Science to Practice

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Science to Practice: Imaging Cancer-associated Fibroblasts: No Innocent Bystanders

Summary:

The era of stromal-based therapies is coming, and methods to image the stroma are likely to become vital to improved understanding of the intricate interrelationships of these cells. Because fibroblasts are so important for the initiation of cancer, stromal-based therapies may serve as preventive regimens in patients who are at high risk for recurrent disease. The method described by Vandsburger et al uses a reporter-gene magnetic resonance (MR) imaging-agent paradigm that withstands dilution from cell division while allowing imaging without ionizing radiation. The requirement for gene transfection makes near-term clinical translation unlikely, but the opportunities for studying cancer-associated fibroblast activity in tumor models and observing and modulating their migratory behavior is an exciting prospect, one that is hoped to bring tangible benefits to patients with cancer.

The Setting

The focus in treatment of solid neoplasms has been on killing epithelial cancer cells and, more recently, inhibiting their blood supply and/or enhancing the host immune response. Although these approaches have improved patient outcomes, most gains in survival have been modest, typically measured in months. A relatively untapped field of therapeutic targets is tumor stromal cells: normal fibroblasts, endothelial cells, and pericytes, which at first glance appear as benign innocent bystanders in

a sea of cancer cells (1). However, these cells, under the influence of neoplastic cells, promote proliferation, neovascularization, invasion, and metastasis while inhibiting host immune response. The latter is perhaps the most insidious, effectively immobilizing the body's immune surveillance, leading to a toleragenic environment (2). Cancer-associated fibroblasts also generate the dense extracellular matrix that characterizes breast, ovarian, and pancreatic cancers and accounts for most of the cells in the tumor (3,4). These cancer-associated fibroblasts are reported to arise from resident fibroblasts, bone marrow-derived progenitor cells, or from epigenetic alterations of endothelial or epithelial cells, leading to mesenchymal cell transition; these varied pathways likely account for the diversity of cancer-associated fibroblasts found in tumors (5). These fibroblasts have unique cell surface markers that distinguish them from normal fibroblasts. Because of their importance in nurturing and sustaining tumor growth, they have become the focus for the development of a new class of therapies: stromal-based drugs (5). Because fibroblasts are found

Conflicts of interest are listed at the end of this article

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throughout the body, very high specificity will be required, and methods to image cancer-associated fibroblasts will be important to the development of highly targeted stromal therapies.

The Science

Vandsburger et al describe a method of monitoring the migration of fibroblasts during tumor growth (6). Fibroblasts were transfected *ex vivo* with the ferritin heavy chain, and the cells were incubated in ferric chloride, which labeled the fibroblasts with iron, making them visible on T2- and T2*-weighted magnetic resonance (MR) images. A coinjection model (labeled fibroblasts injected along with ovarian cancer cells into the thigh of a mouse) or a remote model (cancer cells injected into the thigh while labeled fibroblasts were injected into the peritoneum) was used, making it possible to monitor the recruitment of fibroblasts in the growing tumor. Serial MR imaging revealed that the labeled fibroblasts migrated to the growing rim of the tumor (where the tumor was most vascular), suggesting that the fibroblasts were recruited to the site of the angiogenic niche at the leading edge of the tumor (Fig 3b). By using biexponential relaxometry, quantitative R2 values could be used to estimate the number and percentage of ferritin heavy chain-expressing fibroblasts relative to cancer cells.

The Practice

The results of this study show that fibroblasts are recruited by cancer cells to regions of growth, particularly by assisting in the formation of new blood vessels. The power of the imaging method described by Vandsburger et al is that it uses a reporter gene, ferritin heavy chain, so that cells will remain visible for prolonged periods despite multiple cell divisions (7). This is different from most labeling methods, which

decrease in intensity by about one-half every time the cell divides and eventually become invisible as the remaining label becomes too dilute to detect. Although this reporter gene strategy overcomes the dilution problem (assuming that there is a constant supply of serum iron), it requires the genetic manipulation of a fibroblast, which, from a translational viewpoint, is complicated and expensive and could cause transformation of the fibroblast (7). Therefore, it is unlikely, in the near term, that this diagnostic method would be adopted as a routine clinical test.

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However, this does not invalidate the considerable power of the method as a research tool to investigate the effect of drugs and biologics on the migration of cancer-associated fibroblasts in tumors. Moving forward, it will be important to use highly realistic models, replete with tumor heterogeneity, intact immune systems, and native cancer-associated fibroblasts to better understand the interactions between cancer-associated fibroblasts and cancer and immune cells. Imaging may be crucial in helping to decide which candidate drugs are most effective to interrupt the communications among these cells. In this respect, the MR reporter gene approach may be in competition with similar optical and radionuclide reporter gene methods in

the laboratory (7). Moreover, simple labeling of fibroblasts (with MR imaging, optical methods, or radionuclides) may be more practical in the short term for observation of early fibroblast migration. As cell-based therapies are developed, we may discover that modified fibroblasts are the best way of delivering and monitoring stromal therapy. In that case, these cell-based therapies may justify the use of reporter genes to monitor cancer-associated fibroblasts in humans. Regardless of the approach used, the concept of tracking fibroblasts will be vital to the development of new therapies directed at the stroma.

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