Molecular biologists have focused on mutations in tumor cells in an attempt to understand cancer, but as techniques for manipulating genes and cells improve, researchers are finding that it takes more than just a cancer cell to make a tumor. New research presented in October at the American Association for Cancer Research’s International Conference on Frontiers in Cancer Prevention Research indicate that both stromal cells within and near the tumor influence cancer cell growth, as do the senescent cells within the surrounding tissue.

When Robert Weinberg, Ph.D., a lead scientist at the Whitehead Institute in Cambridge, Mass., and colleagues injected three different types of human cancer cells into mice, they found that the different cell types required different amounts of time to form tumors despite the fact that all three cell lines had been transformed with the same genetic changes. After just 15 days, 100% of the human embryonic kidney cells had formed tumors, whereas the fibroblasts required 20 days to form tumors, and by day 60 only 52% of the human mammary epithelial cell (HMEC) injections had given rise to tumors. The differences in the time required to give rise to tumors implied that something outside of the cancer cells themselves was limiting tumor formation, said Weinberg.

The team uncovered a big clue to what this rate-limiting factor might be when one of the researchers looked in the microscope—a technique, Weinberg said, he generally discourages his trainees from using. The investigator found that, although a single cell type had been injected, the tumors that the cells gave rise to were complex.

“He discovered something pathologists have known for at least 100 years,” said Weinberg. “Tumors are histologically complex.” As many as 80% of the cells within the tumor are stromal cells, including fibroblasts, epithelial cells, mast cells, and macrophages.

Given these data, the team hypothesized that a rate-limiting step in tumor formation following injection of the transformed cells was the recruitment of supporting cells. To test this hypothesis, the investigators mixed the transformed HMECs with either Matrigel (BD Biosciences), which is a mixture of extracellular matrix proteins and growth factors, or with fibroblasts and then injected the combinations into mice. The tumors formed with the HMEC–Matrigel combination in 50 days, whereas the HMEC–fibroblast combination prompted tumors to form in just 25 days.

Although these results clearly support the idea that part of the delay in tumor formation is the time it takes different tumor cell types to recruit support cells, the team began to consider whether the support cells are innocent participants in the tumorigenic process or if, after they have been in close proximity to cancer cells during the long time it takes most spontaneous cancers to arise, they too take on abnormal characteristics.

To test this idea, they isolated fibroblasts from normal human mammary tissue and from tumor biopsies. When they injected the transformed HMECs into mice with either the normal fibroblasts or the fibroblasts from tumors, they found that the fibroblasts from tumor biopsies accelerated the rate of tumor formation relative to the cells from normal tissue. Additionally, fibroblasts taken from outside the margin of the tumor, where tissue is ostensibly healthy, stimulated growth at an intermediate rate, in between that of the tumor-associated fibroblasts and the normal cells. These data, said Weinberg, imply that perhaps tumor inception has something to do with the receptiveness of the tissue surrounding the cancer cells and that, even if the support cells start off relatively normal, they ultimately become part of the problem in cancer growth.

In a separate set of work, Judith Campisi, Ph.D., a senior scientist at the Lawrence Berkeley National Laboratory in Berkeley, Calif., and a professor at the Buck Institute for Age Research in Novato, Calif., reported that senescent cells may be actively promoting tumor formation as well. When healthy cells receive potentially cancer-inducing insults, they enter a state of senescence, or permanent growth arrest. Until recently, researchers regarded senescence as a relatively harmless fate for cells. However, Campisi’s team found that this might not be the case.

“When cells become senescent, they do not simply turn off their cell cycle,” said Campisi. “It turns out that senescence is a very complex phenotype. Importantly, these cells have altered functional capabilities.” In fact, her team has found that senescent cells secrete proinflammatory cytokines, growth factors, and matrix metalloproteinases that appear to have a direct affect on neoplastic cells.

For example, if the scientists plate different types of epithelial cells on a lawn of either presenescent or senescent fibroblasts, they find that neoplastic epithelial cells show a growth advantage in the presence of the senescent cells. “Premalignant cells are stimulated by senescent cells, but there is no preferential stimulation of normal cells grown on presenescent or senescent lawns,” said Campisi.

To see if senescent cells could alter the growth patterns of cells in an animal model, the team injected preneoplastic SCp2 cells into mice, either alone or mixed with presenescent or senescent fibroblasts. Neither the SCp2 cells
injected alone or with the presenescent cells gave rise to tumors, but the cells mixed with senescent fibroblasts formed very aggressive tumors. Thus, it is clear that senescent cells are actively stimulating tumor cell growth, Campisi concluded.

Campisi argued that, because the number of senescent cells increases as a person ages, this type of signaling could be instrumental in driving mutant cells into full-blown tumors. Therefore, she said, if researchers could find a way to kill senescent cells or reverse their phenotype, they might be able to substantially delay the onset of cancer.

Recently her group found that, if they alter the expression levels of p53 and p16 tumor suppressor proteins using lentivirus gene transfection systems, they could force senescent cells to re-enter the cell cycle. “I was skeptical that this would work,” said Campisi. “Until 2 years ago, we didn’t think it was at all possible; we always considered that the senescence phenotype was irreversible.” Now, with the proof of principle in hand demonstrating that the phenotype can be reversed, Campisi thinks it might be possible to work on a way to use the information to slow tumor induction in whole tissues.

“It is really remarkable that senescent cells are secreting something that enhances proliferation of premalignant cells,” said Nancy Colburn, Ph.D., chief of the Gene Regulation Section at the National Cancer Institute’s Center for Cancer Research.

Both Weinberg’s work and Campisi’s fit into a more modern paradigm in which the context of the tumor’s development is as important as looking at the mutations within a tumor cell. “It is important for us to realize that the place where we are going to have the biggest impact in prevention is by altering or limiting gene regulation at the rate-limiting steps in carcinogenesis,” including tumor initiation and promotion, said Colburn. “Based on these talks it is clear that the critical gene expression might not just be in the tumor cells but in the surrounding stroma. And sometimes that communication is going to bounce back and forth between them.”

—Rabiya S. Tuma