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MDA-MB-435 and M14 Cell Lines: Identical but not M14 Melanoma?

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Abstract

A controversy has arisen over the past several years about the true origin of the human MDA-MB-435 cell line. Originally described as a human breast cancer cell line, subsequent expression array studies instead suggested a gene expression profile consistent with a melanoma origin. Subsequent karyotype and comparative genomic hybridization studies supported the idea that current stocks of both MDA-MB-435 cells and M14 melanoma cells must be identical cell lines, and the conclusion was drawn that both cell lines were in fact M14 melanoma cells. However, an alternate conclusion based on these data is that both cell lines are indeed identical, but are in fact MDA-MB-435 breast cancer cells. There is evidence that many cell lines can display "lineage infidelity" and that assignment to tissue type is unreliably made based on expression patterns. Evidence from the literature is presented here that is inconsistent with both lines being of M14 melanoma origin, but rather is consistent with both cell lines being of MDA-MB-435 breast cancer origin. [Cancer Res 2009;69(13):5292–3]

Introduction

The MDA-MB-435 cell line was derived in the late 1970s from the pleural effusion of a female patient with breast cancer (1), and has been shown to be highly metastatic in nude mice (2–4). This cell line has been used for a large number of studies on the biology and molecular biology of breast cancer (see Fig. 1 in ref. 5). However, since 2000, this cell line has been subject to much speculation and controversy, related to the possibility that it is instead the M14 melanoma cell line, due to an early cross-contamination of cell cultures. Here, we revisit the history of this controversy, and present data to suggest that this speculation may not be true.

Evidence which suggests that MDA-MB-435 cells might be melanoma cells. In 2000, Ross and colleagues published a microarray expression study in which they used cDNA arrays to compare 60 human cancer cell lines (the NCI60 panel;[1] ref. 6). In that study, it was found that MDA-MB-435 cells clustered with several melanoma cell lines (6). Interestingly, the other breast lines were found not to cluster together, whereas cell lines from other tumor types showed a stronger clustering based on expression patterns (6). This study led to the speculation that the MDA-MB-435 cell line might be melanoma rather than breast in origin.

"Lineage infidelity", coexpression of melanocytic and epithelial markers in breast tumors, and evidence for cell properties consistent with a melanoma or breast origin of the MDA-MB-435 cell line. MDA-MB-435 cells have been reported to show properties consistent with a breast cancer origin. They can form primary tumors when injected into the mammary fat pad of mice, and can metastasize from these tumors (e.g., refs. 2–4, 7, 8). These cells can also express epithelial markers and secrete milk proteins and lipids (9). When transfected with the *nm23* metastasis suppressor gene, MDA-MB-435 cells show the morphologic features of normal breast epithelial cells, including acinus formation in three-dimensional culture and production of milk components (10).

MDA-MB-435 cells have also been reported to express genes and proteins consistent with a melanoma origin. In addition to the expression array study by Ross and colleagues (6), Sellappan and colleagues have reported that these cells express melanocyte proteins such as tyrosinase and melan A (9). Expressions of S100 and melan A proteins were also shown by immunohistochemistry of tumors growing in the mammary fat pad of mice implanted with MDA-MB-435 cells (11).

Various tumor types have shown "lineage infidelity", expression of markers not usually associated with a particular tumor type. The concept was first described in hematopoietic tumor cells, and has been reported in solid tumors as well (for a review, see ref. 12). Several case reports have shown that breast tumors can coexpress epithelial and melanocytic markers (13–16). In breast cancer, this lineage infidelity is also referred to as metaplastic (melanocytic) differentiation (16). In a recent immunohistochemical study of 100 breast tumor samples, expression of melanocytic markers was frequently found in clinical breast tumor samples, and increased expression of melanocytic markers—notably melan A—was associated with poorly differentiated tumors (17).

Evidence to support the identity of current stocks of MDA-MB-435 and M14 cells. Karyotype studies indicate that multiple MDA-MB-435 derivatives, from sources worldwide, are quite similar, consistent with a common origin and modest karyotypic and phenotypic drift (18). That report showed that the majority of the MDA-MB-435–derived cell lines had two X chromosomes, consistent with a female origin for the cell line (18). In a subsequent study, Rae and colleagues compared stocks of MDA-MB-435 and M14 melanoma cells, using karyotype analyses, comparative genomic hybridization, and microsatellite analyses (5). That study presented evidence that both cell lines were "essentially identical with respect to cytogenetic characteristics as well as gene expression patterns and that the minor differences found can be explained by phenotypic and genotypic drift" (5). These authors concluded that both the MDA-MB-435 and M14 cell lines are identical cell lines, and that "all currently available stocks of MDA-MB-435 cells are derived from the M14 melanoma cell line and can no longer be considered a model of breast cancer" (5). In that study, the authors included DNA fingerprinting analysis of nine individual loci, including the Y chromosome–specific

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[1] http://dtp.nci.nih.gov/docs/misc/common_files/cell_list.html

amelogenin locus. Evidence presented in that study indicates that the lines labeled as MDA-MB-435 and M14 both lacked a Y chromosome and were consistent with a female origin of the cell line(s). The findings presented in that study are consistent with the earlier karyotype analyses, indicating that multiple samples of the MDA-MB-435 lines were of female origin (18).

Evidence that both cell lines cannot be M14 melanoma cells. The M14 human melanoma cell line, also called UCLA-SO-M14, was derived at the University of California Los Angeles (19). Chee and colleagues noted that "the original culture was derived from an amelanotic lesion metastatic to the buttock of a 33-year-old patient" (20). In a subsequent report, Wong and colleagues stated that "a melanoma cell line, UCLA-SO-M14 (M14) was established from a biopsy specimen of a male patient with blood type O" (21). Additional information about the patient from whom the M14 line was derived does not seem to be available in the literature.

If the M14 line was indeed derived from a *male* patient, this is inconsistent with the karyotype results reported years later, which indicate that currently available stocks of M14 cells are of female origin (5, 18).

Conclusions

The controversy surrounding the identity of the MBA-MB-435 and M14 cell lines has generated strong opinions. From the evidence cited above, it seems clear that the MDA-MB-435 cell line is an aggressive cell line that expresses genes associated with both breast cancer and melanoma. Evidence exists in the literature to support the idea that some breast tumors, and especially poorly differentiated ones, can and do exhibit lineage infidelity or "metaplastic melanocytic differentiation" and express markers associated with both epithelial tumors and melanomas. It also seems clear that currently available stocks of the MDA-MB-435 and M14 cell lines are virtually identical at the karyotype level. However, the conclusion that they are identical and both of M14 melanoma in origin is inconsistent with the origin of the M14 cell line from a male patient with metastatic melanoma and the female karyotypes of both cell lines at present. The alternative is more plausible. If the cell lines are indeed now identical due to an early contamination of cell stocks, then it is reasonable to propose that both represent the human MDA-MB-435 breast cancer cell line. [A less likely explanation might involve both the loss of the Y chromosome from the original M14 cell line, coupled with uniparental disomy of the X chromosome, which could be assessed, although a finding of identity of the two X chromosomes in the current cell line(s) would not prove this idea, as uniparental disomy of the X chromosome has also been reported to occur in breast cancer (22, 23).]

The evidence presented above suggests that the idea that the MDA-MB-435 cell line indeed represents a poorly differentiated, aggressive breast tumor line, with expression of both epithelial and melanocytic markers, should be reconsidered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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