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NEWS

Circulating and disseminated tumor cells: many challenges, and even more opportunities, for the cancer and bone field

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A recent *IBMS BoneKEy* webinar focused on the clinical promise of these fascinating cells, as well as the technical difficulties in identifying them

Introduction

The bone field thinks a lot about cancer, not only because bone is the organ to which tumors are most likely to spread, but because some of the most common tumors, especially those of the prostate and breast, have a particularly strong tendency to metastasize to skeletal tissue. Clinicians seeking to prevent and alleviate the often severe bone destruction and pain that patients with metastatic disease exhibit would like to understand which patients are most likely to suffer from the progression and relapse of metastases. They would also like to determine which patients are most likely to benefit from particular treatments, and to be better able to monitor in real time how patients are responding to therapy. Learning why some patients are resistant to certain drugs, identifying better molecular targets for future drug development efforts, and gaining a better understanding of the biology of metastasis—these too are key goals for cancer investigators.

Circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) continue to command keen scientific interest all over the world because they may allow cancer and bone experts, as well as the broader oncology community, to achieve many or even all of these aims.[1] These cells that have broken away from a primary tumor to circulate in the bloodstream (in the case of CTCs) or to enter distant metastatic organs like the bone marrow (in the case of DTCs) were the focus of a recent *IBMS BoneKEy* webinar, 'Disseminated and Circulating Tumor Cells: Their Detection and Clinical Significance' (see the Webinar at http://www.ibmsonline.org/d/do/63). The webinar consisted of a feature presentation by Klaus Pantel (University of Hamburg) and discussion by panelists Evi Lianidou (University of Athens) and Jean-Yves Pierga (Institut Curie and Université Paris Descartes), all leading experts on CTCs and DTCs. Moderated by Philippe Clézardin (INSERM), IBMS BoneKEy Associate Editor for cancer and bone content, the webinar focused not just on the early evidence from clinical studies demonstrating the promise of CTCs and DTCs, but also on the technical challenges in enriching and identifying them, hurdles that must be overcome before these fascinating, yet very elusive, tumor cells can be used to help cancer patients in the clinic.

Finding One Out of Millions

One of the challenges in bringing CTCs and DTCs from the lab into the clinical setting is that they exist only in very low numbers, which vexes efforts to find them. 'This is a considerable technical challenge because we have to design assays that are able to find one disseminated or circulating tumor cell in 10^6 to 10^8 of normal blood or bone marrow cells,' emphasized Dr Pantel at the start of his presentation. CTCs and DTCs are also tough to locate because they are highly heterogeneous entities that differ both in the genes they express and the phenotypic characteristics they exhibit, with some of these tumor cells posing more danger than others.

Currently, CTCs and DTCs can be enriched on the basis of their physical properties like size/deformability, density and electric charge, or on their biological properties, in particular the marker proteins they express. Cancer researchers have focused on one particular marker, epithelial cell adhesion molecule, as it is expressed on a variety of epithelial tumor cells and can be detected with anti-epithelial cell adhesion molecule antibodies. Once they have been enriched by these methods, CTCs and DTCs can then be identified using immunocytochemistry approaches that detect other marker proteins, in particular, by employing antibodies to cytokeratins, cytoskeletal proteins also expressed by a range of malignant epithelial cells, or by using real-time reverse-transcription PCR technology, which can detect messenger RNA transcripts of cytokeratins. Dr Pantel also described a new antibody-based technique developed by Catherine Alix-Panabières (University Medical Center of Montpellier). This cell culture assay, known as epithelial immunospot, measures cytokeratins and other proteins released by viable tumor cells.

Dr Pantel's group has used cytokeratin immunocytochemistry to detect DTCs in the bone marrow of cancer patients,

finding that, although the presence of DTCs in control patients was extremely rare, up to 45% of prostate cancer patients, up to 40% of breast cancer patients, up to 60% of lung cancer patients, and similar percentages of patients with many other types of cancer were positive for DTCs at the time of their primary diagnosis, and before clear evidence of overt metastasis could be observed.[2–4] 'When we looked at a large control group of patients with non-malignant diseases, only 2 out of 191 patients were positive (for DTCs), so it's quite a specific assay,' Dr Pantel stressed.

Because of the ease of taking a blood sample to obtain CTCs, compared with the invasiveness of a bone marrow needle-aspiration procedure used to get hold of DTCs, efforts now focus primarily on the former. In this regard, there is a system called CellSearch that has been approved by the US Food and Drug Administration for detecting CTCs in metastatic breast, colon and prostate cancer. However, despite the acceptance of this technology in the research community, several limitations remain. One weakness is that CellSearch detects CTCs on the basis of epithelial cell adhesion molecule positivity. 'We probably underestimate the number of circulating tumor cells because the CellSearch system uses an antibody that is targeting a specific antigen,' according to Dr Clézardin. One reason this is an obstacle is because CTCs can lose the characteristics of epithelial cells (such as the expression of markers like epithelial cell adhesion molecule) and take on the features of mesenchymal cells, a process known as epithelial to mesenchymal transition. 'We know that epithelial to mesenchymal transition takes place in CTCs, so how can we be sure that we are detecting these cells, even when the CellSearch method is well standardized?' asked Dr Lianidou. Indeed, Dr Pantel described previous work documenting evidence of epithelial to mesenchymal transition in the CTCs from patients with prostate cancer. Consequently, quality assurance, along with the development of new methods that can be standardized across laboratories throughout the world, will be important in giving researchers confidence that they are not overlooking CTCs when they use CellSearch.

Finally, another challenge in identifying CTCs concerns the complicated trafficking of these cells, first from a primary tumor to a distant metastatic organ like the lungs, bone marrow, or liver (that is, where the CTCs become DTCs), and then back into the circulation where they become CTCs once again. There is evidence that the microenvironment of these secondary metastatic sites edits the DTCs, such that they begin to express the genes and take on the phenotype of cells specific to those sites; DTCs in the bone marrow, for instance, begin to resemble osteoblasts. Because of this organ-specific mimetism, tracking the original CTCs that broke off from a primary tumor to enter the circulation in the first place may be very difficult when so many edited CTCs are there to keep them company.

Using CTCs in the Clinic

Researchers hope the technological prowess of modern laboratory techniques can overcome these technical hurdles because CTCs have the potential to fundamentally alter patient care in the oncology setting. Whether or not CTCs fulfill this promise remains uncertain. However, some early returns look favorable.

Indeed, several studies have already shown that CTCs can provide valuable prognostic information that allows clinicians to predict the course that cancer will take in their patients. For instance, studies of advanced breast, prostate and colorectal cancer have demonstrated that patients with higher numbers of CTCs have poorer overall survival compared with patients with lower numbers of CTCs. Findings from a 2008 study of advanced prostate cancer patients by de Bono *et al.*[5] published in *Clinical Cancer Research* also showed that CTC numbers can provide prognostic information on overall survival at an earlier time point (just 2–5 weeks after starting a new chemotherapy treatment) compared with the widely used prostate-specific antigen test (where the prognostic value of that test became statistically significant only 6–8 weeks after initiating therapy).

Similarly, encouraging findings come from studies of early-stage cancer patients, a group of particular interest, as preventing further progression of disease in this population is crucial. Here too, unpublished results from the SUCCESS study revealed by Rack *et al.*[6] at the 2010 San Antonio Breast Cancer Symposium also showed a prognostic benefit of CTC counts. In this phase 3 clinical trial of adjuvant chemotherapy in more than 2000 patients with early-stage breast cancer, CTC numbers were an independent predictor of disease-free survival, and patients with five or more CTCs were at four times the risk for disease recurrence compared with patients with lower numbers of CTCs.

In addition to links between CTCs and survival, several studies have also found correlations between these cells and the presence of bone metastasis. For instance, a 2007 study in *Clinical Cancer Research* by Danila *et al.*[7] found that progressive castration-resistant prostate cancer patients with metastasis to bone only, or with metastasis to both bone and soft tissue had higher CTC numbers compared with patients with metastasis to soft tissue only. With regard to metastasis, a tantalizing possibility that intrigues cancer researchers is that measurements of CTCs could be a simpler alternative to tissue biopsies, but the variety characteristic of CTCs again poses a challenge. 'CTC detection is often presented as a way of having liquid biopsies of metastatic disease,' Dr Pierga noted. 'But since we know there is evidence of heterogeneity between CTCs from different primary sites and perhaps metastatic sites, is there really a future for this application of CTC detection?' Dr Pierga asked. For liquid biopsies to succeed, Dr Pantel emphasized that the oncology field first needs to develop its ability to capture more CTCs, as identifying just a few of them will not provide meaningful information with regard to the heterogeneity of these cells. If this can be accomplished, and further studies can prove that CTCs are in fact a reliable indicator of metastasis, then liquid biopsies could one day become a reality.

Along with providing information about metastasis, CTCs could also be used to monitor patient responses to therapy in real-time and, in doing so, help to identify those who are most likely to respond to specific treatments. This concept has already received support in the case of progressive metastatic castration-resistant prostate cancer. Indeed, a 2010 *Journal of Clinical Oncology* study by Reid *et al.*[8] found that CTC counts declined in most patients taking abiraterone acetate, a drug that inhibits an enzyme responsible for the production of androgens, male steroid hormones that drive prostate cancer.

Heartening results have also been reported in studies of early-stage cancer patients, a population for which there is a glaring need for biomarkers indicating whether drugs are working or not. Here, Dr Pantel discussed findings from the GeparQuattro Study, a randomized, phase 3 clinical trial of early

breast cancer patients without clear evidence of metastasis.[9] In this study, patients received a variety of neo-adjuvant systemic chemotherapy agents, whereas those with epidermal growth factor receptor 2 (HER2)-positive breast cancer also received trastuzumab (Herceptin), a drug that targets this receptor, with the goal of determining how these various therapies affect CTC counts as measured by CellSearch.

Two findings stood out to Dr Pantel and his colleagues running the study. First, they found a statistically significant decrease in the number of CTC-positive patients before and after therapy. In fact, of more than 200 patients, approximately 22% were CTC-positive before therapy, compared with just 11% after therapy, suggesting that treatment did indeed affect the CTC count. Second, the investigators were intrigued to detect HER2-positive CTCs in patients with HER2-negative primary tumors. One possible explanation for this paradox is that small subclones of HER2-positive cells may have been missed in the primary tumors, or that the HER2 status of CTCs evolves over time. Furthermore, after trastuzumab treatment, the researchers could detect both HER2-negative CTCs that may have been selected for by this treatment, and also HER2-positive CTCs that may have resistance to the antibody. 'It could be that patients positive for HER2 on CTCs may profit from anti-HER2 therapy even if they have a HER2-negative primary tumor,' emphasized Dr. Pantel, who is starting a randomized prospective trial in Germany to test this hypothesis. Finally, in addition to stratifying patients to treatment using existing drugs like trastuzumab, CTCs may also help in speeding the development of new drugs; instead of waiting for the results of clinical trials, CTCs could be used as a much earlier indicator of how well a drug is working.

Puzzling Phenomena

Although the clinical promise of CTCs is enticing, many fundamental issues about the characteristics and behavior of these cells, as well as those of DTCs, remain unresolved. Interestingly, the relationship between CTCs and DTCs is unclear. 'We don't know the percentage of CTCs that become DTCs and home to bone marrow or any other distant organ,' Dr Pantel said.

With regard to DTCs, another vexing issue in the cancer arena is that of tumor dormancy, a puzzling feature of the cancer process, whereby a patient may suffer metastatic relapse a decade or more after a tumor has been removed, having had only minimal residual disease and no signs of overt metastasis in the intervening years; DTCs that had remained in a quiescent state somehow finally awaken and pose new danger. However, the factors that stir DTCs from dormancy remain unknown, nor do researchers understand the proportion of cancer patients that possess dormant cells or how current cancer drugs may affect these slumbering entities. Even the pathway that DTCs take to lodge in distant metastatic organs—do they come from the bone marrow, or does the primary tumor directly seed those organs with DTCs?—is uncertain.

Strikingly, yet another open question is whether DTCs actually have, in fact, any future at all. 'Since currently there are many technologies available to detect CTCs, it seems that people are less and less interested in targeting or detecting DTCs. Do they still have a role in this field?' wondered Dr Pierga. Expressing hope that DTCs will not be forgotten as their circulating cousins garner all the fanfare, Dr Pantel noted that DTCs will have much to teach the oncology field. For instance, drugs are already available that target the interaction between tumors and the bone marrow microenvironment, and so knowing the DTC status of patients may enable investigators to better understand which patients are most likely to respond to those drugs, and to learn how they respond to new drugs under development. 'I think the blood analysis and the bone marrow analysis are complementary methods, and should be used in a complementary way in clinical trials,' Dr Pantel said.

One more reason not to overlook DTCs is that they have the potential to provide crucial information about the resistance mechanisms that allow tumor cells to survive the hypoxic conditions of the bone marrow stem cell niches and the ravages of chemotherapy, and to suggest new molecular targets for future drug development efforts. For instance, research using a breast cancer DTC cell line has demonstrated that the cells express proteins mediating the unfolded protein response mechanism that allows cells to survive challenging environmental conditions. In addition, studies of DTCs have already shown that these cells can provide valuable information about the biology of metastasis. For instance, as noted above, DTCs have been found in the bone marrow of patients as early as the time of diagnosis of the primary tumor, suggesting that the metastatic process gets underway very early during the course of cancer. DTCs may provide similarly valuable biological insights in the future.

A final outstanding question is whether DTCs and CTCs have cancer stem cell properties that allow them to found overt metastases, that is, whether among DTCs and CTCs are so-called metastatic stem cells. Several lines of evidence support this hypothesis. For instance, the presence of DTCs in the bone marrow is associated with metastatic relapse. In addition, there is indication that DTCs and CTCs can survive systemic chemotherapy and that they exhibit a low proliferative activity, qualities thought to belong to cancer stem cells, and they may remain in the bone marrow well after the primary tumor has been removed. DTCs and CTCs also respond to and secrete stem cell growth factors, and they express markers indicative of a stem cell phenotype. Proving the stem cell nature of DTCs and CTCs remains a highly active area of investigation.

From the Few (Cells) to the Many (Patients)

CTCs in cancer patients were first noted in 1869 and now, nearly a century and a half later, these cells, and to a lesser extent DTCs, continue to intrigue oncology researchers all over the world. From prognosis to treatment, CTCs and DTCs may have a significant impact in the clinical cancer setting, but just how meaningful will the potential benefit be? Looking only at the United States and at just two of the cancers with a predilection to spread to bone as examples, the American Cancer Society estimates that approximately 230 000 new cases of breast cancer in women and 240 000 new cases of prostate cancer in men, will occur just in 2011.[10] It is a paradox that so many patients could one day be helped by so few CTCs and DTCs, tumor cells so rare and elusive that the challenge for cancer investigators 150 years later is still just (not so) simply to find them.

Conflict of Interest

The author declares no conflict of interest.

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