

Keywords: *TERT*; *MED12*; phyllodes tumor; fibroadenoma; breast

TERT promoter mutations are frequent and show association with *MED12* mutations in phyllodes tumors of the breast

Masayuki Yoshida¹, Reiko Ogawa², Hiroshi Yoshida¹, Akiko Maeshima¹, Yae Kanai², Takayuki Kinoshita³, Nobuyoshi Hiraoka^{1,2} and Shigeki Sekine^{*,1,2}

¹Division of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo 104-0045, Japan; ²Division of Molecular Pathology, National Cancer Center Research Institute, Tokyo 104-0045, Japan and ³Breast Surgery Division, National Cancer Center Hospital, Tokyo 104-0045, Japan

Background: Phyllodes tumors are rare fibroepithelial neoplasms of the breast, which carry the potential risk of local recurrence and metastasis. Phyllodes tumors share several histological features with fibroadenomas, and no widely accepted markers for distinguishing these lesions have been identified.

Methods: We analyzed molecular abnormalities related to telomere elongation in tumors, including *TERT* promoter mutations, as well as loss of expression of *ATRX* and *DAXX*, in a total of 104 phyllodes tumors and fibroadenomas.

Results: Sequencing analyses showed that *TERT* promoter mutations were frequent in phyllodes tumors (30/46, 65%), but rare in fibroadenomas (4/58, 7%). Among phyllodes tumors, the mutations were more frequent in borderline tumors (13/15, 87%), but were also common in benign (9/18, 50%) and malignant tumors (8/13, 62%). Remarkably, all but one *TERT* promoter-mutated tumor also contained *MED12* mutations, indicating that these mutations are strongly associated ($P=8.4 \times 10^{-6}$). Expression of *ATRX* and *DAXX*, as evaluated by immunohistochemistry, was retained in all tumors.

Conclusions: Our observations suggest a critical role of *TERT* promoter mutations, in cooperation with *MED12* mutations, in the development of phyllodes tumors. Because *TERT* promoter mutations are rare among fibroadenomas, their detection may be of potential use in discriminating between phyllodes tumors and fibroadenomas.

Fibroadenomas and phyllodes tumors constitute the major histological types of fibroepithelial tumors of the breast (Brogi, 2009; Tavassoli and Eusebi, 2009; Tan *et al*, 2012). Fibroadenomas are one of the most common benign breast neoplasms, mainly affecting young adults. Phyllodes tumors are rare, accounting for <1% of all breast tumors, and are associated with a risk of local recurrence and metastasis. Despite the distinct clinical behavior of these tumors, there is significant overlap in their morphology, particularly between benign phyllodes tumors and intracanalicular-type fibroadenomas, and biopsy diagnosis of these lesions is often challenging (Tsang *et al*, 2011; Yasir *et al*, 2014).

Recent studies have begun to address the genetic backgrounds of fibroepithelial tumors of the breast. We and other researchers previously reported that *MED12* mutations are very common in both phyllodes tumors and fibroadenomas (Lim *et al*, 2014; Cani *et al*, 2015; Yoshida *et al*, 2015). The identification of a common genetic alteration in these tumors implies a histogenetic relationship. Malignant phyllodes tumors have also been reported to be associated with aberrations in several classic oncogenes and tumor-suppressor genes, including *TP53*, *RB1*, *CDKN2A* and *EGFR* (Kersting *et al*, 2006; Jones *et al*, 2008; Cani *et al*, 2015). However, despite their different biological behavior, no molecular

*Correspondence: S Sekine; E-mail: ssekine@ncc.go.jp

Received 28 April 2015; revised 2 August 2015; accepted 4 August 2015; published online 10 September 2015

© 2015 Cancer Research UK. All rights reserved 0007–0920/15

abnormalities that distinguish benign and borderline phyllodes tumors from fibroadenomas have been reported.

Telomeres are repetitive nucleotide sequences at the end of chromosomes that are required for chromosomal integrity (Aubert and Lansdorf, 2008). In the absence of telomerase activity, telomeres shorten with each cell division, eventually leading to senescence or cell death. Therefore, tumor cells require a mechanism to maintain telomere length to acquire immortality. Most commonly, tumor cells exhibit the increased telomerase activity, which synthesizes telomeric DNA sequences (Kim *et al*, 1994; Shay and Bacchetti, 1997). *TERT* encodes the catalytic subunit of telomerase, which is a major determinant of telomerase activity (Aubert and Lansdorf, 2008). Its transcription is repressed in the most quiescent somatic cells but is activated in the majority of malignant tumors (Shay and Roninson, 2004). Recent studies have shown that mutations in the *TERT* promoter are responsible for overexpression of *TERT* in a range of tumors, including melanomas, hepatocellular carcinomas and urothelial carcinomas (Horn *et al*, 2013; Huang *et al*, 2013; Killela *et al*, 2013; Heidenreich *et al*, 2014). Another subset of tumors uses a different mechanism for telomere maintenance, which is known as the alternative lengthening of telomeres (ALT; Cesare and Reddel, 2010). Interestingly, loss of ATRX and DAXX, both of which are involved in chromatin remodeling, has been shown to be closely associated with the ALT phenotype in several types of tumors, including pancreatic neuroendocrine tumors, glioblastomas and uterine leiomyosarcomas (Cesare and Reddel, 2010; Liau *et al*, 2015).

Although previous studies have reported telomerase activity in a small number of phyllodes tumors and fibroadenomas (Hiyama *et al*, 1996; Mokbel *et al*, 1999), the mechanisms underlying telomere maintenance in these tumors have not been previously examined. In the present study, we analyzed the molecular abnormalities involved in telomere elongation, including *TERT* promoter mutations, as well as loss of expression of ATRX and DAXX, in a series of phyllodes tumors and fibroadenomas.

MATERIALS AND METHODS

Tissue samples. This study was approved by the ethics committee of the National Cancer Center, Tokyo, Japan. All the tissue samples were obtained from the National Cancer Center Hospital, Tokyo, Japan. The resected specimens were routinely fixed in 10% formalin and embedded in paraffin. This study examined 46 phyllodes tumors and 58 fibroadenomas that had previously been analyzed to determine their *MED12* mutation status (Yoshida *et al*, 2015). The phyllodes tumors were subclassified according to the WHO classification as benign, borderline or malignant lesions (Tan *et al*, 2012). Fibroadenomas were classified as intracanalicular-type, pericanalicular-type and complex-type (Tan *et al*, 2012).

Mutational analysis. Ten-micrometer-thick sections of paraffin-embedded specimens were deparaffinized, stained briefly with hematoxylin and subjected to DNA extraction. The lesions were microdissected using sterilized toothpicks under a microscope. The dissected samples were incubated in DNA extraction buffer at 50 °C overnight, heated at 100 °C for 10 min to inactivate proteinase K, and then directly subjected to PCR using a pair of primers encompassing the core promoter region of *TERT* (5'-CAGCGCTGCCTGAACTC-3' and 5'-GTCCTGCCCTTCA CCTT-3') as described previously (Horn *et al*, 2013; Yoshida *et al*, 2015). The PCR products were purified and sequenced bidirectionally using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster, CA, USA). All mutations were confirmed by re-analysis of the respective specimens, including DNA extraction.

A microdissection-based analysis was performed in selected cases with a *TERT* promoter mutation. The epithelial and stromal components were microdissected separately using a laser microdissection system (MMI CellCut system; Molecular Machines and Industries, Glattbrugg, Switzerland). The isolated samples were then examined for the presence of *TERT* promoter mutations, as described above.

Histology and immunohistochemistry. Histological analysis was performed in accordance with WHO classification (Tan *et al*, 2012). Two pathologists (MY and SS) independently scored each case for tumor border, stromal cellularity, stromal atypia, mitosis, stromal overgrowth and malignant heterologous component (Supplementary Table 1). Mitotic counts were quantified per 10 high-power fields with the field size adjusted to 0.196 mm². Discrepant cases were reviewed together to achieve consensus.

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded specimens. Antigen retrieval was performed by autoclaving in a 10 mM citrate buffer (pH 6.0) for 10 min. Anti-ATRX (HPA001906; 1:200 dilution; Sigma-Aldrich, St. Louis, MO, USA) and anti-DAXX (HPA008736; 1:250 dilution; Sigma-Aldrich) antibodies were used as the primary antibodies (Heaphy *et al*, 2011). For staining, we used an automated stainer (Dako, Glostrup, Denmark), according to the manufacturer's protocol. ChemMate EnVision (Dako) methods were used for detection. Tumors showing uniformly negative staining were regarded as showing loss of expression, using lymphocytes and endothelial cells as internal positive controls.

Statistical analysis. Fisher's exact test or an extended Fisher's exact test was used to analyze categorical variables. A Mann-Whitney's *U*-test was used for analysis of continuous variables. *P*-values < 0.05 were considered to indicate statistical significance.

RESULTS

Sequencing analyses revealed *TERT* promoter mutations in 30 of 46 phyllodes tumors (65%) and in 4 of 58 fibroadenomas (7%; Figure 1A, Table 1, Supplementary Table 1), indicating a substantial difference in these two tumor types ($P = 1.5 \times 10^{-10}$). Remarkably, all but one of the *TERT* promoter-mutated tumors concurrently harbored *MED12* mutations ($P = 8.4 \times 10^{-6}$). Non-tumor tissue specimens were available for 17 of the phyllodes tumors and three of the fibroadenomas with *TERT* promoter mutations, and sequencing analyses of these samples did not identify any mutations, indicating the somatic nature of *TERT* promoter mutations.

Among the phyllodes tumors, the presence of *TERT* promoter mutations was significantly associated with older age, but not with tumor size (Table 2). Analysis of tumor grade showed that borderline tumors more frequently possessed mutations, but the difference was not significant when the three different tumor grades were compared. The association with *MED12* mutations was significant among phyllodes tumors. Histological evaluation showed that *TERT* promoter mutational status was significantly correlated with stromal cellularity, but not with the other morphological features (Table 2, Supplementary Table 1). In addition, when a comparison was made among benign phyllodes tumors, lesions with a *TERT* promoter mutation tended to show an edematous stroma, compared with *TERT* mutation-negative tumors, which showed a more fibrous stroma (Figure 1A and B).

All four fibroadenomas with *TERT* promoter mutations, including two intracanalicular-type and two pericanalicular-type lesions, also possessed *MED12* mutations (Supplementary Table 1). Histological re-examination confirmed the original diagnosis of the respective tumors, but we were unable to identify any distinct morphological features that distinguished them from *TERT* promoter mutation-negative tumors (Figure 1C and D).

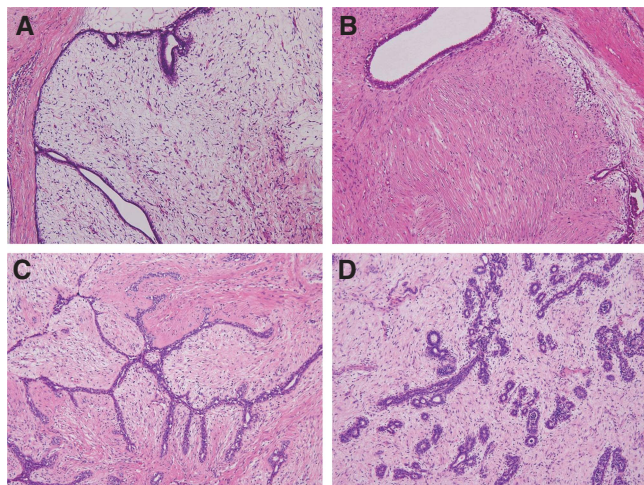


Figure 1. Histology of phyllodes tumors and fibroadenomas. (A) Benign phyllodes tumor with a *TERT* promoter mutation. A distended and compressed duct lined by epithelium and expansion of moderately cellular and edematous stroma. (B) Benign phyllodes tumor without *TERT* promoter mutation. Compressed and partly dilated ducts and relatively fibrous stroma. (C) Intracanalicular-type fibroadenoma with a *TERT* mutation. Slit-like ducts compressed by proliferating stroma. (D) Pericanalicular-type fibroadenoma. Tubular ducts surrounded by edematous stroma.

Table 1. *TERT* promoter mutations in phyllodes tumors and fibroadenomas of the breast

Histology	Number of cases	Total mutated	Nucleotide change	Cases mutated
Phyllodes tumor	46	30 (65%)		
Benign	18	9 (50%)	c.-146C>T c.-124C>T c.-124C>A	3 5 1
Borderline	15	13 (87%)	c.-124C>T	13
Malignant	13	8 (62%)	c.-146C>T c.-124C>T	1 7
Fibroadenoma	58	4 (7%)		
Intracanalicular	32	2 (6%)	c.-124C>T	2
Pericanalicular	20	2 (10%)	c.-124C>T	2
Complex	6	0		

We performed a microdissection-based analysis of five phyllodes tumors and two fibroadenomas with *TERT* promoter mutations, to examine whether the epithelial component also harbored a *TERT* promoter mutation. The epithelial and stromal components were separately microdissected and subjected to sequencing analyses. The results showed that the *TERT* promoter mutations were only present in the stromal components, in all cases (Figure 2B).

Immunohistochemical analysis showed diffuse nuclear expression of ATRX and DAXX in all phyllodes tumors and fibroadenomas (Figure 3A and B), suggesting that inactivation of these factors, which is related to the ALT phenotype, does not play a major role in the tumorigenesis of fibroepithelial tumors of the breast.

DISCUSSION

The present study showed that *TERT* promoter mutations are frequent in phyllodes tumors, regardless of tumor grade. *TERT* promoter mutations are the second most frequent genetic aberration reported to date in phyllodes tumors, following *MED12* mutations, which were identified in 80% of cases in the

Table 2. Correlation between *TERT* promoter mutation status and clinicopathological variables in phyllodes tumors of the breast

	<i>TERT</i> promoter mutation		P-value
	Present	Absent	
Age, year-old	54.5 (34–67)	42.5 (26–58)	0.0051
Tumor size (mm)	50 (10–130)	42 (12–160)	0.39
Grade			
Benign	9	9	0.086
Borderline	13	2	
Malignant	8	5	
Tumor border			
Well-defined	16	9	1.0
Permeative ^a	14	7	
Stromal cellularity			
Low	7	9	0.049
Moderate-to-high	23	7	
Stromal atypia			
None or mild	12	10	0.22
Moderate-to-marked	18	6	
Mitosis/10 HPFs			
0–4	18	10	1.0
≤5	12	6	
Stromal overgrowth			
Absent	18	9	1.0
Present ^b	12	7	
Malignant heterologous component			
Absent	29	14	0.27
Present	1	2	
MED12 mutation			
Absent	1	8	3.6 × 10 ⁻⁴
Present	29	8	

Abbreviation: HPF, high-power field. Age and tumor size are indicated as the median (range).
^aIncludes lesions with focally permeative borders.
^bIncludes lesions with focal stromal overgrowth.

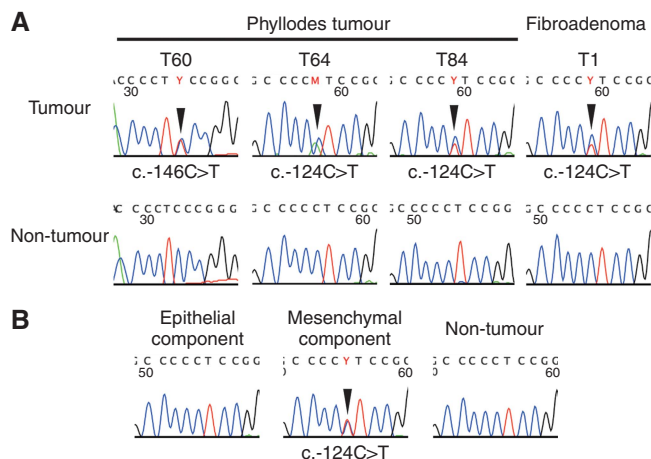


Figure 2. *TERT* promoter mutations in phyllodes tumors and a fibroadenoma. (A) *TERT* promoter mutations in phyllodes tumors and a fibroadenoma. (B) Microdissection-based analysis of *TERT* promoter mutations. Epithelial and stromal components of phyllodes tumors or fibroadenomas were separately microdissected and analyzed for the presence of *TERT* promoter mutations. The arrowheads indicate nucleotide substitutions.

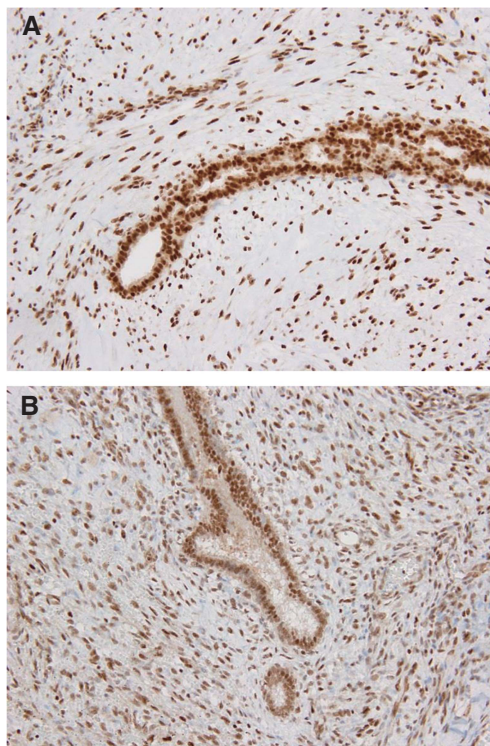


Figure 3. ATRX and DAXX expression in phyllodes tumors. **(A)** ATRX expression in a borderline phyllodes tumor. Diffuse nuclear expression of ATRX in both epithelial and stromal cells. **(B)** DAXX expression in a borderline phyllodes tumor. Nuclear DAXX expression is retained in both epithelial and stromal components.

present cohort (Yoshida *et al*, 2015). These mutations have previously been reported in various tumors and have been shown to enhance promoter activity (Horn *et al*, 2013; Huang *et al*, 2013; Killela *et al*, 2013). Interestingly, *TERT* promoter mutations were identified almost exclusively in tumors with a *MED12* mutation, indicating the synergistic roles of these two genetic abnormalities. *TERT* promoter mutation was also closely associated with older age. A similar association has been reported in thyroid carcinomas (Liu *et al*, 2014). As the telomere shortens with age, *TERT* promoter mutations have been suggested to have more major roles in preventing crisis caused by telomere shortening when oncogenic events take place in aged cells.

In contrast, none of the tumors showed loss of ATRX or DAXX, which are involved in ALT (Heaphy *et al*, 2011; Lovejoy *et al*, 2012). These observations suggest that the activation of telomerase by *TERT* promoter mutations plays a major role in telomere maintenance in phyllodes tumors.

The prevalence of *TERT* promoter mutations in malignant phyllodes tumors was lower than that in borderline tumors. This may simply be a bias due to the limited number of cases; alternatively, this observation might imply that a subset of malignant phyllodes tumors is directly derived from *TERT* mutation-negative precursors, rather than via stepwise progression from borderline tumors. Previous studies have shown that abnormal expression of some tumor-suppressor gene products, including p16, p53 and Rb, is almost exclusively observed in malignant phyllodes tumors (Tse *et al*, 2002; Tan *et al*, 2005; Cimino-Mathews *et al*, 2013). It seems possible that the acquisition of these molecular abnormalities is sufficient to gain malignant phenotypes, even in the absence of *TERT* promoter mutations.

TERT promoter mutations were rare among fibroadenomas. Considering that *MED12* mutations were commonly detected in both fibroadenomas and phyllodes tumors, the difference in the

prevalence of *TERT* promoter mutations may potentially explain the differences in the biological behavior of these tumors. The growth of fibroadenomas is usually self-limited, and they often undergo involution (Tavassoli and Eusebi, 2009; Tan *et al*, 2012). Conceivably, the lack of telomerase activity in fibroadenomas may eventually lead to senescence and/or cell death of tumor cells and prevent continuous growth. On the other hand, continuous growth and local recurrence appear to reflect the immortality of tumor cells, which require a mechanism for telomere elongation, in phyllodes tumors.

The presence of *TERT* promoter mutations in a small subset of fibroadenomas is of potential interest. Although these included two pericanalicular-type fibroadenomas, which are morphologically dissimilar to phyllodes tumors, some of these lesions may represent early-stage phyllodes tumors. Our review failed to identify morphological features specific to *TERT* promoter-mutated fibroadenomas. In addition, *TERT* promoter mutations do not appear to be associated with older age or larger tumor size in fibroadenomas. However, this could be owing to the limited number of cases analyzed, and future studies may determine the clinicopathological significance of *TERT* promoter mutations in fibroadenomas.

Microdissection-based analysis showed that *TERT* promoter mutations were only present in the stromal component, and not in the epithelial component, in both phyllodes tumors and fibroadenomas. This observation is consistent with observations of *MED12* mutations in previous studies (Lim *et al*, 2014; Yoshida *et al*, 2015), and supports the idea that stromal cells are the principal neoplastic component of these fibroepithelial tumors. However, the consistent presence of epithelial components in these tumors implies the requirement for epithelium-derived factors in the proliferation of tumoral stromal cells, as previously suggested (Sawhney *et al*, 1992; Sawyer *et al*, 2002). Phyllodes tumors and fibroadenomas may be developed through interactions between neoplastic stroma and reactive epithelium.

The clinical management of phyllodes tumors and fibroadenomas differs significantly. Because of their self-limited nature, many fibroadenomas are treated by simple excision; follow-up alone, without surgical intervention, is sometimes a valid option (Cant *et al*, 1995; Brogi, 2009). However, phyllodes tumors need to be removed with sufficient margins, to reduce the risk of recurrence (Chen *et al*, 2005; Brogi, 2009). Therefore, preoperative diagnosis has a significant impact on the determination of appropriate clinical management. However, distinction of phyllodes tumors from fibroadenomas is often challenging (Tsang *et al*, 2011; Yasir *et al*, 2014), and there are no reliable molecular markers are currently available to distinguish between these tumors. On the basis of the difference in prevalence observed between these two types of tumors, *TERT* promoter mutations might be of potential use as an ancillary diagnostic tool.

ACKNOWLEDGEMENTS

We are grateful to Ms. Sachiko Miura, Ms. Toshiko Sakaguchi and Ms. Chizu Kina for technical assistance and to Taichi Shimazu for helpful comments on the statistical analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Aubert G, Lansdorp PM (2008) Telomeres and aging. *Physiol Rev* **88**(2): 557–579.

- Brogi E (2009) Fibroepithelial Neoplasms. In: Hoda SA, Brogi E, Koerner FC, Rosen PR (eds) 4th edn. *Rosen's breast pathology*. pp 213–270. Lippincott Williams and Wilkins: Philadelphia, PA, USA.
- Cani AK, Hovelson DH, McDaniel AS, Sadis S, Haller MJ, Yadati V, Amin AM, Bratley J, Bandla S, Williams PD, Rhodes K, Liu CJ, Quist MJ, Rhodes DR, Grasso CS, Kleer CG, Tomlins SA (2015) Next-gen sequencing exposes frequent MED12 mutations and actionable therapeutic targets in phyllodes tumors. *Mol Cancer Res* **13**(4): 613–619.
- Cant PJ, Madden MV, Coleman MG, Dent DM (1995) Non-operative management of breast masses diagnosed as fibroadenoma. *Br J Surg* **82**(6): 792–794.
- Cesare AJ, Reddel RR (2010) Alternative lengthening of telomeres: models, mechanisms and implications. *Nat Rev Genet* **11**(5): 319–330.
- Chen WH, Cheng SP, Tzen CY, Yang TL, Jeng KS, Liu CL, Liu TP (2005) Surgical treatment of phyllodes tumors of the breast: retrospective review of 172 cases. *J Surg Oncol* **91**(3): 185–194.
- Cimino-Mathews A, Hicks JL, Sharma R, Vang R, Illei PB, De Marzo A, Emens LA, Argani P (2013) A subset of malignant phyllodes tumors harbors alterations in the Rb/p16 pathway. *Hum Pathol* **44**(11): 2494–2500.
- Heaphy CM, de Wilde RF, Jiao Y, Klein AP, Edil BH, Shi C, Bettgowda C, Rodriguez FJ, Eberhart CG, Hebbar S, Offerhaus GJ, McLendon R, Rasheed BA, He Y, Yan H, Bigner DD, Oba-Shinjo SM, Marie SK, Riggins GJ, Kinzler KW, Vogelstein B, Hruban RH, Maitra A, Papadopoulos N, Meeker AK (2011) Altered telomeres in tumors with ATRX and DAXX mutations. *Science* **333**(6041): 425.
- Heidenreich B, Rachakonda PS, Hemminki K, Kumar R (2014) TERT promoter mutations in cancer development. *Curr Opin Genet Dev* **24**: 30–37.
- Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, Hiyama K, Piatyszek MA, Shay JW (1996) Telomerase activity in human breast tumors. *J Natl Cancer Inst* **88**(2): 116–122.
- Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, Schandendorf D, Kumar R (2013) TERT promoter mutations in familial and sporadic melanoma. *Science* **339**(6122): 959–961.
- Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA (2013) Highly recurrent TERT promoter mutations in human melanoma. *Science* **339**(6122): 957–959.
- Jones AM, Mitter R, Springall R, Graham T, Winter E, Gillett C, Hanby AM, Tomlinson IP, Sawyer EJ. Phyllodes Tumour C (2008) A comprehensive genetic profile of phyllodes tumours of the breast detects important mutations, intra-tumoral genetic heterogeneity and new genetic changes on recurrence. *J Pathol* **214**(5): 533–544.
- Kersting C, Kuijper A, Schmidt H, Packeisen J, Liedtke C, Tidow N, Gustmann C, Hinrichs B, Wulfing P, Tio J, Boecker W, van Diest P, Brandt B, Buerger H (2006) Amplifications of the epidermal growth factor receptor gene (egfr) are common in phyllodes tumors of the breast and are associated with tumor progression. *Lab Invest* **86**(1): 54–61.
- Killela PJ, Reitman ZJ, Jiao Y, Bettgowda C, Agrawal N, Diaz Jr LA, Friedman AH, Friedman H, Gallia GL, Giovannella BC, Grollman AP, He TC, He Y, Hruban RH, Jallo GI, Mandahl N, Meeker AK, Mertens F, Netto GJ, Rasheed BA, Riggins GJ, Rosenquist TA, Schiffman M, Shih IeM, Theodorescu D, Torbenson MS, Velculescu VE, Wang TL, Wentzensen N, Wood LD, Zhang M, McLendon RE, Bigner DD, Kinzler KW, Vogelstein B, Papadopoulos N, Yan H (2013) TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc Natl Acad Sci USA* **110**(15): 6021–6026.
- Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. *Science* **266**(5193): 2011–2015.
- Liau JY, Tsai JH, Jeng YM, Lee JC, Hsu HH, Yang CY (2015) Leiomyosarcoma with alternative lengthening of telomeres is associated with aggressive histologic features, loss of ATRX expression, and poor clinical outcome. *Am J Surg Pathol* **39**(2): 236–244.
- Lim WK, Ong CK, Tan J, Thihe AA, Ng CC, Rajasegaran V, Myint SS, Nagarajan S, Nasir ND, McPherson JR, Cutcutache I, Poore G, Tay ST, Ooi WS, Tan VK, Hartman M, Ong KW, Tan BK, Rozen SG, Tan PH, Tan P, Teh BT (2014) Exome sequencing identifies highly recurrent MED12 somatic mutations in breast fibroadenoma. *Nat Genet* **46**(8): 877–880.
- Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, Larsson C, Xu D (2014) The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. *Oncogene* **33**(42): 4978–4984.
- Lovejoy CA, Li W, Reisenweber S, Thongthip S, Bruno J, de Lange T, De S, Petrini JH, Sung PA, Jasin M, Rosenbluh J, Zwang Y, Weir BA, Hatton C, Ivanova E, Macconail L, Hanna M, Hahn WC, Lue NF, Reddel RR, Jiao Y, Kinzler K, Vogelstein B, Papadopoulos N, Meeker AK. Consortium ALTSC (2012) Loss of ATRX, genome instability, and an altered DNA damage response are hallmarks of the alternative lengthening of telomeres pathway. *PLoS Genet* **8**(7): e1002772.
- Mokbel K, Ghilchik M, Parris CN, Newbold RF (1999) Telomerase activity in phyllodes tumours. *Eur J Surg Oncol* **25**(4): 352–355.
- Sawhney N, Garrahan N, Douglas-Jones AG, Williams ED (1992) Epithelial-stromal interactions in tumors. A morphologic study of fibroepithelial tumors of the breast. *Cancer* **70**(8): 2115–2120.
- Sawyer EJ, Hanby AM, Rowan AJ, Gillett CE, Thomas RE, Poulson R, Lakhani SR, Ellis IO, Ellis P, Tomlinson IP (2002) The Wnt pathway, epithelial-stromal interactions, and malignant progression in phyllodes tumours. *J Pathol* **196**(4): 437–444.
- Shay JW, Bacchetti S (1997) A survey of telomerase activity in human cancer. *Eur J Cancer* **33**(5): 787–791.
- Shay JW, Roninson IB (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. *Oncogene* **23**(16): 2919–2933.
- Tan PH, Jayabaskar T, Yip G, Tan Y, Hilmy M, Selvarajan S, Bay BH (2005) p53 and c-kit (CD117) protein expression as prognostic indicators in breast phyllodes tumors: a tissue microarray study. *Mod Pathol* **18**(12): 1527–1534.
- Tan PH, Tse G, Lee A, Simpson JF, Hanby AM (2012) Fibroepithelial tumours. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ (eds) *WHO Classification of Tumours of the Breast*. pp 142–147. IARC Press: Lyon, France.
- Tavassoli FA, Eusebi V (2009) Biphasic tumors. In: Tavassoli FA, Eusebi V (eds) vol 10, *Afip Atlas Of Tumor Pathology*. American Registry of Pathology: Washington, DC, USA, pp 315–340.
- Tsang AK, Chan SK, Lam CC, Lui PC, Chau HH, Tan PH, Tse GM (2011) Phyllodes tumours of the breast—differentiating features in core needle biopsy. *Histopathology* **59**(4): 600–608.
- Tse GM, Putti TC, Kung FY, Scolyer RA, Law BK, Lau TS, Lee CS (2002) Increased p53 protein expression in malignant mammary phyllodes tumors. *Mod Pathol* **15**(7): 734–740.
- Yasir S, Gamez R, Jenkins S, Visscher DW, Nassar A (2014) Significant histologic features differentiating cellular fibroadenoma from phyllodes tumor on core needle biopsy specimens. *Am J Clin Pathol* **142**(3): 362–369.
- Yoshida M, Sekine S, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, Ochiai A (2015) Frequent MED12 mutations in phyllodes tumors of the breast. *Br J Cancer* **112**(10): 1703–1708.

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 4.0 Unported License.

Supplementary Information accompanies this paper on British Journal of Cancer website (<http://www.nature.com/bjc>)