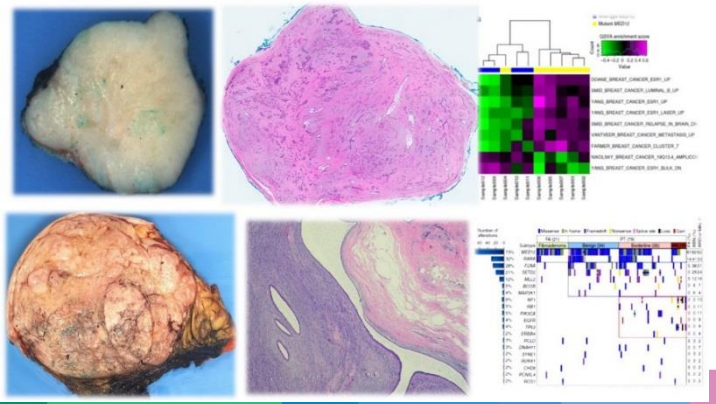
Breast Pathology Course 2019

Division of Pathology
Singapore General Hospital

Genomics of Fibroepithelial Tumours of the Breast



Dr Puay Hoon Tan
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Fibroepithelial breast lesions are biphasic tumours composed of both epithelial and stromal components, and include the common ***fibroadenoma*** and the rarer ***phyllodes tumour***.

Genomics ~ structure, function, evolution, mapping, and editing of genomes. A genome is an organism's complete set of DNA, including *all of its genes*.

Genetics ~ study of *individual* genes and their roles in inheritance.

Not a molecular pathologist!

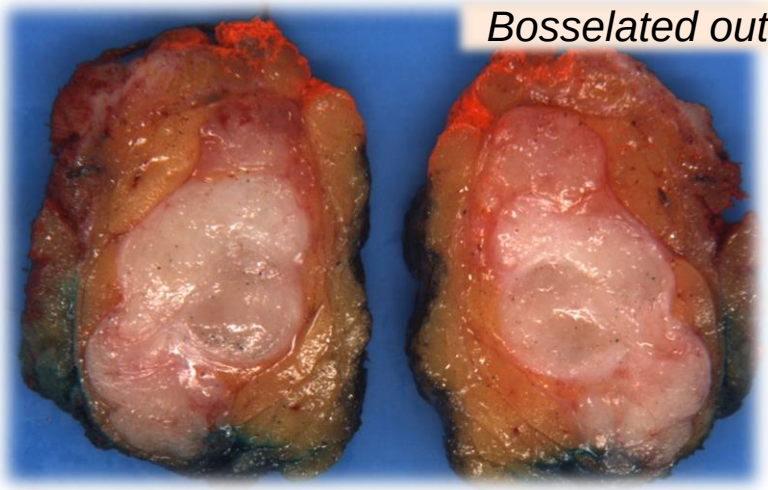
Scope

- Genomics of ~
  - **Fibroadenoma**
  - **Phyllodes tumour**
- Potential clinical applications

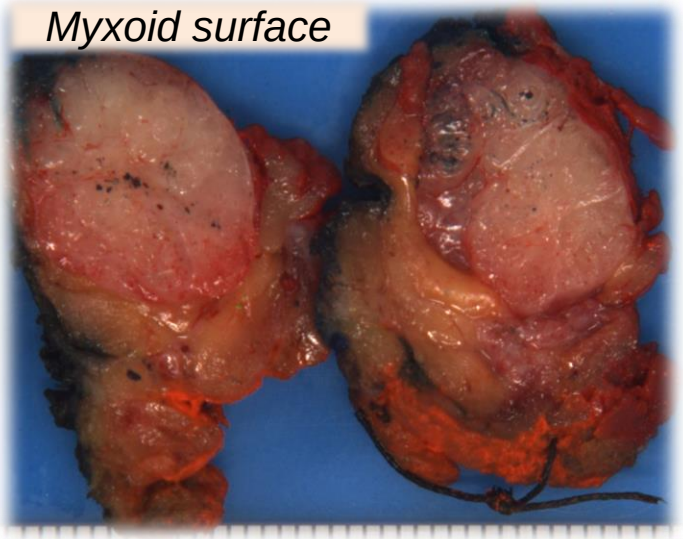
Fibroadenoma

- Common benign biphasic tumour.
- Circumscribed breast neoplasm arising from the terminal-duct lobular unit (TDLU).
- Features a proliferation of both epithelial and stromal elements.
- Occurs most frequently in women of childbearing age, especially those aged < 30 years, although it may be encountered at any age.
- Estimated 10% of women have fibroadenomas.

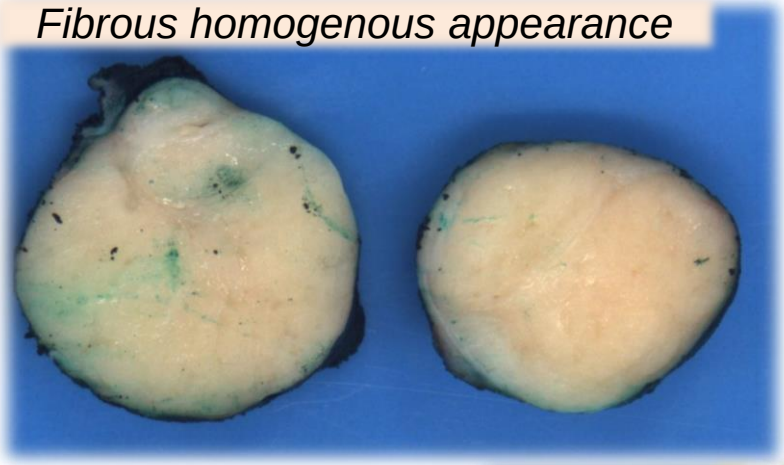
Bosselated outlines



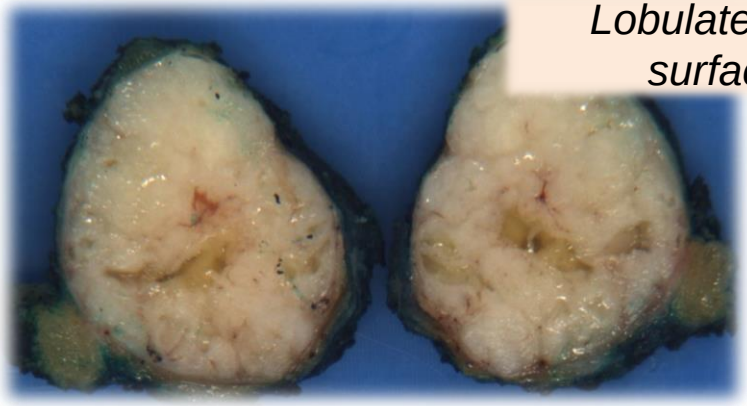
Myxoid surface



Fibrous homogenous appearance

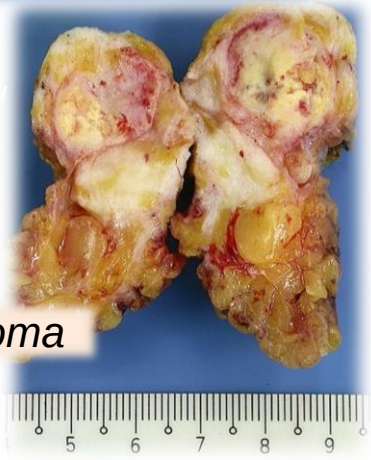


Lobulated cut surface

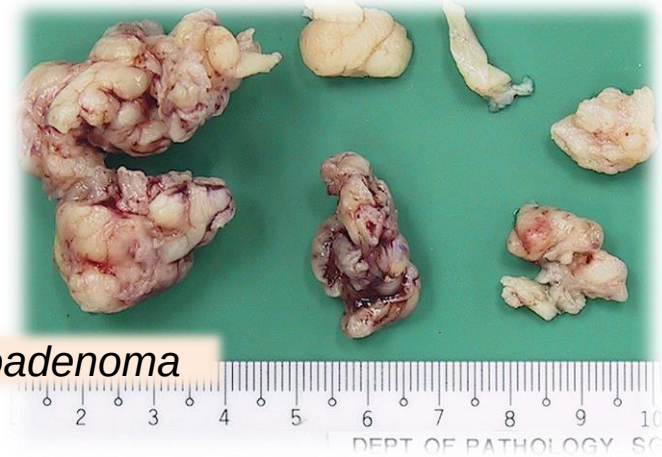


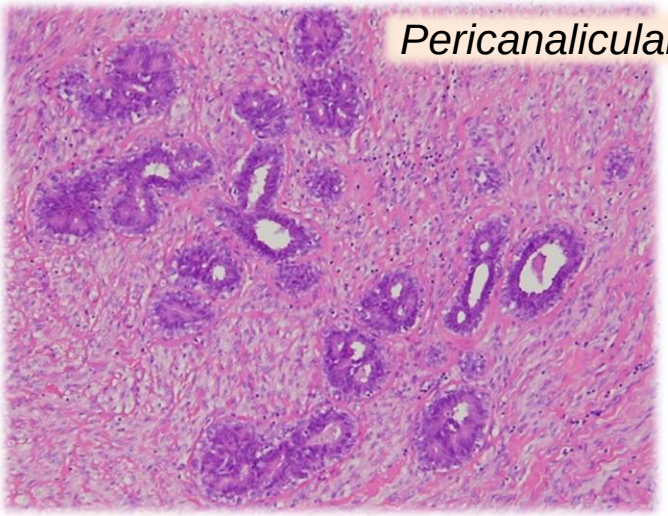
Gross anatomy of fibroadenoma

Ossified fibroadenoma

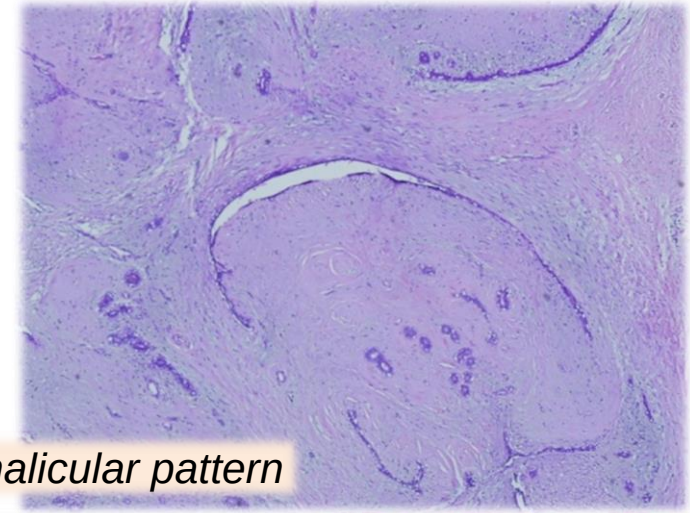


Giant fibroadenoma

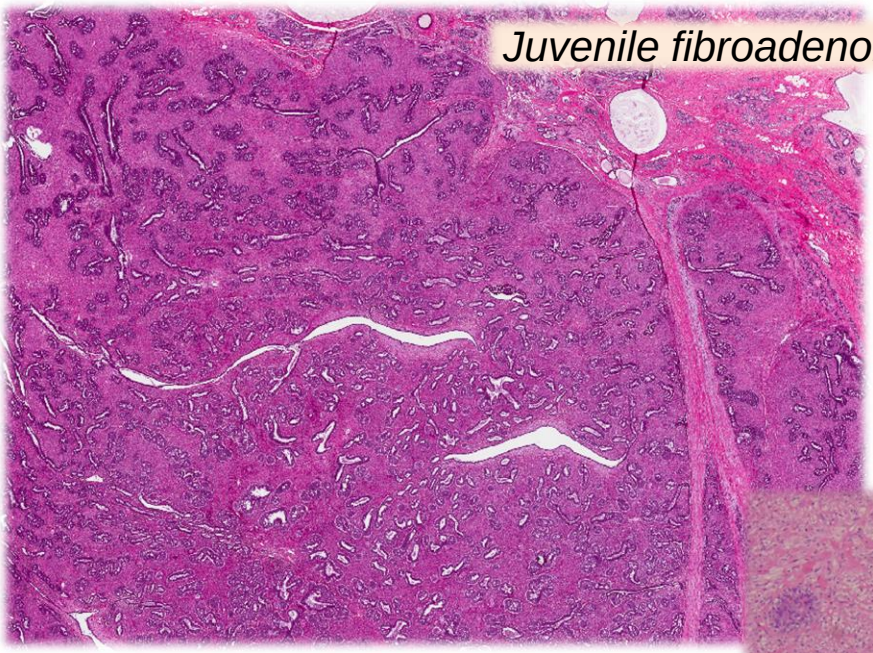




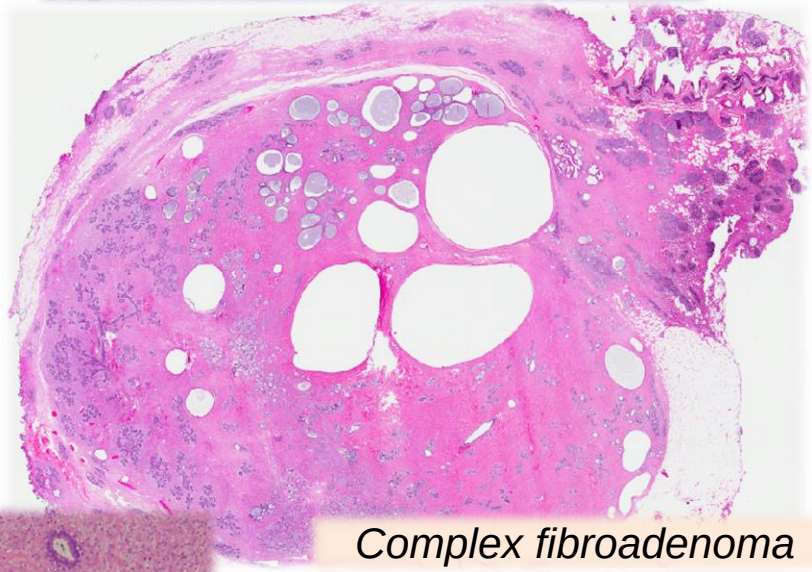
Pericanalicular pattern



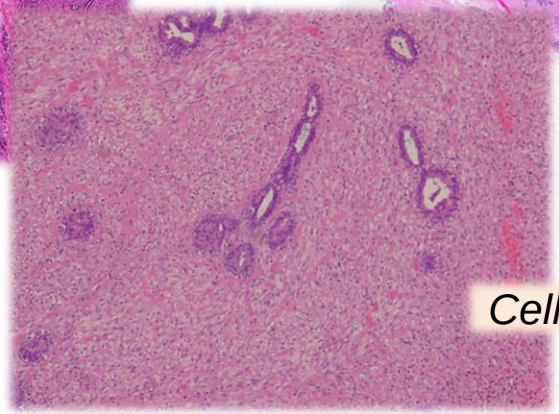
Intracanalicular pattern



Juvenile fibroadenoma



Complex fibroadenoma



Cellular fibroadenoma

Microscopic anatomy of fibroadenoma

Phyllodes tumour

- Uncommon fibroepithelial neoplasm with proliferation of both epithelial and stromal components.
- "Phyllodes"
  - Derived from the Greek word "phyllon" meaning *leaf*, and "eidos" meaning *form*.

φύλλο εἶδο
(leaf form)

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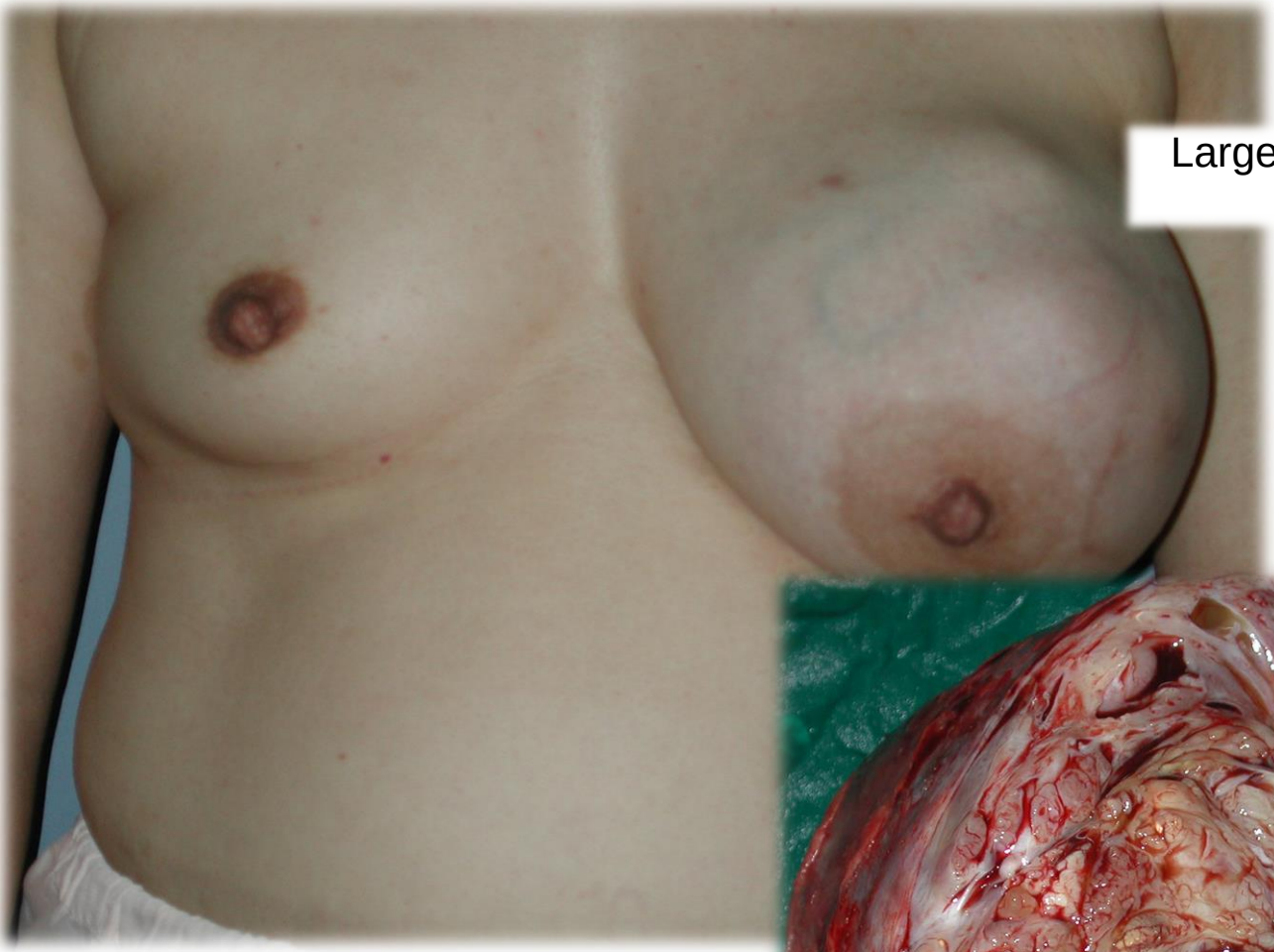
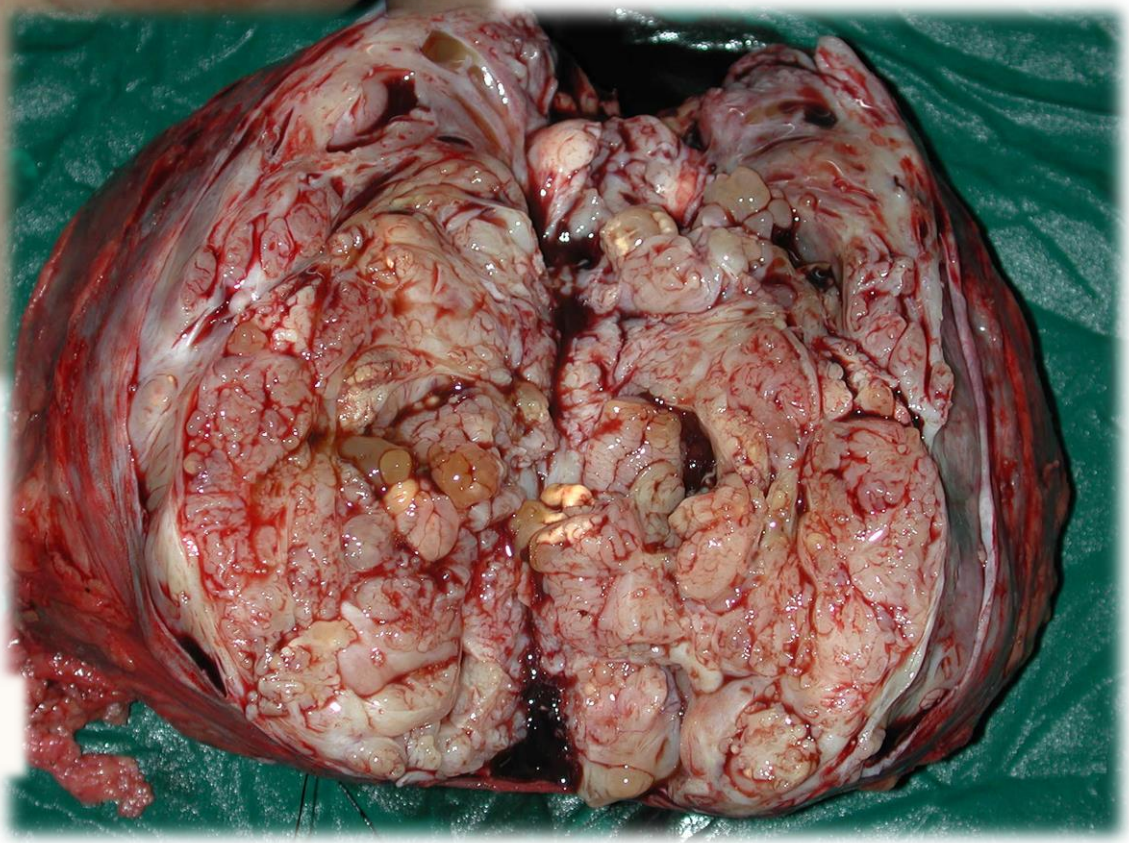
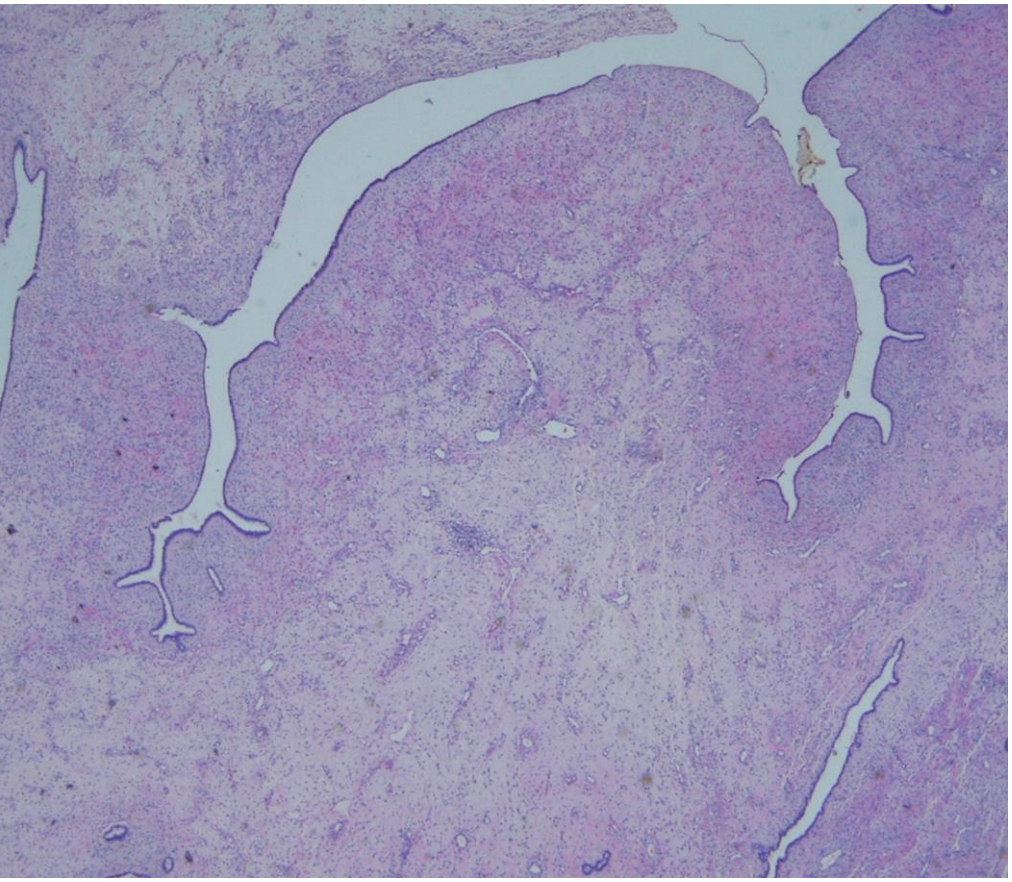


Large tumour stretching skin



Circumscribed bulging mass, mucoid, fleshy, whorled

Phyllodes tumour: *fibroepithelial neoplasm resembling intracanalicular fibroadenoma, but with exaggerated fronded pattern and stromal hypercellularity*



Benign phyllodes tumour

- 0.3-1% of all primary breast tumours.
- Affects mature women (40-50 years).
- Higher incidence in Asians.
- Graded according to histological characteristics.
- Tendency to recur if incompletely excised.

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Molecular genetics & genomics of fibroadenoma

Molecular genetics of fibroadenomas

- Cytogenetic abnormalities in 20% to 30% of fibroadenomas, usually translocations.
- No consistent pattern of specific chromosomal alterations.
- Both epithelial and stromal components are polyclonal. *(Noguchi et al. Cancer Res 1993; 53: 4071-4072)*
- Possible evolution into phyllodes tumors.

(Noguchi et al. Cancer 1995; 76: 1779-1785)

- Low levels of LOH (0% to 1.5%).

(*Wang et al. Breast Cancer Res Treat 2006; 97: 301-309)*

No evidence of recurrent genetic alterations characteristic of fibroadenomas. *Rosen's Breast Pathology 4th edition 2014*

nature genetics

Nat Genet. 2014 Aug;46(8):877-80.

published online 20 July 2014;

Exome sequencing identifies highly recurrent *MED12* somatic mutations in breast fibroadenoma

Weng Khong Lim[1,2,12], Choon Kiat Ong[1,2,12], Jing Tan[1,2,12], Aye Aye Thike[3], Cedric Chuan Young Ng[1,2], Vikneswari Rajasegaran[1,2], Swe Swe Myint[1,2], Sanjanaa Nagarajan[1,2], Nur Diyana Md Nasir[3], John R McPherson[4], Ioana Cutcutache[4], Gregory Poore[5], Su Ting Tay[2], Wei Siong Ooi[6], Veronique Kiak Mien Tan[7], Mikael Hartman[8], Kong Wee Ong[7], Benita K T Tan[9], Steven G Rozen[4], Puay Hoon Tan[3], Patrick Tan[2,10,11] & Bin Tean Teh[1,2,11]

Key findings:

- Exome sequencing of 8 fibroadenomas with matching whole blood samples revealed recurrent somatic mutations solely in MED12 (encodes a Mediator complex subunit).
- Targeted sequencing of an additional 90 fibroadenomas confirmed highly frequent *MED12* exon 2 mutations (58/98, 59%) that are probably somatic, with 71% of mutations occurring in codon 44.
- Using laser capture microdissection, it was confirmed that *MED12* fibroadenoma mutations are present in stromal but not epithelial mammary cells.

MED12 mutations in breast fibroadenoma

- *MED12* is located on the X chromosome.
- Frequent *MED12* exon 2 somatic mutations have been found previously only in uterine leiomyoma (UL).
- *MED12* mutation spectrum observed in fibroadenomas was nearly identical to that of UL in both exon location and variant codon preference.
- Possibility that *MED12* exon 2 mutations could be associated with hormonal expression.
- *MED12 in phyllodes tumours.*

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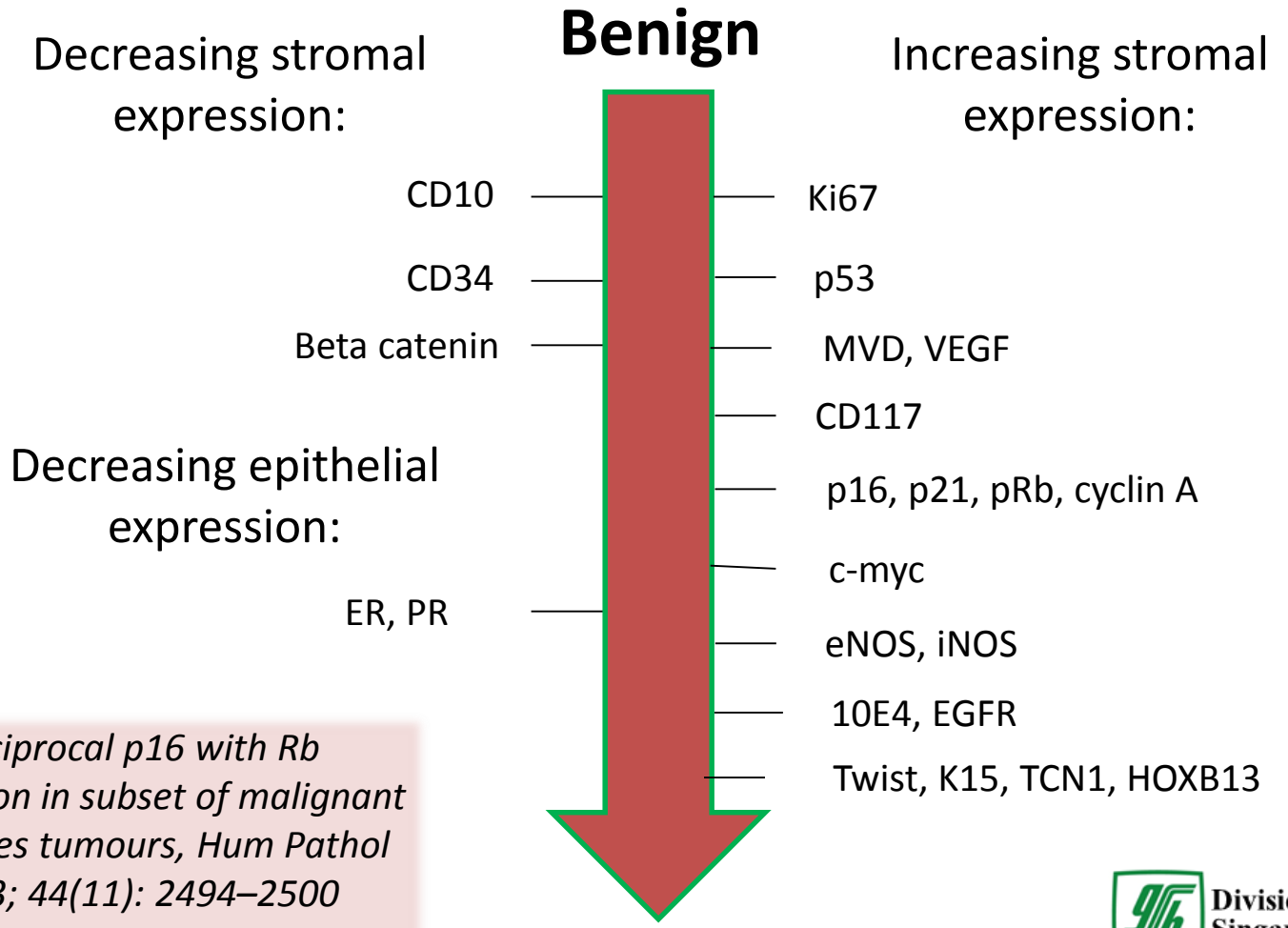
Molecular genetics & genomics of phyllodes tumour

Biomarkers in classification of phyllodes tumours



Reciprocal p16 with Rb expression in subset of malignant phyllodes tumours, Hum Pathol 2013; 44(11): 2494–2500

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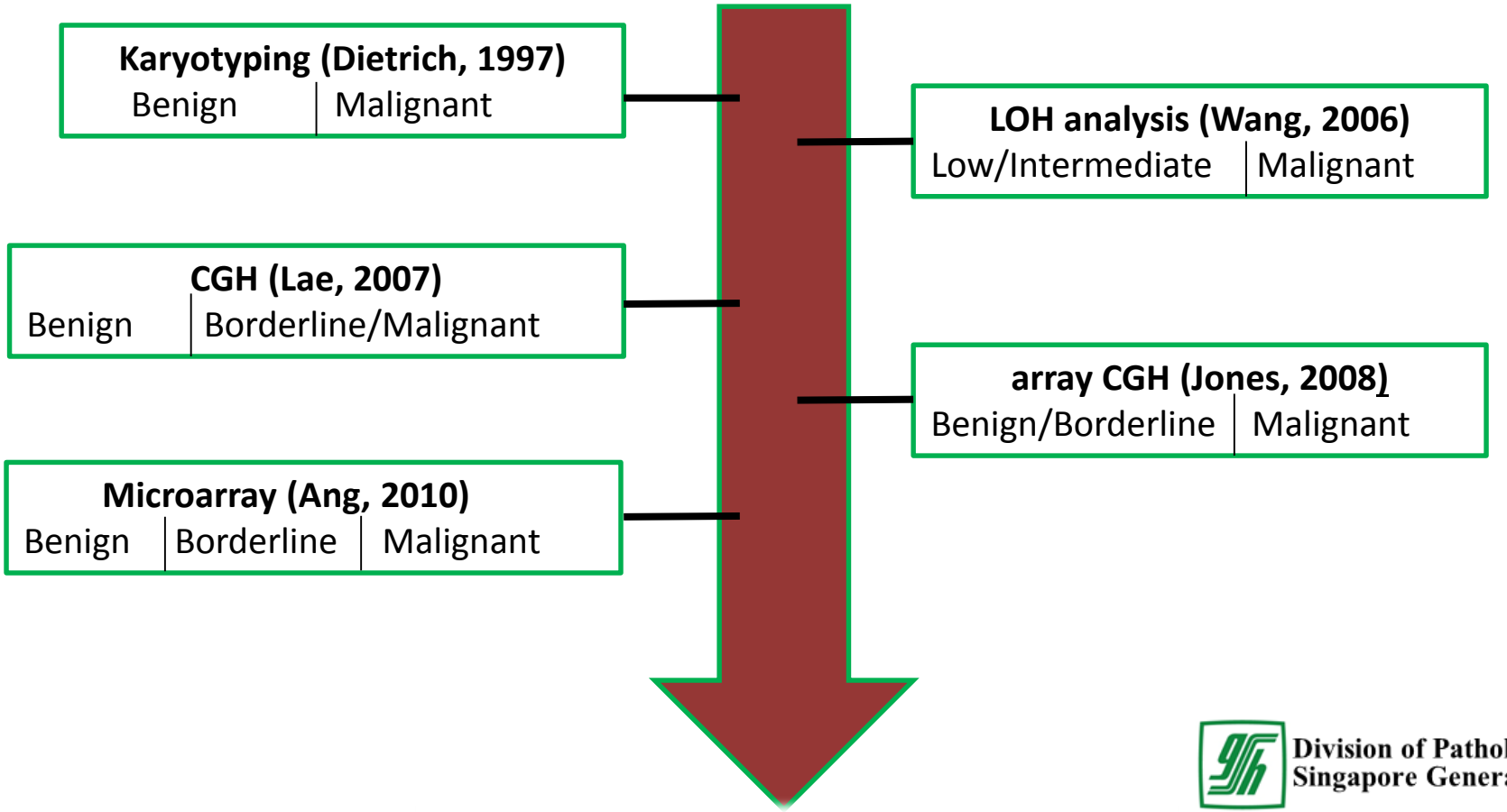
Biomarkers in classification of phyllodes tumours

- Support current histological classification based on stromal characteristics.
- Adjunctive utility in core biopsies.
- Limited role in routine practice.

Molecular classification of phyllodes tumours

Two-tiered and three-tiered grading schemes



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nature genetics

Nat Genet. 2015 Nov;47(11):1341-5.

Published online 5 Oct 2015

Genomic landscapes of breast fibroepithelial tumors

Jing Tan[1,2,16], Choon Kiat Ong[1,2,16], Weng Khong Lim[1,2,16], Cedric Chuan Young Ng[1,2], Aye Aye Thike[3], Ley Moy Ng[4], Vikneswari Rajasegaran[1,2], Swe Swe Myint[1,2], Sanjanaa Nagarajan[1,2], Saranya Thangaraju[1,2], Sucharita Dey[4], Nur Diyana Md Nasir[3], Giovani Claresta Wijaya[1,2], Jing Quan Lim[1,2], Dachuan Huang[1,2], Zhimei Li[1,2], Bernice Huimin Wong[1], Jason Yong Sheng Chan[5], John R McPherson[2], Ioana Cutcutache[2], Gregory Poore[6], Su Ting Tay[2], Wai Jin Tan[3], Thomas Choudary Putti[7], Buhari Shaik Ahmad[8], Philip Iau[8], Ching Wan Chan[8], Anthony P H Tang[8], Wei Sean Yong[9–11], Preetha Madhukumar[9–11], Gay Hui Ho[9–11], Veronique Kiak Mien Tan[9–11], Chow Yin Wong[9–11], Mikael Hartman[8,12,13], Kong Wee Ong[9–11], Benita K T Tan[9–11], Steven G Rozen[2], Patrick Tan[2,4,14], Puay Hoon Tan[3] & Bin Tean Teh[1,2,4,15]

Key findings:

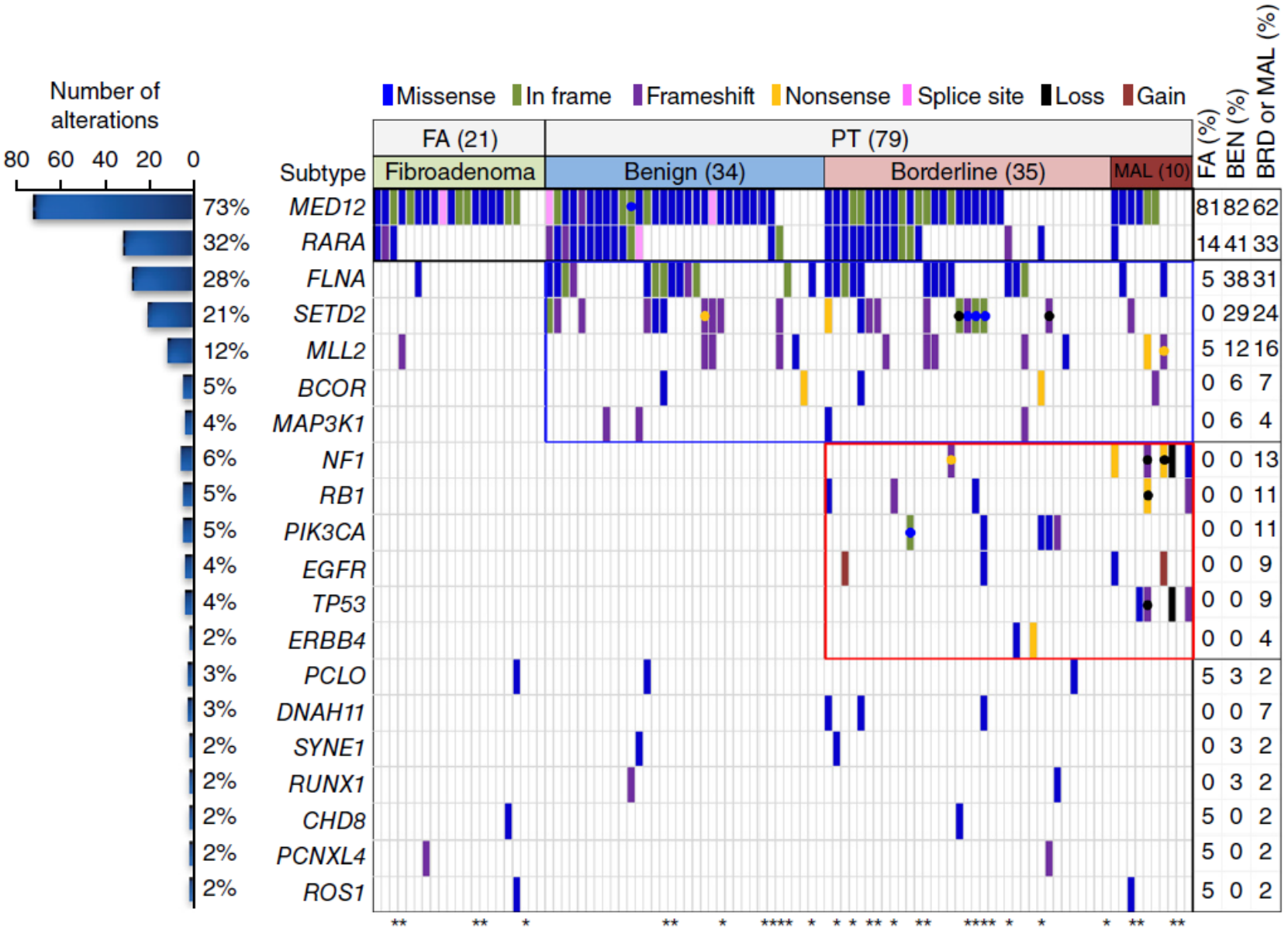
- Exome sequencing of 22 phyllodes tumours followed by targeted sequencing of 100 breast fibroepithelial tumours.
- 3 distinct mutation patterns:

  ~ frequent *MED12* and *RARA* mutations in fibroadenomas and phyllodes tumours.

  ~ phyllodes tumours exhibited additional mutations in *FLNA*, *SETD2*, *KMT2D*.

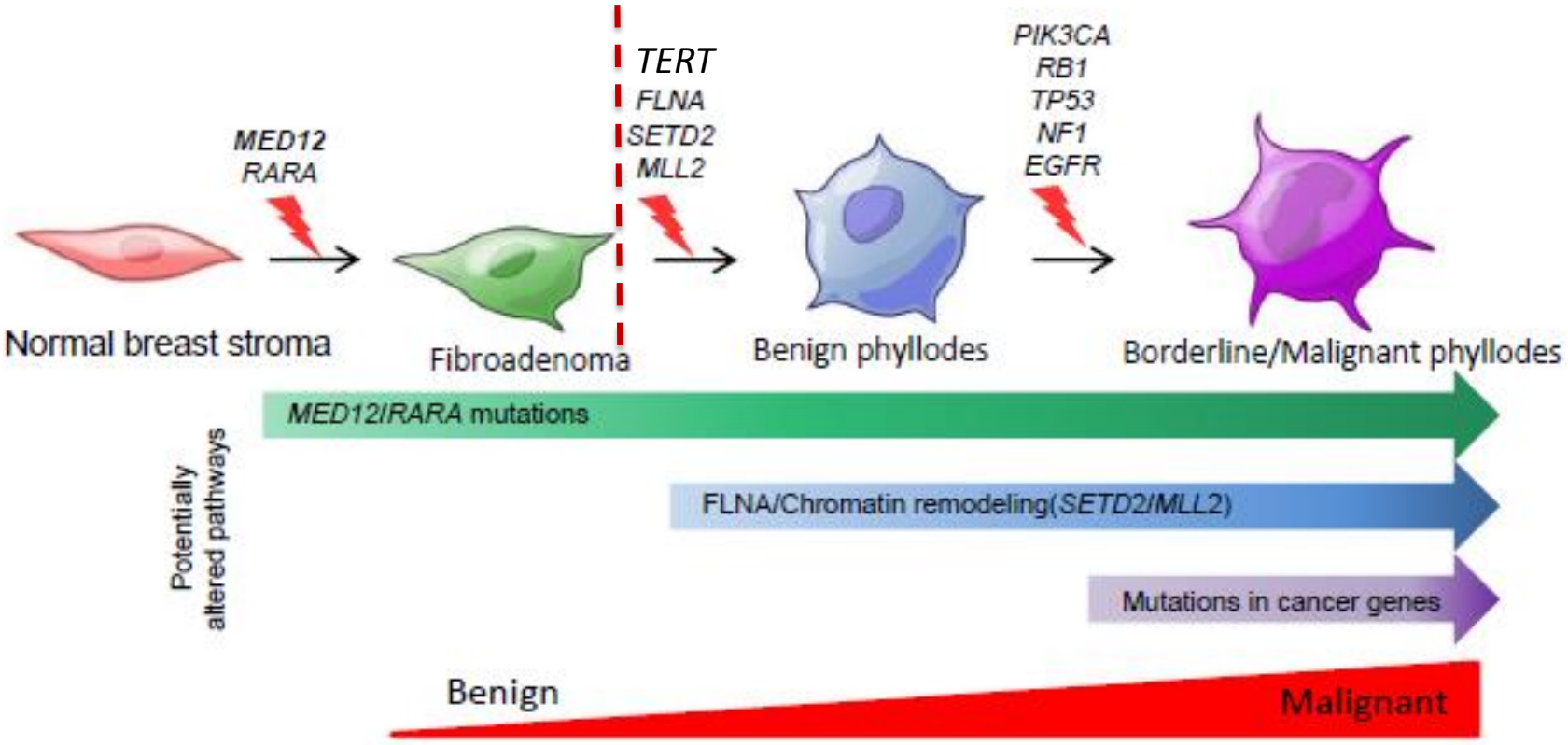
  ~ borderline and malignant phyllodes tumours harboured mutations in cancer associated genes.

Genomic landscapes of breast fibroepithelial tumours



Tan J et al. Nat Genet. 2015 Nov;47(11):1341-5.

A proposed model of the genomic progression of breast fibroepithelial tumours



Tan J et al. Nat Genet. 2015 Nov;47(11):1341-5.

Multiple papers on the genomics of fibroepithelial tumours have been published

FULL PAPER

BJC

Jan 2018

British Journal of Cancer (2018) 118, 277–284 | doi: 10.1038/bjc.2017.450

Keywords: phyllodes tumour; fibroadenoma; RBM15; MED12; TERT; heterogeneity

MED12, *TERT* promoter and *RBM15* mutations in primary and recurrent phyllodes tumours

Diego A Garcia-Dios[1], Dina Levi[1], Vandna Shah[1], Cheryl Gillett[1], Michael A Simpson[2], Andrew Hanby[3], Ian Tomlinson[4] and Elinor J Sawyer[*,1]

[1]School of Cancer and Pharmaceutical Sciences, Guy's Hospital, King's College London, London SE1 9RT, UK; [2]Medical and Molecular Genetics, Guy's Hospital, King's College London, London, UK; [3]Leeds Institute of Cancer and Pathology, Cancer Genetics Building, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK and [4]Institute of Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

- MED12 mutations are common in FAs and benign PTs.
- MED12 mutations can be discordant in recurrent PTs.
- TERT mutations show less temporal heterogeneity.
- RBM15 may be a novel driver mutation in borderline/malignant PTs.

Translational Oncology
www.transonc.com

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Feb 2018

Genetic and Clinical Characteristics of Phyllodes Tumors of the Breast

Ji-Yeon Kim[*,1], Jong Han Yu[†,1], Seok Jin Nam[†], Seok Won Kim[†], Se Kyung Lee[†], Woong-Yang Park[‡,§], Dong-Young Noh[¶], Do-Hyun Nam[§,#,**], Yeon Hee Park[*,§,**], Wonshik Han[¶] and Jeong Eon Lee[†,§,**]

[*]Division of Hematology-Oncology, Department of Internal Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul,06351, Korea; [†]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, 06351, Korea; [‡]Samsung Genome Institute, Samsung Medical Center, Seoul, 06351, Korea; [§]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences & Technology, Sungkyunkwan University, Seoul 06351, Korea; [¶]Department of Surgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 03080, Korea; [#]Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, 06351, Korea; [**]Biomedical Research Institute, Samsung Medical Center, Sungkyunkwan University, Seoul 06351, Korea

MPT harboring *PTEN* and *RB1* copy number deletion showed rapid disease progression.

Histopathology Nov 2018

Histopathology 2018, 73, 809–818. DOI: 10.1111/his.13701

Molecular insights into paediatric breast fibroepithelial tumours

Timothy K Y Tay,[1] Peiyong Guan,[2] Benjamin N Loke,[1] Nur Diana M Nasir,[1] Vikneswari Rajasegaran,[2] Aye Aye Thike,[1] Derrick Lian,[3] Kenneth T E Chang,[3] Bin Tean Teh,[2] Cedric C Y Ng[2] & Puay-Hoon Tan[4]

[1]*Department of Anatomical Pathology, Singapore General Hospital, Singapore,* [2]*Laboratory of Cancer Epigenome, National Cancer Centre, Singapore,* [3]*Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital, Singapore, and* [4]*Division of Pathology, Singapore General Hospital, Singapore*

- MED12 mutations in 53.8% of conventional and 35% of juvenile FAs.
- No TERT promoter mutations.
- Metachronous and synchronous tumours have mutational heterogeneity.

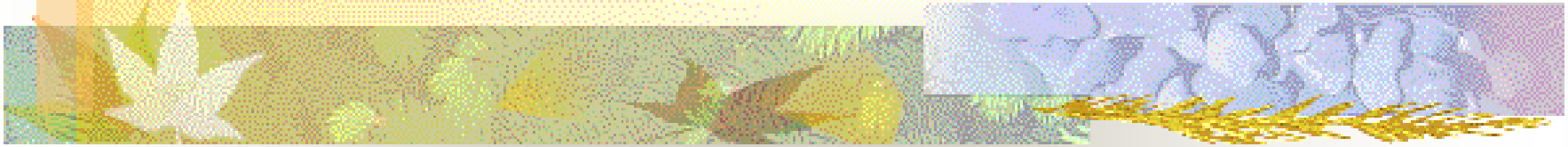
What's the clinical relevance?

- Genomics based classification of breast fibroepithelial lesions, enhancing diagnostic accuracy ~
  - Differentiating FA from PT *(J Pathol 2016;238:508-518)*
  - Differentiating PT from other spindle cell tumors *(APMIS 2016;124:356-364)*
  - Differentiating malignant PT from metaplastic carcinoma *(Pathology 2017;49:786-789)*
  - Refining the grading of PT *(Pathology 2019 Aug;51(5):531-534)*
- Discovery of candidate therapeutic targets in borderline/malignant PT ~
  - PIK3CA activating mutations
  - EGFR amplifications

What's the clinical relevance?

- *MED12 mutations correlated with improved disease free survival* *(J Clin Pathol 2015;68:685-91; Genes,Chromosomes&Cancer 2016;55:495–504)*
- *MED12* and *RARA* mutations linked to hormone receptor signaling.

Phyllodes tumour



WHO Classifications (& Grading)

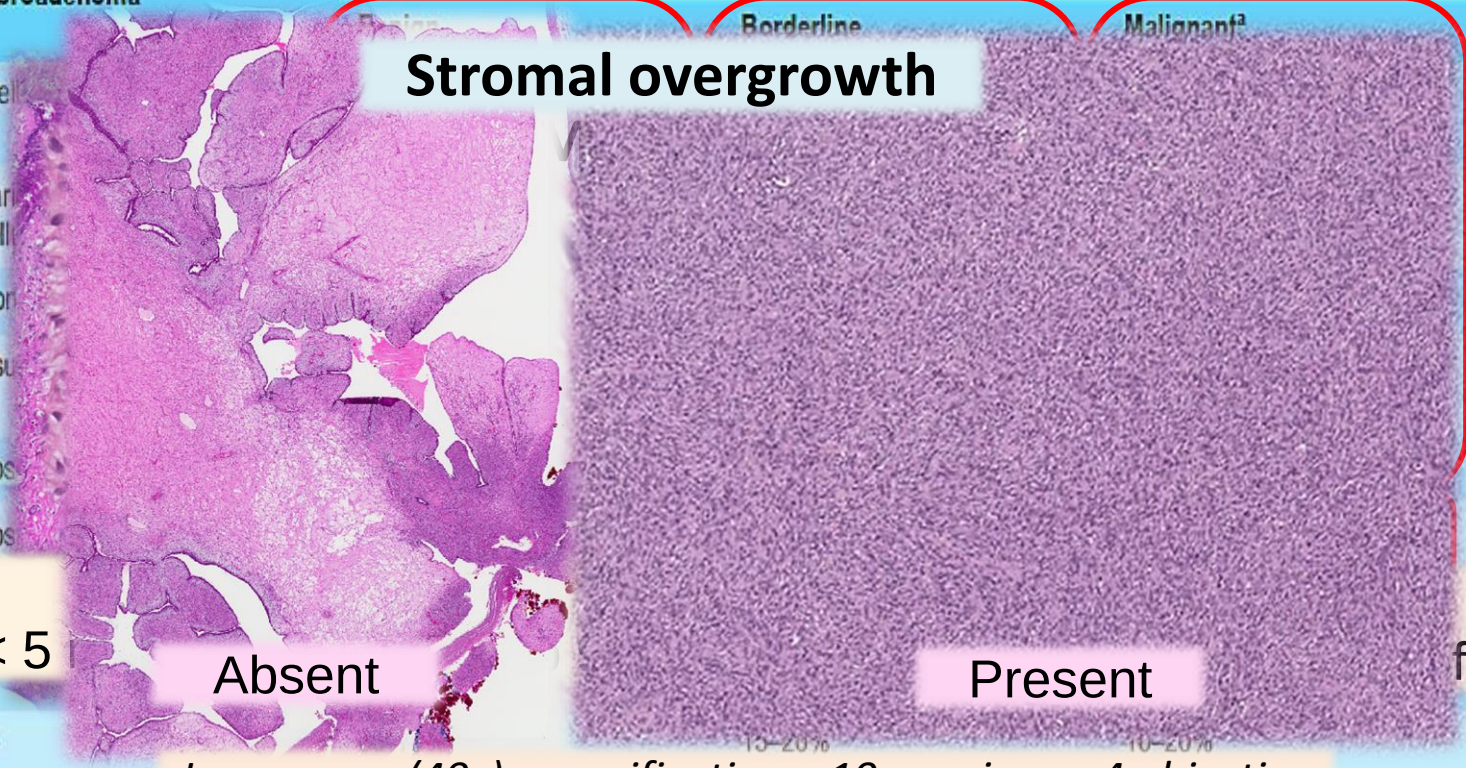
1981, 2003, 2012, 2019

Table 11.01 Histological features of fibroadenoma, benign, borderline and malignant phyllodes tumours

Histological feature	Fibroadenoma	Phyllodes tumour		
		Benign	Borderline	Malignant[a]
Tumour border	Well			
Stromal cellularity	Vari cell			
Stromal atypia	Nor			
Mitotic activity	Usu			
Stromal overgrowth	Abs			
Malignant heterologous elements	Abs			
Distribution relative to all breast tumours		< 5		
Relative proportion of all phyllodes tumours	—			

HPF, high-power fields.

[a] While these features are often observed in combination, they may not always be present simultaneously. Presence of a malignant heterologous element qualifies designation as a malignant phyllodes tumour, without requirement for other histological criteria.



Low power (40x) magnification, x10 eyepiece, x4 objective

WHO classification of breast tumours 2012

Table 11.01 Histological features of fibroadenoma, benign, borderline and malignant phyllodes tumours

Histological feature	Fibroadenoma	Phyllodes tumour		
		Benign	Borderline	Malignant[a]
Tumour border	Well-defined	Well-defined	Well-defined, may be focally permeative	Permeative
Stromal cellularity	Variable, scanty to uncommonly cellular, usually uniform	Cellular, usually mild, may be non-uniform or diffuse	Cellular, usually moderate, may be non-uniform or diffuse	Cellular, usually marked and diffuse
Stromal atypia	None	Mild or none	Mild or moderate	Marked
Mitotic activity	Usually none, rarely low	Usually few (< 5 per 10 HPF)	Usually frequent (5–9 per 10 HPF)	Usually abundant (≥ 10 per 10 HPF)
		< 2.5 mitoses/mm2	2.5 to < 5 mitoses/mm2	≥ 5 mitoses/mm2
Stromal overgrowth	Absent	Absent	Absent, or very focal	Often present
Malignant heterologous elements	Absent	Absent	Absent	May be present
Distribution relative to all breast tumours	Common	Uncommon	Rare	Rare
Relative proportion of all phyllodes tumours	—	60–75%	15–20%	10–20%

HPF, high-power fields.

[a] While these features are often observed in combination, they may not always be present simultaneously. Presence of a malignant heterologous element qualifies designation as a malignant phyllodes tumour, without requirement for other histological criteria.

*

WHO classification of breast tumours 2019

* *Exception ~ liposarcoma*

Phyllodes tumours: *issues with current grading & classification approaches*

- Grade assignment is imperfect:
  - Stromal hypercellularity, atypia, mitoses, overgrowth, borders.

{Singapore nomogram based on stromal **A**typia, **M**itoses, **O**vergrowth, **S**urgical margins

(**AMOS** criteria), validated in other cohorts.}

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SingHealth

Phyllodes Tumour Recurrence

Welcome to the Singapore General ecurrence free likelihood
following a histologic diagnosis of b
This tool is based on a study under 69-76.)
This tool was designed for use by h d to discuss the results with
your doctor. Please read the SGH
Detailed information on this risk as

A : Atypia
M: Mitoses
O: Overgrowth
S : Surgical margin

Risk assessment tool

Does the tumor show stromal cytologic atypia	None or mild	Moderate	Marked
How many mitoses are visible per 10 high powered fields?	Mitoses per 10 hpf 0		
Is there stromal overgrowth seen?	Absent	Present	
Are the margins histologically involved (positive)?	Negative	Positive	

https://mobile.sgh.com.sg/ptrra

Phyllodes tumours: *issues with current grading & classification approaches*

- Distinguishing different entities ~
  - Cellular fibroadenoma vs benign phyllodes tumour.
  - Metaplastic spindle cell carcinoma vs malignant phyllodes tumour vs sarcoma.

Important for accurate grading and diagnosis due to differences in treatment

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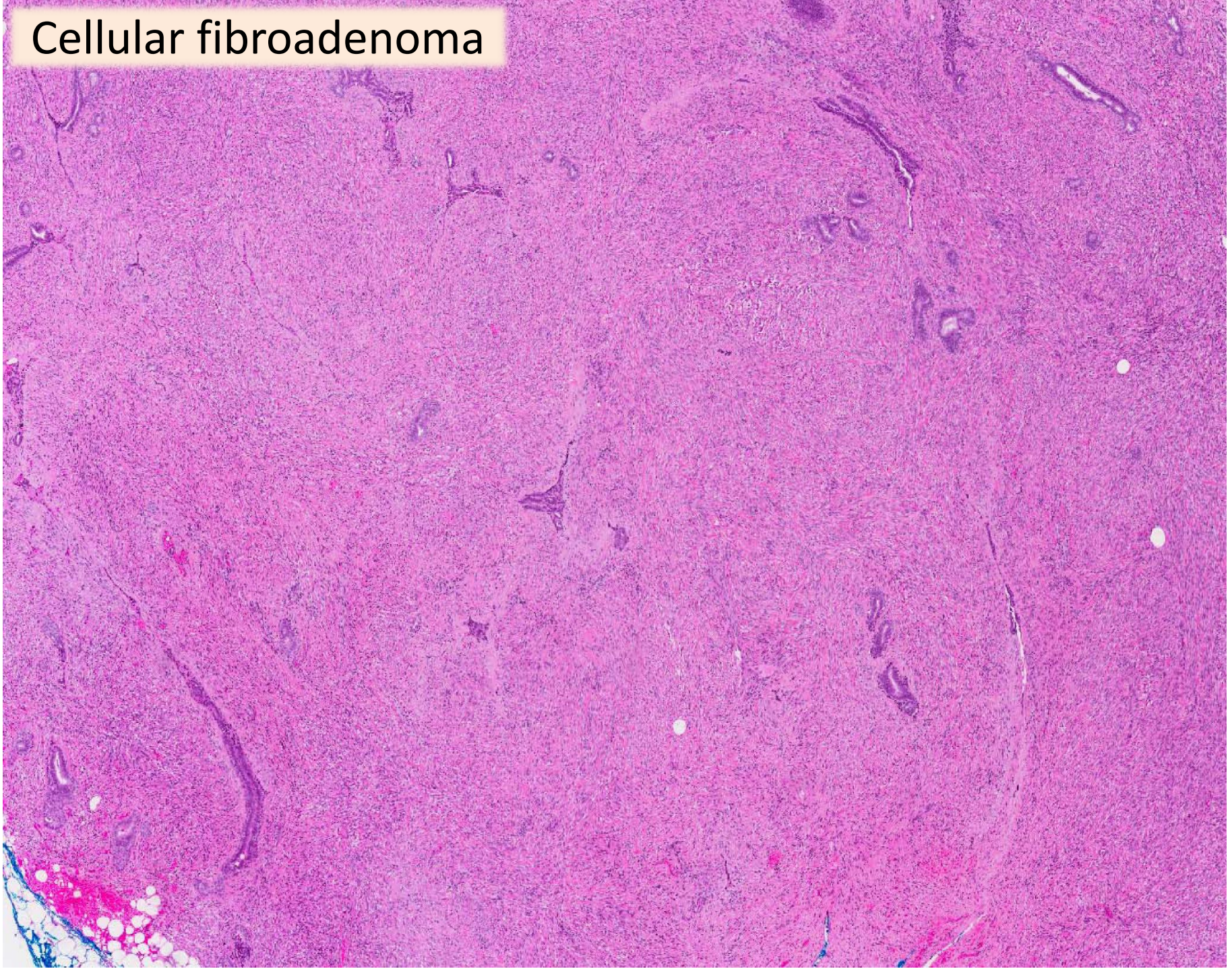
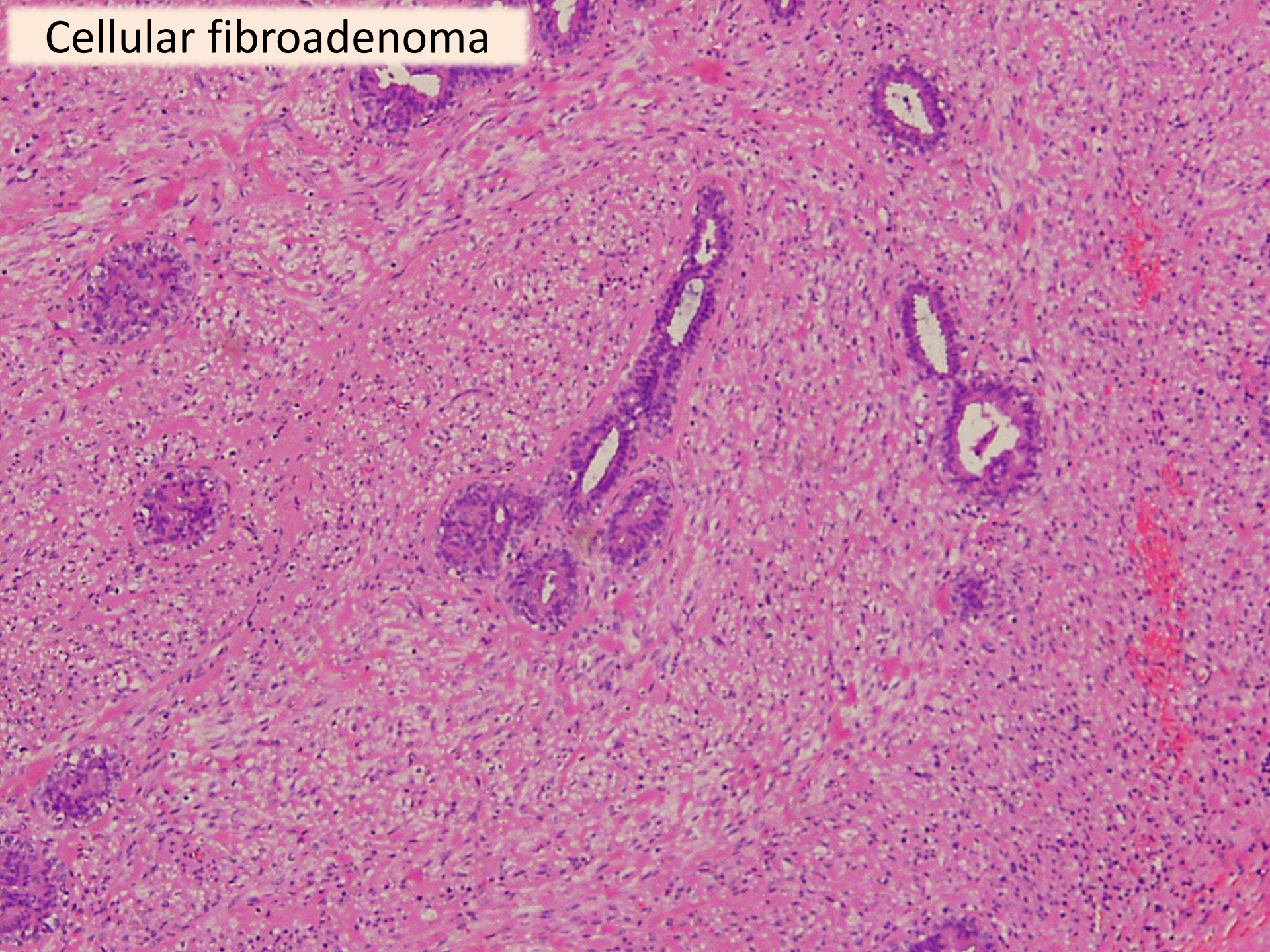
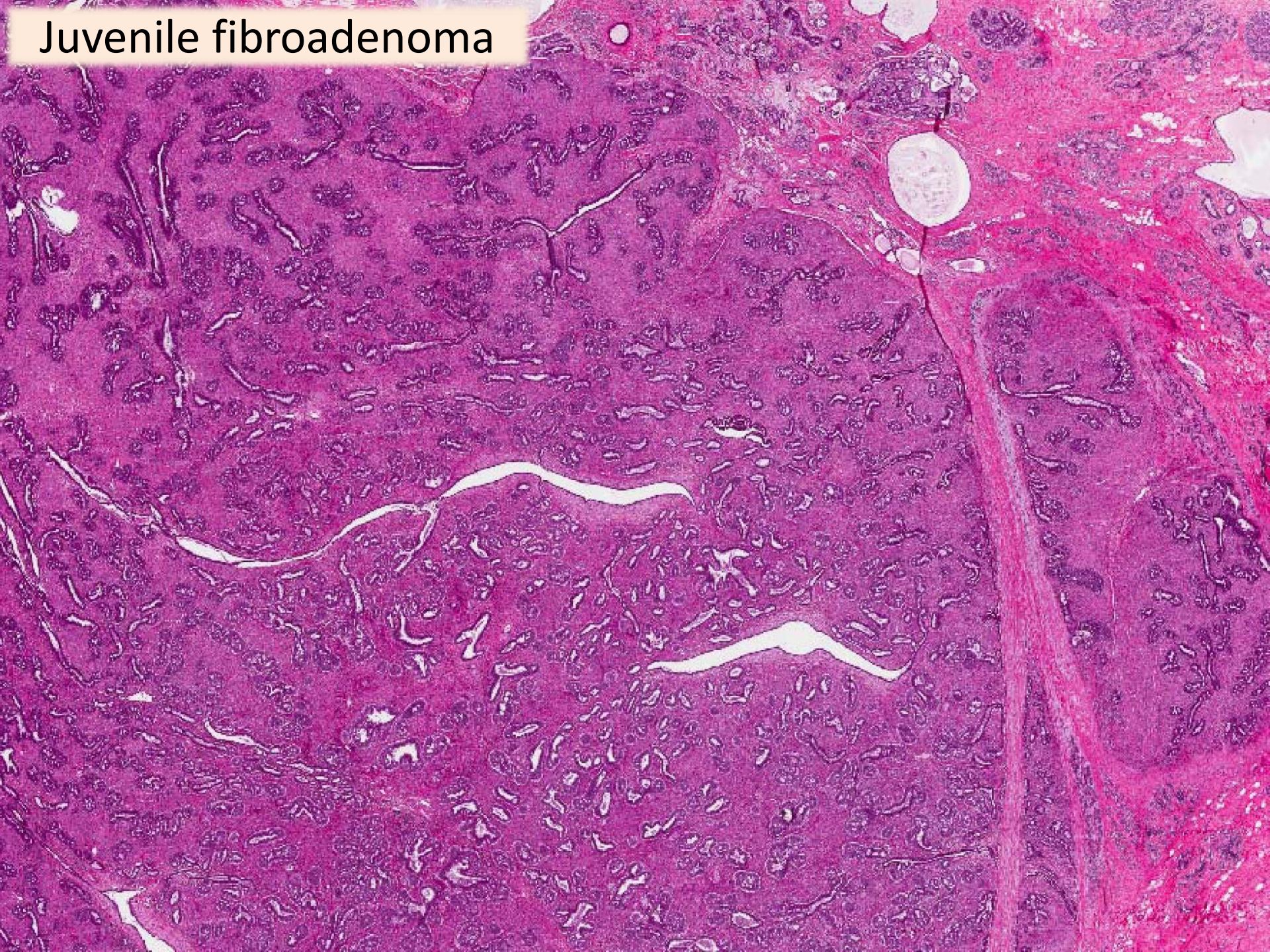
Cellular fibroadenoma vs phyllodes tumour

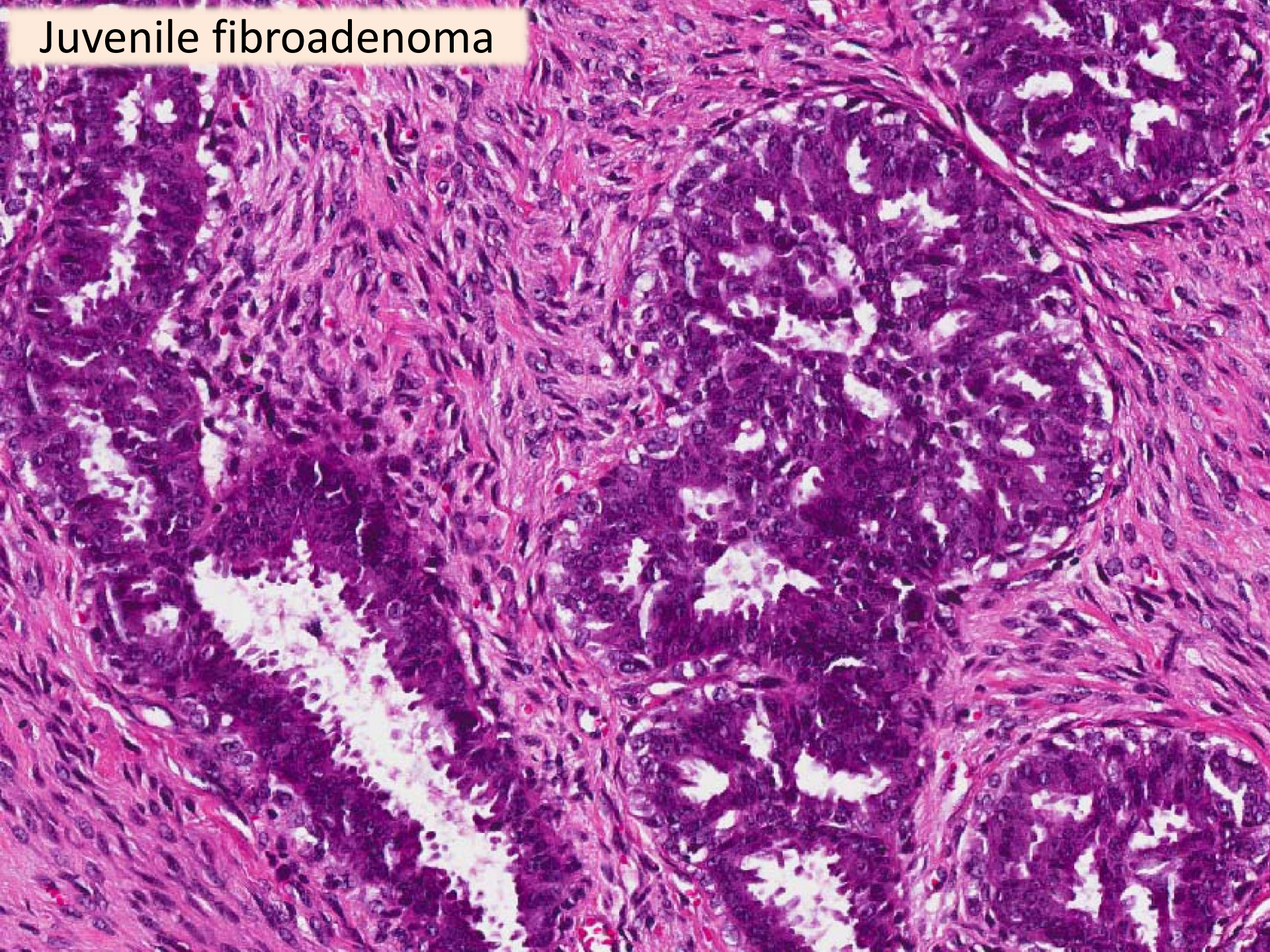
- **Cellular fibroadenoma ~**
  - Typical fibroadenoma but with increased stromal cellularity.
  - Degree of stromal cellularity for this designation is subjective, varying among pathologists.
  - Stromal cellularity tends to be increased in fibroadenomas in the young.
  - Lacks leaf-like fronds of phyllodes tumour.
  - Histological features overlap with the juvenile fibroadenoma.


Cellular fibroadenoma


Cellular fibroadenoma

Juvenile fibroadenoma

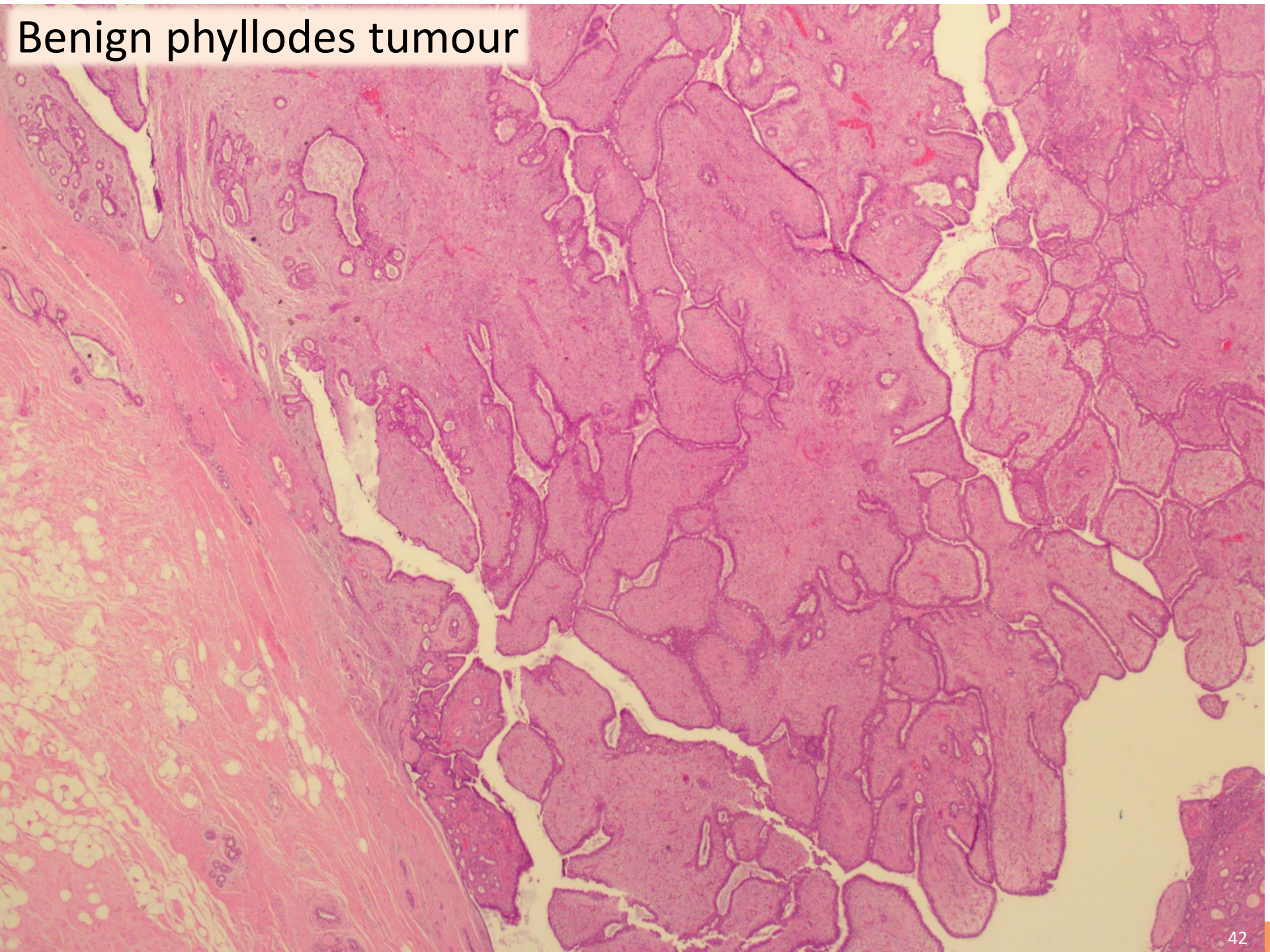



Juvenile fibroadenoma

Cellular fibroadenoma vs phyllodes tumour

- Phyllodes tumour ~
  - Exaggerated intracanalicular growth pattern.
  - Elongated epithelium lined arcs.
  - Broad, well-developed stromal fronds.
  - At least mild stromal hypercellularity.

Benign phyllodes tumour



Cellular fibroadenoma vs phyllodes tumour

- Overlapping histological characteristics ~
  - Stromal hypercellularity
  - Intracanalicular pattern
- Challenging on core biopsy!

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Journal of Pathology
J Pathol 2019
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.5333

ORIGINAL PAPER

Genomic characterisation of breast fibroepithelial lesions in an international cohort

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Division of Pathology
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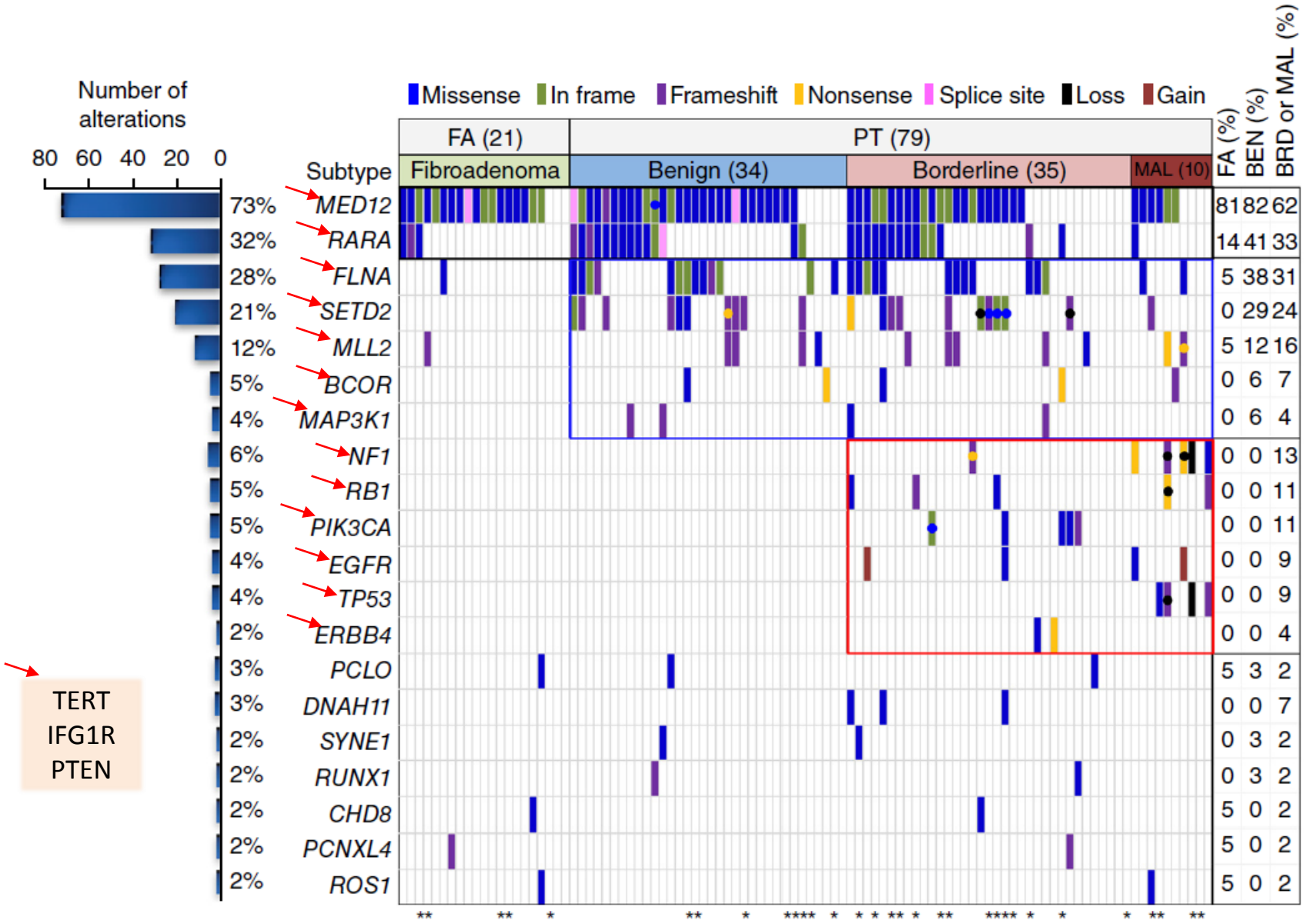
Aims

- Expand our investigation of FELs to a large international multi-institutional cohort, using a customised 16-gene set.
  - Differentiate FAs from PTs
  - Refine grading of PTs
- Compare the genetic profile of Asian with non-Asian FELs.

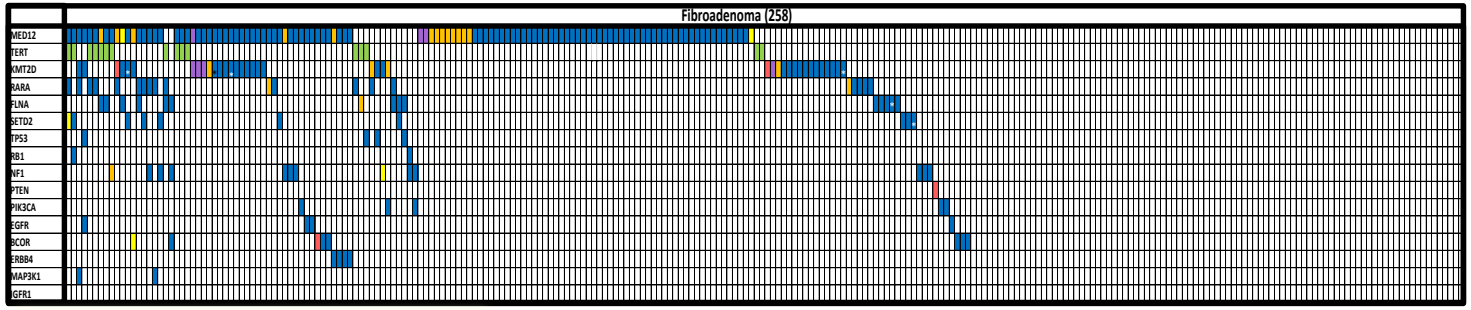
Genomic landscapes of breast fibroepithelial tumours



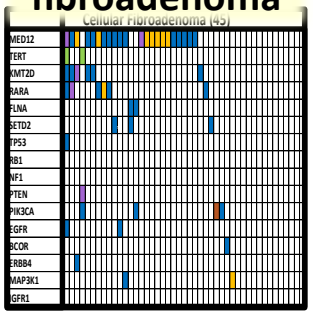
Tan J et al. Nat Genet. 2015 Nov;47(11):1341-5.

Waterfall plot of genetic aberrations in FELs and their mutation types

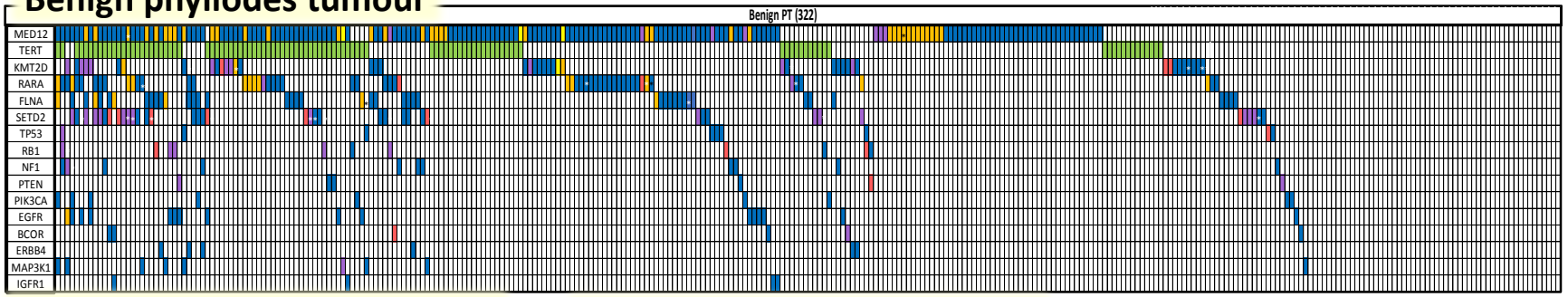
Fibroadenoma



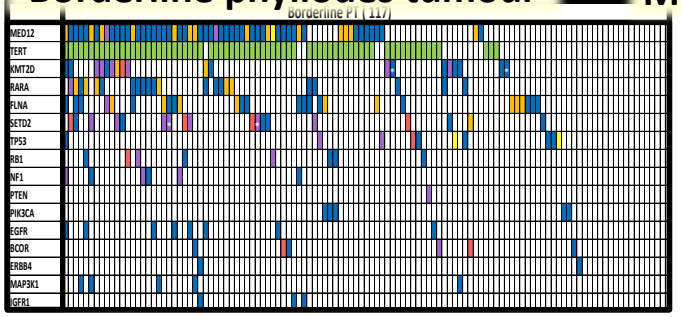
Cellular fibroadenoma



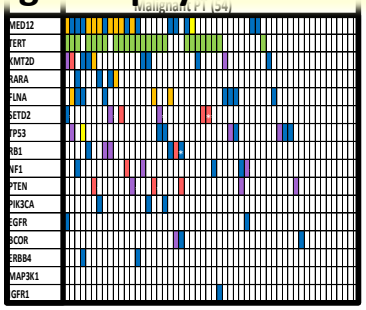
Benign phyllodes tumour



Borderline phyllodes tumour



Malignant phyllodes tumour



Legend:

Nonsense
FrameShift InDel
Stop Loss
InFrame InDel
Missense
Splice Site
Promoter Mutation

*	2 Mutations

796 Samples

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Materials and Methods

Gene	Function
MED12	Subunit of Mediator complex for transcriptional initiation
TERT	Transcriptase for telomere elongation and genomic stability
KMT2D	Methyltransferase for epigenetic regulation and tumour suppression
FLNA	Filamin for cytoskeleton formation and ECM positioning
RARA	Transcriptional factor for gene repression and cellular differentiation
SETD2	Methyltransferase for DNA damage repair and tumour suppression
NF1	Neurofibromin for tumour suppression
ERBB4	Receptor for mitogenesis and differentiation

Materials and Methods

Gene	Function
EGFR	Receptor for cell proliferation and survival
IGF1R	Receptor for cell proliferation and survival
PTEN	Phosphatase for cell division, genomic stability and tumour suppression
BCOR	Transcriptional factor for gene repression
MAP3K1	Kinase regulating apoptosis pathways
RB1	Regulates cell proliferation, DNA replication and tumour suppression
TP53	Regulates cell proliferation, DNA replication and tumour suppression
PIK3CA	Subunit of kinase for cell proliferation, migration, protein production

Results (FA vs PT)

- Targeted sequencing revealed frequent *MED12* mutations across all FELs, and a spectrum of other mutations at varying rates.
- FAs exhibited *MED12* (45%), *KMT2D* (15%) and *RARA* (9%) mutations, while other gene aberrations were much less common, and there was no *IGF1R* mutation.
- **No significant genetic differences were detected between conventional (simple or non-cellular) and cellular FAs.**
- PTs displayed higher variant prevalence than FAs for *MED12* (56% vs 45%, p=0.0017), *TERT* promoter (41% vs 6%, p<0.0001), *RARA* (17% vs 9%, p=0.0013), *FLNA* (16% vs 6%, p<0.0001), *SETD2* (13% vs 4%, p<0.0001), *TP53* (6% vs 2%, p=0.0054), *RB1* (5% vs 1%, p=0.0004), *EGFR* (5% vs 2%, p=0.0248), and *IGF1R* (2% vs 0%, p=0.0271).
- **Non-Asian PTs showed more frequent *KMT2D* mutations (25% vs 14%, p=0.018).**

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Results (FA vs PT)

No. of Mutations	Total FELs (n = 796)	FA (n = 303)	Conventional FA (n = 258)	Cellular FA (n = 45)	Phyllodes Tumours (n = 493)	Benign (n = 322)	Borderline (n = 117)	Malignant (n = 54)	p-value
0	185 (23%)	104 (34%)	91 (35%)	13 (29%)	81 (16%)	54 (17%)	17 (15%)	10 (19%)	<0.001
1	244 (31%)	120 (40%)	102 (40%)	18 (40%)	124 (25%)	93 (29%)	21 (18%)	10 (19%)	
≥2	367 (46%)	79 (26%)	65 (25%)	14 (31%)	288 (58%)	175 (54%)	79 (68%)	34 (63%)	

- The number of mutations was positively correlated with diagnosis, in that **PTs were more likely to harbour multiple mutations than FAs** ($p<0.001$).
- Most borderline and malignant PTs possessed 2 or more mutations.
- FAs had a higher proportion of cases without any mutations or with only a single mutation compared to PTs.

Results (PT grades)

- A **significantly higher number of genetic aberrations observed with increasing grade of PTs**, in particular with regard to *TERT* promoter (32% vs 61% vs 46%, p<0.0001), *FLNA* (13% vs 22% vs 19%, p=0.0289), *TP53* (3% vs 9% vs 17%, p=0.0003) and *RB1* (3% vs 7% vs 11%, p=0.0297) for benign, borderline and malignant PTs respectively.
- *MED12* mutations on the other hand significantly decreased as the PTs progressed
(62% vs 50% vs 37%, p=0.0006).
- A comparison between **borderline and malignant PTs did not show significant differences**, apart from ***PTEN*** (1% vs 11%, p=0.0043).

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Conclusions

- **Potential adjunctive utility** of the 16 gene mutational profile in stratifying FELs that are histologically challenging to characterize.
- *MED12* aberrations common in FAs and PTs, with other gene alterations which affect transcriptional regulation such as through the action of *KMT2D* and *RARA*.
- Involvement of ER and Wnt pathways is plausible given their interaction with *MED12*, and *MED12* mutations may possibly trigger their aberrant signalling.
- ***TERT* promoter mutations** could potentially discriminate between FAs and PTs; while presence of *TERT promoter, FLNA, TP53, RB1, NF1, PTEN, PIK3CA, ERBB4* and *EGFR* aberrations may **implicate higher PT grades**.

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Genomics of Fibroepithelial Tumours of the Breast

~ *Potential clinical applications*

Metaplastic spindle cell carcinoma

Malignant phyllodes tumour

Sarcoma

Metaplastic carcinoma vs malignant phyllodes tumour vs sarcoma

Table 3.4 Distinguishing Features of Sarcomatous Stromal Overgrowth in Malignant Phyllodes Tumour, Spindle Cell Metaplastic Carcinoma, and Primary Breast Sarcoma

Feature	Malignant phyllodes tumour (stromal overgrowth)	Spindle cell metaplastic carcinoma	Primary breast sarcoma
Leaf-like fronds	Present (but may be hard to identify)	Absent	Absent
Peri-epithelial stromal accentuation	Present	Absent	Absent
Carcinoma (in situ and invasive)	Absent	May be present	Absent
Keratins (IHC)	Usually absent, may be focal reactivity	Present, but may be focal	Usually absent
High-molecular-weight keratins	Usually absent, may be focal reactivity	Present, but may be focal	Usually absent
p63, p40	Absent or present	Present, but may be focal	Usually absent

IHC immunohistochemistry

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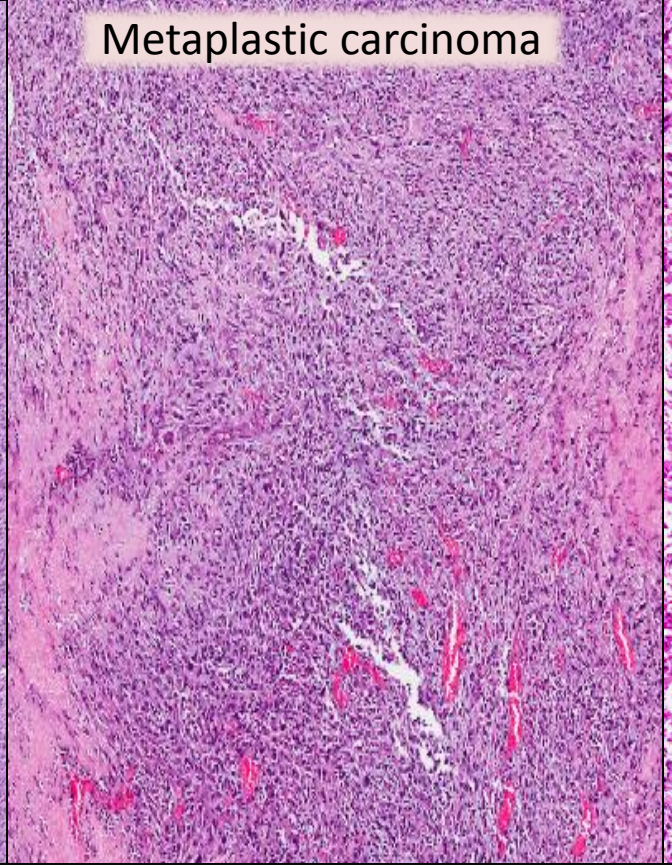
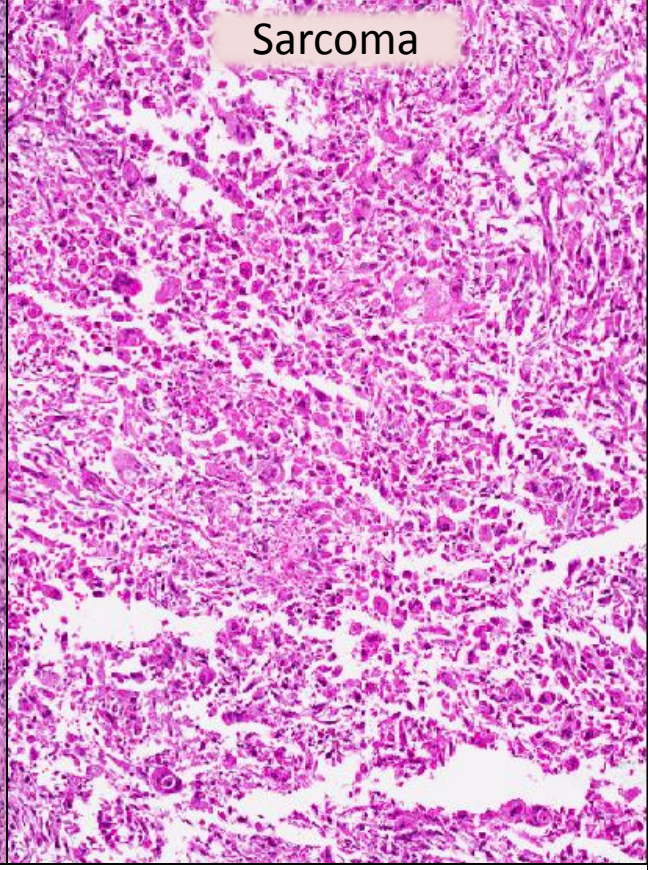
Atlas of Differential Diagnosis in Breast Pathology, Springer 2017


Phyllodes tumour, malignant


Metaplastic carcinoma


Sarcoma

Breast Cancer Res Treat (2016) 159:229–244
DOI 10.1007/s10549-016-3946-1

PRECLINICAL STUDY

Breast sarcomas and malignant phyllodes tumours: comparison of clinicopathological features, treatment strategies, prognostic factors and outcomes

Sue Zann Lim[1] · Sathiyamoorthy Selvarajan[2] · Aye Aye Thike[2] · Nur Diyana Binte Md. Nasir[2] · Benita Kiat Tee Tan[3] · Kong Wee Ong[3] · Puay Hoon Tan[4]

- 17 cases of breast sarcoma and 45 cases of malignant PT.
- No significant difference in survival outcomes.
- Similar clinicopathological features.
- Suggesting shared biological relationship.

Breast Cancer Research and Treatment (2019) 174:365–373
https://doi.org/10.1007/s10549-018-5067-5

PRECLINICAL STUDY

Genomic profile of breast sarcomas: a comparison with malignant phyllodes tumours

Sue Zann Lim[1] · Cedric Chuan Young Ng[2,3] · Vikneswari Rajasegaran[2,3] · Peiyong Guan[4] · Sathiyamoorthy Selvarajan[5] · Aye Aye Thike[5] · Nur Diyana Binte Md Nasir[5] · Valerie Cui Yun Koh[5] · Benita Kiat Tee Tan[1] · Kong Wee Ong[1] · Bin Tean Teh[2,3,6,7] · Puay Hoon Tan[8]

- 9 cases ~ 3 angiosarcomas, 6 non-angiosarcomas (5 undifferentiated pleomorphic sarcoma, 1 osteosarcoma).
- TERT, MED12 mutations common in non-angiosarcomas, whereas angiosarcomas did not demonstrate mutations in these genes.
- Breast sarcomas (non-angiosarcoma) show similar genomic alterations to malignant phyllodes tumours.
- Suggesting shared biological relationship.

CORRESPONDENCE

Pathology. 2017 Dec;49(7):786-789.

A genetic mutation panel for differentiating malignant phyllodes tumour from metaplastic breast carcinoma

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Cedric Chuan Young Ng[3]
Nur Diyana Md Nasir[1]
Kiley Loh[3]
Bin Tean Teh[3]
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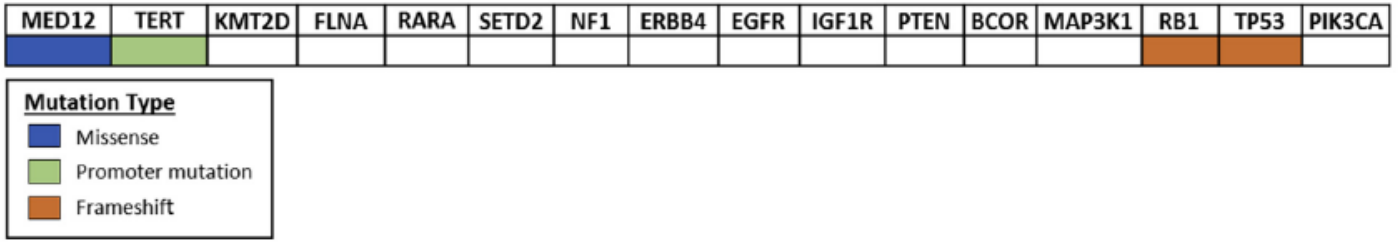
A 55-year-old female presented with a locally advanced breast tumour with metastasis to the right lung and soft tissue of the left lumbar region. The initial biopsy of the breast tumour diagnosed a malignant high-grade tumour (7 cm in the right breast) with a differential diagnosis of either SCMBC or malignant PT. Patient was treated as for

Targeted-sequencing using the FEB assay for the patient

Gene	Transcript ID	Nucleotide (genomic)	Nucleotide (cDNA)	Amino Acid (Protein)	Variant Freq (%)	Mutation Type
RB1	NM_000321	g.chr13: 49030479 delA	c.1954delA	p.K652fs	11.32	Frameshift
TP53	NM_001126115	g.chr17: 7578240-7578241delCA	c.212_213delCA	p.V71fs	15.96	Frameshift
MED12	NM_005120	g.chrX: 70339254 G>T	c.G131T	p.G44V	15.10	Missense
TERT		g.chr5: 1295228 G>A			13.98	Promoter mutation

Schematic showing the mutations of the patient in the 16 genes panel



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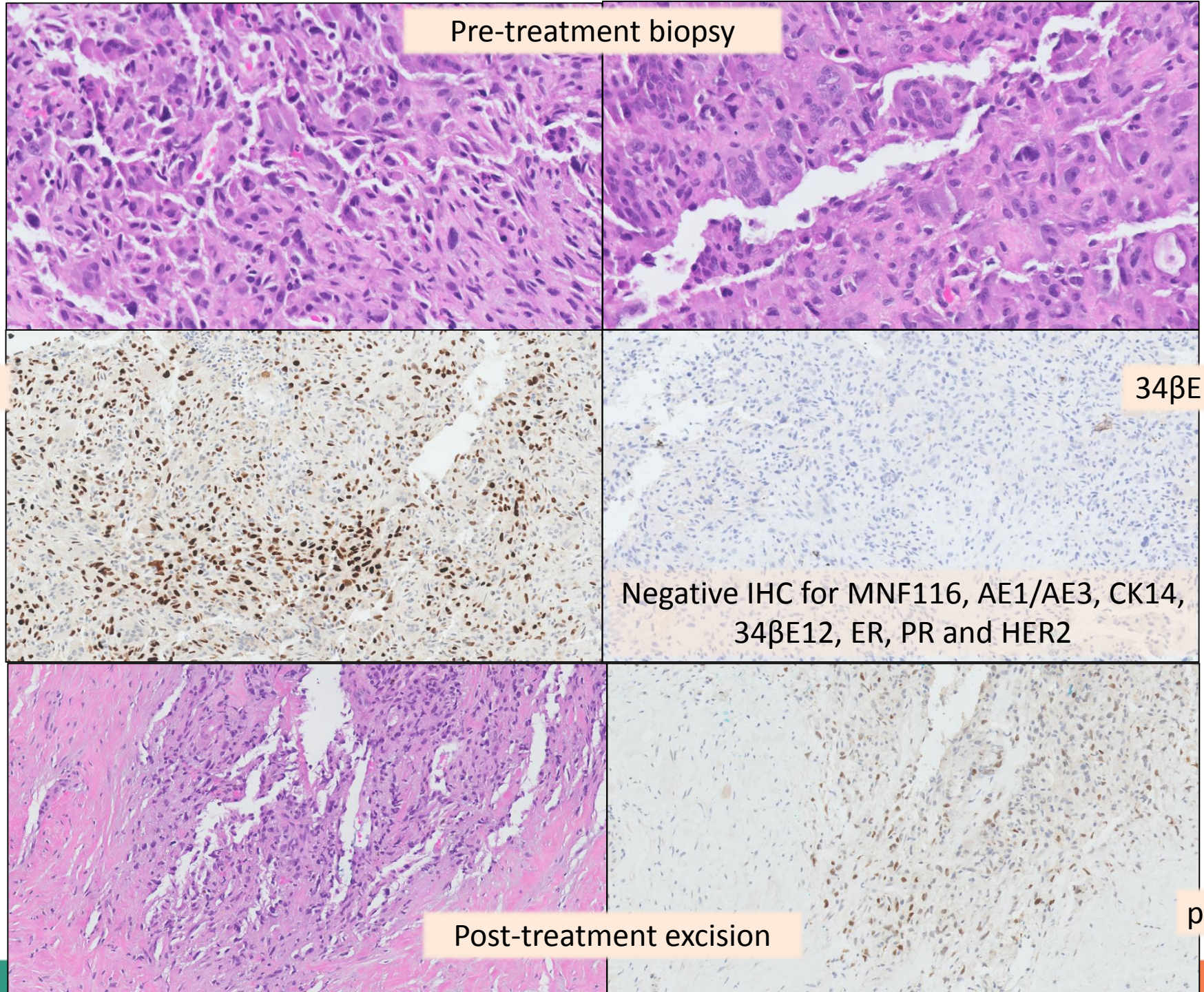
Pre-treatment biopsy
p63
34βE12
Negative IHC for MNF116, AE1/AE3, CK14, 34βE12, ER, PR and HER2
Post-treatment excision
p63

Table 1 Comparison of sequencing results of spindle cell metaplastic carcinomas with malignant phyllodes tumours of the breast, using the 16 gene panel

	Ng *et al.*, 2017[8]	Ross *et al.*, 2014[7]	Liu *et al.*, 2016[10]	Piscuoglio *et al.*, 2016[11]	Cani *et al.*, 2015[12]	Our data
Genes/tumour type	Spindle cell metaplastic carcinoma of the breast	Spindle cell metaplastic carcinoma of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast	Malignant phyllodes tumour of the breast
MED12	0/10, 0%	0/2, 0%	3/10, 30%	4/13, 31%	2/5, 40%	8/19, 42%
TERT	0/10, 0%	0/2, 0%	6/10, 60%	8/13, 62%	3/5, 60%	11/19, 58%
RARA	0/10, 0%	0/2, 0%	1/10, 10%	1/13, 8%	NA	2/19, 11%
PIK3CA	6/10, 60%	0/2, 0%	3/10, 30%	1/13, 8%	NA	1/19, 5%
PTEN	0/10, 0%	0/2, 0%	1/10, 10%	1/13, 8%	NA	2/19, 11%
KMT2D	0/10, 0%	0/2, 0%	2/10, 20%	1/13, 8%	NA	5/19, 26%
RB1	0/10, 0%	0/2, 0%	2/10, 20%	5/13, 38%	1/5, 20%	2/19, 11%
IGF1R	0/10, 0%	0/2, 0%	NA	NA	2/5, 40%	0/19, 0%
TP53	5/10, 50%	0/2, 0%	4/10, 40%	6/13, 46%	3/5, 60%	3/19, 16%
NF1	0/10, 0%	0/2, 0%	NA	3/13, 23%	1/5, 20%	2/19, 11%
ERBB4	0/10, 0%	0/2, 0%	NA	0/13, 0%	NA	1/19, 5%
SETD2	0/10, 0%	0/2, 0%	2/10, 20%	3/13, 23%	NA	6/19, 32%
MAP3K1	0/10, 0%	0/2, 0%	NA	NA	NA	0/19, 0%
EGFR	0/10, 0%	0/2, 0%	0/10, 0%	4/13, 31%	1/5, 20%	2/19, 11%
BCOR	0/10, 0%	0/2, 0%	1/10, 10%	0/13, 0%	NA	3/19, 16%
FLNA	0/10, 0%	0/2, 0%	NA	NA	NA	2/19, 11%

Conclusion ~ Malignant phyllodes tumour

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Refining phyllodes tumour grading

The utility of a targeted gene mutation panel in refining the diagnosis of breast phyllodes tumours

Valerie Cui Yun Koh[1,2], Cedric Chuan Young Ng[3], Boon Huat Bay[2], Bin Tean Teh[3,4], Puay Hoon Tan[2,4,5]

Pathology (2019), **51(5)**, August

In case 1, a 35-year-old Caucasian female, diagnosed with malignant PT of the breast on excision biopsy, sought a second opinion in our institution, where a diagnosis of borderline PT was rendered.

As the initial and reviewed grades differed that impacted on management, with mastectomy recommended by the surgical oncologist for a malignant diagnosis, the customized panel was applied to determine if it could assist in refining grade assignment.

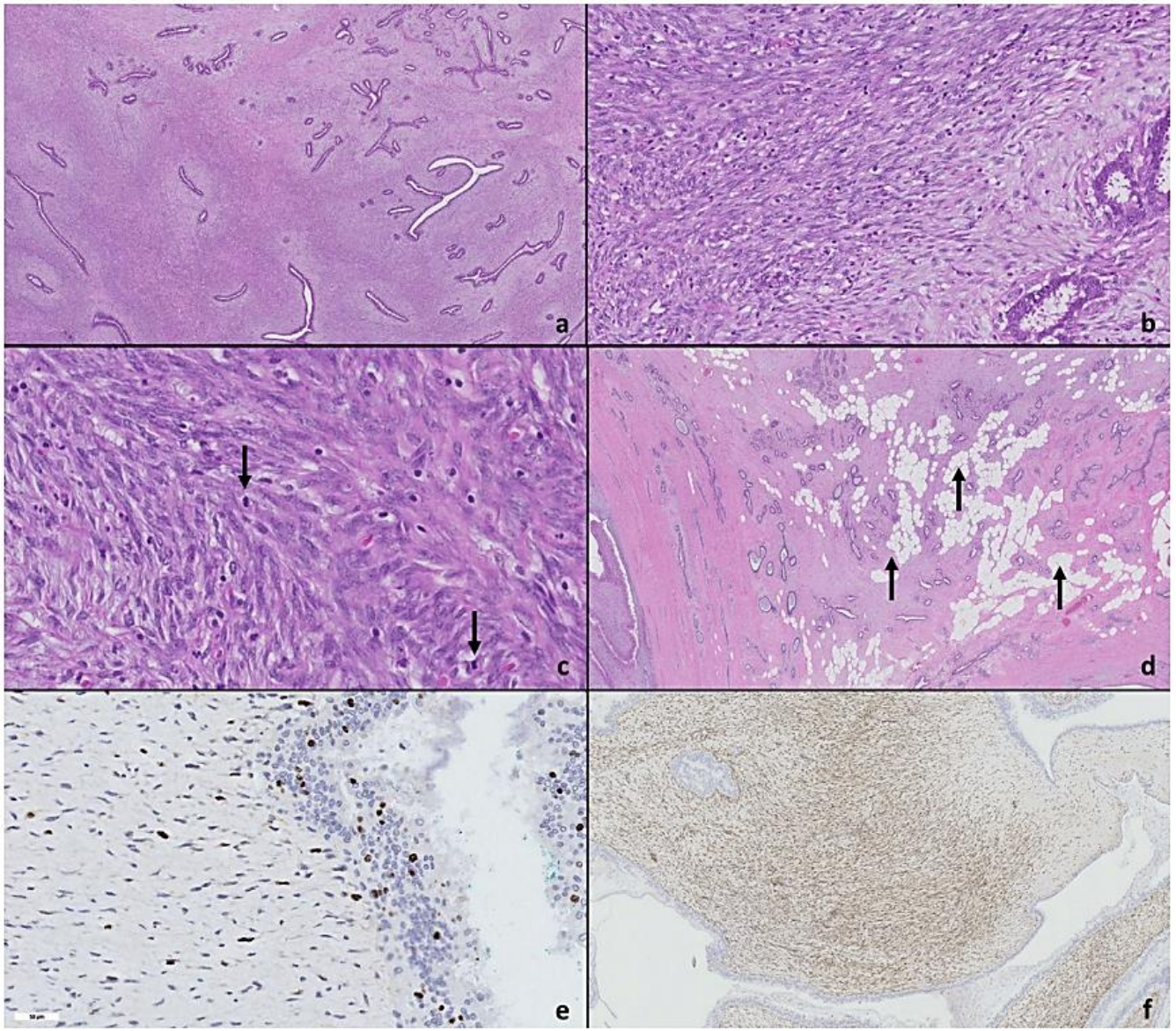


Fig. 1 Light microscopy images of sections from case 1 show (a) increased stromal cellularity in section 2 at low magnification, (b) increased stromal cellularity at medium magnification in section 12, (c) frequent mitoses (arrows) in section 2 at high magnification, and (d) permeative borders (arrows) in section 9 at low magnification. (e) IHC for Ki-67 shows scattered nuclear staining in both stromal and epithelial cells at medium magnification. (f) IHC for CD34 shows diffuse reactivity in the stromal cells of the PT (low magnification).

No stromal overgrowth or overt stromal atypia

Table 1. Variant frequencies (%) of mutations found in cases 1 and 2

Case and section no.	TERT chr5:1295228	RARA chr17:38510601-38510603	MED12 chrX:70,339,215
Case 1 Section 1	20.4	13.6	19.8
Case 1 Section 2	30.7	<1	27.4
Case 1 Section 3	21.5	16.1	23.5
Case 1 Section 4	27.2	14.7	24.5
Case 1 Section 5	22.7	17.4	26.1
Case 1 Section 6	24.9	19.3	25.0
Case 1 Section 7	17.2	16.4	19.4
Case 1 Section 8	26.0	15.4	21.8
Case 1 Section 9	20.6	7.7	21.8
Case 1 Section 10	12.5	<1	10.8
Case 1 Section 11	22.3	22.4	28.7
Case 1 Section 12	19.7	<1	28.5
Case 1 Section 13	26.6	<1	17.7

No cancer driver mutations

Conclusion ~ Favour borderline phyllodes tumour

Arbitrating indeterminate cellular fibroepithelial lesions on core biopsy

Core biopsy diagnosis of cellular fibroepithelial lesions

- Clinico-radiologic-pathologic correlation.
- Pre-operative conclusion is useful to plan treatment approach ~
  - No further treatment for fibroadenoma.
  - Excision for phyllodes tumour.
  - Excision for cellular fibroepithelial lesions.

Can we be more diagnostically precise in this group of tumours?

Core biopsy diagnosis of ***cellular fibroepithelial lesions*** – prediction of phyllodes tumour

Author	Reference	Key findings predicting phyllodes tumour
Jacobs et al	*Am J Clin Pathol 2005; 124: 342-354*	Marked stromal cellularity, mitoses in moderate stromal cellularity, Ki67 & topoisomerase IIα indices
Lee et al	*Histopathology 2007; 51: 336-344*	Stromal cellularity ≥ 50% stroma, stromal overgrowth, fragmentation, adipose within stroma
Resetkova et al	*Breast J 2010; 16:573-80.*	No predictive value of clinical, radiologic or pathologic data Suggested follow-up alone for a patient subset
Jara-Lazaro et al	*Histopathology 2010; 57: 220-232*	Marked stromal cellularity/atypia, stromal overgrowth, mitoses ≥ 2 per 10 hpf, ill-defined lesional borders, Ki67 & topoisomerase IIα indices ≥ 5%, reduced CD34 staining
Yasir et al	*Am J Clin Pathol 2014; 142: 362-369*	Mitoses, stromal overgrowth, fragmentation, adipose infiltration, heterogeneity, subepithelial condensation, nuclear pleomorphism

Tan *et al. Breast Cancer Research* (2016) 18:31
DOI 10.1186/s13058-016-0692-6

Breast Cancer Research

RESEARCH ARTICLE Open Access

A five-gene reverse transcription-PCR assay for pre-operative classification of breast fibroepithelial lesions

Wai Jin Tan[1], Igor Cima[1], Yukti Choudhury[1], Xiaona Wei[1], Jeffrey Chun Tatt Lim[2], Aye Aye Thike[2], Min-Han Tan[1] and Puay Hoon Tan[2,3*]

Methods: We profiled the transcriptome of a training set of 48 formalin-fixed, paraffin-embedded fibroadenomas and phyllodes tumors and further designed 43 quantitative polymerase chain reaction (qPCR) assays to verify differentially expressed genes. Using machine learning to build predictive regression models, we selected a five-gene transcript set (*ABCA8*, *APOD*, *CCL19*, *FN1*, and *PRAME*) to discriminate between fibroadenomas and phyllodes tumors. We validated our assay in an independent cohort of 230 core biopsies obtained pre-operatively.

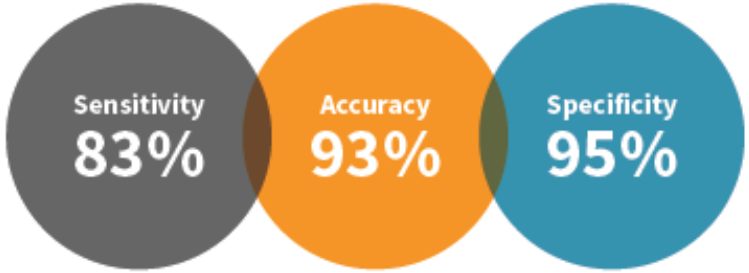
Results: Overall, the assay accurately classified 92.6 % of the samples (AUC = 0.948, 95 % CI 0.913–0.983, $p = 2.51E-19$), with a sensitivity of 82.9 % and specificity of 94.7 %.

FibroPhyllo™ Tissue Test

The performance of the FibroPhyllo™ Tissue Test in pre-operative classification of breast fibroepithelial lesions was validated in a cohort study of 230 core biopsies with at least 2 years of follow-up[5].

LUCENCE DIAGNOSTICS

TEST PERFORMANCE



TEST REQUIREMENTS

1) FFPE Tissues - Core / Excisional Biopsies
   - Minimum 50 microns equivalent (e.g. 10 slides of 5 micron-thickness or 5 slides of 10 micron-thickness)
   - Slides uncharged and uncoated

2) Matched H&E slide with tumor region marked out

3) Matched histology report of tissue biopsy

4) Tissue curls are not be accepted

TURNAROUND TIME

7 days

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LUCENCE DIAGNOSTICS

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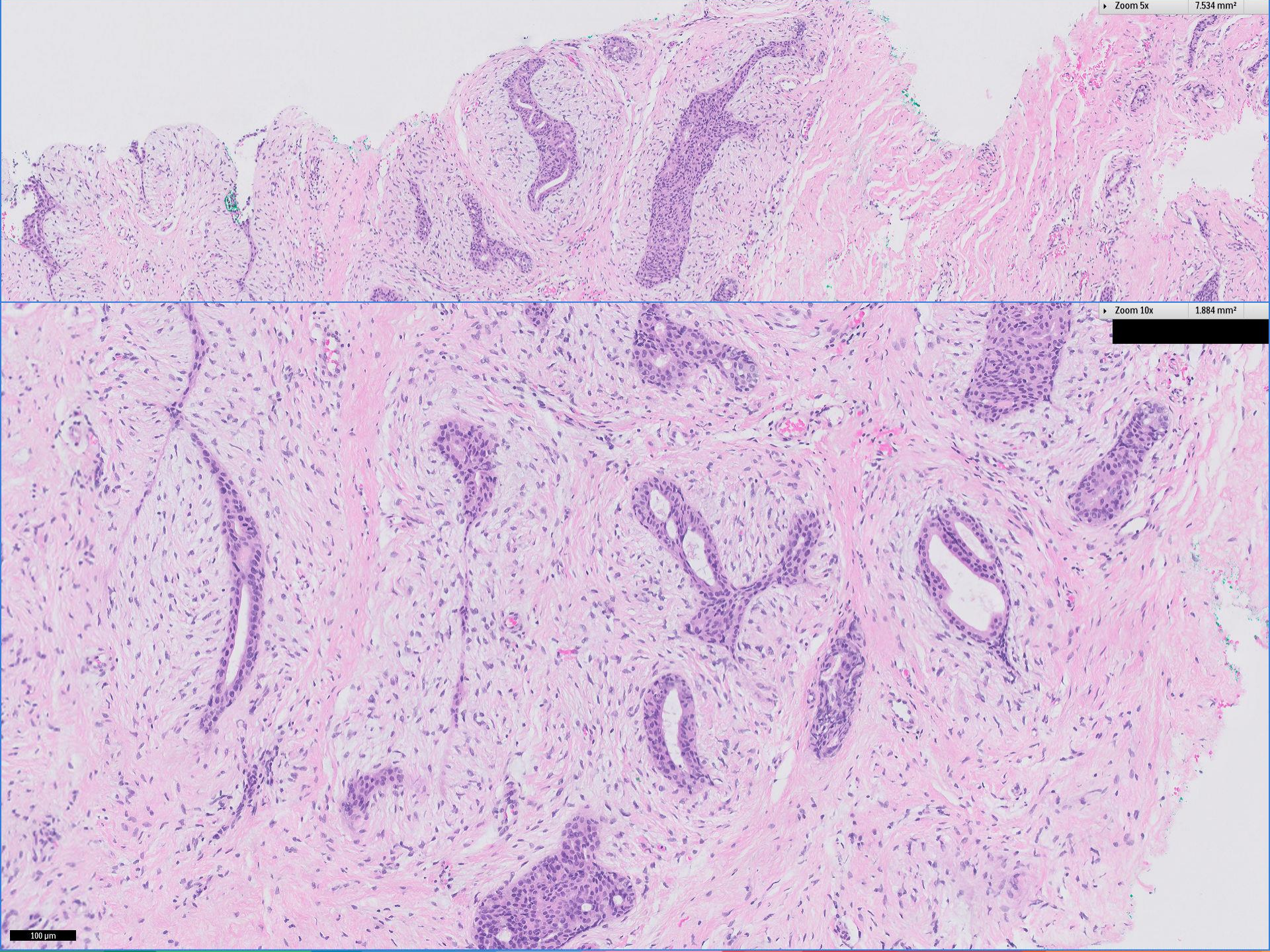
Dr Min-Han Tan, Founder & CEO, Lucence Diagnostics

Launched 31 October 2018


Zoom 5x
7.534 mm²
Zoom 10x
1.884 mm²
100 µm

TEST(S) PERFORMED

FIBROPHYLLO™ TISSUE ASSAY

GENES EVALUATED: Expression of 5 genes (*ABCA8, APOD, CCL19, FN1 and PRAME*) in FFPE breast fibroepithelial tissue

CLINICAL DIAGNOSIS

CELLULAR FIBROEPITHELIAL LESION. A PHYLLODES TUMOR CANNOT BE EXCLUDED

TREATMENT HISTORY

NONE

TEST INFORMATION

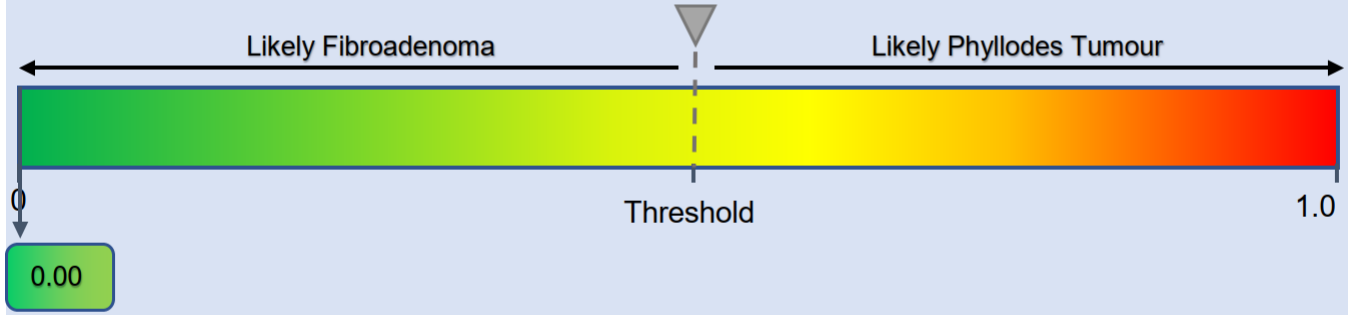
The FibroPhyllo™ Tissue assay is a multigene assay that predicts pre-operative breast fibroepithelial lesions as either fibroadenoma or phyllodes tumor.

SAMPLE INFORMATION

SPECIMEN TYPE: SLIDE
HISTOLOGY NUMBER:
TUMOR PERCENTAGE IN TISSUE : Tumor region marked out
TOTAL SURFACE AREA EXCISED : ~5.07 cm^2

RESULTS SUMMARY

FIBROPHYLLO™ score = 0.00



The FibroPhyllo™ Tissue assay uses quantitative real time-PCR to determine the expression of a panel of 5 genes in breast fibroepithelial tissues. A FibroPhyllo™ probability score (p-score) is calculated from the gene expression results using a validated prediction algorithm. P-score of 0.5 has been determined as a threshold for classification into likely fibroadenomas (≤ 0.5) and likely phyllodes tumor groups (≥ 0.5).

In a validation cohort of 230 pre-operative core biopsies (including biopsies with malignant phyllodes tumor), the FibroPhyllo™ Tissue assay was able to accurately predict classification of 213 (92.6%) biopsies[1]. Prediction accuracy rates for fibroadenomas and phyllodes tumors were 94.7% (179/189) and 82.9% (34/41) respectively with positive (PPV) and negative (NPV) prediction values of 77.3% and 96.2%[1].

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BMC Medical Genomics

RESEARCH ARTICLE Open Access

A novel genomic panel as an adjunctive diagnostic tool for the characterization and profiling of breast Fibroepithelial lesions

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- Targeted sequencing of a 16 gene panel on 275 formalin-fixed paraffin embedded fibroepithelial lesions.
- ***241 core biopsies*** and 34 surgical excisions ~ 212 FAs, 35 benign, 21 borderline and 7 malignant PTs.
- Mutations were observed in all 16 genes across FELs, except for a lack of *PTEN* mutations in FAs and an absence of *MAP3K1* and *IGF1R* mutations in PTs.
- Common to all grades of PTs were mutations in *MED12*, *TERT* promoter, *FLNA* and *RB1*.
- Predictive scoring system that classified FELs on core biopsy as low or high risk of being PTs ($p < 0.001$).

Table 8 The scorecard describing the weightage points of each predictor that was derived through their beta coefficients and the cut-off points required for a lesion to be classified as either a fibroadenoma or a phyllodes tumor

Predictors	Score
Genes	
Presence of mutations in TP53 gene	
Yes	1
No	0
Mutation types	
Presence of promoter mutation	
Yes	1.22
No	0
Presence of nonsense mutation	
Yes	1.14
No	0
Risk groups	
Low risk of being a phyllodes tumor	< 1
High risk of being a phyllodes tumor	≥ 1

Genomic predictive scoring system

ARTICLE OPEN

Phyllodes tumors with and without fibroadenoma-like areas display distinct genomic features and may evolve through distinct pathways

Fresia Pareja[1], Felipe C. Geyer[1], Rahul Kumar[1], Pier Selenica[1], Salvatore Piscuoglio[1,2], Charlotte K. Y. Ng[1,2,3], Kathleen A. Burke[1], Marcia Edelweiss[1], Melissa P. Murray[1], Edi Brogi[1], Britta Weigelt[1] and Jorge S. Reis-Filho[1]

npj Breast Cancer (2017)3:40

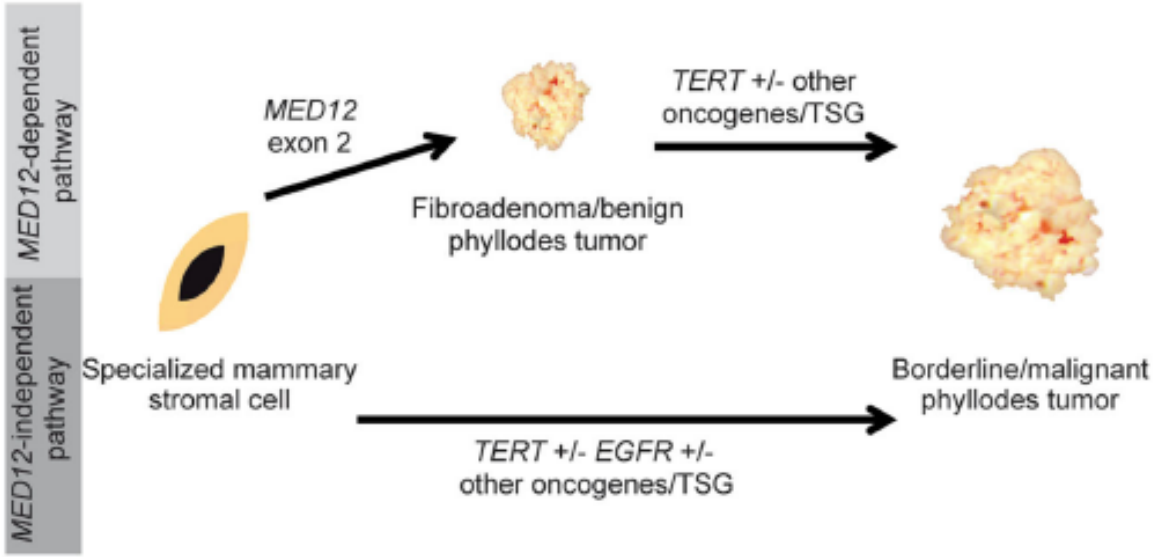

Fig. 4 Proposed model of the evolutionary origin of borderline and malignant phyllodes tumors. Phyllodes tumors might follow two different evolutionary pathways. (i) In the *MED12*-mutant pathway, *MED12* exon 2 mutations are posited to lead to the development of a benign fibroepithelial lesion, which upon the occurrence of additional genetic alterations affecting *TERT* and/ or other cancer genes may progress to a borderline or malignant phyllodes tumor. (ii) In the *MED12*-independent pathway, borderline or malignant phyllodes tumors might arise *de novo*, through the acquisition of genetic alterations targeting cancer genes, such as *TERT* and/ or *EGFR*. TSG tumor suppresor genes

Summary

- Genomics of fibroepithelial tumours ~ fibroadenoma, phyllodes tumour.
- Findings of the International Fibroepithelial Consortium study.
- Potential clinical applications.

Future work

- Non-MED12 mutated pathogenetic pathway.
- Malignant and metastatic phyllodes tumours.
- Malignant heterologous elements.
- Role of the epithelium, and epithelial-stromal interactions.

- *Enlarging the International Fibroepithelial Consortium (**IFC**)*
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- Dr Min-Han Tan
- Dr Mihir Gudi

Join the International Fibroepithelial Consortium

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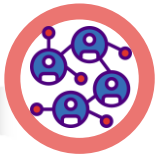

To build a network of pathologists interested in fibroepithelial tumours.

To increase the scientific knowledge on this fascinating group of tumours.

To collaborate on genomic research in fibroadenomas and phyllodes tumours.

To exchange interesting and challenging cases.

To make pathologist friends all over the world!

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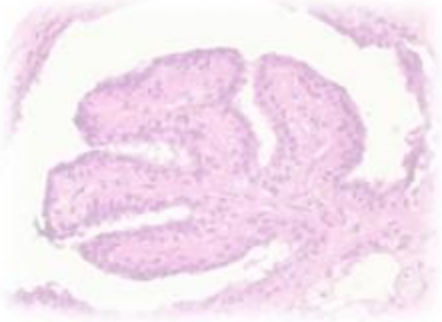
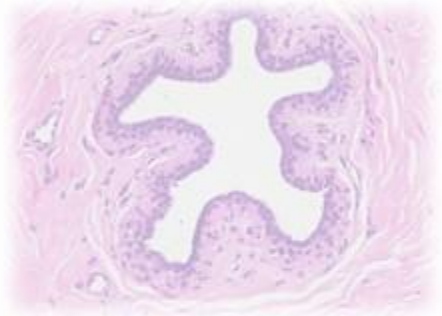
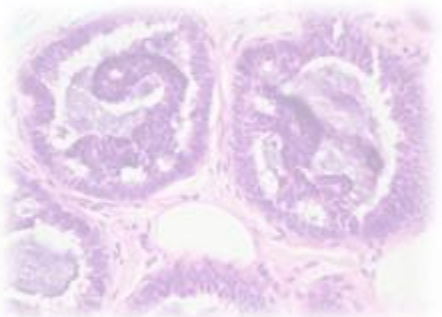
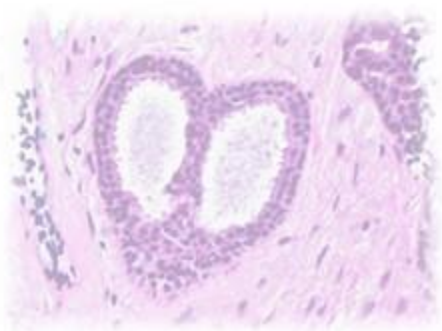
Thank you!









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