-1 by transfection in LNCaP-C33 cells induces aggressive cell growth, whereas knocking down MIC-1 in LNCaP-derived subclones (C81 and LNCaP-Ln3: LNCaP cells highly metastasis to lymph nodes) using antisense oligonucleotides inhibits cell growth and proliferation (14). However, in the androgen-independent prostate cancer cell line PC3, gene transfection studies have shown that forced expression of MIC-1 in PC3 reduces the growth of the tumor in nude mice (28). Similarly, treatment of the androgen-insensitive prostate cancer cell line DU145 with recombinant MIC-1 showed loss of adhesion and induction of apoptosis (29). Also, MIC-1 overexpression in colon cancer cell lines reduces the growth of xenograft tumors (30), but serum MIC-1 levels were positively correlated with tumor stage and metastasis (31). Despite ambiguous observations in various tumor cell types, data obtained from clinical studies has established increased serum MIC-1 levels with the progression of disease to metastasis, implicating the role of MIC-1 in prostate tumorigenesis and metastasis.

In prostate cancer, MIC-1 may play its role as a paracrine and autocrine factor for the abnormal proliferation of androgen receptor–positive prostate cancer cells (14) and may influence the microenvironment in favor of prostate cancer growth. Due to its reductive effect on cell adhesion, MIC-1 may have a role in tumor dissemination, an event which is essential for the development of metastatic cancer cells (29).

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3 D. Karan, unpublished observations.
Prospective Modulation of MIC-1 Function for Tumor Disadvantage

Because macrophages are the key components of the inflammatory responses and function as key regulators of the activities of many of the other cell types involved in inflammation, they can be educated toward the construction of a microenvironment in favor of tumor suppression. Macrophages are effector cells in the innate immune system and play an important role in antitumor immune reactions (32). Macrophage-mediated antitumor effects could be augmented using toll-like receptor (TLR) agonists, which have been shown to induce the regression of tumors through the engagement of both innate and adaptive immune systems (33). It has been suggested that systemic injection of TLR agonists can induce an antitumor effect via macrophages in the absence of natural killer and T cells (32). Such an effect could be possible by modifying the tumor microenvironment. Therefore, direct or indirect activation of macrophages might regulate the physiologic environment as a consequence of inflammation, leading to modulation of the function of MIC-1, and ultimately, tumor growth suppression. In Fig. 1, we describe the summary of potential events controlling the regulation of MIC-1 by educating the macrophages. It has been shown that treatment of macrophages with TLR9 agonist (CpG ODN) activated the nuclear factor-κB signaling cascade, which is important in cancer-related inflammatory response. CpG-induced nuclear factor-κB activation in tumor-associated macrophages increased the antitumor activity of these cells and reduced their M2 polarization (34, 35). Given the complexity of the immune system network and the multidimensionality of host-tumor interactions, use of MIC-1 as a “signature molecule” can elucidate the molecular kinetics of inflammatory responses. Studies using animal models are needed to validate that targeting key inflammatory components such as MIC-1 to modulate the physiologic environment in favor of tumor disadvantage can delay the initiation of prostate cancer. Therefore, targeting macrophages via TLR agonists to modulate MIC-1 function may catalyze the development of new treatments to prevent prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Macrophage Inhibitory Cytokine-1: Possible Bridge Molecule of Inflammation and Prostate Cancer

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