

A case of mistaken identity

A cell line used in more than 650 published breast cancer studies may not be a breast cancer cell line at all

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Some breast cancer researchers may be studying the wrong type of cancer. A growing body of evidence suggests a cell line that's been a cornerstone of metastatic breast cancer research over the last 25 years is in fact derived from melanoma cells.

This case of mistaken identity is causing concern among researchers working with the cells, reviewers, and cell suppliers, with some already reclassifying the cells as melanoma.

It began with a [Nature Genetics](#) paper in 2000. Reading it, James Rae, then a doctoral student at Georgetown University, thought the National Cancer Institute had made an error. The paper examined gene expression in 60 human cancer cell lines at the NCI, including one Rae was using -- MDA-MB-435, the most widely used model for metastatic breast cancer. The first figure in the paper, a colored tree diagram, showed the 435 cell line in pink, but it was not grouped with the other pink [breast cancer](#) lines. It lay sandwiched between brown [melanoma](#) cell lines.

Two years later, scientists at AstraZeneca investigated the incongruence further. Using RT-PCR and immunohistochemistry, they compared expression of breast and melanoma genes in 435 and nine other breast cancer and melanoma cell lines. The 435 cell lines expressed none of the genes characteristic of breast cancer cells, but did express several genes commonly expressed by melanocytes -- [a finding](#) that prompted the study's authors to recommend that 435 not be used as a primary line in breast cancer research.

By then [Rae](#) was a professor at the University of Michigan Medical School, and he decided to track down the mix-up to see just how many 435 lines looked like melanoma. In 2004, he and colleagues at Michigan [reported](#) that all lines of 435 they tested from various repositories -- including the cells' original source, the MD Anderson Cancer Center in Texas -- expressed melanoma-specific genes. "It was all the same cell line, and it was probably melanoma," said Rae.

Not all data pointed in the same direction. That same year, researchers at Anderson reported that 435 cells express breast-specific markers and can be induced to secrete milk lipids, a characteristic of well-established breast cancer cell lines. The Anderson authors [concluded](#) that 435 was a breast cancer model that simply showed heterogeneity, and its melanoma-like phenotype was probably the result of dedifferentiation due to genetic instability.

But Rae wasn't convinced. In 2006, he and colleagues from Georgetown examined 435 cell lines using karyotype analysis, comparative genomic hybridization, single nucleotide polymorphism data, and [gene expression studies](#). They reached one definite conclusion, which they [published](#) in *Breast Cancer Research and Treatment*: The true origin of MDA-MB-435 cells is a melanoma cell line called M14. A 2005 Nature paper from researchers at the Dana-Farber Cancer Institute in Boston had reached the same conclusion, also using SNP array analysis: 435 is derived from the same individual as melanoma cell line M14.

Today, Rae has no doubts about the claim: "I'm absolutely confident to say this is a melanoma," he said. What's more, Rae's 2006 paper, as well as NCI DNA fingerprinting of samples of 435, suggest that the misidentification of 435 occurred before 1982, meaning that 25 years of breast cancer research on 435 may be based on an incorrect model system. Although the cells phenotypically resemble breast cancer cells, causing cancer and metastasizing when injected into mouse breast tissue, the melanocytic origin of the cells means that the molecular profile of the tumors are potentially very different. Breast cancer hypotheses based on studies of the cell line may be incorrect, and treatments developed from it may ultimately be ineffective.

Even [Janet Price](#), an associate professor at Anderson who distributed the cell line in the 1990s, now doubts its identity. "We thought it was a breast cancer," said Price, "but it does have certain characteristics of melanoma." It's impossible to say where it came from without tracing it back to the original patient, said Price. She believes 435 can still be used as a valid model for metastatic cancer, but no longer advises people to use it as a model for breast cancer. "Colleagues will probably be disappointed that I'm not saying what I used to," she said. "I suggest they find other cell lines if they can."

Since Rae's 2006 paper, cell line distributors have taken action. The [NCI](#) and the [Berkeley Lab](#), a breast cancer cell line repository, have reclassified the cell line to melanoma. [ATCC](#), a leading cell line distributor, stopped selling the line in March, and is testing the cell line's identity, according to Brian Douglass, product manager for cell biology at the company.

Misidentification of cell lines is not rare; a short tandem repeat analysis of 100 human cell lines last year [found](#) 18 of the lines were incorrectly designated. But with 435, misidentification may be especially problematic. Because of the cell line's unrivaled metastatic ability in mice, more than 650 studies using 435 as a breast cancer model have been published (including more than 60 so far this year). Despite growing doubts about its identity, with nothing to replace the model line, researchers have been unwilling to let it go. "There are people vested in using the cell line because they have grants involved," said Rae.

[Alison Allan](#), an oncologist at the London Health Sciences Center in Ontario, recently attended the Joint Metastasis Research Society-AACR Conference on Metastasis. "A good 40% of the presentations used [435] in studies," said Allan. "Nobody even questioned that they weren't breast. They're carrying on as usual."

Allan uses 435 cells to study metastasis in breast cancer, but, she said, "It certainly makes me nervous to use them." Although she isn't convinced it's a melanoma line -- "There are 500 papers that say it's breast and five that say it's melanoma" -- reviewers have now begun questioning its use as a breast cancer model. She now adds a caveat statement to methods sections of papers, citing the debate over the origins of the cell line.

At the same time, some of her work in progress is already invested in 435, and she wants to continue to use that data. But, Allan is concerned with keeping

her cancer models as clinically relevant as possible, and she is currently testing several new cell lines that could provide alternative metastatic models. Researchers have enough problems extending the relevance of their studies to different subtypes of breast cancer, she said. "Then, when you introduce the possibility it's not even a breast cancer, and you look at molecular mechanisms and different proteins we think might affect metastasis, metastasis of melanoma might be controlled by a whole of different set of proteins."

Researchers who stick with 435 as a breast cancer model don't just jeopardize their own scientific results, said Rae; there are rumors of young PIs being turned down for publishing and grant funding based on incorrect use of the line. What's more, Rae believes researchers turning a blind eye to the controversy are preventing the field from directing effort toward finding a replacement to 435. "It's an overall shame," he said. But Allan noted that aside from her own interest, many of her colleagues at the recent metastasis conference spoke informally about wanting alternative models. "Everyone is in the same boat," she said. "Everyone was saying, 'If you find one, let me know.'"

Not all researchers are as concerned. [Selvarangan Ponnazhagan](#), a pathologist at the University of Alabama at Birmingham, injects the cells into bone, where they cause osteolytic bone lesions characteristic of strongly metastatic breast cancer. When reviewers of a paper he published earlier this year raised the issue of the cell line's identity, he responded that he didn't want to mention the controversy in the paper, stating that 435 is a well-known breast cancer cell line, and that it behaves as such in bone lesions. "I'm truly not very interested in finding out what cell line it is," he said. "This is an ideal model [for what I'm studying]."

Rae believes all researchers will eventually come around and stop using 435 once a new model is found. And although he is adamant against using 435 in breast cancer studies, Rae, who is a breast cancer researcher himself, agrees it remains a valuable system for studying metastases. He hopes it can be a "boon" for melanoma research, providing years of extensive literature on M14 to the melanoma community. "The efforts spent over the years on studying MDA-MB-435 have not been wasted," he wrote in the conclusion of his 2006 analysis of 435. "The many studies published using MDA-MB-435 as a model for breast cancer could now conceivably be reinterpreted as studies using M14 as a model for melanoma."

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