Breast cancer is the most common cause of death of middle-aged American women and few preventable risk factors have been identified. Identifying breast carcinogens could lead to risk reduction, but this has not been a focus of toxicological research. High throughput testing methods, such as those being developed in US EPA ToxCast and NTP Tox21 programs, have the potential to identify carcinogens more quickly and cheaply than traditional rodent bioassays; the predictive power of these methods is still being evaluated. We used high content screening to measure the responses of two human mammary epithelial cell types, MCF7 (derived from a tumor) and MCF10A (derived from normal cells), to 24 and 72 hour exposures to a set of 111 chemicals relevant to breast cancer. Endpoints included measurements of DNA damage repair (P53, pH2AX), mitochondrial health, and cytoskeletal integrity (tubulin), among others, but no endocrine-related pathways. Because few human breast carcinogens have been identified, we tested 74 rodent mammary carcinogens (MCs), 19 "non-carcinogens" that did not induce any tumors in NTP bioassays, and 18 chemicals that disrupt mammary gland development in rodents. Comparing the two time points, assays run with 72 hours of exposure were more sensitive than those measured after 24 hours of exposure in either cell line, with more chemicals showing activity and more of the active chemicals showing activity at lower doses. Chemicals previously demonstrated to be genotoxic without metabolic activation in the Ames assay did not show more activity than Ames-negative chemicals in the two assays reflecting DNA damage repair (p53 and pH2AX). Among chemicals with high content screening data in HepG2 liver cells in ToxCast Phase II, Hepg2 results were moderately concordant with both breast cell lines. Additional research is required to relate high content screening endpoints to standard toxicity tests such as genotoxicity batteries and Ames, and to understand the importance of cell line and exposure conditions.