Cell Cycle 14:6, 798; March 15, 2015; © 2015 Taylor & Francis Group, LLC

The immortality two-step

Comment on: Garbe JC, et al. Immortalization of normal human mammary epithelial cells in 2 steps by direct targeting of senescence barriers does not require gross genomic alterations. Cell Cycle 2014; 13(21):3423-35; http://dx.doi.org/10.4161/15384101.2014.954456

James B Lorens*; Department of Biomedicine and Center for Cancer Biomarkers; University of Bergen; Bergen, Norway; *Email: jim.lorens@biomed.uib.no ; http://dx.doi.org/10.1080/15384101.2015.1006560

Current deep sequencing technologies have provided an unprecedented view of the mutational burden of human cancer. The staggering assortment of mutations in the most common class of tumor, carcinoma, reveals a complex clonal evolution and vast intratumoral heterogeneity.[1] As we come to grips with this reality, it is worthwhile to recall that our understanding of the rate-limiting step toward malignancy, immortalization, remains comparatively poorly understood and surprisingly neglected. The paper by Garbe, Stampfer and colleagues in a recent issue of *Cell Cycle*[2] show how readily epithelial cells can attain immortality and delineate a cell culture model system to study this rate-limiting process.

Cultured human mammary epithelial cells (HMEC) have 2 distinct tumor suppressive, senescence barriers that rely on activation of the retinoblastoma protein (Rb) pathway and absence of telomerase activity.[3] The first barrier, denoted *stasis*, is a consequence of accumulated cellular stresses that lead to increased levels of the cyclin-dependent kinase inhibitor p16INK4a and inactivation of the Rb tumor suppressor function. Importantly, in contrast to other cell types such as fibroblasts, stasis in HMEC does not require p53, p21Cip1 or p14ARF. The second, well-recognized, barrier is replicative senescence, which is triggered when telomeres become critically shortened as a result of cell divisions in the absence of telomerase. Interestingly, in p53 wild-type HMEC, this telomere dysfunction leads to a largely viable growth arrest (termed agonescence), while in cells with mutant p53, more familiar crisis and cell death occur.[4] These results reveal important cell type-specific differences between how HMEC and for example keratinocytes attain immortality, emphasizing that the path to malignancy is dependent on how cellular senescence barriers are regulated, an aspect likely reflected in the recurrent mutations of cognate tumors.

Overcoming these tumor suppressive barriers in HMEC entails 2 steps: inactivation of Rb function and reinstatement of telomerase activity. Garbe et al show that when HMEC are cultured under optimized "low stress" conditions,[3] silencing of p16 followed by overexpression of c-Myc are sufficient to overcome these tumor suppressor barriers, respectively. This protocol generates immortal HMEC at very high efficiency while maintaining a normal karyotype context. The latter point will be important to model recurrent mutations that are hypothesized to drive immortality via telomerase reactivation (for example using CRISPR-Cas9) in a normal genomic background. This aspect also provides a unique model system to screen pharmacological inhibitors of telomerase reactivation in a defined context and without confounding genomic instability. As the reactivation of telomerase activity also reduces vulnerability to oncogene-induced senescence (OIS), malignant transformation of immortalized cells by activated oncogenes is facilitated.[5] In other words, once immortality is attained, the main tumor suppressive barriers have been conquered, and malignancy is far more likely to occur. Hence defining common molecular mechanisms that surmount these proliferative barriers is key to early cancer detection and intervention strategies.

It is intriguing that a breast cancer cell could be created and sustained without gross genetic changes. This indicates that acquisition of immortality does not require genetic instability *per se*. Indeed, recent tumor genome sequence analysis reveals mutational landscapes spanning several orders of magnitude.[6] However, the inherent mutagenic consequences of telomere dysfunction could create many of the genomic errors detected in primary breast cancers. Once an HMEC suffers a genomic change affecting Rb regulation that allows bypass of stasis, hyper-proliferation can ensue until the telomere dysfunction threshold is reached. The replicative senescence barrier will arrest most of these post-stasis cells, but rare cells that acquire mutations allowing telomerase reactivation could immortalize, and perpetuate all the other genomic errors accumulated to that point. Consequently, the genomic instability incumbent with lack of telomerase activity in pre-malignant cells could be an intrinsic source of many mutations present in early carcinomas.

In summary, the cell systems described by Garbe et al are a useful resource to study several critical aspects of epithelial tumorigenesis.

References

1. Cancer Genome Atlas Network. Nature 2012; 490:61-70; PMID:23000897; http://dx.doi.org/10.1038/nature11412
2. Garbe JC, et al. Cell Cycle 2014; 13:3423-35; PMID:25485586; http://dx.doi.org/10.4161/15384101.2014.954456
3. Garbe JC, et al. Cancer Res 2009; 69:7557-68; PMID:19773443; http://dx.doi.org/10.1158/0008-5472.CAN-09-0270
4. Garbe JC, et al. Cell Cycle 2007; 6:1927-36; PMID:17671422; http://dx.doi.org/10.4161/cc.6.15.4519
5. Suram A, Herbig U. Aging Cell 2014; 13:780-6; PMID:25040628
6. Lawrence MS, et al. Nature 2013; 499:214-8; PMID:23770567; http://dx.doi.org/10.1038/nature12213