Cancer Stem Cell Hypothesis and Trastuzumab in HER2-Negative Tumors

By Rabiya S. Tuma

Trastuzumab (Herceptin) is approved for the treatment of breast cancer patients whose tumors express a high level of the drug’s target HER2/neu. Yet at least two retrospective analyses of prospective trials suggest the drug may also improve survival in women with early breast cancer whose tumors do not overexpress HER2/neu. A plausible mechanism for those controversial observations has been lacking. Now, Max Wicha, M.D., and colleagues at the University of Michigan think the cancer stem cell hypothesis provides adequate explanation, but not everyone is ready to call the case closed so quickly.

Her-2 Positive Subpopulation

Wicha, professor of internal medicine and director of the University of Michigan Comprehensive Cancer Center in Ann Arbor, is a major proponent of the cancer stem cell hypothesis, which asserts that only a subpopulation of cells within a tumor – referred to as cancer stem cells – can give rise to new tumors or metastases. In the case of HER2-positive breast cancer, Wicha and colleagues reported several years ago that HER2 appeared to regulate the cancer stem cell population. When they engineered several HER2-normal breast cancer cell lines to overexpress the protein, they found the proportion of cells that express the stem cell marker aldehyde dehydrogenase (ALDH) increased. Similarly, the proportion of cells that form spheroids in culture, a trait associated with cancer stem cells, increased. By contrast, when the researchers did the opposite experiment and used trastuzumab to block HER2 activity in HER2-amplified cell lines, they saw the proportion of ALDH-positive cells dropped. Based on those experiments, the team concluded that trastuzumab’s clinical efficacy is due to its ability to reduce the cancer stem cell population in patients with HER2-positive tumors.

Following on that work and the clinical data hinting that women with HER2-negative tumors benefit from adjuvant trastuzumab, Wicha’s group started looking for HER2-expressing cells in breast cancer cell lines and tumors clinically characterized as HER2 negative. By definition, these tumors express little or no HER2 and lack gene amplification. However, in some of those cell lines, the team uncovered a subpopulation of cells that expressed HER2, and many of those HER2-expressing cells also expressed the stem cell marker ALDH.

As would have been expected previously, trastuzumab treatment had little effect on the overall cell line population. However, when the team examined the effect of trastuzumab using cancer stem cell criteria, they revealed a different story: The treated cell lines lacked the rare ALDH-expressing cells and the ability to form spheroids in culture.

Similarly, when the team injected MCF-7 breast cancer cells — which are frequently used as HER2-negative control cells — into the mammary fat pad of mice and allowed them to form established tumors, trastuzumab had little effect. If, however, the team started treating the mice with trastuzumab just one day after injecting the cells into the fat pad, tumor growth was completely blocked, according to Wicha, who presented the work at the AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics Conference in San Francisco last fall.

Numerous breast cancer researchers are looking for variation in HER2 expression between primary and metastatic tumors, but Wicha thinks that isn’t quite the right question. Rather, he thinks subpopulations are the key. If he uses short-hairpin RNAs to shutdown HER2 expression in MCF-7 cells and then injects them into the leg bone of a mouse, no tumor forms. By contrast, control cells that have the HER2-positive/ALDH-positive subpopulation of cells readily form tumors in the bone. “HER2 is absolutely necessary for cells to grow in metastatic sites like the bone,” Wicha said.

Looking at tumor samples from patients, Wicha’s group again sees that some clinically HER2-negative have a subset of cells that do, in fact, express the protein, and many of those cells also express ALDH, as was seen in the cell lines. “What we find in primary luminal tumors is the distribution of HER2 and the stem cell marker is not uniform throughout the tumor, but on the invasive edge of tumor you see induction of the HER2-positive cells,” Wicha said. He notes, however, that they see this pattern in luminal breast cancer, but not basal-type tumors, suggesting that the team’s hypothesis only applies to certain types of HER2-negative breast cancers.

Finally, when Wicha’s team looked at matched primary and metastatic tumors from 10 patients, the investigators saw an increase in HER2 expression in metastases from luminal tumors in the bone.
microenvironment, but not in metastases from basal tumors. Further experiments suggest that HER2 is upregulated in response to the bone resorption factor RANK ligand, hinting that early bone metastases might be sensitive to both trastuzumab and the RANK ligand inhibitor denosumab, according to Wicha.

“These data provides a biological explanation for the puzzling clinical result for patients whose tumors are Her-2 negative,” Wicha concluded.

Mixed Reviews

“I think it is a very interesting concept, if it is real,” said Dennis Slamon, M.D., Ph.D., director of clinical and translational research at the Jonsson Comprehensive Cancer Center at the University of California, Los Angeles, who helped develop HER2-targeted therapy. “If HER2-normal patients are responding this is one way to explain it. But my opinion — it might be the minority opinion — is that the jury is still out about whether HER2-normals are responding. It is just as much a likelihood of [HER2] testing error as it is of real biology.”

Additionally, he thinks Wicha’s group needs to do a real metastasis assay in preclinical models before they push their hypothesis too far. He notes that while results in the bone experiment are interesting, it is an artificial assay in which the cells are injected straight to the bone and asked to establish a tumor. The process by which metastases arise in patients is much more complex, requiring movement from the primary tumor to the new site, as well as establishment of a new lesion.

Furthermore, Slamon sees inconsistencies in Wicha’s arguments. For example, Wicha says that once a metastatic lesion is established the HER2-positive, ALDH-positive expressing cells are no longer critical and that is why women with clinically HER2-negative tumors might benefit from trastuzumab or lapatinib treatment in the adjuvant setting, but not in the metastatic setting. “I don’t understand how the stem cell would become less important in metastatic disease that responds to chemotherapy but then comes back pretty quickly,” Slamon said, noting that the proponents of the stem cell hypothesis suggest that when chemotherapy kills off the bulk of the tumor, the stem cells remain and repopulate the tumor. “The data are tantalizing but we can explain them in more than one way,” Slamon continued.

Additionally, Slamon notes that normal stem cells in a variety of tissues, including the breast and colon, express low levels of HER2. If anti-HER2 therapy blocks the cancer stem cells, one might expect them to also inhibit normal stem cells. Yet the side effect profiles associated with trastuzumab and lapatinib do not suggest that is the case.

Despite his current skepticism, Slamon emphasizes that Wicha’s new observations are intriguing and should be pursued. In fact, the two groups have established a collaboration to follow-up on the work.

Meanwhile, Soonmyung Paik, M.D., director of the Division of Pathology at the National Surgical Adjuvant Breast and Bowel Project, whose group first published data suggesting that HER2-normal patients benefit from trastuzumab, is a bit more optimistic about Wicha’s hypothesis. “I think it fits, but obviously it may not be the only explanation,” he said. Furthermore, he notes, a group of researchers from the Barbara Ann Karmanos Cancer Institute and Wayne State University in Detroit have also reported isolating a subpopulation of HER2-positive cells from clinically HER2-negative tumors. And like Wicha, the Detroit group found that these cells had the ability to initiate new tumors in animal models, suggesting they are cancer stem cells.

To confuse the matter further, Paik’s own recent work indicates that the degree of response to trastuzumab does not directly correlate with the level of HER2 expression. Because not all patients whose tumors are clinically HER2-positive respond to anti-HER2 therapy, Paik’s group has been trying to develop a predictive marker for HER2-positive patients who don’t benefit from the therapy. “We show that responses do not follow what common sense would tell us: They do not follow HER2 level in linear way,” Paik said “It actually turns out that patients with intermediate levels are the ones who do not benefit, not the real low ones. So that is also suggestive of the fact that HER2-negative patients may derive significant benefit.”

Along the same lines, he notes, work in his lab as well as others indicates that the HER2 expression level in a primary tumor is not predictive of the protein’s expression level in circulating tumor cells. “So it is possible that there is always a subset of HER2-positive cells in tumors in a subset of patients,” he said.

Trial Could Resolve Doubts

Of course preclinical experiments and retrospective analyses can only go so far in solving the current conundrum. The real key is what happens in patients, and a prospective randomized trial investigating the question is already underway. Paik and others in the National Surgical Adjuvant Breast and Bowel Project launched a phase III trial comparing adjuvant chemotherapy with and without trastuzumab in women with clinically HER2-negative invasive breast cancer. The trial aims to enroll more than 3000 patients, but has accrued just 500 or so since it opened in January 2011.

Paik attributes the relatively slow enrollment to the community’s skepticism about the benefits. In that way, Wicha’s work, which Paik says is more mechanistic than the Detroit group’s work, could be beneficial. If physicians and patients can see a plausible reason for the drug to work, they might be more enthusiastic about participating in the trial.