Syringomatous tumour of the nipple and low-grade adenosquamous carcinoma: evidence for a common origin

Werner Boecker and Igor Buchwalow
University of Münster, and Hamburg, Germany

boecker@me.com
Syringomatous tumor
- Nipple tumor with sweat duct differentiation.
- Branching cords of cells, glandular structures and keratinous cysts.
- Recurrences after incomplete resection
- Pathogenesis: Sweat duct origin

Low-grade adenosquamous carcinoma
- Metaplastic carcinoma with well developed glandular formation and nest of squamous cells in spindle cell background.
- Largely indolent, locally aggressive tumor
- Association with sclerosing lesions and adenomyoepithelioma
- Pathogenesis: Myoepithelial origin.

Material and Methods

Material:
- 12 Syringomatous tumors
- 9 nipple adenomas

Methods

Multicolor Immunofluorescence experiments (Double- or Triple Immunofluorescence) for
- p63 (p53 homologue)
- Basal keratins K5 and K14
- Luminal keratins K7, K8/18, (K19)

Gel electrophoresis and immunoblotting
qRT PCR

Basal and luminal keratins

Salivary gland; Excr. Duct

- Basal keratins K5, K14, (K17) expressed in cells sitting in a basal position
- Luminal (glandular) keratins K7, K8/18 expressed in glandular cells

Squamous epithelium

- Basal keratins K5, K14, (K17) expressed in cells sitting in a basal position
- Squamous keratins K10 expressed in more differentiated squamous cells
**Breast duct**

Triple Immunofluorescence for K5, K8/18, and SMA
Syringomatous tumor

- **12 cases**: 3 core biopsies, 9 excision biopsies

- Macroscopy: 0,4- 3,0 cm size (Literature: 1-3,5 cm, mean 1,7)

- Microscopy: tubular to solid nests or columns with comma or tail-like extension and small squamous cysts. Infiltration into smooth muscle bundles of nipple.

Low-grade adeno-squamous carcinoma

- **9 cases**: All 9 tumors located deep in the parenchyma outside the nipple.

- Macroscopy: 1,0- >3,0 cm size (Literature: 0,5-3,4cm, average about 2.0cm)

- Microscopy: tubular to solid nests or columns and occasional small squamous cysts in a spindle cell stroma. Infiltration between and around ducts and lobules.
Low grade adeno-squamous carcinoma (n=9)

All 9 tumors located deep in the parenchyme outside the nipple.
Macroscopy: 1,0- >3,0 cm size (Literature: 0,5-3,4cm, average about 2.0cm)
Microscopy
Microscopy

Syringomatous tumor

Low-grade adeno-squamous carcinoma
Immunohistochemistry

Syringomatous tumor

Low-grade adeno-squamous carcinoma
Syringomatous adenoma
In-situ Triple Immunofluorescence for K5, K8/18 and K10

K5+ cells give rise to squamous and glandular cells

K5
K8/18
K10
Progenitor cells give rise to squamous cells.
LG AdSC: Triple Immunofluorescence for p63, K5 and SMA

SMA+ myoepithelial cells are not observed in these tumors!
Conclusion:
Syringomatous tumors and low-grade adenosquamous carcinoma contain p63+K5+ progenitor cells that give rise to either K10+squamous or K8/18+glandular cells.
Ontogeny and breast tumors

Normal breast epithelium

Two cells concept
- Basal/myoepithelial
- (K5/14+; SMA+, p63+ etc)
- Luminal
- (K7;K8/18;K19)
- ER

Perou, Sorlie et al, 2000; Sorlie et al, 2001

The two-cell concept does not provide an explanation for the histogenesis of these non-classical basal type tumors!

Stem cells in breast epithelium
Smith (1996); Chepko and Smith (1997); Clarke et al. (2003); Dontu et al. (2003); Smalley and Clarke (2005); Villadsen (2005); Sleeman et al. (2006); Stingl et al. (2006); Asselin-Labat et al. (2008); Eirew et al. (2008); Van Keymeulen et al. (2011); Tosoni et al. (2012); Fu et al. (2014); Visvader and Stingl (2014) (Boecker et al., 2002); Lim et al, 2009
P63+K5+ progenitor cells in breast ducts give rise to glandular and myoepithelial cells

Breast duct

Salivary excretory duct
P63+K5+ bipotent cells give rise to K8/18+ glandular and SMA+ myoepithelial cells
Conclusions

(1) Syringomatous tumors and low-grade adeno-squamous carcinomas contain p63+K5+ progenitors, K8/18+ glandular and K10+ squamous cells.

(2) In both tumors the p63+K5+ progenitors differentiate through intermediary cells towards the glandular or squamous lineage.

(3) A transdifferentiation from myoepithelial to squamous or glandular cells can be excluded.

(4) Thus syringomatous tumor and Low-Grade adeno-squamous carcinoma seem to be immunophenotypically identical lesions.

(5) Normal breast ducts contain p63+K5+ physiological progenitors which through intermediary cells differentiate towards the K8/18+ glandular and SMA+ myoepithelial lineage.

(6) We conclude that the p63+K5+ physiological progenitors might be the cells of origin for both tumors.
Thank you for your attention
Lim et al, 2009: Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers:

Lim et al, 2009
Eirew et al, 2008
• Flow cytometry
• Cell culture
• Transplantation assays
• Immunohistochemistry

Fluorescence activated cell sorting for surface markers CD49f and EpCAM of normal human mammary epithelium revealed:
• CD49f+EpCAM- subpopulation of cells enriched in cells positive for p63, K5/6 and K14 which were shown to have “stemness” features in regard to their bipotent differentiation potency in transplantation studies, albeit without showing which exact cell type was responsible for the formation of the structures.
Associated lesions

- Syringomatous tumors
- 1/9 hybrid lesion of syringomatous tumor and adenosis.
- 2/9 lesions of syringomatous and adenomatous adenoma
- Low-grade adeno-squamous carcinoma*
- 6/9 hybrid lesions of low-grade adenosquamous carcinoma and sclerosing lesions.
- 3 cases showed transitions to squamous or metaplastic carcinoma.

*It is nor unusual to find... a(n associated) papilloma or sclerosing adenosis. ...rarely transition to..... squamous carcinoma) Rosen, 2009
Basal-like tumors of the breast unlike classical basal-like tumors

Pleomorphic adenoma (n=4)
Adeno-myoepithelial tumors (n=7)
Adenoid-cystic carcinoma (n=6)
Squamous carcinoma breast (n=8)
Syringoma (n=12)
Adeno-squamous carcinoma (n=8)

Reis-Filho and Schmitt, 2002 (p63..reliable stem cell marker)
Boecker et al, 2012, 2013, 2014a, b
K5/14+ P63+

progenitor cell

glandular lineage

K 5/14
K 5/14
K 8/18

myoepithelial lineage

K 5/14
SMA
K 5/14
K 8/18
SMA

squamous lineage

K 5/14
K 10

K10
progenitor cell

- **Glandular lineage**
  - K5/14+
P63+

- **Myoepithelial lineage**
  - K5/14
  - K8/18
  - SMA

- **Squamous lineage**
  - K5/14
  - K8/18
  - K10
Ontogeny

Knowledge of the human adult mammary gland epithelium is a requirement for understanding physiologic regeneration and for developing concepts of abnormal proliferative disease. Recent cell culture studies and transplantation assays have provided profound evidence for the existence of stem cells, pointing to the existence of an epithelial differentiation hierarchy in both human and mouse mammary gland epithelium.
Ontogeny

However, different definitions and partly opposing concepts on the nature of mammary progenitor cells have evolved based on type of experimental study and on tools used to identify the cells such as FACS with cell surface markers, molecular stem cell-and lineage markers, Hoechst 33342 dye efflux, and multicolor immunofluorescence experiments.
Invasive carcinoma, basal-like

- HE: high grade
- IH: K5+ a/o EGFR+
- Molecular signature
- bad prognosis

Current classification: Two cells concept

- basal/myoepithelial (K5/14+ and/or EGFR+; triple negative)
- luminal (K7+; K8/18+; K19+)
  - ER+/-
  - Her2+

Ontogeny and breast tumors

Normal breast epithelium

Invasive carcinoma

Two cells concept
- Basal/myoepithelial
- (K5/14+; SMA+, p63+ etc)
- Luminal
- (K7;K8/18;K19)
- ER
- Her2

Perou, Sorlie et al, 2000; Sorlie et al, 2001
Ontogeny: Hypothetical Models of the Cellular Hierarchy of Human Breast Epithelium I

**Putative common Progenitor**
- K5/6+
- K14+

**Intermediary cells**
- Luminal
  - K5/6+
  - K8/18+
- K5/6+
  - SMA+
  - Myoepithelial

**Differentiated Cells**
- K8/18+

**Boecker et al, 2002**
- *In situ Double Immunofluorescence*

**Dontu et al, 2003**
- *Cell culture*
- *Immunohistochemistry*

**Rios et al, 2014**
- clonal cell-fate mapping studies

**Stingl et al, 2003**
- *Flow cytometry*
- *Immunofluorescence*
- *Cell culture*
- *Transplantation assays*

**Gudjonsson et al, 2002**

**Villadsen et al, 2007**
- *Flow cytometry*
- *Immunofluorescence*
- *Cell culture*
- *Transplantation assays*
Hypothetical Models of the Cellular Hierarchy of Human Breast Epithelium II

**Putative common Progenitor**
- EpCAM<sup>−</sup>  
- CD49<sup>high</sup>  
- P63<sup>+</sup>  
- K5<sup>+</sup>  
- K14<sup>+</sup>  
- Vimentin<sup>+</sup>

**Restricted Progenitors**
- Luminal
  - EpCAM<sup>+</sup>  
  - CD49<sup>+</sup>  
  - K5<sup>+</sup>  
  - K8/18<sup>+</sup>  
  - MUC1<sup>+</sup>
- Myoepithelial
  - EpCAM<sup>−</sup>  
  - CD49<sup>f</sup>  
  - K8/18<sup>+</sup>  
  - K19<sup>+</sup>  
  - MUC1<sup>+</sup>  
  - ER<sup>+</sup>  
  - ER<sup>−</sup>

**Differentiated Lineage Cells**
- K14<sup>+</sup>  
- SMA<sup>+</sup>

---

**Lim et al, 2009**
- Flow cytometry
- Cell culture
- Transplantation assays
- Immunohistochemistry

**Eirew et al, 2008**
- Flow cytometry
- Cell culture
- Transplantation assays
- Immunohistochemistry

**Clarke et al, 2005**
- Immunomagnetic sorting
- Immunofluorescence
- Cell culture
- Transplantation assays

**Shehata et al, 2012**
**Liu et al, 2014**
**Deng et al, 2014**
Basal-like tumors of the breast unlike classical basal-like carcinomas

- Pleomorphic adenoma breast (n=4)
- Adeno-myoepithelial tumors breast (n=7)
- Adenoid-cystic carcinoma breast (n=6)
- Squamous carcinoma breast (n=8)
- Syringoma breast (n=12)
- Adeno-squamous carcinoma (n=8)

- ERneg, PRneg, Her2neg, K5+
- Low-grade o benign
- p63-pos