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Keratin 6 is not essential for mammary gland developmentSandra L Grimm¹, Wen Bu², Mary Ann Longley¹, Dennis R Roop¹, Yi Li² and Jeffrey M Rosen¹¹Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA²Breast Center, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

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Breast Cancer Research 2006, **8**:R29 (doi:10.1186/bcr1504)This article is online at: <http://breast-cancer-research.com/content/8/3/R29>© 2006 Grimm *et al.*; licensee BioMed Central Ltd.This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**Abstract**

Introduction Keratin 6 (K6) has previously been identified as a marker of early mammary gland development and has also been proposed to be a marker of mammary gland progenitor cells. However, the function of K6 in the mammary gland was not known, so we examined the expression pattern of the protein during both embryonic and postnatal mammary development, as well as the mammary gland phenotype of mice that were null for both K6a and K6b isoforms.

Method Immunostaining was performed to determine the expression pattern of K6a throughout mammary gland development, from the embryonic mammary bud to lactation. Double immunofluorescence was used to co-localize K6 with known markers of mammary gland development. Wild-type and K6ab-null mammary tissues were transplanted into the cleared fat pads of nude mice and the outgrowths were analyzed for morphology by whole-mount staining and for markers of mammary epithelium by immunostaining. Finally, progesterone receptor (PR) and bromodeoxyuridine co-localization was quantified by double immunofluorescence in wild-type and K6ab-null mammary outgrowths.

Results Here we report that K6 is expressed earlier than described previously, by embryonic day 16.5. K6a is the predominant isoform expressed in the mammary gland, localized in the body cells and luminal epithelial cells but not in the cap cells or myoepithelial cells. Co-localization studies showed that most K6a-positive cells express steroid receptors but do not proliferate. When both the K6a and K6b genes are deleted, mammary gland development appears normal, with similar expression of most molecular markers examined in both the pubertal gland and the mature gland. Loss of K6a and K6b, however, leads to an increase in the number of steroid-receptor-positive cells, and increased co-localization of steroid receptor expression and proliferation was observed.

Conclusion Although K6a was not essential for mammary gland development, loss of both K6a and K6b resulted in an increase in PR-positive mammary epithelial cells and decreased proliferation after exposure to steroid hormones. There was also increased co-localization of PR and bromodeoxyuridine, suggesting alterations in patterning events important for normal lobuloalveolar development.

Introduction

The mammary gland is unique in that its development primarily occurs postnatally. However, the tissue is initially formed during embryonic development (reviewed in [1]). A milk line first appears at about embryonic day 10.5 (E10.5). At E11.5, five pairs of placodes have formed at specific positions along the milk line, and by E12.5 mammary buds invaginate from the ectoderm, surrounded by a specialized mammary mesenchyme. These mammary anlagen begin to form a lumen by E16.5 and sprout into the underlying fat pad. Branching mor-

phogenesis then occurs, to give rise to a rudimentary ductal tree in the newborn pups.

Any piece of the mammary gland, from the embryonic mammary bud to the differentiated gland, can be transplanted into a cleared fat pad to generate another ductal structure containing all the epithelial cell types that make up the mammary gland, supporting the idea that progenitor cells are dispersed throughout the tissue [2]. Although lineage markers have been identified in the hematopoietic system and epidermis, a clear picture of mammary lineage markers is still evolving [3-5].

BrdU = bromodeoxyuridine; C/EBP = CCAAT-enhancer binding protein; E = embryonic day; E + P = estrogen and progesterone; ER α = estrogen receptor α ; K5 = keratin 5; K6 = keratin 6; K8 = keratin 8; MEC = mammary epithelial cell; PR = progesterone receptor; SMA = smooth muscle α -actin; SP = side population; TEB = terminal end bud; WT = wild-type.