PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Normal mammary epithelial cells escape of senescence

ArticleInfo		
ArticleID	:	3762
ArticleDOI	:	10.1186/bcr-2001-68444
ArticleCitationID	:	68444
ArticleSequenceNumber	:	34
ArticleCategory	:	Paper Report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 2001–8–20 Received : 2001–3–2 Accepted : 2001–8–20 OnlineDate : 2001–9–12
ArticleCopyright	:	Biomed Central Ltd2001
ArticleGrants	:	
ArticleContext	:	1305833

Valerie Speirs,[Aff1]

Aff1 Molecular Medicine Unit, University of Leeds, UK

Keywords

Mammary epithelial cells, senescence, telomeres

Context

Senescence is believed to represent an important natural barrier which prevents cells that have reached the end of their replicative lifespan from becoming malignant. Human fibroblasts undergo a limited number of population doublings before undergoing senesence, which is thought to be an irreversible process. In this paper, the characteristics of human mammary fibroblasts (HMFs) and epithelial cells (HMECs) at senescence were compared.

The authors analysed the *in vitro* characteristics of HMECs and HMFs to assess cell-specific differences in growth and senescence.

Significant findings

Both HMECs and HMFs underwent a limited number of population doublings before reaching plateau. Morphologically, the cells enlarged, adopted a flattened shape with vacuolated cytoplasm and expressed the senescence-associated ?-galactosidase. Both cells types had low proliferative and apoptotic rates, as shown by BrDU incorporation and annexin V staining, respectively. In contrast to HMFs, which failed to produce proliferating cells from senescent cultures (even after 5 months), the senescent growth plateau in HMECs was transient. Small refractile proliferative cells emerged from this plateau to undergo a second period of exponential growth followed by a second plateau growth phase. Cells at the second plateau were quite different from those at the first, (senescent) plateau, showing greater heterogeneity and continuing to incorporate BrDU. A proportion (~20%) were also annexin V positive. They also lost expression of p16, a feature frequently associated with the development of epithelial malignancies. Analysis of metaphase spreads revealed many abnormalities at the cytogenetic level, including translocations, deletions, polyploidy, aneuploidy and telomeric associations. Telomere erosion, not seen in the senescent HMECs at the first plateau, was a feature of cells at the second

plateau. This was also accompanied by a reduction in the number of telomeric repeats identified by FISH.

Comments

The traditional view of normal cell growth was that cells underwent a finite number of doubling times before reaching plateau, at which point the cells underwent senescence. While this still remains true for fibroblasts, the authors have shown that for normal mammary epithelial cells senescence is transient and followed by a second exponential growth phase. During the second phase of proliferation, at least *in vitro*, cells acquire many of the features associated with early stage tumourigenesis, including reduced telomere erosion and genetic aberrations. Under the *in vitro* conditions described, tissue integrity is obviously lost, and it will be important to determine if these changes also occur *in vivo*where the cells are under structural, biochemical and physiological control. Overall, an interesting and thought-provoking paper which provides potential new insights into mechanisms of neoplastic transformation.

This work has shown that HMECs can escape senescence and go on to develop the types of genetic abnormalities often associated with early breast cancers. Growth past the senescent barrier may be a critical event associated with the evolution of a malignant tumour. Defining the differences between fibroblasts and epithelial cells at senescence should facilitate our understanding of the neoplastic phenotype. This may have important clinical implications by identifying not only markers that predict neoplastic transformation but also novel therapeutic or preventative targets which prevent epithelial cells evolving beyond the growth plateau.

Methods

HMECs and HMFs were established in vitro from reduction mammoplasty material and cultured under conditions which supported each specific cell type. Growth curves were performed to determine population doublings and cell morphology was assessed over time. For chromosome analysis, metaphase spreads were prepared from colcemid treated cultures and G-banding karyotyping was performed. Cell cycle analysis was conducted on BrDU treated cultures by flow cytometry and cell death was assessed by TUNEL. Telomere length was determined by TRAP assay and telomeric repeats were visualised by FISH analysis of metaphase spreads. Cell cycle associated proteins were determined by western blotting of whole-cell extracts.

Additional information

References

1. Romanov SR, Kozakiewicz BK, Holst CR, Stampfer MR, Haupt LM, Tlsty TD: Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes . Nature . 2001, 409: 633-637.