A sequential acquisition of genetic events is critical in tumorigenesis. A key step is the attainment of infinite proliferative potential. Acquisition of this immortalization requires the activation of telomerase in addition to other activities, including inactivation of TP53 and the retinoblastoma family of tumor-suppressor proteins. However, the importance of the order in which these genetic events occur has not been established. To address this question, we used a panel of normal mammary fibroblasts and endothelial cultures that were immortalized after transduction with the catalytic subunit of telomerase (hTERT) and a temperature-sensitive mutant of the SV40 large-tumor (tsLT) oncoprotein in different orders in early- and late-passage stocks. These lines were maintained in continuous culture for up to 90 passages, equivalent to >300 population doublings (PDs) post-explantation during 3 years of continuous propagation. We karyotyped the cultures at different passages. Cultures that received hTERT first followed by tsLT maintained a near-diploid karyotype for more than 150 PDs. However, in late-passage stocks (>200 PDs), metaphase cells were mostly aneuploid. In contrast, the reverse order of gene transduction resulted in a marked early aneuploidy and chromosomal instability, already visible after 50 PDs. These results suggest that the order of genetic mutations is a critical determinant of chromosome count and structural
aberration events.