



A Common Case of Cell Line Misidentification

Linger, R.M.A., D. DeRyckere, L. Brandao, K.K. Sawczyn, K.M. Jacobsen, X. Liang, A.K. Keating, and D.K. Graham. 2009. "Mer receptor tyrosine kinase is a novel therapeutic target in pediatric B-cell acute lymphoblastic leukemia." *Blood* no. 114 (13):2678-87. doi: 10.1182/blood-2009-03-209247.

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Everyone agrees that cancer is an ugly beast of a disease, and when a child is diagnosed with cancer it is exceptionally unjust. The ultimate goal of the research described in this paper was to identify new therapies for pediatric leukemia. Although cure rates have dramatically improved in recent decades, childhood cancer survivors still face devastating consequences of their former disease, such as effects on endocrine, reproductive, musculoskeletal, and neurologic function as well as increased risk of new cancer in adulthood ([Winick, Carroll, and Hunger 2004](#)). The focus of this research study was to validate a specific protein, Mer receptor tyrosine kinase, as a novel therapeutic target in pediatric B-cell acute lymphoblastic leukemia (B-ALL).

Mer was originally cloned from a B lymphoblastoid library in the early 1990s ([Graham et al. 1994](#)). Later studies revealed that Mer is not normally present on B lymphocytes during any stage of maturation, but that Mer is frequently found on the surface of B-ALL cancer cells ([Graham et al. 2006](#)). One study used microarray technology to demonstrate that a subset of B-ALL, those in which the cancer cells contain a specific gene translocation called E2A-PBX1, exhibit particularly high expression of Mer ([Yeoh et al. 2002](#)). These discoveries lead us to hypothesize that the presence of Mer on B-ALL cancer cells was a driving factor in the abnormal expansion of these cells. The 697 cell line was identified as an E2A-PBX1-containing B-ALL cell line that expressed high levels of Mer. Our approach was to reduce Mer expression in the 697 cell line by transducing the cells with short hairpin RNA sequences complementary to *MerTK*. Once clonal populations were isolated by limiting dilution, we began generating in vitro data to support our hypothesis. We also tested our ideas in a mouse model of B-ALL. We did not encounter any unusual hurdles during the project and were generally very excited by our data and the resulting publication.

Our work investigating Mer continued to move in a positive direction after the original publication. Mer-selective tyrosine kinase inhibitors (TKIs) were developed in collaboration with a team at the University of North Carolina ([Christoph et al. 2013](#); [Schlegel et al. 2013](#)), and these molecules have recently demonstrated promising anti-tumor effects in mouse models. The trouble began a few years after publication when it became routine practice in our laboratory to perform short tandem repeat (STR) analysis of all cell lines. Through this testing, we discovered that some clonal derivatives of the wild type 697 cell line were not, in fact, derived from 697. They were progeny of a different, non-E2A-PBX1 B-ALL cell line, REH.

Our first reaction to this data was disbelief that the misidentified cells could possibly have been used to generate the data in the paper. The REH cell line had also been used for some experiments in the original paper. We had used STR analysis to authenticate the identity of the wild type 697 and REH cell lines prior to the original publication, but the shRNA-containing derivatives had never been tested before. In 2009, STR analysis was not new, but was still relatively expensive, and almost never required by journal publication guidelines. We thought we were ahead of the game by testing our wild type lines. Our mistake was not conducting the STR analysis on all cell lines, including the shRNA-containing clones derived from the verified wild type.

Subsequent to our unfortunate discovery of the misidentified cell lines, we tested numerous frozen stock vials. Because meticulous records had been kept during production of clonal derivatives, we were able to specifically identify the point in time when newly isolated REH clones were mislabeled as 697 clones. We were devastated by the realization that the misidentified cell lines had been used for experiments published in the original article. At this point, we self-reported the error to the journal, which ultimately resulted in retraction.

Despite the inaccuracy of the data in the retracted paper, we still believed our hypothesis to be true. And additional data from the Mer-selective TKIs indicated that our conclusions were valid. Working from a polyclonal population of verified 697 cells containing Mer-targeted shRNA, we generated new clonal 697 cell lines and repeated most of the experiments from the original paper. Although this was an arduous task, it was well worth the effort as we validated our original findings and even extended our observations with a new publication in the same journal ([Linger et al. 2013](#)).

Our story is just one example of a preventable, but common, problem. Cell line misidentification was first documented in the late 1960's, just 15 years after isolation of the first human cancer cell line, HeLa. In the decades since, numerous reports have suggested that between 15-36% of human cell cultures are misidentified ("[Identity crisis](#)" 2009; [Drexler et al. 2003](#)). The misidentification error we made was a simple case of mislabeling. Other types of misidentification errors include cross-contamination and errors of the tissue, or species, of origin. Despite decades of evidence, cell line misidentification continues to be a pervasive problem in biological research. For example, since publication of a DNA microarray study in 2000, numerous other studies have suggested that the human cell line MDA-MB-435, originally classified as breast cancer, is actually of melanoma origin ([Ross et al. 2000](#); [Sellappan et al. 2004](#); [Rae et al. 2004](#); [Ellison et al. 2002](#)). A PubMed search for MDA-MB-435 indicates that 1073 articles investigating this cell line have been published since 1982. Although striking evidence demonstrating that MDA-MB-435 is derived from the melanoma cell line M14 was published in 2007 ([Rae et al. 2007](#)), papers identifying MDA-MB-435 as a breast cancer cell line have continued to infiltrate the literature, including at least six publications in 2014.

Human cell lines are an essential part of cancer research. So, how do we proceed without compromising the integrity of the research conducted? Widespread changes are necessary including modification of laboratory standard operating protocols and requirements for both funding and publication. Individual laboratories must take responsibility by installing safety nets to ensure quality control. While not without challenges, STR profiling is a valuable tool for preventing misidentification errors ([Eltonsy et al. 2012](#)). In our case, we have instituted a standard laboratory practice such that every time a cell line is obtained, and every time a stock vial of cells is thawed, a sample is sent for STR analysis. In addition, a new vial of cells is thawed periodically in order to reduce the number of cell passages, which can lead to genetic drift and unstable STR profiles. This method prevents new errors of mislabeling and cross-contamination. Free resources are available online, including searchable STR profile databases on the [American Type Culture Collection \(ATCC\)](#) and [German Collection of Microorganisms and Cell Cultures \(DSMZ\)](#) websites, and a [database of cross-contaminated or misidentified cell lines](#) curated by the International Cell Line Authentication Committee (ICLAC).

While the role of publishers is more hotly contested than the responsibility of individual researchers,

many experts on cell line authentication advocate journal-mandated cell line testing as a prerequisite of publication. Some publishing bodies, such as [Nature publishing group](#) and the [American Association for Cancer Research](#) currently require submitting authors to state whether or not cell lines used in the described work were authenticated. A smaller number of publishers currently mandate cell line authentication.

Ultimately, the burden of responsibility must fall on all who seek to elucidate new scientific truths. In the words attributed to Socrates in Diogenes Laertius' *The Lives of Eminent Philosophers*, "The only good is knowledge and the only evil is ignorance."

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