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Detection and characterization of circulating and disseminated prostate cancer cells

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TABLE OF CONTENTS

1. ABSTRACT

The dissemination of prostate cancer cells to secondary sites appears to be an intermediate step in the formation of tumor metastases. However, the significance of tumor cell dissemination into the blood and bone marrow as well as the characteristics of these cells remains largely unknown. In attempts to correlate the presence of disseminated tumor cells with disease prognosis, studies have utilized a range of molecular and histologic techniques. The results of this research have been largely inconclusive in terms of clinical utility. Nevertheless, they have demonstrated that these cells are detectable and present much more often than would be expected based on the rate of prostate cancer recurrence. Further research has thus begun to focus on the isolation of individual disseminated tumor cells which can then be analyzed with techniques such as gene expression microarrays and comparative genomic hybridization in order to better characterize the cells. This review paper will examine the various methods of detecting disseminated tumor cells in patients with prostate cancer and the results of studies correlating these cells with clinical variables. Additionally, we discuss the isolation and analysis of disseminated cells and examine their potential value in helping to understand the relationship between these cells and tumor metastasis.

2. INTRODUCTION

Prostate cancer is not only an important malignancy from a clinical standpoint, but it provides special opportunities to study metastatic mechanisms. It is the most common malignancy in men, with an estimated 234,460 new cases in 2006 in the United States. This constitutes one-third of all new male cancer diagnoses, and the age-adjusted trend has been steadily rising over the past 30 years (1). Additionally, although 91% of cases are considered "localized" compared with 5% staged as "distant" (4% "unreported"), a significant number suffer so-called "recurrence." For example, after radical prostatectomy an estimated 20-30% of patients suffer recurrence, which is really persistent disease after the prostate is removed. The characteristics of this persistent disease are especially evident because PSA, which, after the prostate is out, can generally only be elevated in the blood if cancer cells remain. Numerous studies have demonstrated that PSA elevations—and thus evidence of prostate cancer "recurrence"—are most common and of the worst prognostic significance in the initial post-operative period. For example, after radical prostatectomy, a PSA doubling time less than 3 months is associated with a significantly shorter time to death (2, 3). However, PSA elevations may first occur as long as ten to fifteen years

after surgery, suggesting a prolonged dormant period for the tumor cells. Studies looking at PSA recurrence after prostatectomy have shown that the probability of recurrence steadily increases years after surgery at all stages and grades (4, 5). Moreover, there is often great delay between PSA elevation and metastasis. Thus, in patients with a PSA recurrence after prostatectomy, the median time from recurrence to metastasis (which almost always is to the bone) has been estimated at 8 years, and from metastasis to death at 5 years (6). The phenomenon of "cancer dormancy" is therefore evident and observable in prostate cancer. We will discuss dormancy later on in this review.

The presence, location, and characteristics of the tumor cells that give rise to these recurrences are therefore of great interest from both a biologic and clinical standpoint. Whether these metastases arise from pre-existing micrometastases or from persistent local disease has not been clearly elucidated. However, the dissemination of the cancer cells to distant sites via the vasculature is certainly a pre-requisite for metastasis. The search for cancer cells in the vasculature is not new—these cells were being investigated over fifty years ago (7, 8)—but only in the last fifteen years has technology allowed significant progress in the field. The literature utilizes primarily two terms, circulating tumor cells (CTC) and disseminated tumor cells (DTC), somewhat interchangeably to define these cells. For this review, CTC will refer to the tumor cells in the peripheral blood and DTC will refer to tumor cells in lymph nodes or bone marrow. Here, we review the current literature regarding CTC and DTC in prostate cancer. The characterization of these cells using molecular, genetic, and histologic techniques has yielded many insights while resulting in additional questions regarding the nature of metastasis in prostate cancer. With further investigation and analysis, the hope is that these cells will not only reveal prognostic information for patients with prostate cancer but also provide biological and molecular targets for further therapy.

3. METHODS OF STUDYING CIRCULATING AND DISSEMINATED TUMOR CELLS

A variety of approaches have been employed in the study of CTC and/or DTC. These methods include immunohistochemistry, flow cytometry, and reverse transcriptase polymerase chain reaction (RT-PCR). Usually, these studies involve some degree of tumor cell enrichment. For example, using density gradient centrifugation to isolate cells, the mononuclear cells (MNC) and apparent disseminated tumor cells are separated from the other cells in the specimen (9, 10). Most commonly, Ficoll-Hypaque, a solution with a density of 1.077 g/ml, is employed. Since red blood cells and granulocytes have densities >1.077 g/ml and MNCs have a density <1.077 g/ml, centrifugation utilizing Ficoll-Hypaque helps create a layered separation of these cell types. The layer containing the MNCs—and the disseminated tumor cells, if present—can then be used for further study. However, the density separation process is not perfect and one should expect to loose some tumor cells as aggregation can result in cell densities exceeding 1.077 g/ml.

3.1. Immunohistochemistry

In the late 1980s, the study of CTC/DTC was initially performed using immunohistochemistry. A key issue was—and still is—the choice of cell marker for targeting. In most cancers there is no known tumor specific marker, and often there is no organ-specific one. Consequently, investigators were forced to use markers of embryonic origin, such as epithelial markers for adenocarcinomas. This necessity left one with no assurance that the detected cells are in fact cancer cells. However with evolving sophistication in the subsequent characterization of these cells and the wealth of data showing significant differences between controls and cancer patients, the scientific community is, generally, embracing the assumption that the cells detected by these non-specific approaches are malignant. This is why discussions of these studies fall under the rubric of CTC/DTC. The goal of these initial immunohistochemistry studies has been to confirm the presence of these CTC/DTC in individuals with prostate cancer and, in turn, to determine whether their detection offered any prognostic significance. A discussion of specific relevant cell characterization studies will be presented subsequently.

By far the most common immunohistochemistry approach to detect CTC and DTC has been to target the cytokeratins. Of course, cytokeratins are expressed abundantly in the cytoplasm by epithelial cells and therefore do not offer any cancer type specificity. Other examples of markers common to epithelial cells are human epithelial antigen (HEA) and epithelial cell adhesion molecule (EpCAM). In theory, studies involving prostate cancer patients have the advantage of more organ-specific markers, and some investigators have attempted to utilize PSA as a target (11). However, in part due to the variability in PSA expression PSA staining has not been as successful as hoped and most immunohistochemistry studies have focused on cytokeratin staining. These studies, however, have had varying success and yielded somewhat disparate results as we will now briefly review.

Bretton *et al.* (12) studied 20 prostate cancer patients using a panel of three monoclonal antibodies directed against cytoskeletal and membrane antigens. Twenty-two percent of their patients with localized prostate cancer stained positive for epithelial cells in their bone marrow, and 36 percent of their patients with known metastatic disease were positive for these cells. Notably, in patients with localized disease, the serum PSA was significantly higher in those who tested positive compared with those who tested negative (26.6 ng/ml vs. 12.3 ng/ml). Pantel *et al.* (13) studied 44 patients with stage C prostate cancer and found that 54.5 percent had cytokeratin 18 (Ck 18) expressing cells in their bone marrow.

Weckermann and colleagues (14) also used a Ck 18 immunostain and found positive cells in the bone marrow of 23.7% of the 266 pre-radical prostatectomy

patients studied. Additionally, they found no correlation to other risk factors including Gleason score, pathologic stage, ploidy, and preoperative serum PSA. And in a subset of 169 patients with local disease and a median follow-up of 32 months, there was no demonstrable difference in the rate of biochemical relapse between the patients with and without cytokeratin-positive cells (15). However, this same group assayed bone marrow specimens from 82 pre-radical prostatectomy patients with an antibody recognizing cytokeratins 8 and 19 in addition to Ck 18 (16). Since Ck 18 has been found to be downregulated in many tumors, this method offered increased sensitivity of the test (17). At a median follow-up of four years, cytokeratin-positive individuals were found to have a significantly earlier biochemical progression than patients without positive cells preoperatively. In fact, cytokeratin staining was seen to be an independent prognostic factor when compared with standard parameters such as PSA, stage, and Gleason score. Additionally, Lilleby *et al.* (18) employed a pan-cytokeratin antibody and found a correlation between cytokeratin staining in patients two years status-post external beam radiotherapy and progression-free survival.

3.2. Flow cytometry

Flow cytometry has provided another important method for studying CTC/DTC, affording the ability to analyze thousands of cells per second and measure individual cells according to multiple different parameters. Initially in prostate cancer, flow cytometry focused on tumor ploidy (19-21). Subsequently, this technique grew to be utilized in the detection of surface antigens on prostate cancer cells (22, 23). These investigations suggested that the cell surface marker phenotype of prostate cancer cells is closest to secretory prostate epithelial cells rather than basal cells, and that the expression profiles differ between primary tumors and metastases.

Further investigations have attempted to utilize flow cytometry to correlate CTC/DTC with disease prognosis. Moreno and colleagues (24, 25) have utilized a technique based on immunomagnetic enrichment of blood targeting the epithelial cell adhesion molecule (EpCAM) followed-by fluorescent labeling with anti-cytokeratin and anti-CD45 antibodies. Using flow cytometry, they were able to show some correlation between the level of CTC and PSA progression in a group of 10 patients with metastatic disease who were tested at serial intervals (25). In another study of 37 patients with metastatic prostate cancer and using a threshold of 5 or more tumor cells per 7.5 ml of peripheral blood, they found that the 23 patients who tested positive had significantly worse survival than the thirteen who tested negative (median survival 0.70 years vs > 4 years) (24). It remains to be seen, however, whether the use of cytokeratin as a target for flow cytometry can offer consistent and reproducible results in the evaluation of CTC/DTC presence and clinical meaning.

In fact, due primarily to limits in sensitivity, flow cytometry has come to be seen as increasingly inferior to newer techniques such as RT-PCR. De la Taille and colleagues (26, 27) found the detection limit of flow cytometry to be 1 LNCaP cell per one-thousand lymphocytes compared with RT-PCR which had a detection limit of 1 LNCaP cell per ten million lymphocytes. Other studies showed detection of PSA-positive cells by flow cytometry in the peripheral blood of patients who had no PSA mRNA transcripts by RT-PCR (28, 29). A proposed explanation for this phenomenon was that monocytes may have expressed PSA after either phagocytosis of tumor or binding of free PSA. For these reasons, most efforts in studying CTC/DTC over the past fifteen years have focused on RT-PCR.

3.3. Reverse transcriptase polymerase chain reaction (RT-PCR)

The advent of RT-PCR and its increasing use in the 1990's led to numerous investigations into its ability to detect CTC/DTC in the blood, bone marrow, and lymph nodes. The most commonly employed target has been the mRNA of PSA. Given that RT-PCR is a sensitive test for gene expression, it is well-suited to detect a marker that is both relatively specific to prostate cells and variably expressed in these cells (30). Two additional genes thought to meet these criteria and studied closely as well are prostate specific membrane antigen (PSMA) and human glandular kallikrein (hK2). Notably, hK2 is a serine protease and shares 80% homology with PSA; RT-PCR primers must therefore be able to distinguish between these transcripts in studies targeting hK2.

Overall, the use of RT-PCR to study CTC/DTC in prostate cancer has fulfilled its technical promise if not its clinical one. Most studies have utilized the PSA transcript and some have indicated that RT-PCR enables detection of a single PSA-expressing cell in up to 100 million background cells *in vitro* (31). *In vivo*, this technique has been shown to enable detection of a single PSA-positive cell among 10^6 to 10^7 hematogenous cells (32).

From a clinical standpoint, RT-PCR for PSA has yielded conflicting results. Reassuringly, PSA mRNA transcripts have been detected in the peripheral blood of up to 88% of patients with metastatic prostate cancer (33). In studies of patients with varying disease stages, positive samples have ranged from 24% to 80% of the patients studied (34, 35). A study with one of the highest positivity rates for local disease was that of Gao *et al.* (35) who evaluated the peripheral blood of patients prior to either radical prostatectomy or prostate biopsy. Eighty percent of the pre-radical prostatectomy patients were positive and 82% of the biopsy patients who had prostate cancer were positive. In that study and in many others, the false positive rate was very low, with 98% of their controls testing negative (32, 34, 36). Despite the considerable sensitivity and specificity seen in this study, no significant correlations could be demonstrated with clinically significant factors such as pathologic stage or biochemical recurrence.

There are also a variety of studies of PSA RT-PCR in bone marrow. In general, bone marrow aspirates have produced higher detection rates. For example, such aspirates from patients with metastatic disease have been positive in 77-

100% of patients, and studies of localized disease have shown positivity rates ranging from 45-71% (37, 38). Notable in these results is the far higher rate of PSA transcript positivity relative to the proportion of patients with localized prostate cancer who are likely to suffer progression of disease. This suggests that dissemination of prostate cancer cells from the primary tumor is an early event and that dissemination alone is not sufficient for true clinical metastasis or disease progression. There is some question, however, as to the overall frequency of PSA expression among CTC and DTC during their transition in blood and early seeding of the bone marrow as it has been observed that PSA expression can be significantly diminished or lost when the epithelial cells loose contact with stromal cells. Studies continue to examine new markers, such as HER-2/neu which has been detected in the CTC of 10% of patients with localized disease and 54% of patients with metastatic disease (39).

4. CIRCULATING AND DISSEMINATED TUMOR CELLS AND DISEASE PROGNOSIS

4.1. Prostate cancer

Whether or not the presence of CTC/DTC can provide useful information in the clinical management of cancer patients has been the focus of much attention. As already mentioned, efforts to link the presence of these cells with disease prognosis utilizing immunohistochemistry and flow cytometry have demonstrated mixed results. Specifically, cytokeratin immunostaining was not associated with clinical progression in some studies, while the investigation by Lilleby and coworkers did find that cytokeratin staining correlated with progression-free survival in a select group of patients (14, 18). The reduced survival observed in patients with metastatic prostate cancer who had 5 or more CTC per 7.5 ml of blood by flow cytometry lends support to the possibility that these cells may be a useful tumor marker (24). Bianco *et al.* (40) used a combined approach, performing immunohistochemistry only in patients who were PSA RT-PCR positive. Bone marrow samples were stained for both cytokeratin and MIB-1, an indicator of proliferation, in 58 pre-radical prostatectomy patients who were RT-PCR positive. They found a significant correlation between disease-free survival and the presence of proliferating circulating tumor cells, suggesting that further characterization of these cells may yield more prognostic information.

Indeed, most of the research on disease prognosis and CTC/DTC has focused on RT-PCR. Although the reports of PSA RT-PCR have often shown a trend towards increased detection in patients with more advanced disease, few have demonstrated any prognostic capacity of the test. Wood *et al.* (38) found that patients who tested negative by PSA RT-PCR had a significantly better disease-free survival. And several reports out of Columbia University have reported that the presence of PSA transcripts in the peripheral blood of patients prior to radical prostatectomy independently predicts postoperative treatment failure (27, 36, 41). However, numerous studies from other institutions on the bone marrow or peripheral blood of large numbers of patients failed to find a significant, independent correlation between pre-operative PSA RT-PCR and pathologic or clinical outcome (42-44). It therefore appears that these cells are shed into the circulation and seed the bone marrow from prostate tumors regardless of tumor severity. Collectively, these results suggest that PSA RT-PCR cannot be reliably utilized to predict biochemical failure. However, some believe that the use of quantitative RT-PCR for PSA transcripts may yet demonstrate some clinical value for this technique (45).

4.2. Breast cancer

While there is no prognostic role at this point for the simple detection (i.e. presence or absence) of CTC and DTC in prostate cancer, there is increasingly strong evidence that CTC may have a prognostic role in breast cancer. One study employed RT-PCR for cytokeratin 19 to evaluate peripheral blood samples in 167 patients with node-negative breast cancer prior to adjuvant chemotherapy. Notably, patients who were positive for this assay had markedly reduced disease-free survival (46). A meta-analysis of CTC/DTC studies in breast cancer has been performed as well. In a report encompassing 4703 patients, Braun *et al.* (47) showed that detection of DTC in the bone marrow of patients with breast cancer was a significant predictor of both disease-specific and overall survival. In a separate study, Braun and colleagues (48) extended their inquiry to partially characterize the circulating cells. Thus, they showed that over-expression of erbB2 by DTC of patients with breast cancer independently predicted poor clinical outcome.

Other studies in breast cancer have utilized the CellSearch system, a promising proprietary technology for the detection of CTC. This system immunomagnetically enriches blood samples for CTC with the use of ferrofluids coated with epithelial cell-specific EpCAM antibodies. After magnetic separation, cells are stained with antibodies to anti-cytokeratin and anti-CD45 antibodies, each conjugated to phycoerythrin for fluorescent labeling. Cells are then viewed under a fluorescence microscope and counted; or, in the newest version, the stained cells are presented on a computer screen for visualization (49). This semi-automated technique allows consistent and reproducible detection of CTC and has been employed in multiple breast cancer studies of note. Cristofanilli *et al.* (50) performed a prospective study of 177 patients with metastatic breast cancer and found that patients with fewer than 5 circulating cells per 7.5 ml of blood had a significantly better progression-free and overall survival. Additionally, after undergoing therapy, patients who had changed from the ≥ 5 CTC group to the < 5 CTC group had significantly prolonged survival compared with those who continued to have ≥ 5 CTC per 7.5 ml of blood. Further follow-up of this population has shown that throughout the course of treatment the detection of ≥ 5 CTC per 7.5 ml of blood remains an independent predictor of disease progression and mortality (51). A recent study compared the CellSearch system with conventional imaging in 138 patients with metastatic breast cancer and found that CTC elevation was an earlier and more consistent predictor of disease progression (52). This technique is now FDA

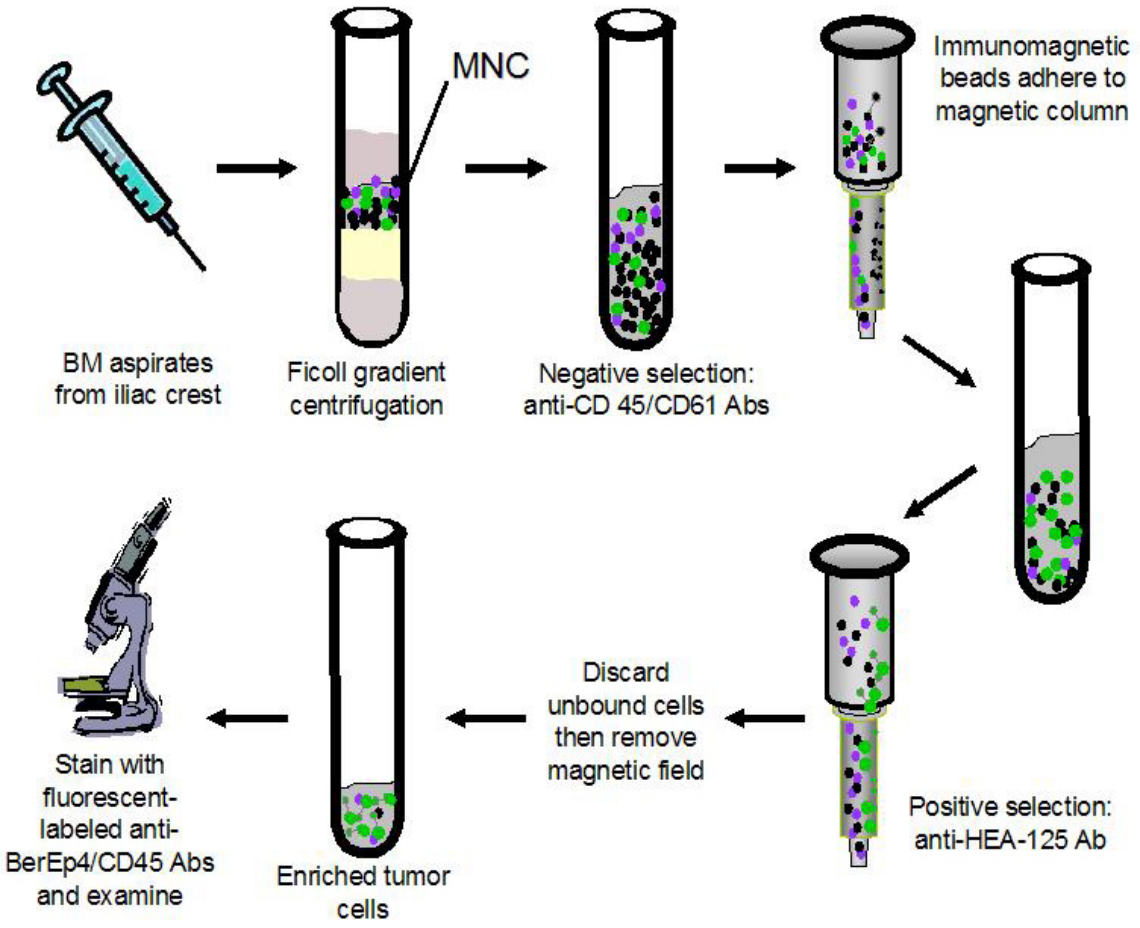

Figure 1. Procedure for enriching and isolating disseminated tumor cells from the bone marrow of prostate cancer patients.

approved and is beginning a Phase III trial to evaluate whether there is benefit to changing therapy in patients with metastatic breast cancer found to have elevated CTC. Whether or not similar prognostic information will be gleaned from prostate cancer CTC remains to be seen.

5. CELL ENRICHMENT AND ISOLATION

Whether using the CellSearch system or other techniques, the isolation of CTC/DTC requires enrichment of these cells from the initial bone marrow or blood specimen. As previously discussed, density gradient centrifugation, typically employing Ficoll-Hypaque, is often performed as an initial step. Further separation and enrichment of the tumor cells involves recognition of cell-specific antigens with monoclonal or polyclonal antibodies. Since prostate cancer cells are epithelial in origin, epithelial cell-specific markers are utilized for this purpose. Antibodies reactive to cytokeratins 8, 18, and 19 have been used, but, more commonly, attempts are made to target cell surface antigens such as HEA/EpCAM (53-56). With coupling of the antibody either to small particles susceptible to magnetic fields or to a fluorescent label, the cells can be enriched with immunomagnetic beads or by flow cytometry, respectively (57-59). This type of epithelial cell targeting is an example of a positive selection step.

Negative selection may be utilized as well as—or instead of—a positive selection step. This involves targeting and removing cells other than the ones of interest. Typical examples are targeting of the CD45 antigen expressed on leukocytes and the CD61 antigen expressed on megakaryocytes and platelets (34, 59). Various enrichment protocols have been successfully employed that include negative selection, positive selection, or both. Despite these steps, however, tumor cells still typically constitute < 0.1% of the total number of cells in the enriched population.

In order to isolate these cells further for more specific study, we have utilized a method for selecting individual CTC/DTC. After Ficoll-Hypaque centrifugation as well as negative and positive selection with immunomagnetic beads, the enriched population is labeled with FITC-conjugated anti-BerEP4, an antibody specific for HEA. This epithelial cell fluorescent stain allows these cells to be visualized under ultraviolet light. The steps involved in the enrichment and isolation of these cells are illustrated in "Figure 1". Using a micromanipulator pipette system, the individual tumor cells are then harvested and either combined for pooled analysis or studied as single cells (Figure 2).

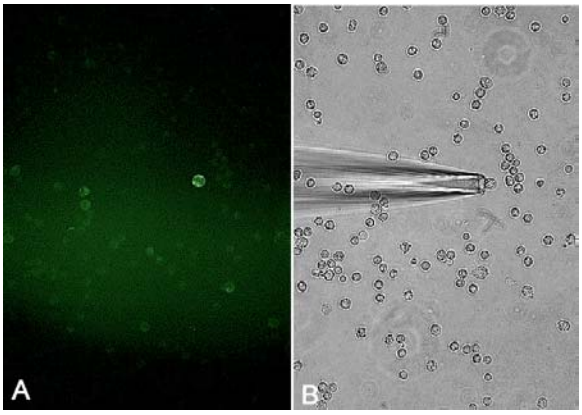

Figure 2. A. After tumor cell enrichment and labeling with anti-BerEP4-FITC, a brightly stained cell is visualized using fluorescent microscopy (X40). B. Under light microscopy the same cell is harvested from among numerous background cells by using a micropipette system (X40).

6. CHARACTERIZATION OF ENRICHED CELLS

The enriched cell populations—or the pure, harvested cells—may be characterized by a variety of techniques. Whereas the presence or absence of these cells in patients with prostate cancer has not yet yielded clear prognostic information, further analysis of these cells may lend significant insight into the mechanisms of metastasis. Numerous genetic techniques have been utilized in order to study these cells on molecular levels that span from chromosomes to DNA to specific proteins. These techniques include comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), gene expression microarrays, and study of telomerase activity.

Comparative genomic hybridization has begun to offer significant information in characterizing these cells. CGH was employed by Klein *et al.* (60) on single isolated disseminated cells showing detection of more chromosomal aberrations than when pooled tumor cells were tested. Kraus and coworkers (61) evaluated micrometastatic prostate carcinoma cell lines with array CGH. Their data demonstrated specific chromosomal regions which were gained or lost in these cells and allowed correlation with FISH studies. These and similar results provide strong evidence that the isolated cells are indeed malignant and not simply shed normal epithelial cells. Further use of these techniques may help elucidate key genes that enable prostate cancer metastases. Using pools of 10-20 disseminated tumor cells harvested with the micromanipulator pipette, we are currently able to study chromosomal changes in these cell populations. Comparing these changes across high and low risk populations as well as comparing primary tumors with their metastases may yield significant insight into the molecular distinctions between aggressive and non-aggressive tumors.

The use of FISH to study CTC/DTC began in the mid 1990s. Muller *et al.* (62) utilized a HER-2/neu DNA probe to study CTC in breast and prostate cancer patients. While two of eight patients with breast cancer had HER-2/neu amplifications, none of the patients with prostate cancer had amplification of this gene. In prostate cancer FISH has been used to confirm that the CTC do, in fact, originate from the primary tumor. In a group of eight men with prostate cancer, Ts'o *et al.* (63) found the CTC to be aneuploid and thus consistent with the cells being cancerous. Wang and colleagues (64) analyzed CTC in prostate cancer patients with FISH in order to determine the number of androgen receptor gene copies. They found two copies of the receptor present in a large percentage of their patients suggesting that these cells were prostatic in origin.

Gene expression microarrays offer a complementary technique for detecting the specific genes that may play a role in prostate cancer metastasis by identifying those whose expression level is up or down. With the use of these "gene chips," thousands of genes can be assessed at a time to determine the genetic changes in prostate cancer cells. This has been demonstrated in primary prostate tumors, such as in detecting variations in gene expression between tumors of different grades (65). Efforts to characterize gene expression in patients with disseminated breast cancer cells have identified distinct expression profiles in these patients (66). Work is ongoing in our lab to characterize disseminated prostate cancer cells using gene microarrays. By coupling these data with information gleaned from CGH, the goal is to translate knowledge about chromosomal abnormalities into an understanding of the actual genes involved.

On a protein level, telomerase may be of significant interest in the study of CTC/DTC. An enzyme which prevents the loss of the telomeric repeats at the 3' end of chromosomal DNA, telomerase may help characterize the tumorigenic potential of the disseminated cells. Normal somatic cells do not usually express telomerase, and telomerase activity is seen as a cancer cell indicator with detection in over 90% of primary bladder and prostate tumors (67). Studies of DTC have found telomerase-positive cells in 73% of patients with stage IIB and IV non-small cell lung cancer and 72% of patients with Duke's stage C and D colon cancer (68). We examined these cells in patients with prostate cancer and found telomerase present in 17 of 35 patients (49%) who had DTC isolated from bone marrow aspirates (69). While it remains to be seen whether telomerase status has any correlation with disease recurrence, the capacity to evaluate specific proteins in these DTC is an important step in developing a better understanding of their biology.

With time, characterization of the CTC/DTC using the above techniques will hopefully yield new insights into important aspects of tumor biology. For one, identifying dormant metastatic cells remains a central goal of DTC research. As in breast cancer, circulating and disseminated tumor cells may persist in some patients with a history of prostate cancer many years after surgery and with no evidence of recurrence (70, 71). These cells appear to have either withdrawn from the cell cycle or to have struck a balance between proliferation and apoptosis. The

cells are then capable of prolonged survival and may start proliferating at a later time, leading to overt metastasis (72). Muller *et al.* (73) found that the proliferation marker Ki-67 was not expressed in DTC in the bone marrow of breast cancer patients. This is suggestive of a dormant state of cell cycle arrest in disseminated breast tumor cells and may indicate that a similar process is occurring in disseminated prostate cancer cells. Further study may elucidate the role these individual dormant cells play in tumor recurrence.

Additionally, further characterization will help determine how the CTC/DTC relate to the primary tumor. Certainly, the large percentage of patients with prostate cancer who are found to have DTC at the time of radical prostatectomy points toward early dissemination of these cells. Klein *et al.* (74) put forth chromosomal evidence for early dissemination of these cells in a study of over 500 bone-marrow, lymph-node, and serum samples from patients with a variety of cancers including prostate. Using CGH, they found significant heterogeneity in DTC within individual patients, suggesting early diversification of these lineages. More support for early dissemination was seen in patients with breast cancer as DTC were observed to be genetically distinct from their primary tumor (75). These results are consistent with early separation of the DTC from the primary tumor leading to independent evolution. Whether the same holds for DTC in prostate cancer remains to be seen.

7. SUMMARY AND PERSPECTIVE

As with any cancer, a full understanding of the metastatic cascade in prostate cancer remains an important goal both in terms of tumor biology and clinical significance. In particular, the cells which appear to interface between the primary tumor and the distant metastases may offer unique and valuable information in delineating the process of tumor metastasis. While these CTC and DTC in prostate cancer have been studied for years without a clear understanding of their significance, improved molecular techniques are now beginning to shed light on the biology these cells. With these novel techniques, we can now examine the genetic changes occurring in this tumor cell population, and, with time, understand how these cells relate to the primary tumor and the clinical metastases. From a clinical standpoint, it is apparent that DTC exist in the bone marrow of a large percentage of patients prior to primary treatment and that they can persist for years after prostatectomy even in patients with no evidence of disease. Despite extensive study with immunohistochemistry and RT-PCR, there remains no conclusive correlation between the presence of these cells and tumor stage or progression. With tumor cell enrichment and isolation coupled with techniques such as gene expression microarrays and array CGH, the genetic characteristics of disseminated cells may be elucidated. One of the central ideas behind this research is the thought that these cells may eventually serve as both prognostic and therapeutic targets. To date, this has not been realized and researchers continue to seek new approaches to identify biomarkers that can help predict the course of disease (76-79). However, molecular characterization of CTC/DTC may not only provide new insights into the biology of metastases, but may reveal differences among these cells that allow them to serve as markers of tumor progression. Given the tremendous heterogeneity of prostate cancer both in terms of tumor pathology and disease course, the molecular characterization of the disseminated cells may offer an important mechanism for stratifying the treatment and prognosis of patients with prostate cancer.

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Abbreviations: CGH: Comparative genomic hybridization, CTC: Circulating tumor cells, DTC: Disseminated tumor cells, MNC: mononuclear cells, PSA: Prostate specific antigen, RT-PCR: Reverse transcriptase polymerase chain reaction

Key Words: Disseminated Tumor Cells, Circulating Tumor Cells, Prostate Cancer, PSA, Tumor Dormancy, Review

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