

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

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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.*

WHO-Classification of Tumors of the Breast, Eusebi et al, 1012; 2012 Carter and Dyess, 2004; Doctor and Sirat, 1971; Jones et al, 1989; Rosen, 1984; Suster et al, 1991; Raju, 1990; Reis-Filho et al, 2012; Drudis et al, 1994; Rosen and Emsberger, 1987, Van Hoeven et al, 1993

Material and Methods

Material:

- 12 Syringomatous tumors
- 9 nipple adenomas

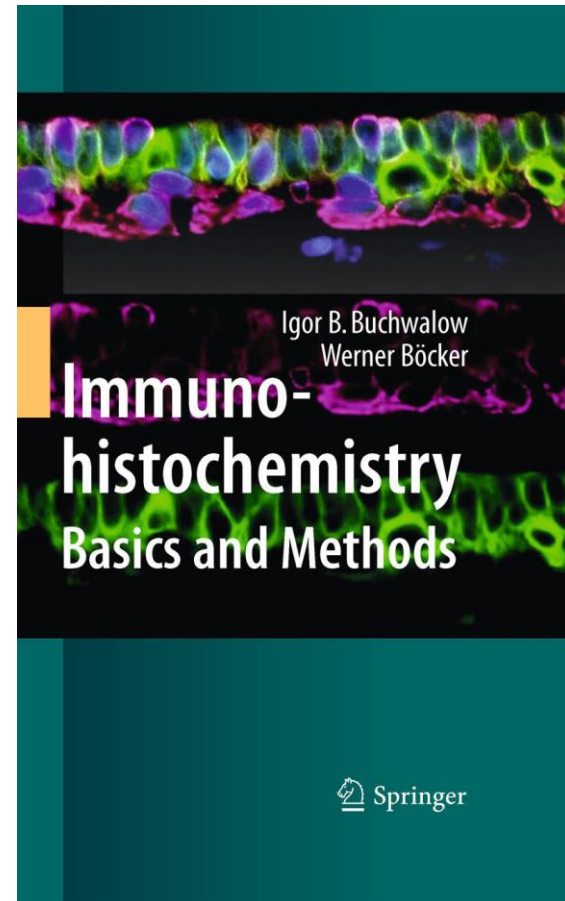
Methods

Multicolor Immunofluorescence experiments (Double- or Triple Immunofluorescence) for

- **p63** (p53 homologue)
- **Basal keratins K5 and K14**
- **Luminal keratins K7, K8/18, (K19)**

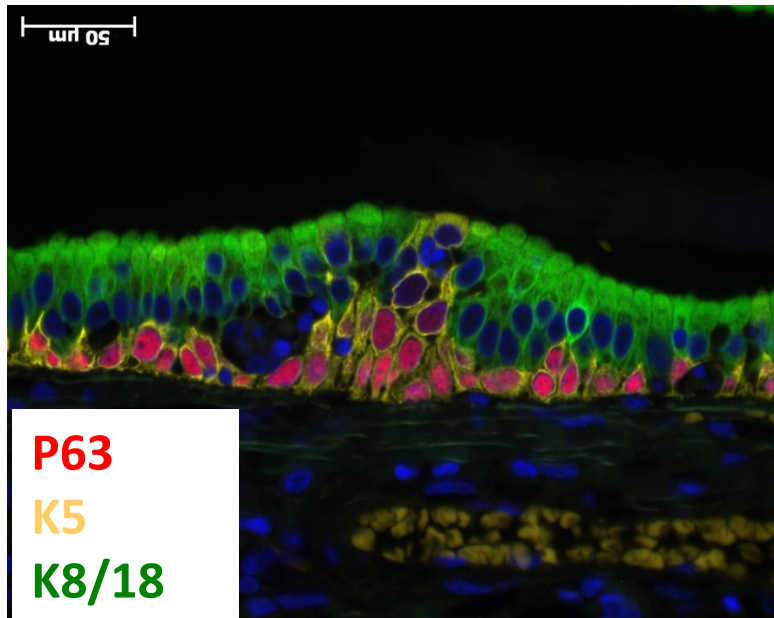
Gel electrophoresis and immunoblotting

qRT PCR



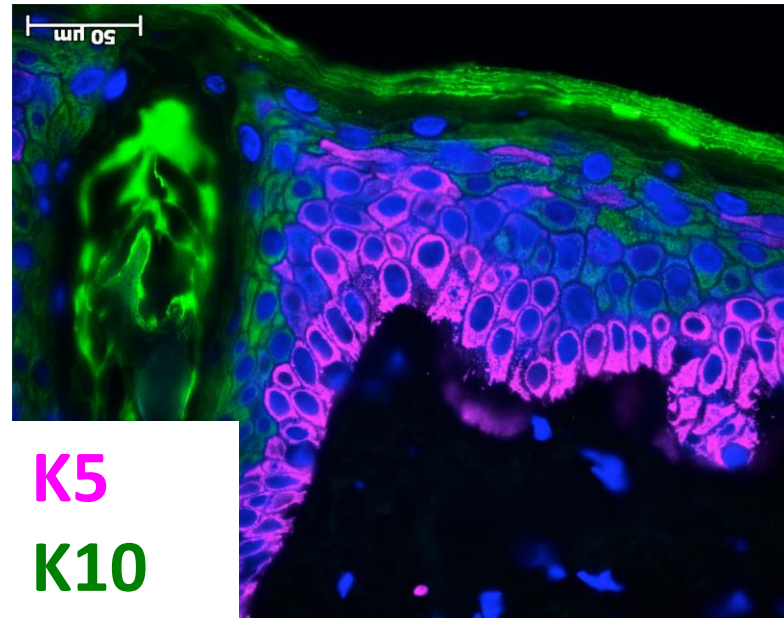
Nagle et al, 1986; Boecker et al, 1992, 1993, 2012, 2014; Buchwalow and Boecker, 2006, Reis-Filho et al, 2002, 2006; Moll, 2008,

Basal and luminal keratins



Salivary gland; Excr. Duct

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

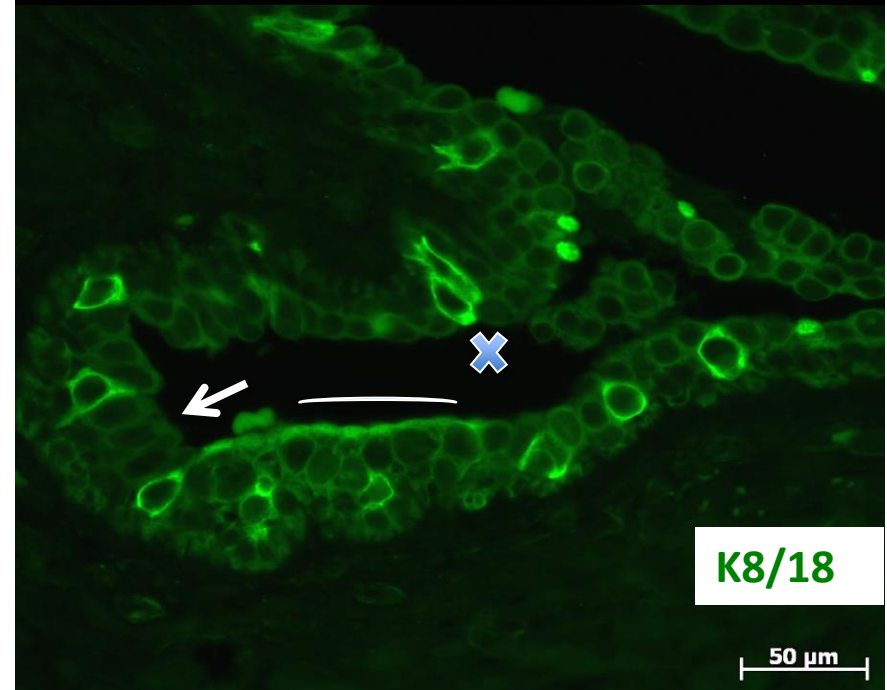
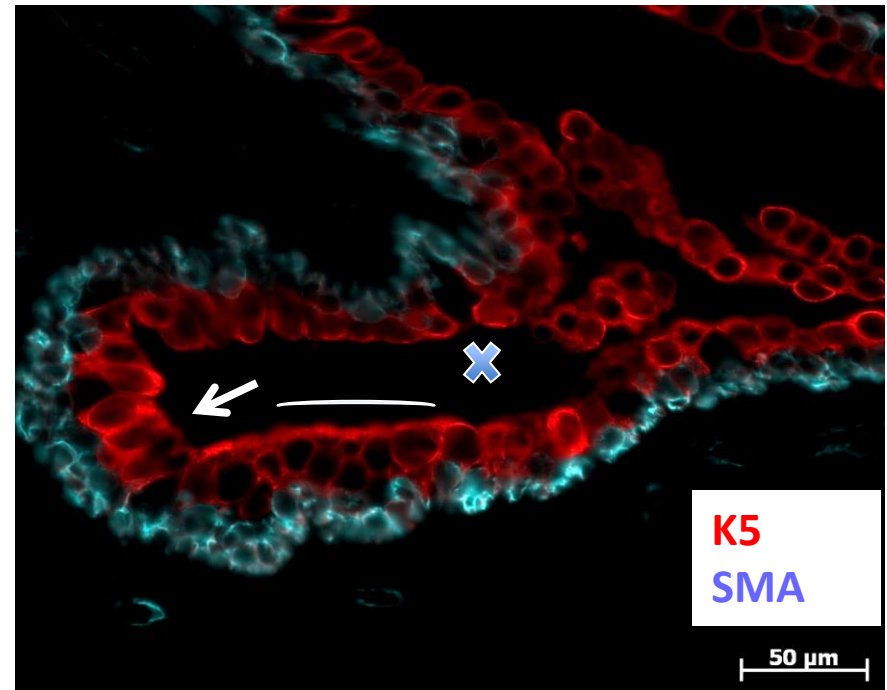


Squamous epithelium

- **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.

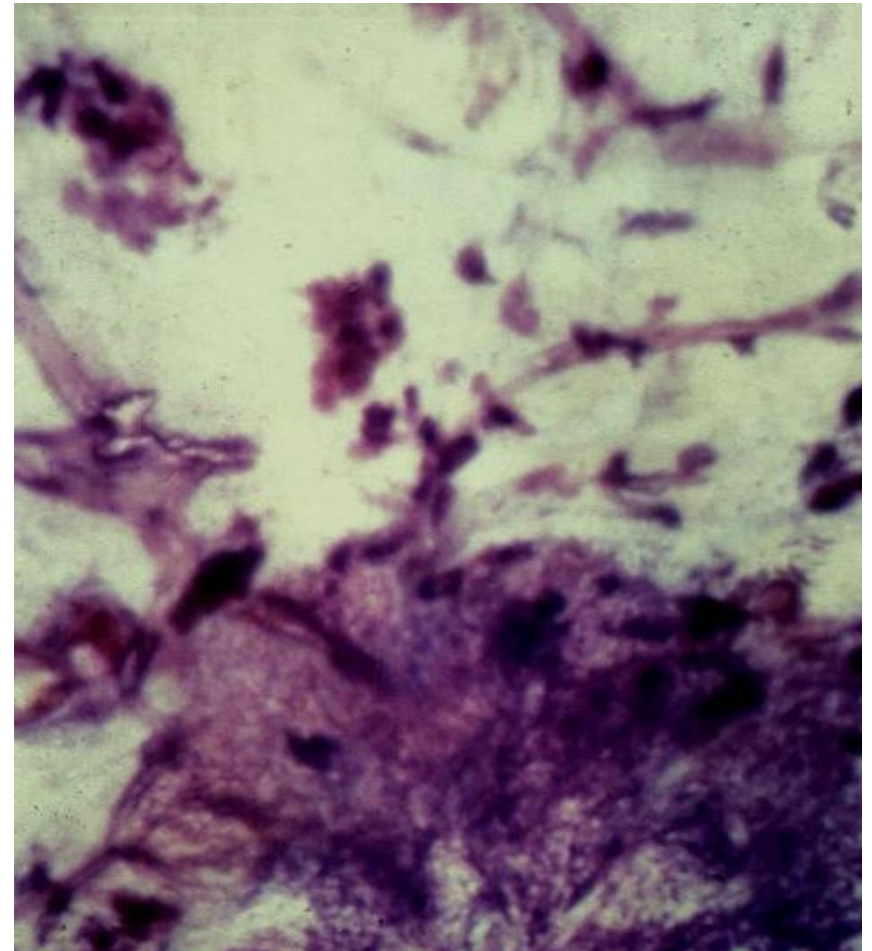
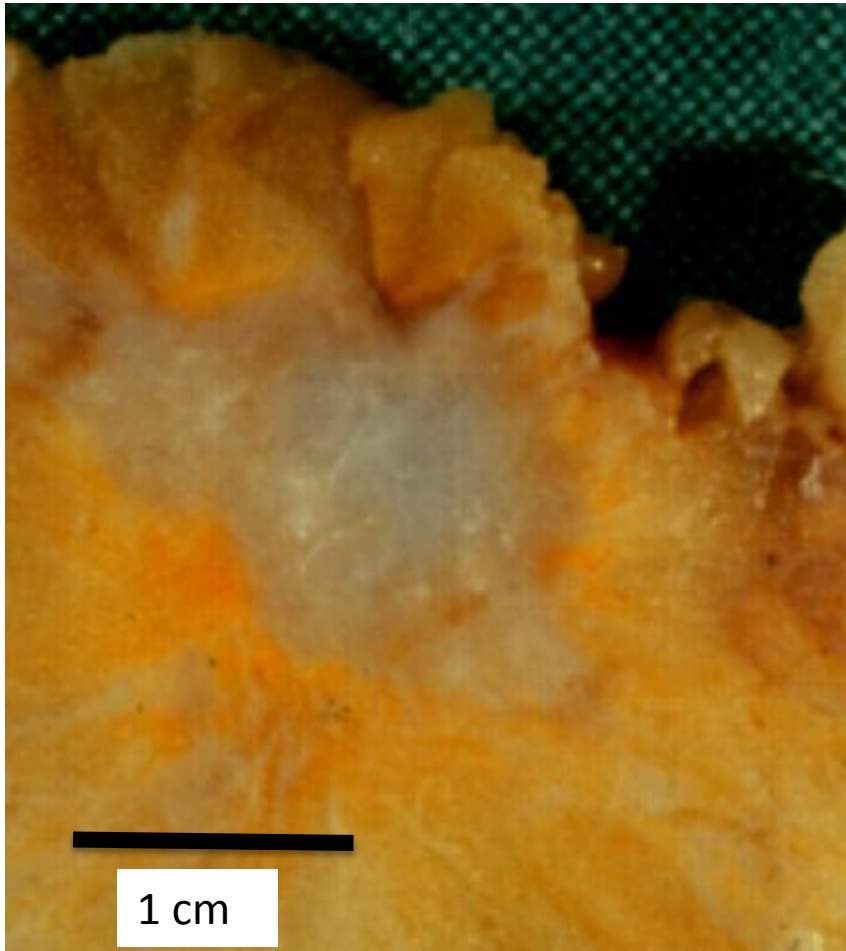
Low-grade adeno-squamous carcinoma

- **9 cases:** All 9 tumors located deep in the parenchyme 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.

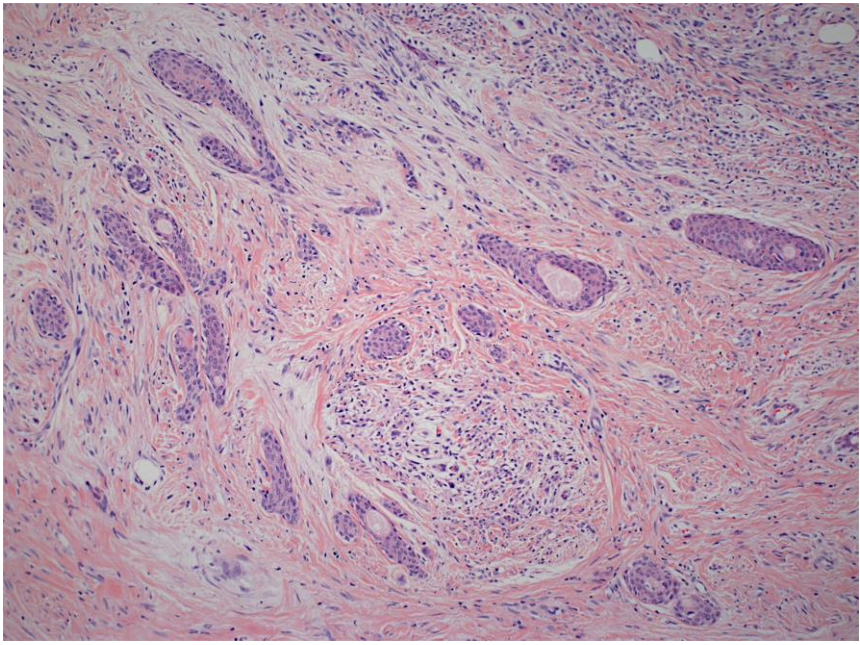
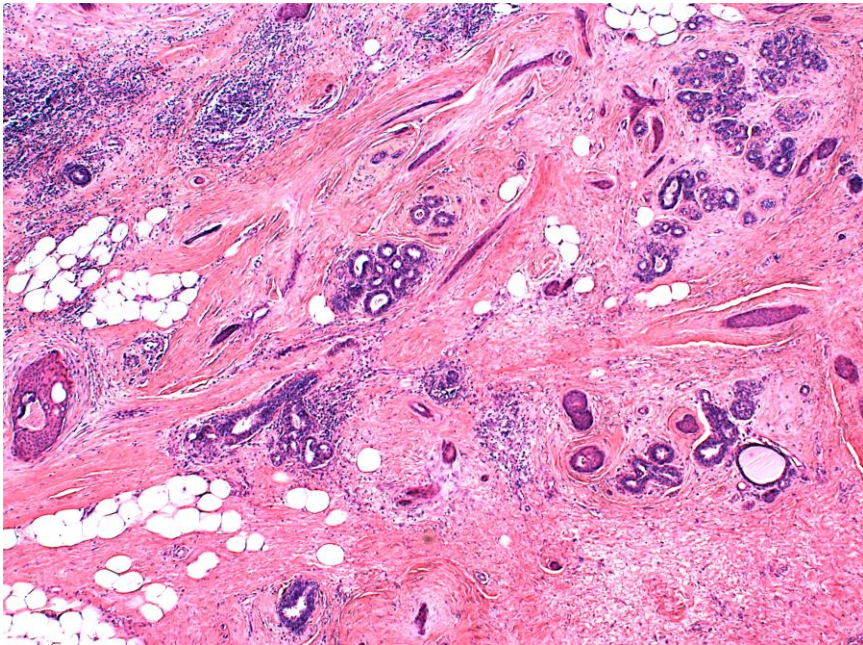
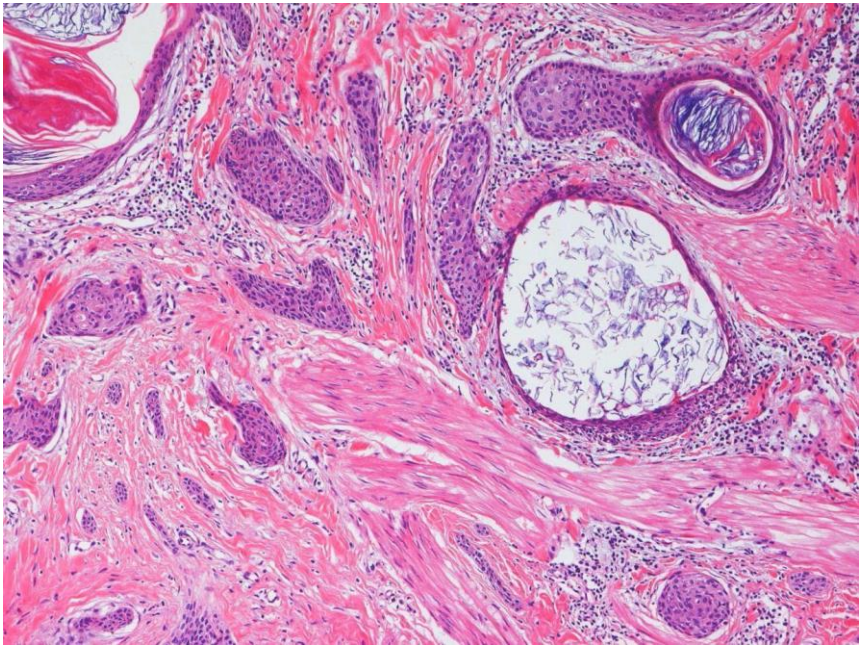
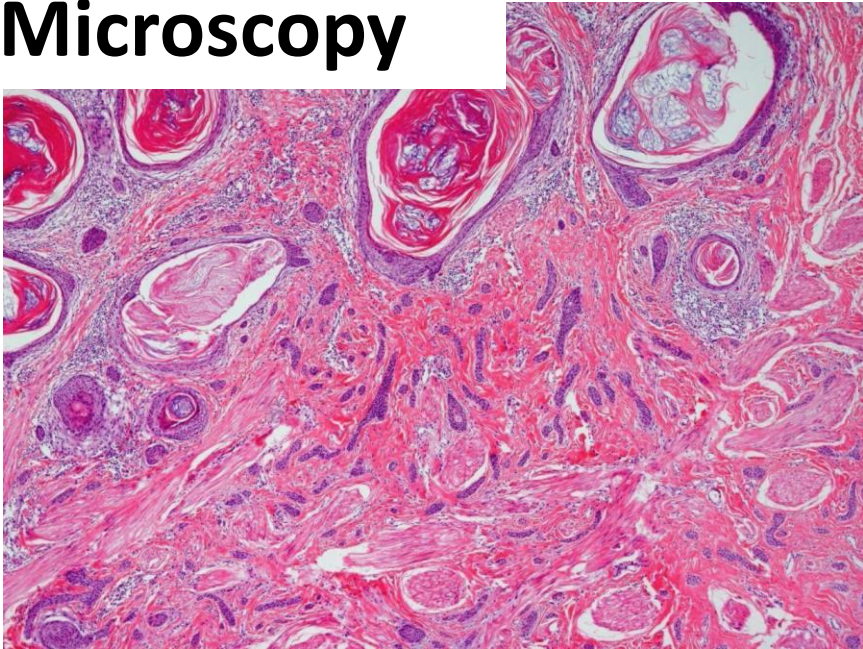
Low grade adeno-squamous carcinoma (n=9)

All 9 tumors located deep in the parenchyme outside the nipple.

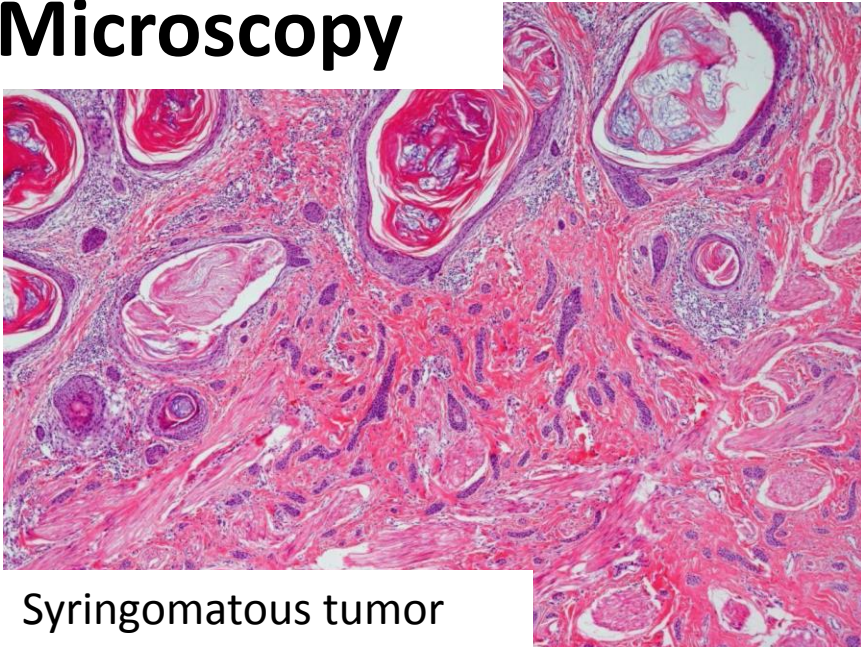
Macroscoopy: 1,0- >3,0 cm size (Literature: 0,5-3,4cm, average about 2.0cm)



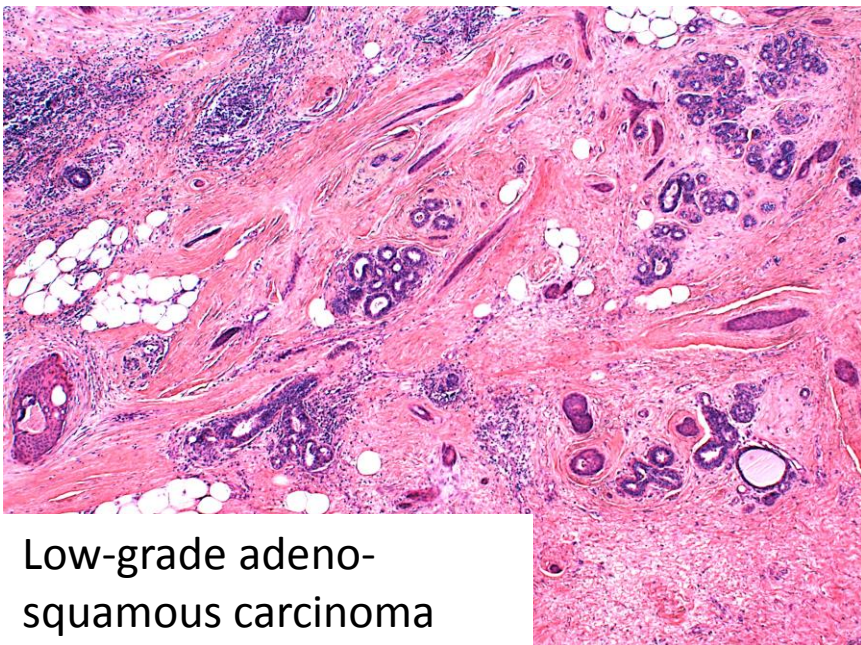
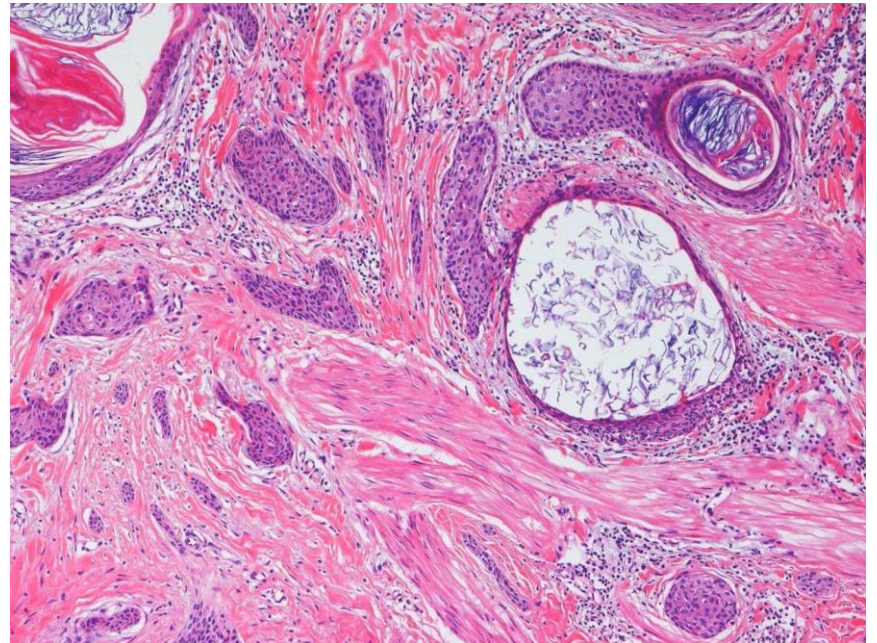
Microscopy



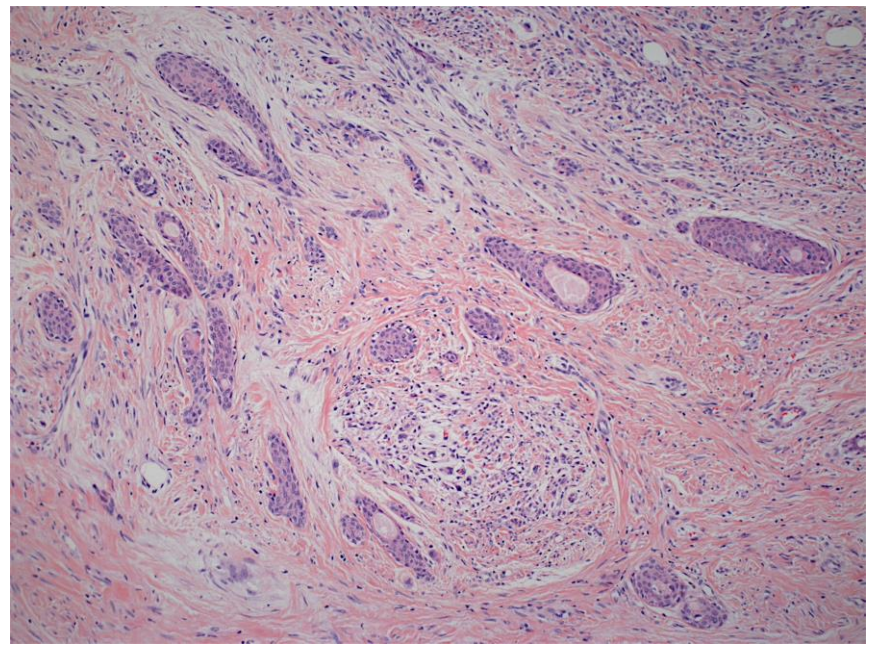
Microscopy



Syringomatous tumor

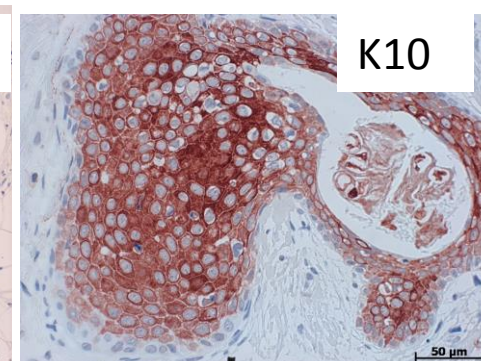
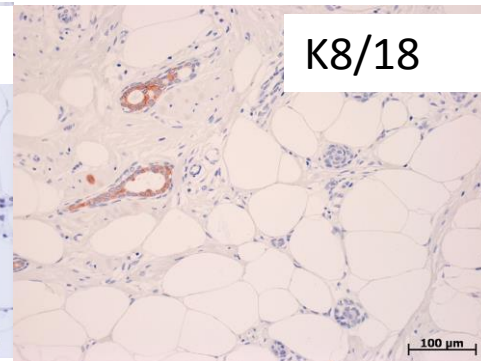
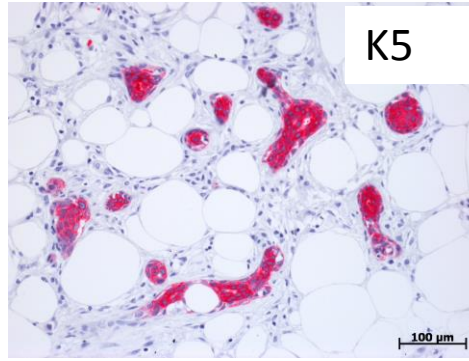
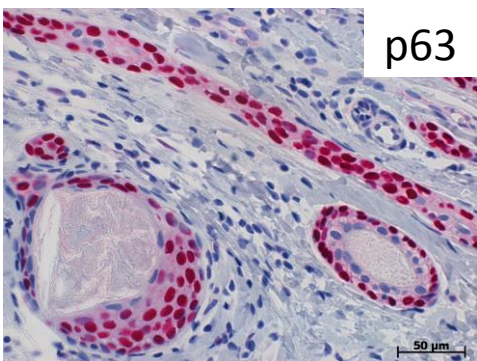


Low-grade adeno-squamous carcinoma

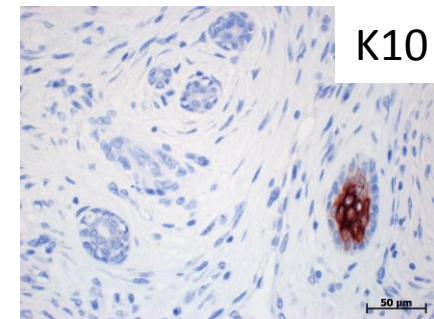
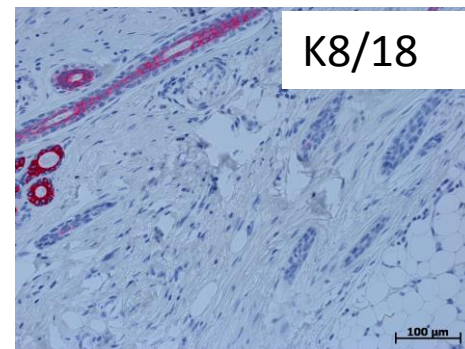
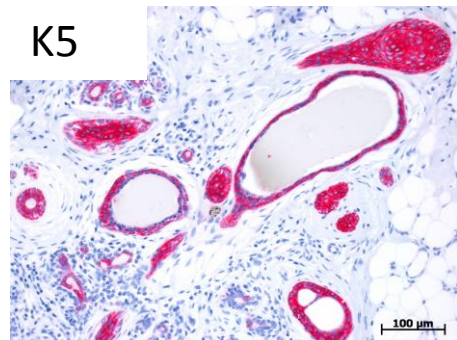
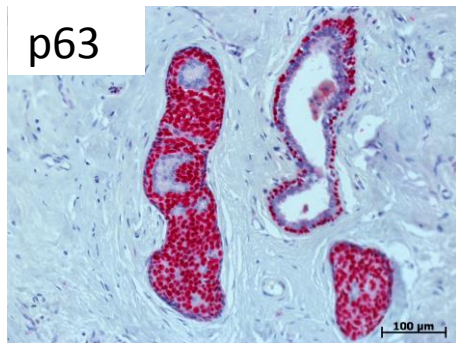


Immunohistochemistry

Syringomatous tumor

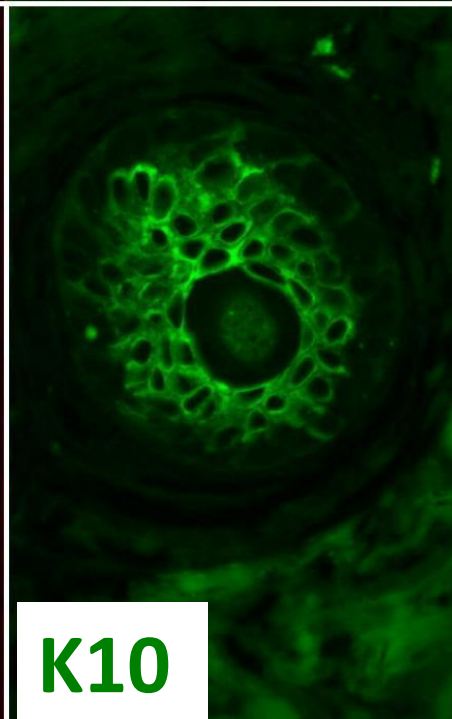
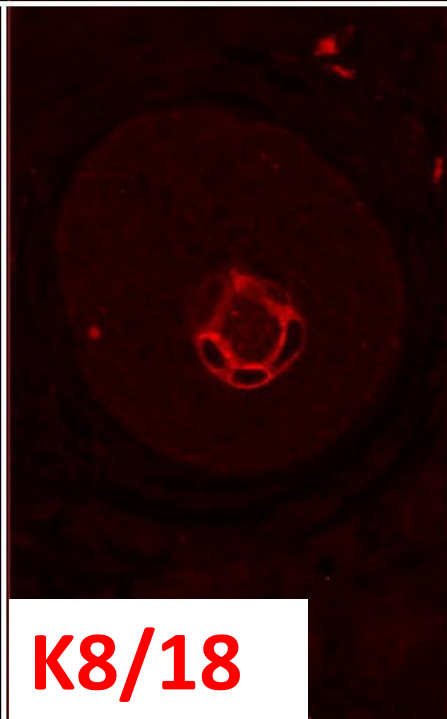
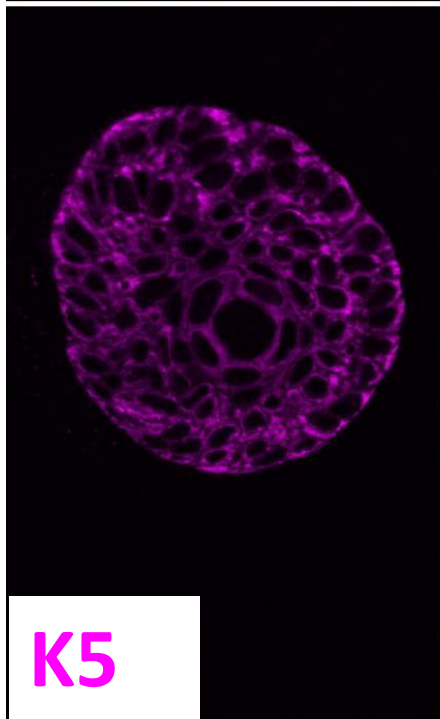
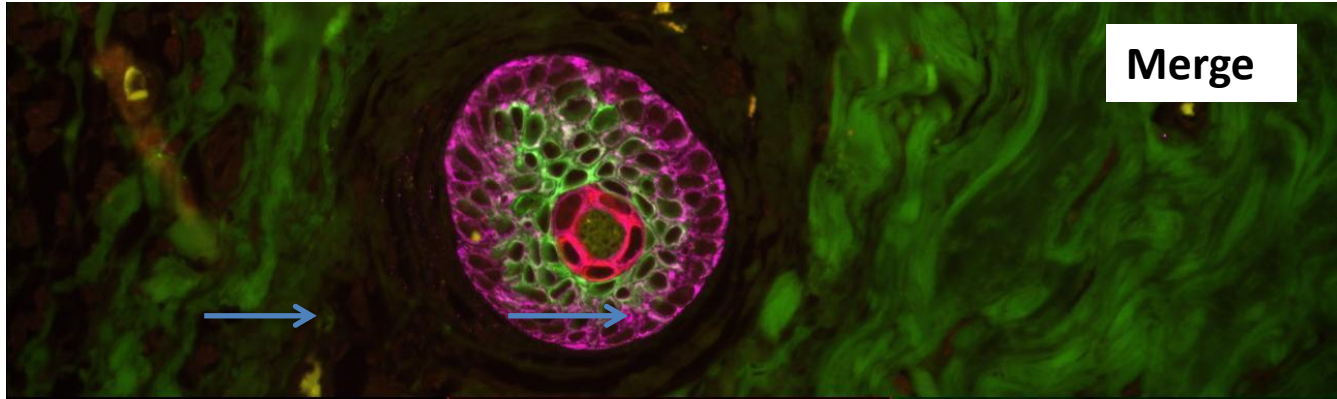


Low-grade adeno-squamous carcinoma

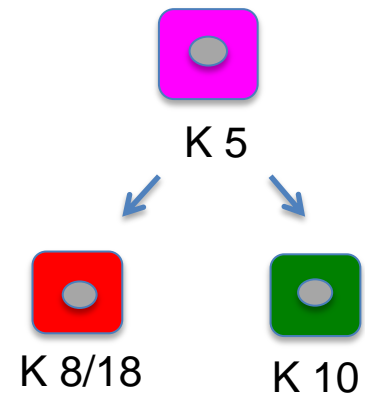


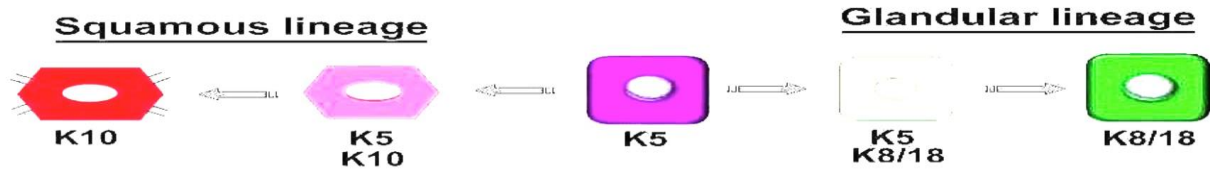
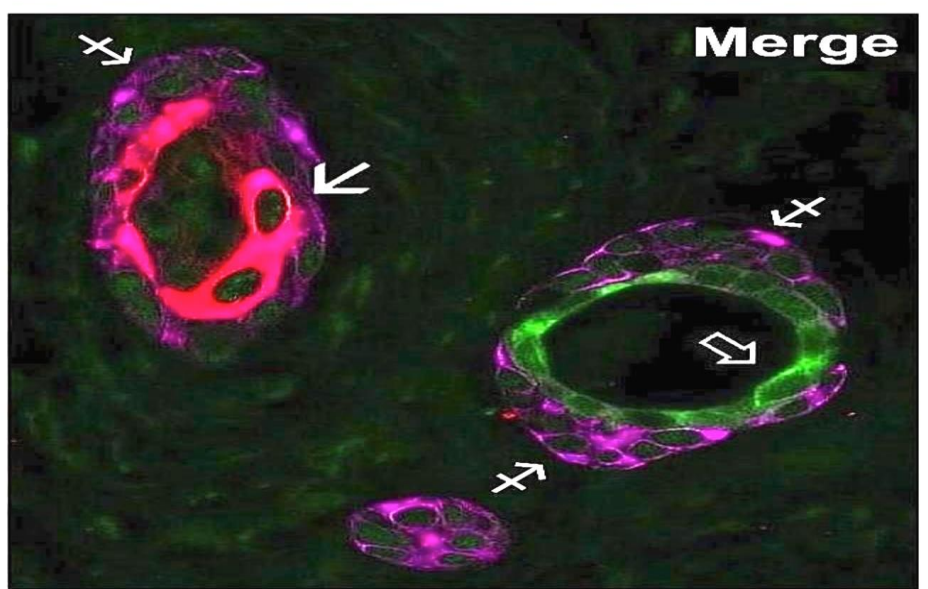
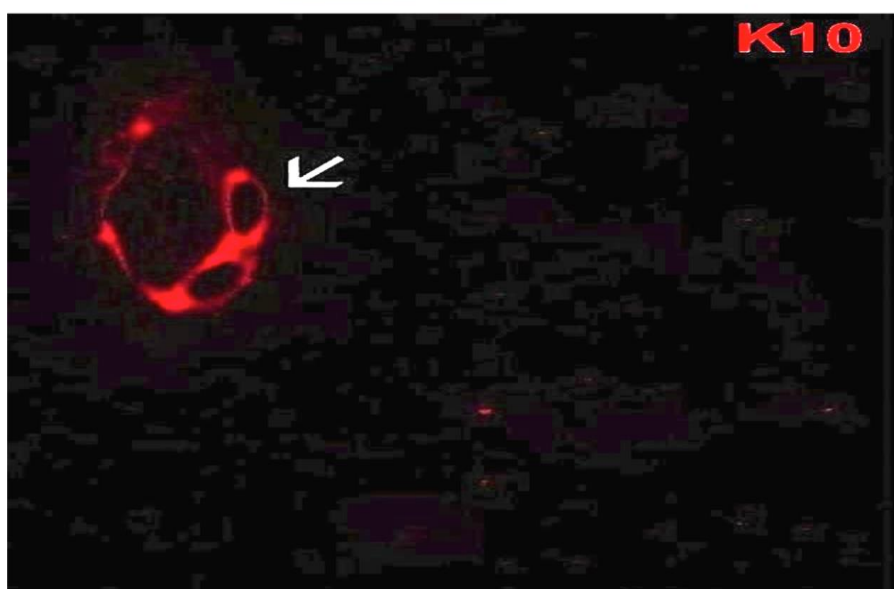
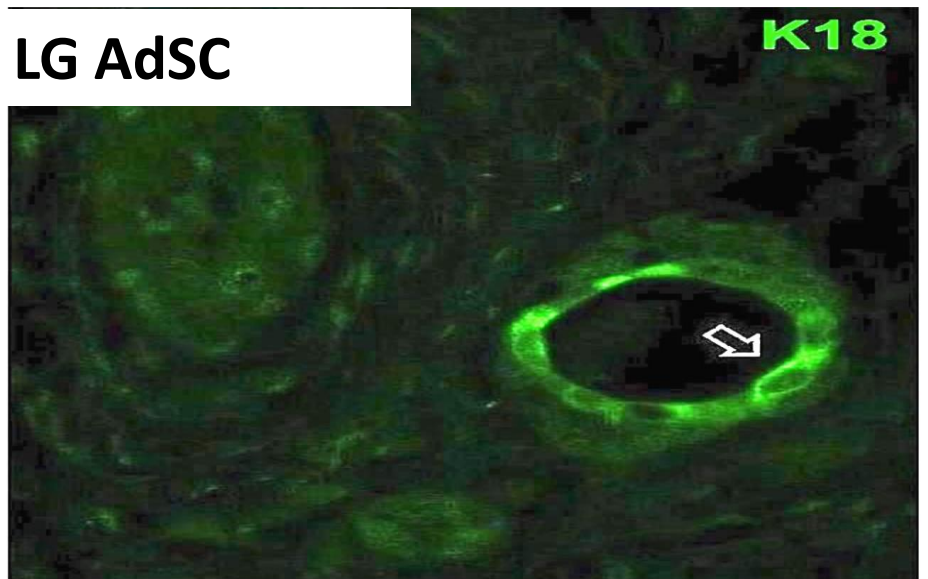
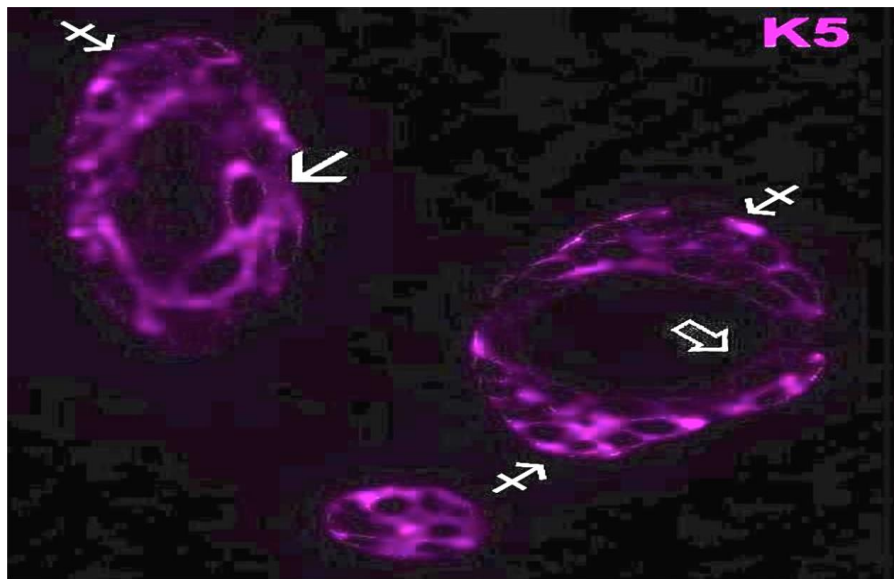
Syringomatous adenoma

In-situ Triple Immunofluorescence for K5, K8/18 and K10



K5+ cells
give rise to
squamous
and
glandular
cells





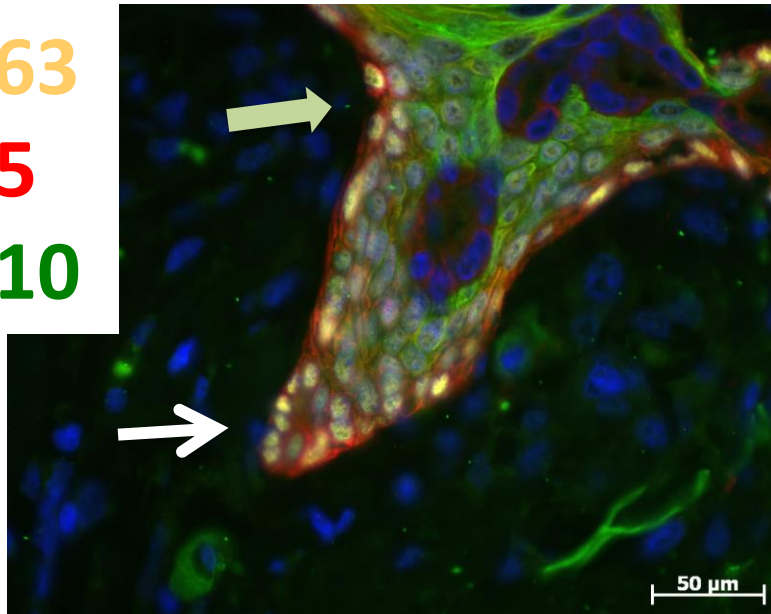
Syringomatous adenoma

In-situ Triple Immunofluorescence for p63, K5, and K10

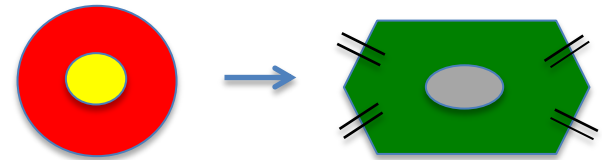
P63

K5

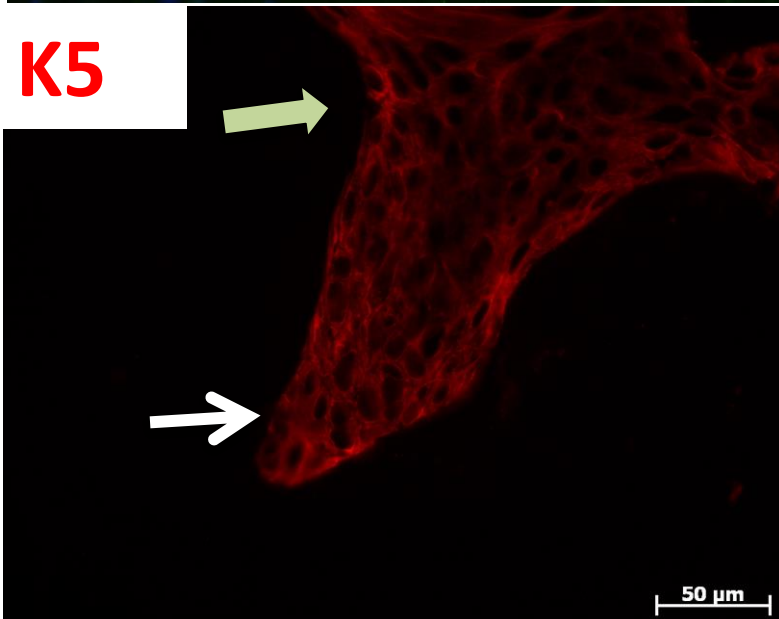
K10



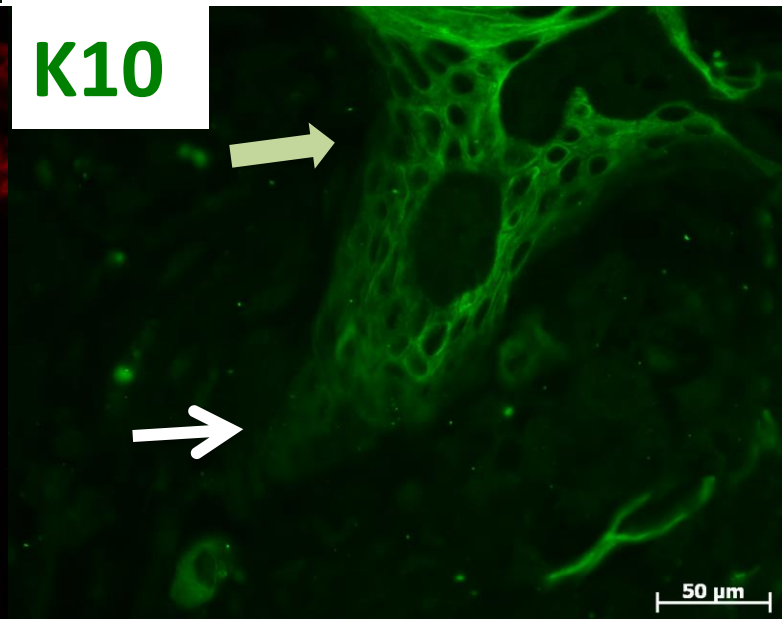
P63+K5+ progenitor cells give rise to squamous cells



K5



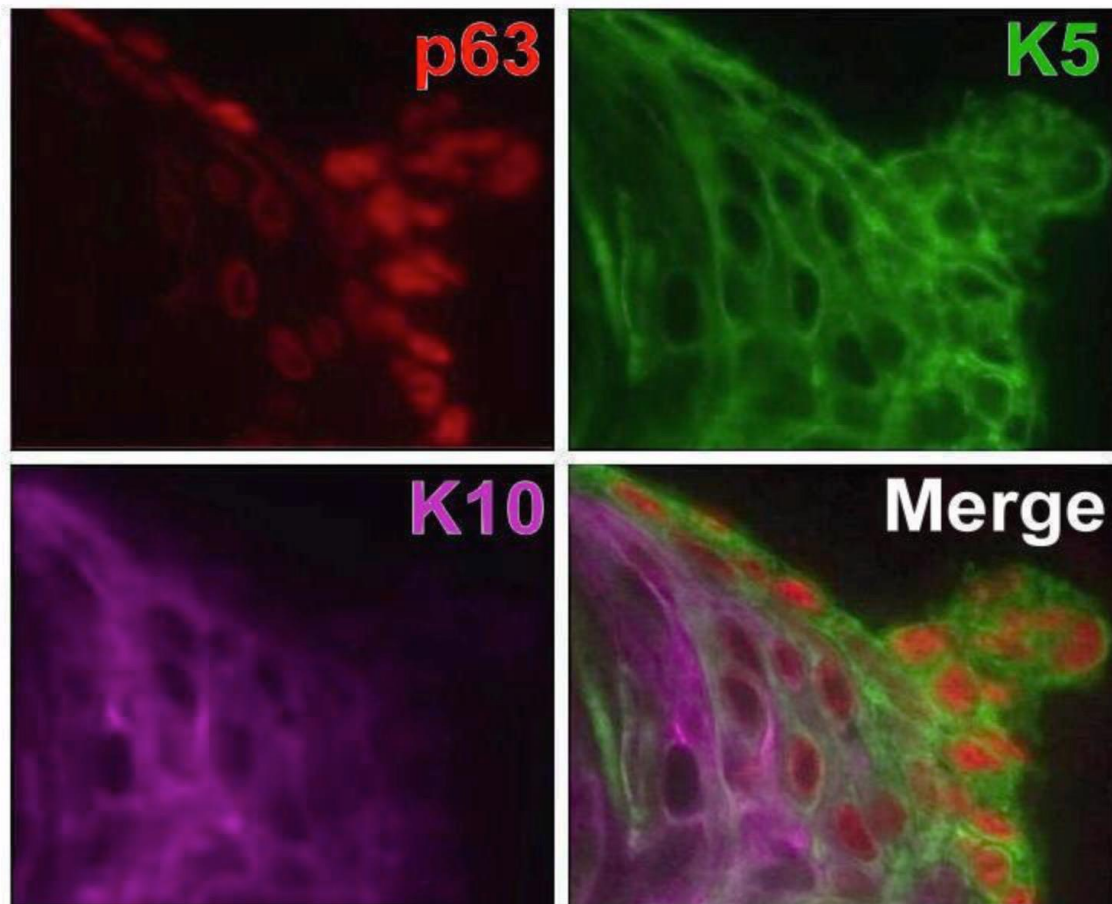
K10



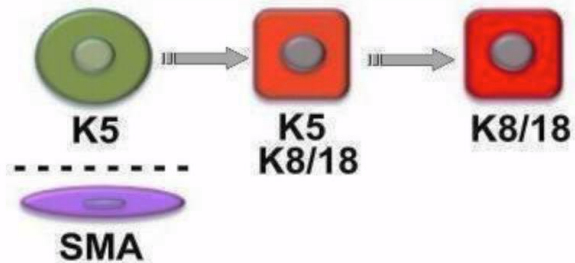
a LG AdSC



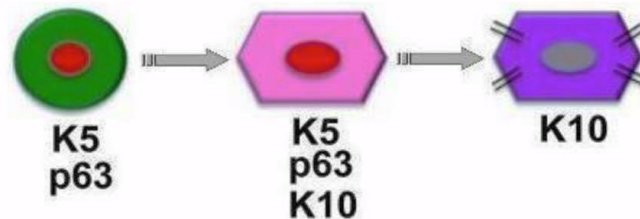
b



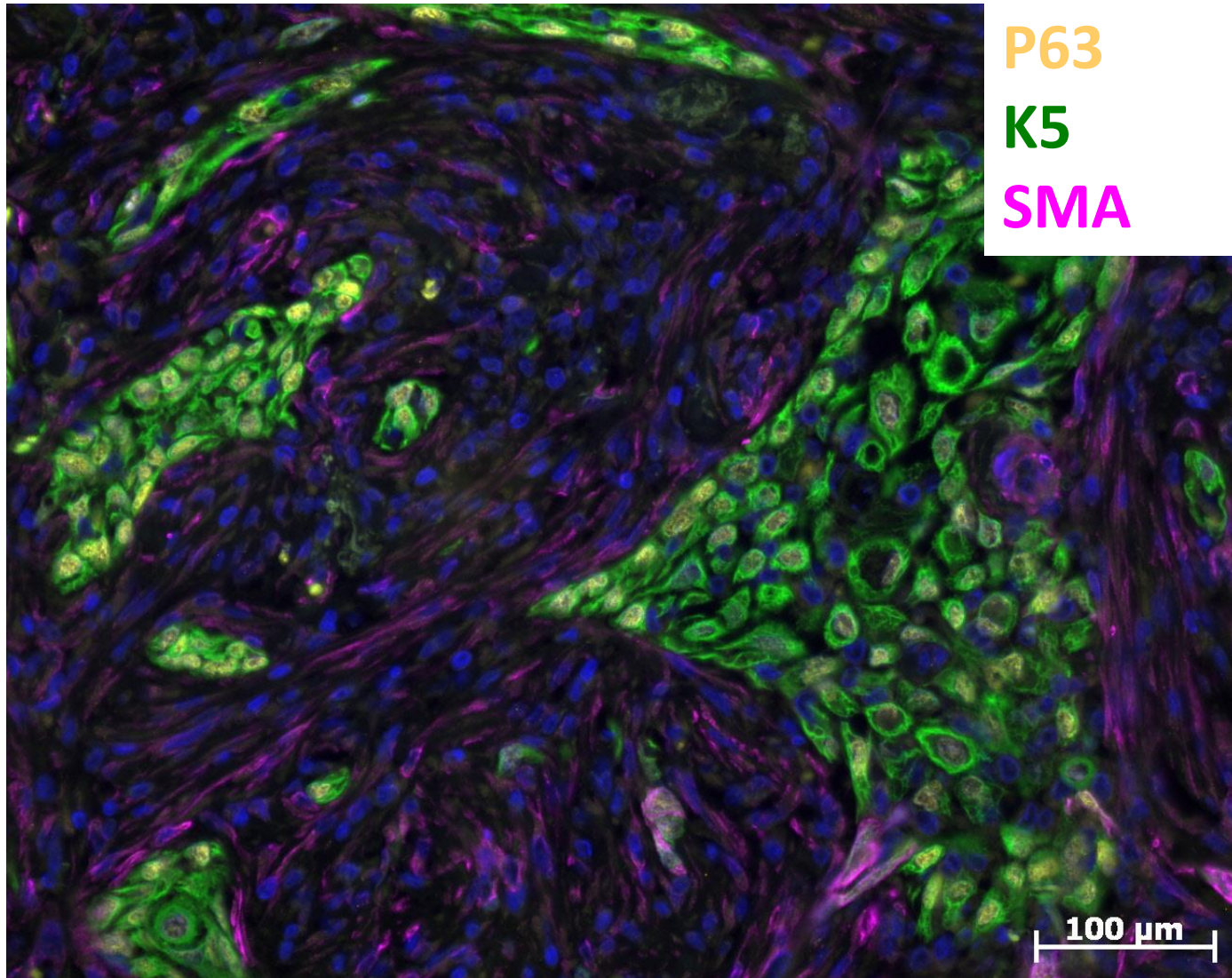
Glandular lineage



Squamous lineage

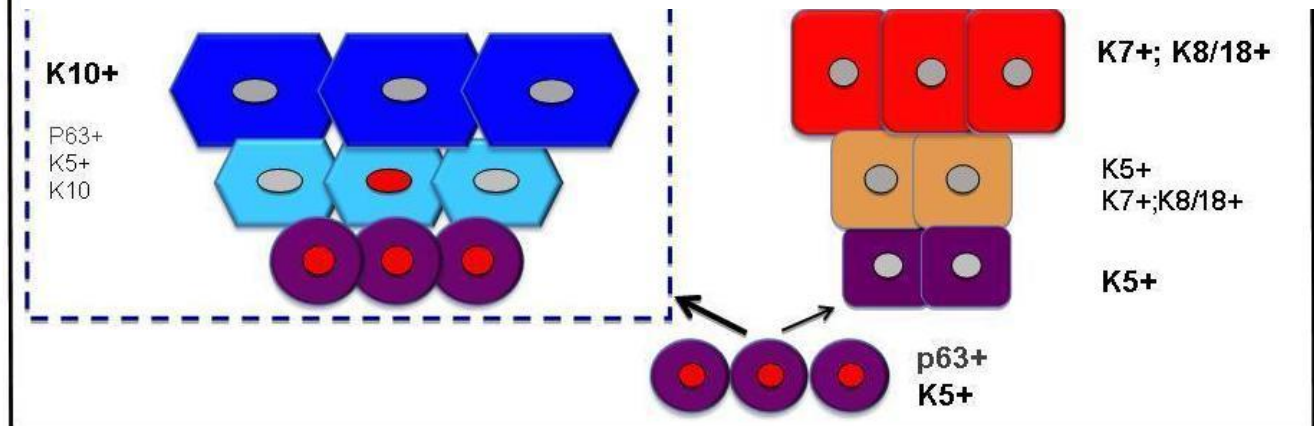


LG AdSC: Triple Immunofluorescence for p63, K5 and SMA



SMA+ myoepithelial cells are not observed in these tumors!

Syringomatous tumor/ Low-grade adenosquamous carcinoma

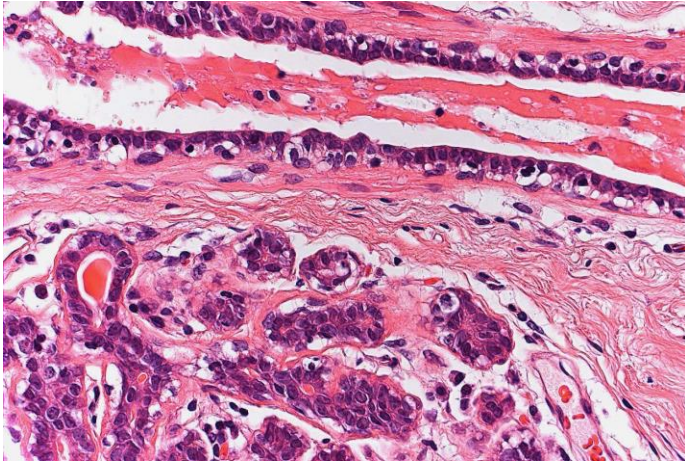


Conclusion:

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



HE
IH
Molecular

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!

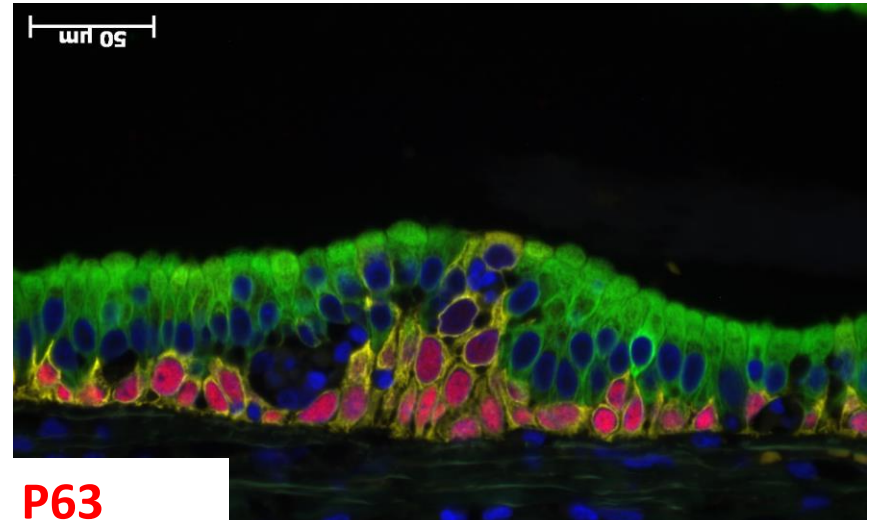
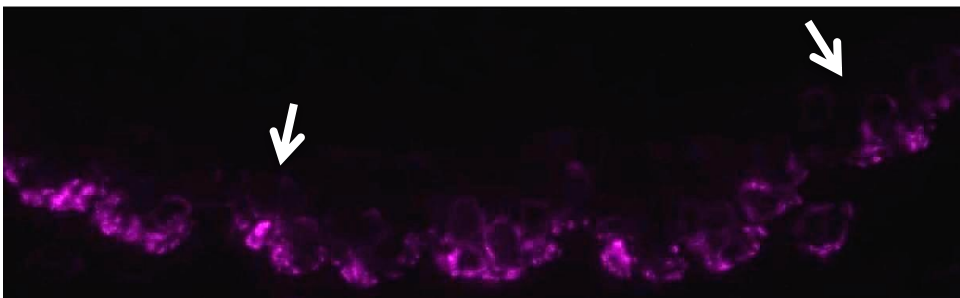
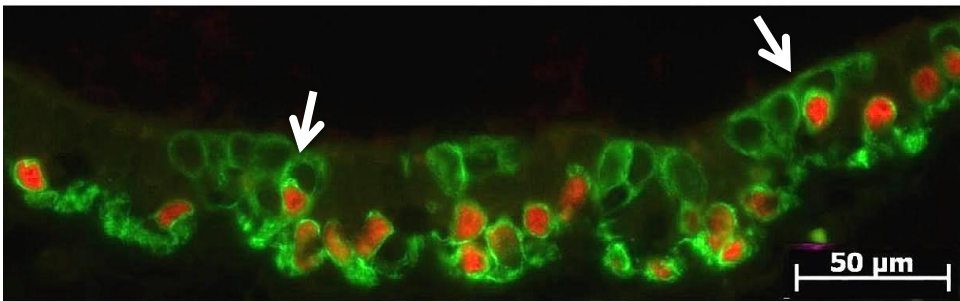
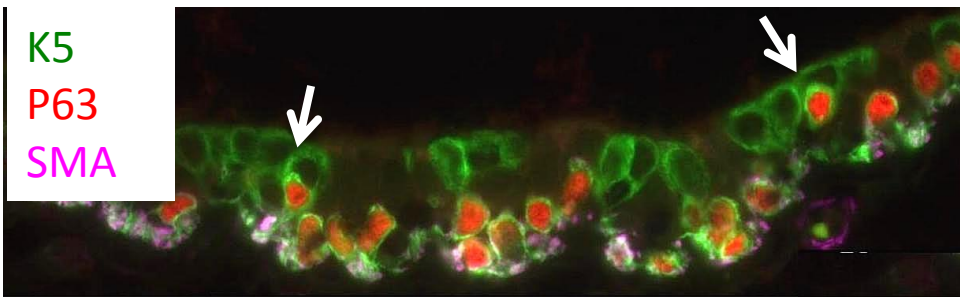
Stem cells in breast epithelium

(Eirew et al., 2008) (L) [5] [4].Lui et al (Dontu) 2005, (Kordon and Smith, 1998) (Novelli et al., 2003; Tsai et al., 1996) [4]. (SP) (Goodell et al., 1997; Welm et al., 2002, Smith and Chepko, 2001) (Chepko and Smith, 1997),(Smalley and Clarke, 2005) (Sleeman et al., 2007).

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**

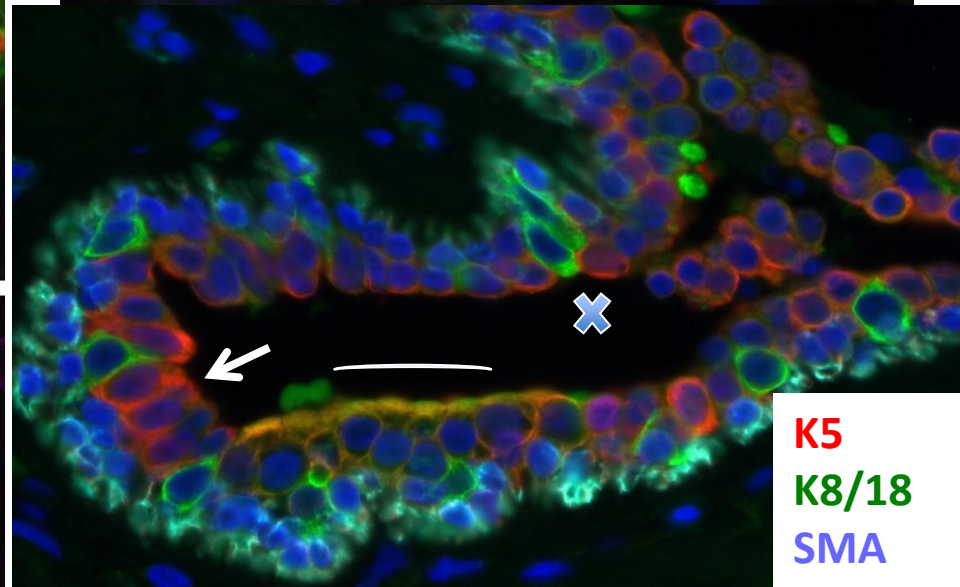
P63+K5+ progenitor cells in breast ducts give rise to glandular and myoepithelial cells

Breast duct



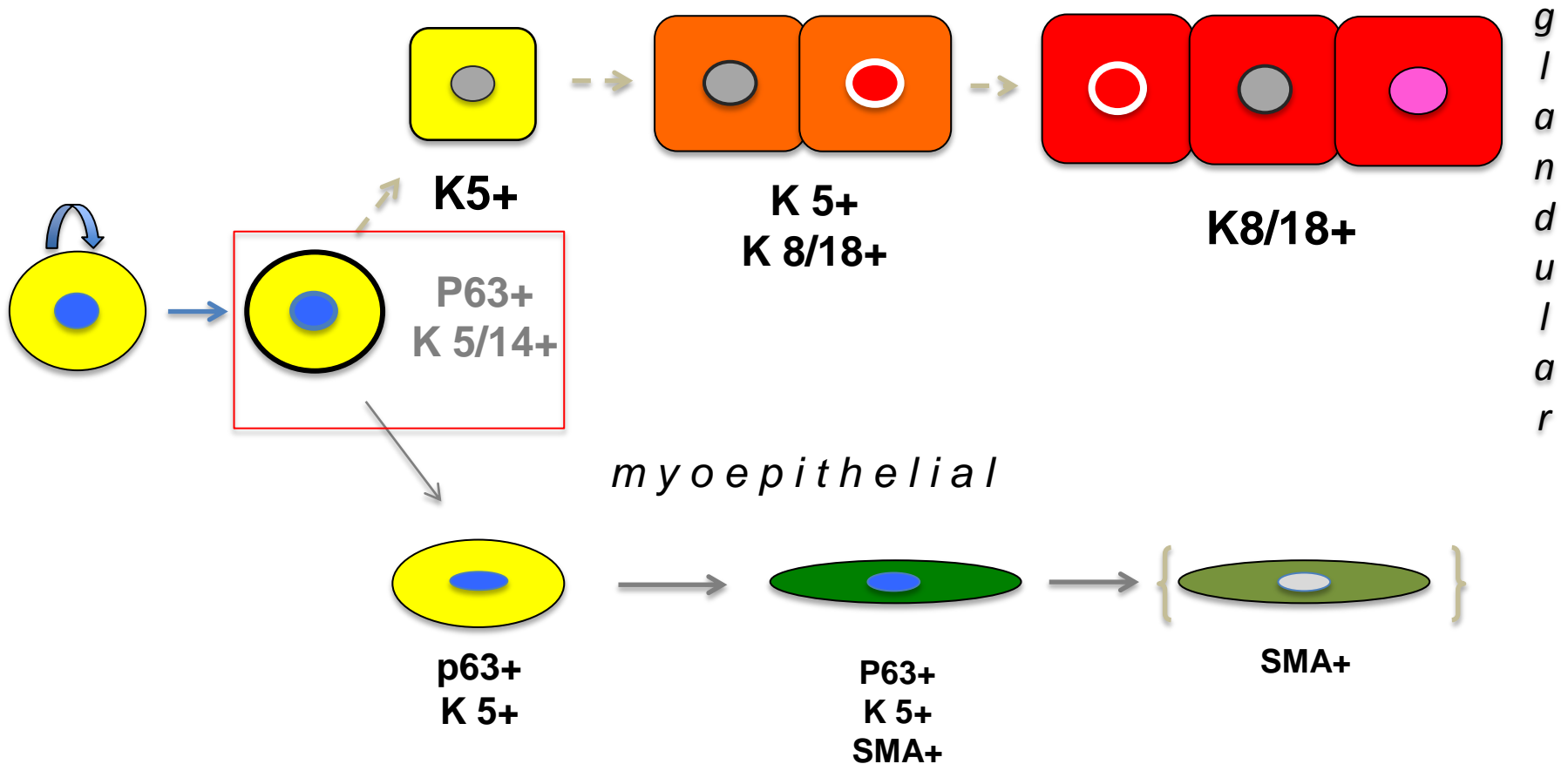
P63
K5
K8/18

Salivary excretory duct



K5
K8/18
SMA

Breast Duct Epithelium



P63+K5+ bipotent cells give rise to K8/18+ glandular and SMA+ myoepithelial cells

● p63

● Estrogen receptor

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.

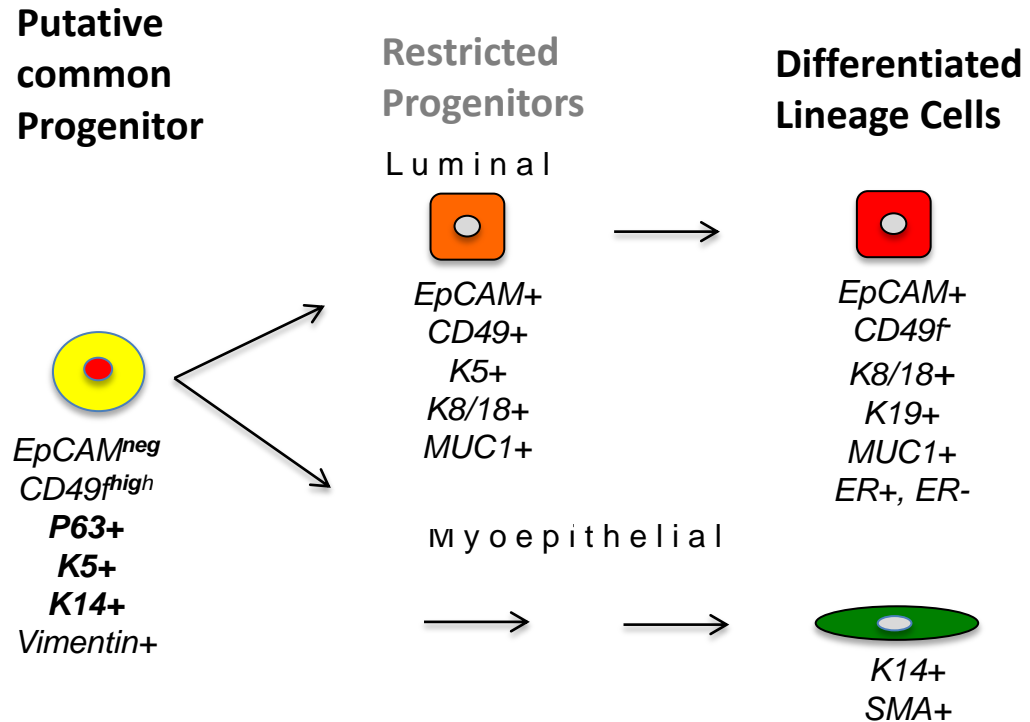
**Thank you
for your
attention**

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 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)

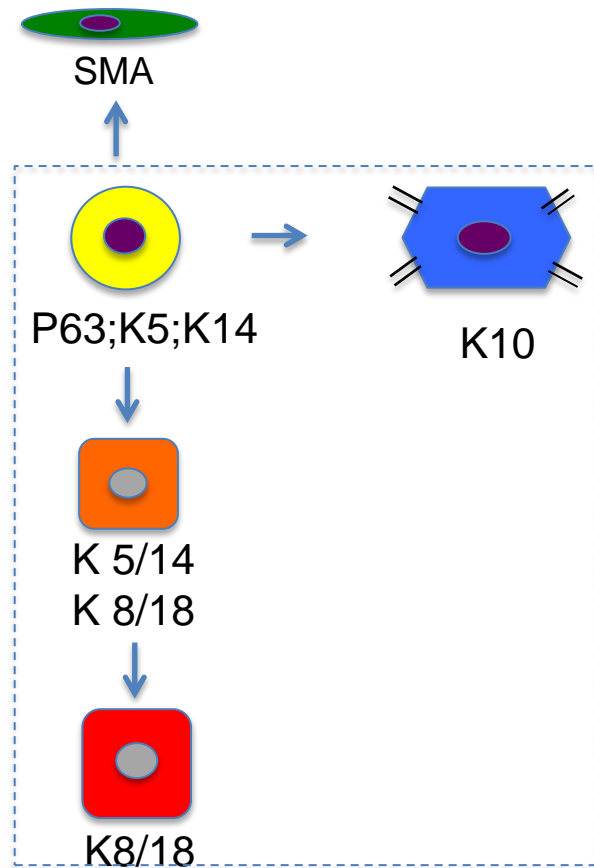
Adeno-myoepithelial tumors (n=7)

Adenoid-cystic carcinoma (n=6)

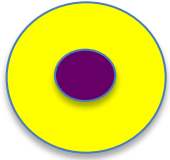
Squamous carcinoma breast (n=8)

Syringoma (n=12)

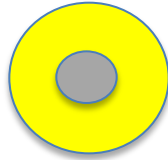
Adeno-squamous carcinoma (n=8)



Chang et al, 2003; Foschini and Eusebi, 1989; Loose et al,1992; Marchio et al, 2010;Shin and Rosen, 2002;Slaughter et al, 1992; Suster et al, 1991; Pia Foschini and Reis-Filho, 2003; Rosen, 1989; Trendell-Smith et al, 1999; Wetterskog et al, 2012
Reis-Filho and Schmitt, 2002 (p63..reliable stem cell marker)
Boecker et al, 2012,2013,2014a,b



K5/14+
P63+



progenitor cell

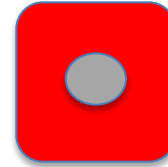
glandular lineage



K 5/14

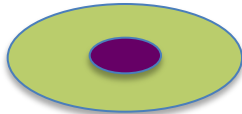


K 5/14
K 8/18

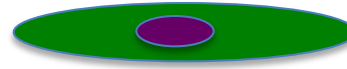


K 8/18

myoepithelial lineage

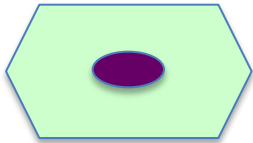


K 5/14
SMA

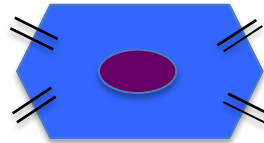


SMA

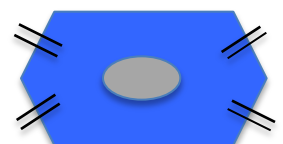
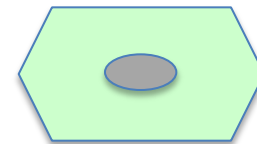
squamous lineage

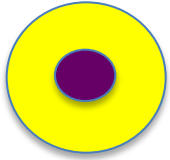


K 5/14
K10

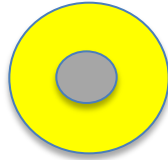


K10





K5/14+
P63+



progenitor cell

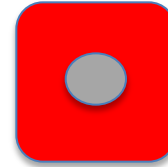
glandular lineage



K 5/14

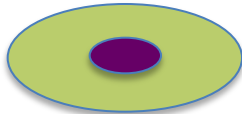


K 5/14
K 8/18



K 8/18

myoepithelial lineage

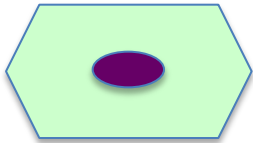


K 5/14
SMA

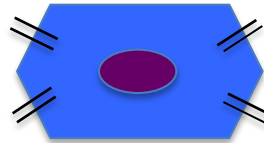


SMA

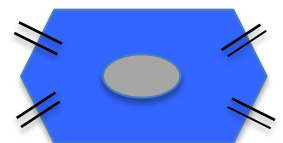
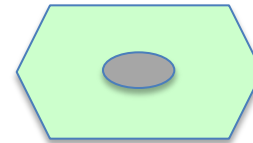
squamous lineage



K 5/14
K10



K10



Ontogeny

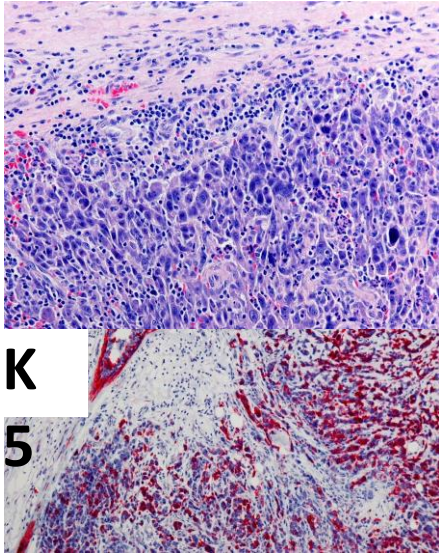
Knowledge of the human adult mammary gland epithelium is a requirement for understanding physiologic regeneration and for developing concepts of abnormal proliferative disease.

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**

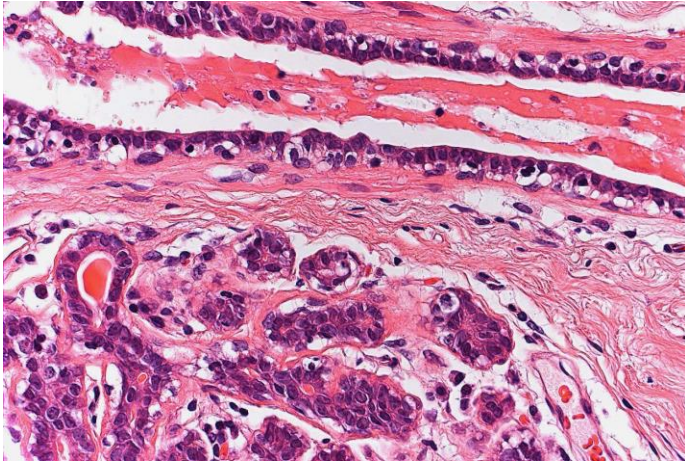
Current classification: Two cells concept

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

Perou, Sorlie et al, 2000; Sorlie et al, 2001, Abd El Rehim , Pinder et al,2004; Banerjee, Reis-Filho et al, 2006, Fulford, Reis-Filho et al, 2007; Haupt et al, 2010, Jumppanen et al, 2007, Kim et al, 2006; Korsching et al, 2008; Laakso et al, 2006, Lavasani, Moinfar, 2012, Lerma et al, 2009, Linn, Van `t Veer, 2009; Livasi et al, 2007, Moinfar, 2008, Nassar et al, 2012; Natrajan et al, 2010, Nielsen et al, 2004; Polidoro et al, 2009; Rakha et al, 2008; Reis-Filho et al, 2006, Reis-Filho and Tutt, 2008; Tan et al,2008; Tuner and Reis-Filho, 2006; Yamashita et al, 2013

Ontogeny and breast tumors

Normal breast epithelium



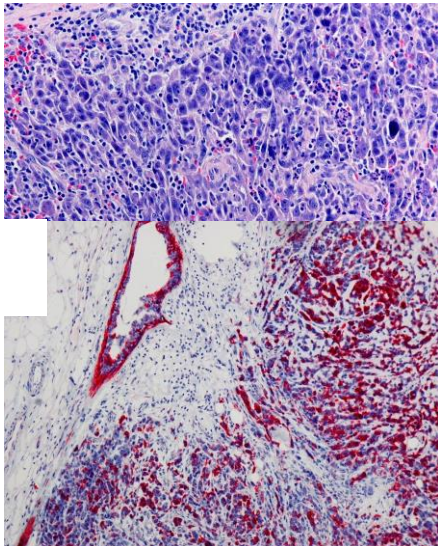
HE
IH
Molecular

Two cells concept

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



Invasive carcinoma



HE
IH
Molecular

Two cells concept

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

Ontogeny: Hypothetical Models of the Cellular Hierarchy of Human Breast Epithelium I

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

Gudjonsson et al, 2002

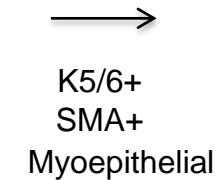
Villadsen et al, 2007

- *Flow cytometry*
- *Immunofluorescence*
- *Cell culture*
- *Transplantation assays*

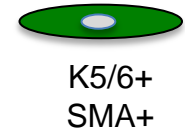
Putative common Progenitor



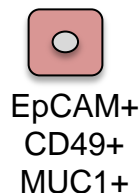
Intermediary cells



Differentiated Cells



Luminal



Myoepithelial



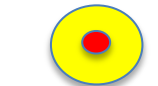
Hypothetical Models of the Cellular Hierarchy of Human Breast Epithelium II

Lim et al, 2009

Eirew et al, 2008

- Flow cytometry
- Cell culture
- Transplantation assays
- Immunohistochemistry

Putative common Progenitor



EpCAM^{neg}
CD49^{high}
P63+
K5+
K14+
Vimentin+

Restricted Progenitors

Luminal



EpCAM+
CD49+
K5+
K8/18+
MUC1+

myoepithelial

Differentiated Lineage Cells



EpCAM+
CD49^f
K8/18+
K19+
MUC1+
ER+, ER-



K14+
SMA+



EpCAM+
MUC1+
ER+ o ER-



K14+
CD10+

Clarke et al, 2005

- Immunomagnetic sorting
- Immunofluorescence
- Cell culture
- Transplantation assays



ER+
Musashi+
or p21+
K19-

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-myoeithelial 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**

Chang et al, 2003; Foschini and Eusebi, 1989; Loose et al,1992; Marchio et al, 2010;Shin and Rosen, 2002;Slaughter et al, 1992; Suster et al, 1991; Pia Foschini and Reis-Filho, 2003; Rosen, 1989; Trendell-Smith et al, 1999; Wetterskog et al, 2012
Boecker et al, 2012,2013,2014a,b